Interactive effects of thiourea and phosphorus on clusterbean under water stress

U. BURMAN*, B.K. GARG and S. KATHJU

Division of Soil-Water-Plant Relationship, Central Arid Zone Research Institute, Jodhpur-342003, India

Abstract

Effects of phosphorus and thiourea application (either alone or in combination) were studied on clusterbean (*Cyamopsis tetragonoloba* Taub.) plants subjected to water stress by withholding irrigation at pre- and post-flowering stages in pot culture trial. Water stress significantly decreased shoot water potential, relative water content of leaves, net photosynthetic rate, contents of total chlorophyll, starch and soluble proteins as well as nitrate reductase activity at both the growth stages. Application of phosphorus and thiourea or combined application increased most of these parameters. Results revealed synergistic effects of P and thiourea in enhancing net photosynthesis, leaf area, chlorophyll content and nitrogen metabolism leading to significant improvement in plant growth and seed yield under water stress condition.

Additional key words: Cyamopsis tetragonoloba, nitrate reductase, photosynthetic rate, plant water status, yield.

Introduction

Clusterbean (*Cyamopsis tetragonoloba* Taub.), a drought resistant crop, is widely grown under rainfed conditions in western Rajasthan, in India, though growth and yield is often restricted due to low and erratic rainfall and poor soil fertility conditions (Garg and Burman 2002). Thiourea has been reported to significantly improve growth, yield and water use efficiency of wheat (Sahu and Singh 1995), pearl millet (Parihar *et al.* 1998) and clusterbean (Garg *et al.* 2003) under arid and semi arid conditions. Similarly, phosphorus application is reported to increase drought tolerance in a number of crops (Singh *et al.* 1997, Gutierrez-Boem and Thomas 1998). However, information on the combined application of P and thiourea on clusterbean is lacking. Therefore, the present investigation was undertaken to study the interaction of P nutrition and thiourea application on water relations, photosynthesis and nitrogen metabolism of clusterbean under simulated water stress conditions.

Materials and methods

The 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). The soil contained 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 either soaked for 4 h in distilled water (T_0) or in 500 µg g^{-1} thiourea (T_1) and dried in the shade for 1 h. The seeds were sown in pots containing 0 and 40 kg P ha^{-1}, designated as P_0 and P_{40} applied through super phosphate single (as 16 % P_2O_5) at the time of sowing. The plants were given a foliar spray of water (T_0) or 1000 µg g^{-1} thiourea (T_1) at 25 and 40 d after sowing (DAS).

One set of plants under each of the above four treatments (P_0T_0, P_0T_1, $P_{40}T_0$ and $P_{40}T_1$) were subjected to two cycles water stress by withholding irrigation for 8 d

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Abbreviations: Chl - chlorophyll; d.m. - dry matter; NR - nitrate reductase; P_N - net photosynthetic rate; RWC - relative water content; ψ_w - shoot water potential.
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* Fax: (+91) 0291 2740706, e-mail: uburman@cazri.raj.nic.in

at 30 DAS (pre-flowering) and at 45 DAS (post flowering). Another set of well- watered plants served as control. There were 8 treatments with 25 replicates (pots) under each treatment.

Just prior to the termination of water stress at the pre-flowering (38th day after sowing) and post-flowering (53rd day after sowing) stages, observations were recorded, in quadruplicate, on shoot water potential using Pressure Chamber (*PMS Instrument Company*, Oregon, USA) and relative water content of leaves as per standard procedure (Slatyer and Mcllroy 1961). At the same time net photosynthetic rate was measured in two upper most fully expanded leaves using *LICOR-6200* (Lincoln, USA) portable photosynthetic system, while leaf area was measured using *LICOR-3000* leaf area meter. These measurements were made on four plants under each treatment between 10:00 to 11:00. Just before rewatering of the water stressed plants at both the growth stages, two upper most fully expanded leaves of 8 plants (from 4 pots), under each treatment, were also analyzed for total chlorophyll (Arnon 1949), soluble protein (Lowry *et al.* 1951), free amino acids (Yemm and Cocking 1955) contents and nitrate reductase activity (Jaworski 1971). Data on seed yield and dry matter of shoot were recorded at harvest from 8 pots for each treatment. The significance of the data was adjudged through analysis of variance adopting completely randomized factorial design.

Results and discussion

Plant water status: Water stress, irrespective of P and thiourea application, significantly decreased plant water potential (ψ_w) and relative water content (RWC) at both the growth stages (Table 1). This decrease was marginally higher in P and thiourea treated plants probably due to higher leaf area development under these treatments. More reduction in ψ_w and RWC in fertilized than unfertilized plants under water limiting conditions has also been reported in cotton (Radin and Parker 1979) and pearl millet (Kathju *et al.* 2001).

Photosynthesis and leaf area development: Water stress drastically reduced the net photosynthetic rate (P_N) at both the growth stages and this reduction was 77.1 % at the pre-flowering and 72.1 % at the post-flowering stage (Table 2). P application, however, significantly increased P_N in water stressed plants by 26.7 % at pre-flowering and 27.2 % at post-flowering stage. Evidences in the literature suggest that photosynthetic rate of water stressed plants was more at high than at low P levels (Sharma 1995, Chaudhary and Fujita 1998). Similarly thiourea application increased P_N to the extent of 28.2 to 16.3 %. Thiourea application has also been reported to significantly enhance P_N in clusterbean under rainfed conditions (Garg *et al.* 2003). However, combined application of P and thiourea increased P_N by 62.2 % at the pre-flowering and 45.8 % at the post-flowering stage in water stressed plants. This indicates that application of P and thiourea has positive and synergetic effect in alleviating water stress induced decrease in photosynthesis. Increase in P_N due to thiourea and P was, however, less pronounced in case of control plants. Association of drought tolerance with maintainance of high leaf water potential, relative water content and photosynthetic rate in *Sorghum* have been reported earlier (Tsuji *et al.* 2003).

Leaf area was reduced by 42.8 % at pre-flowering and 35.5 % at the post-flowering stage under drought. This adverse effect on leaf growth was considerably overcome by P and thiourea application, more so when used in

Table 1. Effects of water stress (D), P and thiourea (TH) application on shoot water potential (ψ_w) and relative water content (RWC) of clusterbean at pre-flowering and post-flowering stages.

Treatments		ψ_w [-MPa] pre-flowering		post-flowering		RWC [%] pre-flowering		post-flowering	
		P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}
Control	–TH	1.30	1.36	1.35	1.40	84.0	82.8	81.3	78.2
	+TH	1.30	1.40	1.40	1.65	84.2	81.6	79.5	78.0
Drought	–TH	3.10	3.30	3.40	3.60	55.7	53.3	52.9	50.7
	+TH	3.55	3.65	3.50	3.66	52.4	50.8	49.6	48.2
$LSD_{0.05}$ D			0.13		0.11		1.3		7.5
$LSD_{0.05}$ P			0.13		0.11		1.3		NS
$LSD_{0.05}$ TH			0.13		0.11		1.3		NS
D × P			NS		NS		NS		NS
D ×TH			NS		0.15		1.9		NS
P × TH			NS		NS		NS		NS

Table 2. Effects of water stress (D), P and thiourea (TH) application on leaf area, total chlorophyll and net photosynthetic rate of clusterbean at pre-flowering and post-flowering stages.

Treatments		Leaf area [cm^2 $plant^{-1}$]				Chlorophyll [mg g^{-1} (d.m.)]				P_N [µmol m^{-2} s^{-1}]			
		pre-flowering		post-flowering		pre-flowering		post-flowering		pre-flowering		post flowering	
		P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}
Control	–TH	119.2	137.5	234.7	264.8	7.59	9.04	7.39	7.90	11.46	12.44	9.34	10.76
	+TH	136.6	158.1	262.8	289.0	8.40	9.52	7.63	8.32	12.58	13.62	11.20	11.92
Drought	–TH	66.3	76.9	143.1	167.7	6.38	7.69	6.36	7.15	2.22	2.80	2.64	3.12
	+TH	78.6	93.7	169.4	198.7	7.03	7.97	6.67	7.69	2.83	3.60	2.84	3.85
$LSD_{0.05}$ D			6.4		13.1		0.24		0.17		0.25		0.65
$LSD_{0.05}$ P			6.4		13.1		0.24		0.17		0.25		0.65
$LSD_{0.05}$ TH			6.4		13.1		0.24		0.17		0.25		0.65
D × P			NS		NS		0.34		0.24		NS		NS
D ×TH			NS		NS		NS		NS		0.35		NS
P × TH			NS		NS		0.34		NS		NS		NS

combination as leaf area of water stressed plants increased to the extent of 41.3 and 38.8 % at pre-flowering and post-flowering stages, respectively. The role of P and thiourea in increasing the leaf area is well documented (Sahu and Solanki 1991, Gutierrez-Boem and Thomas 1998, Garg *et al.* 2003), but the present study indicates their additive or synergistic interactive effects, particularly under water stress conditions. Water stress also decreased the chlorophyll (Chl) content by 15.8 to 10.7 % at pre- and post-flowering stages, respectively (Table 2). P application, however, increased Chl content more at the pre-flowering stage (16 %). Thiourea marginally (6 - 7 %) increased Chl content. Combined application of P and thiourea, however, showed a synergistic effect (Table 2). Sharma (1995) reported a favourable influence of P nutrition on chlorophyll concentration and photosynthesis in mulberry plants. Likewise, increased Chl content due to thiourea application has been reported in several crops including clusterbean (Garg *et al.* 2003). Maintenance of higher Chl content may delay leaf ageing and senescence, a role already attributed to thiourea by earlier investigators (Sahu and Singh 1995) as it exhibits cytokinin like activity (Vassilev and Mashev 1974). Positive influence of thiourea on P_N in the present study is also pointer towards its cytokinin like activity similar to the effect of N_6-benzyladenine observed in water stressed bean and sugarbeet plants (Rulcová and Pospíšilová 2001, Vomáčka and Pospíšilová 2003).

Nitrogen metabolism: A marked reduction in the activity of nitrate reductase (NR), a key enzyme for nitrate reduction in higher plants, was observed under water stress. (Table 3). The reduction was more than 75 % at both the growth stages indicating higher sensitivity of NR to water deficits as reported earlier (Garg *et al.* 2001). Combined application of both P and thiourea increased enzyme activity by 113.3 % at pre-flowering and 88.0 %

Table 3. Effects of water stress (D), P and thiourea (TH) application on soluble protein, free amino acids and nitrate reductase activity of clusterbean at pre-flowering and post-flowering stages.

Treatments		Soluble protein [mg g^{-1} (d.m.)]				Free amino acids [mg g^{-1} (d.m.)]				Nitrate reductase activity [µg(NO_2) g^{-1}(d.m.) s^{-1}]			
		pre-flowering		post-flowering		pre-flowering		post-flowering		pre-flowering		post-flowering	
		P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}	P_0	P_{40}
Control	–TH	27.96	34.49	45.06	58.82	9.13	11.47	4.08	4.84	0.083	0.100	0.111	0.127
	+TH	38.62	44.82	47.12	59.69	13.08	13.45	4.67	5.20	0.092	0.112	0.123	0.137
Drought	–TH	13.57	16.39	34.79	45.47	12.05	11.92	9.75	9.90	0.014	0.026	0.022	0.029
	+TH	21.90	24.43	37.73	46.92	13.81	13.71	8.71	8.91	0.020	0.030	0.026	0.041
$LSD_{0.05}$ D			0.64		1.03		0.40		0.40		0.007		0.003
$LSD_{0.05}$ P			0.64		1.03		0.40		0.40		0.007		0.003
$LSD_{0.05}$ TH			0.64		1.03		0.40		NS		0.007		0.003
D × P			0.90		1.46		0.56		NS		NS		NS
D ×TH			0.90		NS		0.56		0.57		NS		NS
P × TH			NS		NS		0.56		NS		NS		NS

at the post-flowering stages in water stressed plants. The maintenance of higher NR activity under P and thiourea application indicates efficient N metabolism in treated plants as plant N balance, growth and NR activity are closely related (El-Komy *et al.* 2003). Positive relationship between P, plant growth and N metabolism in bean plants (Al-Karaki *et al.* 1996) further strengthens the view that in legumes role of phosphorus nutrition is critical. This contention was further supported by the data on soluble protein and free amino acid contents (Table 3). The water stress induced decrease in soluble protein was comparatively less in P supplied plants than those grown without P application. Furthermore, thiourea application also enhanced concentration of soluble protein in both control and water stressed plants though the effects were more pronounced at the pre-flowering stage. When thiourea and P were applied together there was a synergistic effect, especially in the water stressed plants where soluble protein increased by 80 % at the pre-flowering and 34.8 % at the post-flowering stage. The data on free amino acids further indicate that application of thiourea with or without P application overcame the adverse effects of water stress and decreased the accumulation of free amino acids. Though favourable influence of P or thiourea application on protein concentration has been reported (Azcon *et al.* 1996, Garg *et al.* 2003) but the present study suggests that their combined application could also promote N metabolism in a synergistic manner under water stress conditions.

Plant performance: Water stress significantly decreased seed yield and shoot dry mass at both the P contents, irrespective of thiourea treatment (Table 4). On an average the reduction due to water stress was 27.5 % in seed yield and 30.2 % in shoot dry mass. However, the decline in seed yield was consistently less in P treated plants as compared to those grown without P application.

Table 4. Influence of phosphorus and thiourea (TH) on seed yield and shoot dry matter of clusterbean under water stress (D).

Treatments		Seed yield [g plant^{-1}] P_0	P_{40}	Shoot d.m.[g plant^{-1}] P_0	P_{40}
Control	−TH	3.71	4.38	11.41	13.20
	+TH	4.46	4.91	12.72	13.94
Drought	−TH	2.65	3.15	7.77	9.02
	+TH	3.28	3.42	9.01	9.92
$LSD_{0.05}$ D			0.27		0.54
$LSD_{0.05}$ P			0.27		0.54
$LSD_{0.05}$ TH			0.27		0.54
D × P			NS		NS
D ×TH			NS		NS
P × TH			NS		NS

Likewise the application of thiourea increased the seed yield of both control and water stressed plants but more so in the latter. The combined application of P and thiourea showed synergistic effects as seed yield and shoot dry mass increased by 34.2 and 27.3 %, respectively, in case of water stressed plants. The results thus indicate that both P nutrition and thiourea application had a significant and favourable influence on plant growth and yield under drought conditions. Number of reports indicates that P nutrition under water deficits increased drought resistance and improved growth and yield of plants (Gutierrez-Boem and Thomas 1998, 1999, Singh and Sale 2000). Thiourea induced increase in seed yield due to better partitioning of photosynthates and increased harvest index has also been reported earlier (Sahu and Solanki 1991, Parihar *et al.* 1998, Garg *et al.* 2003). Evidences presented here indicated that favourable effects of both thiourea and P application could be synergistically realized by their combined application under water-limited conditions.

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