Annals of Arid Zone 47(2): 177-184, 2008

Influence of Pre- and Post-Drought Application of Thiourea on Growth, Net Photosynthesis and Nitrogen Metabolism of Clusterbean

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Abstract: Clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.) plants, grown with and without thiourea (seed treatment with 500 ppm + one foliar spray with 1000 ppm of thiourea at 28 days after sowing), were subjected to moderate (5 days drought) and severe water stress (10 days drought) at the critical pre-flowering stage (40 DAS) where well-watered plants served as control. After 4 days of rewatering one set of plants under each treatment was sprayed with 1000 ppm thiourea and another with water, to study the post-drought effects of thiourea application. Thiourea pre-plants maintained higher relative water content, net photosynthesis, levels of different leaf metabolites and nitrate reductase (NR) activity than control plants under all intensities of water stress. The recovery of all the above-mentioned parameters on rewatering was also better in thiourea-than control plants. Furthermore, post-drought foliar spray of thiourea resulted in higher levels of total chlorophyll, starch, soluble protein, NR activity and net photosynthesis as compared to water- sprayed plants one week after their application. This led to significantly higher seed yield and shoot dry matter production in thiourea-applied plants even after the drought event. Results also revealed that pre- and post-drought application of thiourea displayed additive effects on plant performance and yield due to cumulative favorable effects on leaf metabolism and photosynthesis.

Key words: Clusterbean, thiourea, foliar spray, net photosynthetic rate, nitrate reductase, water stress.

Clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.), a drought-hardy crop, is widely grown in arid and semi-arid regions receiving an annual rainfall of 200-600 mm. Growth and yield of the crop is, however, limited due to low precipitation and recurring drought under rainfed conditions of western Rajasthan, India (Garg and Burman, 2002). Thiourea, a sulphydral compound (NH_2-CS-NH_2) known for breaking dormancy and stimulating germination, has also been reported to significantly improve growth and yield of wheat (Sahu and Singh, 1995) and pearl millet (Parihar *et al.,* 1998) under arid and semi-arid conditions. As clusterbean often experiences drought under arid conditions, the need was felt to explore the possibility of drought amelioration through thiourea application. Garg *et al.* (2006) recently reported that seed treatment with thiourea followed by foliar spray could significantly increase growth, seed yield and water use efficiency of rainfed clusterbean due to enhanced photosynthesis and more efficient nitrogen metabolism. However, the merit of thiourea application after experience of drought by crops has not been investigated so far. Therefore, the present study was undertaken to explore the possibility of pre- and post-drought application of thiourea for yield improvement of clusterbean under different intensities of water stress and to understand the associated physiological processes.

Materials and Methods

Clusterbean (*Cyamopsis tetragonoloba* (L.) Taub. cv. RGC-936) plants were grown in earthen pots containing 10 kg loamy sand soil (Typic Camborthids having 7.1% clay, 5.6% silt, 63.1% fine sand and 24.1% coarse sand) having 0.28% organic carbon, 0. 023% total nitrogen, 80 kg ha^{-1} available N, 12 kg ha^{-1} available P and 120 kg ha^{-1} available K. Before sowing, seeds were soaked for 4 h in either distilled water (T_o) or in 500 ppm solution of thiourea (T_1) and dried in the shade for 1 h. Two uniform plants were maintained in each pot. After 28 days of sowing, T_1 plants were given a foliar spray of 1000 ppm thiourea while T_0 plants received a foliar spray of water. Plants under each set were subjected to drought at the pre-flowering stage (40 DAS) by withholding of irrigation for 0 (D_0-control), 5 (D_1-moderate stress) and 10 days (D_2-severe stress). Thus there were six treatments with 40 pots under each treatment. Just prior to the termination of stress at the pre-flowering stage (on 50^{th} day after sowing) and 2 days after rewatering, observations were recorded, in quadruplicate, on plant water potential (ψ_{plant}) using Pressure Chamber (PMS Instrument Company, USA) and relative water content (RWC) of leaves as per standard procedure (Slatyer and Mcllroy, 1961). At the same time net photosynthetic rate (P_N) and stomatal conductance (CS) was measured in two uppermost fully expanded leaves of four plants under each treatment between 10.00 to 11.00 hours using LICOR-6200 portable photosynthetic system. Two uppermost fully expanded leaves were also analyzed for the contents of total chlorophyll (Arnon, 1949), starch (Yemm and Willis, 1954), soluble protein (Lowry *et al.,* 1951), free amino acids (Yemm and Cocking, 1955) and nitrate reductase activity (Jaworski, 1971) just before termination of water stress and 2 days after rewatering.

To study the effects of post-drought foliar application of thiourea, one set of plants under each of the above six treatments was sprayed with 1000 ppm thiourea solution (F_1) and another set with water (F_o) four days after re-watering of water stressed plants (i.e., on the 54^{th} day of sowing). All the above-mentioned measurements and estimations were made in each of the 12 treatments, seven days after the foliar spray of thiourea or water. Data on seed yield and dry matter of shoot were recorded at harvest from eight pots (replicates) for each treatment. The significance of the data was adjudged through analysis of variance adopting factorial design.

Results and Discussion

Increasing water stress significantly and progressively decreased plant water potential and relative water content (Table 1) as well as rate of net photosynthesis and stomatal conductance (Table 1). However, at all intensities of water stress, plants with thiourea prior to water stress (pre-drought treatment) maintained higher RWC, P_N and CS. Plants with thiourea on an average maintained 8.1% higher RWC and 24.5% higher net photosynthesis over unplants. Furthermore, higher RWC, net photo- synthetic rate and stomatal conductance two days after

Table 1. Influence of thiourea on plant water potential and relative leaf water content, net photosynthetic rate and stomatal conductance, nitrate reductase activity, chlorophyll, starch, and soluble protein contents conductance of clusterbean under control (D_0), moderate (D_1) and severe (D_2) levels of stress and 48 h after rewatering

Drought intensity	Stressed		Rewatered		Stressed		Rewatered	
	Control	Thiourea	Control	Thiourea	Control	Thiourea	Control	Thiourea
	ψ (-MPa)				RWC (%)			
Control (D_0)	1.2	1.1	1.0	0.9	88.0	94.0	87.2	88.2
Moderate (D_1)	2.2	2.2	1.2	1.4	66.2	71.6	82.4	83.4
Severe (D_2)	2.9	3.0	1.7	1.6	30.6	37.9	70.4	78.2
Mean	2.1	2.1	1.3	1.3	61.6	66.6	80.0	82.3
LSD (P=0.05)	D	0.1	D	0.1	D	2.1	D	1.6
	TH	NS	TH	NS	TH	1.8	TH	1.3
	DxTH	NS	DxTH	NS	DxTH	3.0	DxTH	2.2
	Net photosynthetic rate (μmol m^{-2} s^{-1})				Stomatal conductance (cm s^{-1})			
Control (D_0)	12.50	15.20	13.15	15.30	1.16	1.04	1.24	1.20
Moderate (D_1)	4.28	5.29	12.08	13.78	0.27	0.29	1.02	1.12
Severe (D_2)	1.50	2.32	9.50	11.40	0.10	0.12	0.86	0.96
Mean	6.10	7.60	11.58	13.49	0.51	0.48	1.04	1.09
LSD (P=0.05)	D	0.83	D	0.65	D	0.04	D	0.06
	TH	0.68	TH	0.54	TH	NS	TH	0.04
	DxTH	1.18	DxTH	NS	DxTH	0.05	DxTH	0.08
	Chlorophyll (mg g^{-1} dw)				Starch (mg g^{-1} dw)			
Control (D_0)	8.17	8.48	7.73	8.13	46.6	47.7	44.1	46.1
Moderate (D_1)	7.18	7.84	7.44	7.94	26.3	28.8	42.4	42.9
Severe (D_2)	4.70	5.58	5.48	6.33	12.6	14.0	21.4	29.7
Mean	6.68	7.29	6.88	7.46	28.5	29.8	35.9	39.5
LSD (P=0.05)	D	0.34	D	0.21	D	1.9	D	2.4
	TH	0.17	TH	0.17	TH	1.5	TH	2.0
	DxTH	0.48	DxTH	0.29	DxTH	2.7	DxTH	3.4
	Nitrate reductase activity (μg NO_2 g^{-1} dw h^{-1})				Soluble protein (mg g^{-1} dw)			
Control (D_0)	791.3	832.7	715.7	839.0	96.9	102.2	96.5	102.2
Moderate (D_1)	219.0	264.8	532.3	623.3	72.8	75.4	94.7	98.9
Severe (D_2)	26.7	40.7	400.9	562.7	41.6	50.2	68.2	80.7
Mean	352.3	376.1	549.6	675.0	70.4	74.9	86.4	93.4
LSD (P=0.05)	D	17.1	D	55.7	D	2.9	D	2.1
	TH	13.9	TH	45.5	TH	2.4	TH	1.7
	DxTH	24.2	DxTH	NS	DxTH	4.1	DxTH	3.0

rewatering at all stress levels in thiourea-as compared to control plants revealed better recovery from drought in thiourea-plants (Table 1). For instance net photosynthetic rate was 16.5% higher in thiourea-as compared to control plants and similar trend

Table 2. Influence of post-drought foliar application of thiourea on net photosynthetic rate and contents of chlorophyll and starch in leaves of clusterbean plants

Treatment	Drought intensity	Net photosynthetic rate (μmol m^{-2} s^{-1})		Total chlorophyll (mg g^{-1} dw)		Starch (mg g^{-1} dw)	
		F_0	F_1	F_0	F_1	F0	F_1
Control plants (T_0)	D_0	10.02	10.70	7.25	7.41	33.4	36.1
	D_1	9.64	9.98	6.95	7.43	32.4	35.0
	D_2	9.28	9.60	6.86	7.40	26.1	28.0
	Mean	9.65	10.09	7.02	7.41	30.6	33.0
Thiourea-plants (T_1)	D_0	11.02	11.50	7.30	7.42	33.3	36.1
	D_1	9.96	10.46	7.16	7.36	32.7	35.7
	D_2	9.46	9.85	7.07	7.29	29.6	32.8
	Mean	10.15	10.60	7.18	7.36	31.9	34.9
	Overall mean	9.90	10.35	7.10	7.39	31.2	33.9
LSD (P=0.05)	Drought	0.38		NS		1.01	
	Thiourea	0.31		NS		0.89	
	Foliar spray	0.31		0.18		0.89	

F_0-Foliar spray of water, F_1-Foliar spray of thiourea (1000 ppm).

was observed for stomatal conductance and RWC. Thiourea application has been reported to maintain favorable plant water status and to significantly enhance net photosynthesis in clusterbean under water stress (Burman *et al.*, 2004). Maintenance of high leaf water potential, RWC and photosynthetic rate under soil moisture deficit has been reported to be associated with better drought tolerance in sorghum (Tsuji *et al.*, 2003), possibly due to increased activities of antioxidant enzymes under water stress, as reported in wheat (Nathawat *et al.,* 2007)

Total chlorophyll and starch (Table 1) as well as soluble protein and nitrate reductase (NR) activity (Table 1) significantly decreased due to water stress in control as well as thiourea-plants. However, thiourea-plants maintained higher contents of leaf metabolites and NR activity compared to control plants, irrespective of water stress intensity. This indicated that thiourea helped the plants to maintain better metabolic status despite water stress, possibly due to antioxidant protection as mentioned earlier (Nathawat *et al.*, 2007). This trend was further obvious from the fact that recovery upon rewatering in all metabolic parameters was also more in thiourea-as compared to control plants since these plants were exposed to a less intense stress. Thiourea has been known to suppress the speed of chlorophyll decrease during senescence (Liu *et al.*, 2002) that may be responsible for maintenance of higher chlorophyll concentration as reported in wheat (Sahu and Singh, 1995) and clusterbean (Burman *et al.*, 2004). This appears logical because thiourea exhibits cytokinin like activity (Vassilev and Mashev, 1974) that is known to delay leaf senescence (Gzik *et al.*, 1987). Furthermore, close linkage between cellular SH content and ADP glucose starch synthetase as observed by Pande *et al.* (1983) might also favor starch synthesis in thiourea-plants. Besides favorably modulating the carbohydrate metabolism, thiourea has been reported to significantly increase nitrate reductase activity and concentration of soluble protein, indicating its positive role in enhancing nitrogen metabolism (Burman *et al.*, 2004; Garg *et al.*, 2006).

Post-drought application of thiourea (1000 ppm) as foliar spray (F_1) to water-stressed clusterbean plants significantly affected leaf metabolism and photosynthetic rate as compared to control plants that were sprayed with only water (F_0). Post-drought thiourea spray increased net photosynthetic rate in both T_0 and T_1 compared to water sprayed clusterbean plants (Table 2). Similarly, total chlorophyll and starch contents were significantly higher in plants sprayed with thiourea as compared with water spray after re-watering, irrespective of drought intensity experienced earlier. Thus, clusterbean plants with thiourea at both pre- and post-drought stages recorded maximum net photosynthetic activity, probably due to high chlorophyll content that was also reflected in high starch content (Table 2). The results, thus, indicate that thiourea application, even after the drought event, was beneficial in crop recovery from water stress. Post-drought thiourea application, on an average, enhanced net photosynthesis by 4.5%, chlorophyll content by 4.1% and starch content by 8.6% over simple water application (Table 2). It appears that thiourea helps plants to maintain photosynthetic efficiency and delay chlorophyll degradation, besides providing antioxidant protection and stabilizing protein structure as reported by earlier investigators (Deneke, 2000; Garg *et al.*, 2006).

The above mentioned contention is further supported by the data recorded on nitrate reductase activity and soluble protein concentration, one week after the foliar spray (Table 3). Post-drought thiourea spray (F_1) increased NR activity by 3.8% and 4.5% in water and thiourea pre-plants, respectively, over water-sprayed plants (F_0) after drought. The maintenance of higher NR activity in thiourea-sprayed plants even at the belated stage is a pointer towards

Table 3. Influence of post-drought foliar application of thiourea on nitrate reductase activity and contents of soluble protein and free amino acids in leaves of clusterbean plants

Treatment	Drought intensity	Nitrate reductase ($\mu gNO_2\ g^{-1}$ dw h^{-1})		Soluble protein (mg g^{-1} dw)		Free amino acids (mg g^{-1} dw)	
		F_0	F_1	F_0	F_1	F0	F_1
Control plants (T_0)	D_0	550.4	562.4	38.4	40.8	5.5	6.4
	D_1	543.9	564.9	38.3	42.6	6.6	7.0
	D_2	532.1	560.4	34.2	36.6	6.9	7.2
	Mean	542.1	562.2	37.0	40.0	6.3	6.9
Thiourea-plants (T_1)	D_0	556.9	576.8	39.2	41.2	5.4	5.7
	D_1	549.9	573.4	39.2	42.6	5.5	5.9
	D_2	536.4	567.0	38.6	40.2	6.2	6.8
	Mean	547.7	572.4	39.0	41.3	5.7	6.1
	Overall mean	544.9	567.5	38.0	40.7	6.0	6.5
LSD (P=0.05)	Drought	19.9		1.8		0.3	
	Thiourea	NS		1.4		0.2	
	Foliar spray	16.2		1.4		0.2	

F_0-Foliar spray of water, F_1-Foliar spray of thiourea (1000 ppm).

higher nitrogen assimilation. Further, both pre- and post-drought application of thiourea resulted in maximum increase in nitrate reductase activity. This might have contributed towards higher concentrations of soluble protein and free amino acids in the thiourea-sprayed plants observed in the present study (Table 3). On an average soluble protein concentration increased by 7.1% and that of free amino acids by 8.3% over the water-spray, suggesting a beneficial role of thiourea on nitrogen metabolism of water stressed plants. The maintenance of higher NR activity and protein concentration under thiourea application indicates efficient N metabolism as plant N balance, growth and NR activity were closely related in wheat plants (El-Komy *et al.*, 2003).

It is noteworthy that application of thiourea before the imposition of water stress (as seed treatment+foliar application at 28 DAS) and after the termination of drought had additive beneficial effects on overall carbohydrate and nitrogen metabolism (Tables 5 and 6). This gain was ultimately reflected in significant improvement of seed yield and dry matter production. On an average either pre- or post-drought application of thiourea enhanced seed yield by 17.8% over control whereas both pre- and post-drought thiourea application increased seed yield to the extent of 30.4% over control (Table 4), notwithstanding a decreased seed yield with increasing drought intensity in all cases. Similarly shoot dry matter increased to the extent of 20.3% both by pre- and post-drought thiourea application over no application, whereas pre- or post-drought treatment alone could increase DMP by 12.4% and 5.8%, respectively, indicating synergistic effects of thiourea application before and after the drought event. Better performance of thiourea-plants, in spite of experiencing moisture stress of varying intensity, could be due to stabilization of lipoprotein structure in thiourea-plants due

Table 4. Influence of pre- and post-drought application of thiourea on seed yield and shoot dry weight of clusterbean under different intensities of water stress

Treatment	Drought intensity	Seed yield (g plant^{-1})		Dry weight shoot (g plant^{-1})	
		F_0	F_1	F_0	F_1
Control plants (T_0)	D_0	3.25	3.80	15.96	16.67
	D_1	2.83	3.25	14.08	14.96
	D_2	1.34	1.59	7.35	7.91
	Mean	2.47	2.91	12.45	13.18
Thiourea-plants (T_1)	D_0	4.13	4.51	17.24	18.61
	D_1	3.17	3.43	15.33	16.38
	D_2	1.44	1.73	9.43	9.95
	Mean	2.91	3.22	14.00	14.98
	Overall mean	2.69	3.07	13.30	14.08
LSD (P=0.05)	Drought	0.17		0.61	
	Thiourea	0.14		0.50	
	Foliar spray	0.14		0.50	

F_0-Foliar spray of water, F_1-Foliar spray of thiourea (1000 ppm).

to less malondialdelyde production compared to control (Mojtaba and Karr, 2001). As some enzymes related to carbohydrate, nitrogen and protein metabolism are embedded in lipoprotein membranes (Benson and Jokela, 1976), its stable structure in thiourea-plants restricts lipid peroxidation and thus account for better plant performance to some extent.

The merit of pre-drought thiourea application is well documented earlier, but the present study revealed that both pre- and post-drought thiourea application gave the best response. Furthermore, thiourea application even after drought event significantly improved seed yield. Thus, a resource-deficient farmer of the arid zone (where rain events are unpredictable) may achieve good recovery and seed yield in clusterbean by a foliar spray of thiourea (1000 ppm) after the drought.

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