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# Phosphorus, a potential nutrient for alleviating water deficit stress in clusterbean( *Cyamopsistetragonoloba*L.Taub)

Shubhra Barwa<sup>1</sup>, Balaram Pani<sup>2</sup> and Leena Shakya<sup>3</sup>

<sup>1</sup>Assistant Professor, Department of Biomedical sciences, Bhaskaracharya College of Applied Sciences, University of Delhi, India

<sup>2</sup>Associate Professor, Department of Chemistry, Bhaskaracharya College of Applied Sciences, University of Delhi, India

<sup>3</sup> Assistant Professor, Department of Botany, Kirorimal College, University of Delhi, India

\*corresponding author: [leena.shakya@gmail.com](mailto:leena.shakya@gmail.com)

**ABSTRACT:** Clusterbean (*C. tetragonoloba* L. Taub) locally called Guar, is an annual summer legume grown as a important forage crop. The crop has ability to recover from water stress. Clusterbean cultivar HG 365 was used for the present study. Three level of phosphorus were supplied (i.e 15, 30 and 60 mg/kg of the soil in two split doses at weekly intervals in the form of  $\text{KH}_2\text{PO}_4$  one week after sowing of the crop. Water stress was created by withholding irrigation at different growth stages of the crop. Plants were sampled at permanent wilting point (PWP) with soil moisture content  $3.5 (\pm 0.5)$ . Water stress decreased stem height (14-20%) at all the stages of growth. Stem height improved with increasing level of phosphorus however higher level of phosphorus (P2 and P3) increased height significantly in control as well as stressed plants at all three growth stages. Water deficit decreased RWC from 85.1 % (control) to 69.1% (stress) as well as leaf area (24-27%) relative to control at all stages of the crop, vegetative stage being the highly sensitive stage. Significant decline in the chlorophyll content was also observed at all the growth stages of the crop under stress relative to control plants. Phosphorus application improved the chlorophyll content (3-8%) relative to untreated control.

**KEYWORDS:** water deficit stress, phosphorus nutrition, clusterbean, photosynthesis, leaf area

### I. INTRODUCTION

Clusterbean (*C. tetragonoloba* L. Taub) locally called Guar, is an annual summer legume grown as a important forage crop, as a green manure and as a vegetable for human consumption. The word guar represents its derivation from the Sanskrit word “GAU AAHAR” which means cow fodder or fodder for the livestock. The crop is also known by the local names as Khutti, Dararietic, Guari, etc. Basically it is a drought resistant leguminous crop of the arid zone. Agro-ecological conditions of India and Pakistan are ideal for guar cultivation but it can also be grown successfully in USA, South Africa, Brazil, Australia, Zaire and Sudan. Being a leguminous crop it adds to the fertility of the soil by fixing atmospheric nitrogen (Iver Jacobsen, 1985). Guar seed has attained an important place in industry because of its Galactomannan rich endosperm which constitutes guar gum and forms 25-35% of whole seed (Bhadoria and Tomar, 1997). Guar gum became commercially important substitute of locust bean gum during World War 2 (Joshi, 2004). The hull and the germ portion of guar seed is termed Guar meal and is a rich source of proteins. The economic importance of Guar seeds is more because of seed quality rather than seed quantity. Guar gum finds international market for its ever increasing demand in industries like Textiles, paper, food processing, cosmetics, mining, pharmaceuticals, petroleum and oil industry, photography and refining. India earns foreign exchange worth more than Rs 20 crore

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through the export of guar gum only. Guar gum has a medicinal value in treatment for diabetes and cholesterol cases (Arora et al, 1998). A further improvement in seed quality will be a future deciding fate of this crop and gum industry. In face of global scarcity of water resources, abiotic stress as water deficit is a major limiting factor for plant growth and is likely to become more severe as desertification covers more and more of the world's terrestrial area (Delmer 2005). The primary symptom of water deficit is the inhibition of shoot growth and to lesser extent root growth, stomatal closure followed by reduction in transpiration, CO<sub>2</sub> uptake for photosynthesis, uptake of mineral ions and their translocation, interrupted reproductive development, premature leaf senescence, wilting and under severe conditions desiccation and death of the plant (Achard et al, 2006). Plant adapt to water deficit at the cellular level by accumulating excess of osmolytes and at the molecular level by synthesizing proteins involved in stress tolerance (Yamaguchi-Shinozaki and Shinozaki, 2007). Phosphorus is an important plant nutrient essential for plant growth, development and protein synthesis. It plays an important role in combating water deficit stress. In cluster bean phosphorus application lead to efficient nodulation (Lynd and Ansman, 1989), root development (Singh et al, 2002), growth, leaf area index and nutrient uptake (Tiwana and Tiwana, 1994). It also improved the yield attributing parameters as plant height, number of branches, pods/plant, podlength and test weight significantly. It may be attributed to phosphorus induced plant growth and efficient nutrient translocation to potential sinks.

Our limited knowledge of the stress associated metabolism is still a major gap in understanding stress tolerance. Thus a comprehensive profiling of stress associated metabolism and the efficiency of phosphorus as a nutrient for combating the water stress conditions was the main objective of the present investigation.

## II.MATERIAL AND METHODS

In the present study seeds of clusterbean cultivar HG 365 were taken and surface sterilized with 1% sodium hypochlorite solution and then inoculated with mixed rhizobium culture. Seeds were sown under natural conditions in the screen house in earthen pots filled with 5 kg of dune sand. In each pot five seeds were sown and after germination only two healthy plants were retained. Irrigation was given as and when required. Each pot was supplied with nitrogen free nutrient solution (Wilson and Reisenauer, 1963) at fortnightly intervals. Plant protection measures were taken by spraying 0.02% malathion at regular intervals. Three level of phosphorus were supplied (i.e 15, 30 and 60 mg/kg of the soil in two split doses at weekly intervals in the form of KH<sub>2</sub>PO<sub>4</sub> one week after sowing of the crop. Equal concentration of potassium was maintained in control and phosphorus treated plants by adding K<sub>2</sub>SO<sub>4</sub> and CH<sub>3</sub>COOK in 1:1 ratio. Water stress was created by withholding irrigation at different growth stages of the crop. Plants were sampled at permanent wilting point (PWP) with soil moisture content 3.5 (±0.5). For each sampling three replicates were taken. Leaf area was determined using portable leaf area meter (Model Li 3000 LICOR, USA). Relative water content (RWC) was determined by the method of Wetherley, 1950. Total chlorophyll content, soluble sugars and free amino acids were determined as per the method given by Hiscox and Israelstam, 1979; Dubois et al, 1956; and Yemm and Cocking, 1955 respectively. All the biochemical parameters were measured in third fully expanded leaf from the top. Gum content, a very important attribute of the seed quality was determined by using method of Das et al, 1977. All the parameters were statistically analyzed using Complete randomized design (CRD) and critical difference was calculated at 5% level of significance.

## III.RESULTS

One of the important indicator of healthy vegetative growth, stem height increased progressively with the age of the plant. Water stress decreased stem height (14-20%) at all the stages of growth both in untreated as well phosphorus treated plants but decrease was significant only with P3 level of the phosphorus at vegetative stage relative to control plants. Stem height improved with increasing level of phosphorus however P2 and P3 level of phosphorus increased height significantly in control as well as stressed plants at all three growth stages (Table 1). Among the different growth stages, highest RWC was observed at vegetative and lowest at pod-filling stage. RWC decreased significantly under water deficit stress from 85.1 % (control) to 69.1% (water deficit) at vegetative stage (Table 1). Similar decrease in RWC under water deficit was seen at other two plant growth stages relative to control. Lesser decline in this parameter was seen with phosphorus treatment. RWC increased from approximately 4-8% (control) and approximately 10-16% (water stress) by phosphorus treatment at successive growth stages (Table 1). Water stress induced decrease in RWC

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was reported in cluster bean (Kuhad and Sheoran 1986; Shubhra et al 2004), chickpea (Singh 1995), Arabidopsis (Rizhsky et al 2004), and wheat (Nagarajan et al 1999; Foulkes et al 2007). Phosphorus improved RWC under stress in clusterbean (Shubhra 2001) wheat (Basak and Dravid 1997), and cherry (Centritto et al 1999). Water stress decreased the leaf area (24-27%) relative to control at all stages of the crop, vegetative stage being the highly sensitive stage (Table 1). This parameter improved significantly by P treatment at each growth stage in control as well stressed plants. Highest increase in leaf area was observed at the pod filling stage. Phosphorus treatment lessened the decline in this parameter under stress conditions at vegetative and flowering stages but the effect was non significant in case of the pod filling stage.

Table 1: Impact of water deficit and phosphorus application on stem height(cm), RWC(%) and leaf area(cm<sup>2</sup>) in clusterbean.

Treatment	P content	Vegetative stage			Flowering stage			Pod-filling stage		
		S.H	RWC	L.A	S.H	RWC	L.A	S.H	RWC	L.A
Control	P0	12.00	85.10	54.00	18.30	81.86	63.60	24.60	79.45	74.76
	P1	15.20	86.89	74.60	20.30	83.87	78.00	28.01	81.00	84.54
	P2	21.00	87.42	81.66	24.00	85.89	110.30	33.30	83.89	126.00
	P3	23.00	90.05	94.33	28.00	88.44	128.30	37.60	87.00	176.00
Stress	P0	10.33	69.10	39.33	14.66	61.24	48.50	20.60	55.65	56.70
	P1	12.80	76.55	50.34	16.00	65.56	53.60	25.00	58.82	68.60
	P2	15.66	83.82	54.00	19.30	68.37	83.33	28.00	63.60	104.00
	P3	19.53	87.11	72.00	24.30	71.72	103.30	33.30	67.43	118.33
CD at 5%	S	2.89	2.06	6.61	NS	3.78	5.71	NS	6.50	6.04
	P	4.08	2.91	9.35	6.23	5.35	8.08	5.08	NS	8.54
	S*P	NS	4.12	NS	NS	NS	NS	NS	NS	12.08

Among the biochemical parameters total chlorophyll content increased up to flowering stage and decreased thereafter in control as well stressed plants (Table 2). Water stress decreased the chlorophyll content at all the growth stages of the crop relative to control plants. Maximum decrease in this parameter was observed at flowering stage (20%) relative to other two growth stages. Phosphorus application improved the chlorophyll content (3-8%) relative to untreated control. On the contrary, free amino acid content in the leaves decreased progressively with the growth of the plant under control as well as stress conditions (Table 2). Water stress decreased the free amino acid content relative to control at each of the growth stages. Maximum decrease in this parameter was seen at the vegetative stage (32%). The parameter also decreased (2-12%) by phosphorus treatment at each growth stage.

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Treatment	P content	Vegetative stage			Flowering stage			Pod-filling stage		
		T.Chl.	TSC	FAA	T.Chl.	TSC	FAA	T.Chl.	TSC	FAA
Control	P0	4.25	25.26	4.71	4.45	36.05	4.23	4.21	20.48	4.00
	P1	4.36	29.20	4.63	4.61	38.34	4.03	4.25	21.60	3.77
	P2	4.47	30.82	4.38	4.67	40.45	3.95	4.31	22.70	3.65
	P3	4.58	32.70	4.25	4.74	43.63	3.79	4.39	24.16	3.51
Stress	P0	3.49	28.26	6.20	3.58	38.78	4.91	3.40	22.31	4.70
	P1	3.62	32.06	6.09	3.71	41.20	4.72	3.45	23.80	4.47
	P2	3.69	33.00	5.53	3.80	42.30	4.63	3.49	25.11	4.38
	P3	3.73	35.20	5.40	3.86	45.50	4.52	3.53	26.61	4.27
CD at 5%	S	0.25	2.48	0.99	0.21	NS	0.34	0.29	NS	0.13
	P	NS	3.51	NS	0.29	NS	NS	0.31	NS	0.18
	S*P	NS	NS	NS	0.40	NS	NS	0.42	NS	NS

Table 2: Impact of water deficit and phosphorus application on Total chlorophyll (mg/g dry wt.), total soluble carbohydrates (mg/g dry wt.) and free amino acids (mg/g dry wt.) in clusterbean

Yield determining parameters such as pod number/ plant decreased 30-40% due to water deficit stress relative to control. Flowering stage was the most sensitive to stress indicating maximum decline in this parameter ( Table 3).P treatment improved this parameter in control as well as stressed plants at each of the growth stages. Maximum improvement in pod number was observed at the flowering stage (59%) with highest P level. Similarly test weight declined significantly in stressed plants at different stages of growth relative to control (Table 3). P treatment improved the test weight (5-12%) relative to untreated control plants. The results were significant except with P1 level at vegetative and flowering stage and with both P1 and P2 level of phosphorus treatment at pod filling stage under stress conditions.

Gum content , an important attribute for seed quality decreased under water deficit stress in P treated as well as untreated plants (Table 3). Pod filling stage was the most susceptible as maximum (7%) decrease in gum content was observed at this stage. Gum content improved (15-25%) by P application in control as well as stressed plants at each of the growth stages. These results are in conformity with those given by Bhadoria and Tomar,1997; Garg et al,1998 and Shubhra et al,2004.

Table 3: Impact of water deficit and phosphorus application on Number of pods, test weight (g) and gum content (%) in clusterbean

Treatment	P content	Vegetative stage			Flowering stage			Pod-filling stage		
		T.Chl.	TSC	FAA	T.Chl.	TSC	FAA	T.Chl.	TSC	FAA
Control	P0	4.25	25.26	4.71	4.45	36.05	4.23	4.21	20.48	4.00
	P1	4.36	29.20	4.63	4.61	38.34	4.03	4.25	21.60	3.77
	P2	4.47	30.82	4.38	4.67	40.45	3.95	4.31	22.70	3.65
	P3	4.58	32.70	4.25	4.74	43.63	3.79	4.39	24.16	3.51
Stress	P0	3.49	28.26	6.20	3.58	38.78	4.91	3.40	22.31	4.70
	P1	3.62	32.06	6.09	3.71	41.20	4.72	3.45	23.80	4.47
	P2	3.69	33.00	5.53	3.80	42.30	4.63	3.49	25.11	4.38
	P3	3.73	35.20	5.40	3.86	45.50	4.52	3.53	26.61	4.27
CD at 5%	S	0.25	2.48	0.99	0.21	NS	0.34	0.29	NS	0.13
	P	NS	3.51	NS	0.29	NS	NS	0.31	NS	0.18
	S*P	NS	NS	NS	0.40	NS	NS	0.42	NS	NS

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## IV.DISCUSSION

The present investigation was carried out in cluster bean cultivar HG-365 under screen house conditions. The purpose of the study was to ameliorate the deleterious effect of water stress using phosphorus as a fertilizer. Attempts were also made to probe into physiological and biochemical basis for sensitivity and tolerance of clusterbean under water deficit condition.

Growth is one of the best characteristics for evaluating plant responses. Water stress led to decrease in plant height and leaf area (Table 1) whereas plant dry matter increased appreciably under water deficit (Data not given). The decline in plant height was due to reduced water supply leading to loss of turgor and inhibition of the assimilatory process. With an increase in soil moisture deficit stress leaf area gets reduced (Flavio et al 1998; Rana and Singh, 1998). This could be due to limitation of moisture availability required for leaf expansion and due to abscission of leaves (Shubhra 2001). Decrease in leaf area is positively correlated with decreased photosynthetic capacity of the plant which ultimately gets reflected in terms of decreased growth and yield parameters of the plant (Table 3). Phosphorus treatment improved the leaf area in control as well as stressed plants at each of the three growth stages (Table 1). The results presented here are in confirmation of the earlier reports in chickpea (Liang et al, 1996) and clusterbean (Shubhra 2001, Kuhad and Sheoran 1986).

RWC is among the best plant water relation parameter studied to ascertain the severity of water deficit stress. Water stress lead to significant decrease in RWC at each growth stage of the plant, lowest value seen at pod filling stage, in phosphorus untreated as well as treated plants (Table 1). The decrease in RWC results in decreased turgor of the plant however to maintain it the solute potential becomes more negative leading to accumulation of solutes. Phosphorus treatment resulted in significant decline in  $\Psi_s$  but improved the water potential to lesser extent pointing to more role of the organic solutes in maintaining turgor which gets reflected in terms of increased RWC. Such enhanced RWC help the plant to carry out its metabolism more efficiently even under low moisture conditions (Scotti Campo et al 1999, Nagarajan et al 1999 and Shubhra 2003).

Total chlorophyll content increased up to flowering stage and thereafter declined in control as well as stressed plants (Table 2). Water stress lead to decrease in this parameter at all the three growth stages of the crop relative to control. Flowering stage was most susceptible to stress resulting in decrease in chlorophyll content (20%) relative to other two stages of the crop. Phosphorus treatment significantly improved the chlorophyll content (3-8%) compared to untreated control. Free amino acid content decreased progressively with the growth of the plant in control as well as stressed plants (Table 2). Water stress led to decrease in free amino acids at each growth stage relative to control, maximum decrease seen at vegetative stage (32%). Free amino acid also declined by phosphorus treatment (2-12%). Soluble sugars play an important role as they are building blocks of plant and an important source of energy both for physiological as well as biochemical processes. Sugar accumulated in P treated as well as untreated plants under water deficit (Table 2). Its accumulation increased 25-26% in P untreated and upto 32% in P treated plants under control conditions at vegetative stage. Similar trend was noted at the other two stages as well. Further increase in sugar accumulation was noted due to P application which may either be due to inhibition of their normal utilization and translocation under stress or due to hydrolysis of starch.

Water stress adversely affect the yield of crop but the magnitude of damage depends both on the intensity of stress and stage of the plant. Number of pods/plant decreased (30-40%) by water deficit stress and improved by phosphorus treatment at each of the three sampling stages of the crop (Table 3). Flowering stage was most sensitive to stress treatment indicating highest decrease in this parameter. With the highest phosphorus treatment level (P3) maximum improvement in this parameter was observed at flowering stage (59%) compared to other two growth stages. Significant decrease in test weight was observed in plants given stress at different growth stages. Phosphorus treatment improved this parameter (5-12%) relative to untreated control plants (Table 3). The results were significant except with P1 level at vegetative and flowering stage and with P1 and P2 level of phosphorus treatment at pod filling stage under stress conditions. Beneficial effect of fertilizer treatment were attributed to increase in number of pods/plant and size of grain (Table 3). One of the important seed quality parameter gum content decreased under water deficit stress in phosphorus treated and untreated plants (Table 3). Pod filling stage was the most susceptible to water deficit as the



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reduction in gum content was maximum (upto 7%). The parameter improved by phosphorus treatment in control as well as stressed plants at all the three sampling stages. This may be due phosphorus induced polysaccharide synthesis which is the main component of seed and contribute to the gum content (Table3). These results were in coformation to that reported by Garg et al (1998), Bhadoria and Tomar (1997), Shubhra et al (2004).

## V.CONCLUSION

In the present study phosphorus played a decisive role in combating the disastrous effects of the water deficit stress on the physiology and yield parameters in cluster bean. Cluster bean being an arid region crop faces water deficit conditions quite frequently, thus treatment with phosphorus as a nutrient may play a important role in alleviating the stress induced disturbance in the physiology, grain yield and quality in clusterbean.

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