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EFFECT OF PHOSPHORUS AND BIO FERTILISERS ON LEGHAEMOGLOBIN AND NITROGEN FIXATION OF CHICKPEA[1]

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The effect of different levels of phosphorus and biofertilisers on the leghaemoglobin and nitrogen fixation of chickpea was studied under field conditions at the Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, during 1978-79 and 1979-80 *rabi* seasons. Fresh weight of nodules increased significantly with increasing levels of phosphorus as well as with Rhizobium inoculation. Rhizobium inoculation had marked influence on leghaemoglobin content of nodule tissue. The total leghaemoglobin content in the nodules is directly related to the total nitrogen fixed by the chickpea.

Ever since the recognition of root nodule pigment as haemoprotein, its funtion has been the subject of biochemical investigation. Kabo (1939) recognised the red pigment of nodule as leghaemoglobin which enhanced the respiration of Rhizobia in nodules functioning in the transport system of oxygen. Virtanen (1947) suggested that the haemoglobin might function directly in N2 fixation by forming hydroxylamine from dinitrogen. A correlation has been observed between the ability of the nodule to fix nitrogen and leghaemoglobin contents (Smith, 1949) suggesting that fundamentel role of leghaemoglobin in dinitrogen fixation. It is universally accepted that phosphate fertilisation to legumes increased nitrogen fixation. Beneficial effects of Rhizobium inoculation on nitrogen fixation and leghaemoglobin content of legume root nodules have been reported from time to time. Leghaemoglobin content of the nodules along with net nitrogen fixed by chickpea with different fertiliser levels was estimated so as to findout the relationship between the leghaemoglobin and the effectiveness of nodules in nitrogen fixation.

METERIAL AND METHODS

A field experiment was laidout in a randomised block design with three replications during the *rabi* seasons of 1978-79 and 1979-80 under irrigated conditions. Chickpea variety T3 was sown in a sandy clay loam soil which has low available nitrogen (160.4 kg ha^{-1}) medium phosphorus (12.6 kg ha^{-1}) and potassium (194.8 kg ha^{-1}) with 7.1 pH. Four levels of phosphorus (0, 20, 40 and 60 kg ha^{-1}) and three

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bacterial cultures (no culture, Rhizobium culture and Phosphobacterin culture) were compared to study their effect on leghaemoglobin and nitrogen fixation. The desired quantity of seeds for each plot was weighed and mixed with specific strain of Rhizobia (H-45) and *Pseudomonas striata* Var. phosphobacterin with the help of a sticker. Seeds were sown after little drying by spreading the seeds in the shade. Five plants were dug out carefully from each treatment with a ball of mud along with intact root nodules. The dug out plants were soaked thoroughly with water and thereafter soil was washed out gently. The nodules were separated from the roots with the help of a forceps and were washed with cold distilled water and the fresh weight of the nodules were recorded after drying on a filter paper. Composite samples of fresh nodules were subjected to leghaemoglobin following, Proctor (1963). Total nitrogen content of the soil from 0-30 cm depth was estimated before and after harvest of the crop as per the method suggested by Jackson (1973). Nitrogen fixation was worked out by using the formula of Saxena and Tilak (1975).

$$X + U - A \quad \text{where,}$$

X – gain or loss of soil nitrogen after the crop harvest i. e. calculated by substracting the initial nitrogen content of the soil from the final nitrogen content of the soil

U – Total nitrogen uptake by the crop

and

A – Nitrogen added to the soil if any through the uninoculated crop.

RESULTS AND DISCUSSION

Fresh weight of nodules

It is evident from the data (Table 1) that an increase in the level of phosphorus resulted in significant increase of the fresh weight of nodules. Thus, with 60 kg P_2O_5 ha^{-1} a maximum fresh weight of nodules was produced per plant but it was at par with 40 kg P_2O_5 ha^{-1} at all stages during both the years. Most prabably phosphorus stimulated the nodulation more through its effects on bacteria than on the host. In the presence of adequate phosphorus, the bacterial cells became motile and flegellate, the perrequisite for bacterial migration, where as in the absence of phosphorus or with inadequate supply' the infection remains latent, leading to the poor development of the nodules (Diener, 1950). Rhizobium inoculation also had the significant effection nodule development. The nodule fresh weight tended to decrease sharply at 120th day after planting, which synchronises the pod filling stage presumably because carbohydrates and nitrogen reserves of plants and nodules are being utilised extensively by the developing seeds (Pate, 1958) or due to decay of bacterial population and lysis of nodules found earlier (Sahu, 1973).

LEGHAEMOGLOBIN

An examination of the data (Table 2) indicate that marked differene due to phosphorus levels were not found in leghaemoglobin content of nodules at any stage of the crop, where as Rhizobium inoculation had relatively higher values of leghaemoglobin. Sidhu *et al.*,

(1967) also reported similar results. It may be inferred that although the leghaemoglobin per gram of nodule does not appear to bear to the yield of plant yet there is a direct relationship between the total haemen content of nodule per plant and seed yield per plant.

The correlation between the total leghaemoglobin content of nodule per plant and seed yield per plant as well as net nitrogen fixed was worked out and r = 0.843 and 0.927 at 1% level, respectively indicating the direct relationship of total leghaemoglobin with seed yield and nitrogen fixation. Several workers have also reported that inoculation of legume seeds with Rhizobium increased the leghaemoglobin content in nodule tissue (Sethi and Rao, 1975) there by increased the seed yield (Simhadri and Tilak, 1976) and nitrogen fixation in the soil (Saxena and Tilak, 1975).

From these observations it can be concluded that the amount of leghaemoglobin contained in the nodule of a legume but not the haemoglobin per gram of nodule is directly related to the total nitrogen fixed. Despite the fact that application of superphosphate had no positive effect on the leghaemoglobin concentration per gram of fresh nodules yet, it increased the total haemen content per plant by giving higher fresh nodule weight and total soil nitrogen indicating the role of phosphorus in the mechanism of nitrogen fixation and also for the growth of nodule bacteria. These findings closely corroborate with the results of Sidhu *et al.* (1967)

It was interesting to note that leghaemoglobin content of nodule tissue increased from the beginning and reached to a maximum level at 90 days after sowing which coincided with flowering and there after decreased sharply. Iyer (1976) found maximum haemen content in legume root nodules sharply prior to flowering when nodules attain their peak activity in nitrogen fixation.

It was evident from the data Table 2) that in legume root nodules under uninoculated plots also had haemen content which is attributed to the presence of many native Rhizobia in the soil belonging to the same strain that might induce nodule development but fix little or no nitrogen. This would explain as to why the uninoculated crop gave lower yield than inoculated inspite of the nodulation.

Net Nitrogen Fixation

Data (Table 3) on net nitrogen fixation clearly indicate that nitrogen fixation was affected markadly not only by phosphorus application but also by Rhizobium inoculation.

According to Vyas and Desi (1953), addition of phosphorus increased symbiotic nitrogen fixation power and in turn stimulated the growth of plants. On an average 91.9 and 85.5 kg of net nitrogen was fixed by chick-pea crop during first and second year respectively. Higher amount of nitrogen 2 2.1 and 196.0 kg ha-1 along with Rhizobium inoculatton was recorded during first and second years respectively.

Further, Phosphobacterin culture had no marked influence on any characters studied.

From these observations it can be concluded that leghaemoglobin content of nodules per plant runs parallel to the total nitrogen fixed by the legume crop. Hence, leghaemoglobin content can be used as guide to symbiotic effectiveness. These findings are in confirmation with those of Singh *et al.*, (1977) who reported similar results while studying some aspects in root nodulation in tropical legumes.

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Table 1 Fresh Nodule Weight (g) Plant ¹ of Chickpea as Affected by Phosphorus and Bio-Fertilisers

Treatment	Days after sowing							
	30		60		90		120	
	1978-79	1979-80	1978-79	1979-80	1978-79	1979-80	1978-79	1979-80
Level of P_2O_5 kg ha^{-1}								
0	1,095	0 993	5,460	8,923	10.009	10,241	6.734	6,148
20	1,541	1 652	9,542	9,696	12.239	13,465	8.514	8.574
40	2.073	2.218	12 458	12,912	16.629	17.684	11,661	12.073
60	2,253	2,433	14,284	13,607	18,096	19 391	12 682	13,230
Sem ±	0,103	0.114	0 937	0,804	0,736	0,858	1,032	0.812
C. D. 5%	0.302	0,334	2.749	2,359	2.158	2 518	3.045	2.382
Bio-fertilisers								
No culture	1.500	1 661	8,624	9,834	12.573	13 723	8 128	7.485
Rhizobium culture	2.184	2,201	14 181	13.246	17,864	17·478	12,862	12.116
Phosphobacterin culture	1.519	1.712	9:148	10 365	13·182	14 302	8.711	8.171
Sem ±	0,089	0.099	0.811	0.696	0 6.7	0.743	0 894	0.703
C. D. 5%	0. 62	0,269	2 380	2.043	1 868	2 181	2,622	2 062
Phosphorus x culture								
Sem ±	0.178	0.197	1 623	1 392	1.275	1.486	1.788	1.406
C. D. 5%	N. S.	N. S.	N. S.	N. S.	N S	N. S.	N. S.	N. S.

N. S. Not Significant

Table 2 Leghaemoglobin Content (mg. g^{-1} fresh weight) of Chickpea Root Nodules as Affected by Phosphorus and Bio-Fertilisers

Treatment	Days after sowing								Seed yield (g) plant⁻¹	
	30		60		90		120			
	1978-79	1979-80	1978-79	1979-80	1978-79	1979-80	1978-79	1979-80	1978-79	1979-80
Level of phosphorus (kg ha⁻¹)										
0	3.402	3.393	3.772	3.782	3.956	3.967	3.612	3.639	16.3	16.6
	(3.725)	(3.369)	(20.595)	(22.401)	(39.596)	(40.626)	(24.323)	(22.372)		
20	3.451	3.427	3.801	3.865	3.993	3.996	3.658	3.667	18.7	20.1
	(5.318)	(5.661)	(36.854)	(37.572)	(48.859)	(53.442)	(31.144)	(31.441)		
40	3.504	3.469	3.874	3.839	3.984	4.012	3.683	3.645	20.9	23.0
	(7.264)	(7.694)	(50.021)	(49.569)	(66.283)	(70.948)	(42.947)	(44.006)		
60	3.511	3.480	3.867	3.862	4.012	4.005	3.692	3.705	21.8	23.2
	(7.910)	(8.467)	(52.618)	(52.550)	(72.061)	(77.661)	(47.822)	(49.017)		
Sem ±									0.36	0.78
C. D. 5%									1.06	2.29
Bio-fertilizers										
No culture	2.774	3.027	3.157	3.503	3.147	3.541	2.903	3.164	18.9	20.4
	(4.161)	(5.029)	(27.226)	(34.449)	(39.567)	(48.596)	(29.596)	(23.682)		
Rhizobium culture	4.940	4.351	4.143	4.851	5.638	5.827	4.712	4.692	20.5	23.1
	(10.710)	(9.577)	(50.735)	(59.405)	(99.702)	(87.862)	(60.606)	(66.848)		
Phosphobacteie culture	2.853	3.042	3.204	3.347	3.345	3.537	2.997	3.184	19.0	20.9
	(4.419)	(5.208)	(29.310)	(34.692)	(44.094)	(60.586)	(26.107)	(26.016)		
Sem ±									0.31	0.67
C. D. 5%									0.91	1.97
Phosphorus x Culture										
Sem ±									0.62	1.5
C. D. 5%									N. S.	N S

Figures in the parenthesis indicate the total leghaemoglobin content per plant : N. S. = Not Significant

Table 3 Net Nitrogen (kg ha^{-1}) Fixation by Chickpea as Affected by Phosphorus and Bio-Fertilisers

Bio-fertilisers	No culture		Rhizobium culture		Phosphobacterin culture	
Leave of P_2O_5 (kg ha^{-1})	1978-79	1979-80	1978-79	1979-80	1978-79	1979-80
0	—	—	40.2	38.0	5.2	3.4
20	52.4	51.2	121.8	113.5	60.9	55.9
40	91.9	87.6	179.1	164.7	94.7	89.2
60	118.4	111.4	212.1	196.0	126.6	114.6