Effect of *Pseudomonas striata* and *Glomus fasiculatum*, phosphorus nutrition and mode of phosphorus placement to proline content, root dry weight and grain yield of Chickpea (*Cicer arietinum* L.) under rainfed condition

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The field experiment was conducted in two consecutive *rabi* seasons at the Indian Agricultural Research Institute, New Delhi. The experiment was laid out in balanced confounded asymmetrical factorial design with three replications in which four levels of biofertilizers (no inoculation, *Pseudomonas striata, Glomus fasiculatum* and *Pseudomonas striata + Glomus fasiculatum*), three levels of phosphorus (0, 30 and 60 kg P_2O_5 ha^{-1}) and two mode of phosphorus application (dry placement and aqua placement) with 20,000 litres of water in blocks per replication and 12 plots per block. The result showed that interaction between *Pseudomonas striata + Glomus fasiculatum* with 60 kg P_2O_5 ha^{-1} recorded minimum proline accumulation, maximum dry weight of roots. Dual inoculation of *Pseudomonas striata + Glomus fasiculatum* enhanced the dry weight of nodules $plant^{-1}$ in both the years. The percentage increases with *Pseudomonas striata, Glomus fasiculatum* and *Pseudomonas striata + Glomus fasiculatum* as compared to no inoculation was 7.68, 10.79 and 17.78 in the first year and 9.19, 12.96 and 16.23 in the second year respectively. Thus, the results of the present investigation confirmed the beneficial effects of microbial inoculants in combination with phosphorus to increase drought tolerance, root nodulation, root dry weight and yield.

Key words: Chickpea, proline, PSB, rainfed, root, yield, VAM

INTRODUCTION

Pulses occupy an unique position not only in Indian agriculture but also occupy a vital position for solving the protein malnutrition problem in India. Among rabi pulses, chickpea (*Cicer arietinum* L.) occupies an important position because it is grown and consumed all over the world, especially in the Afro-Asian countries. It is a good source of carbohydrates and protein, and protein quality is considered to be better than other pulses. Chickpea has significant amounts of all the essential amino acids except sulphur-containing amino acids, which can be complemented by adding cereals to the daily diet. Ca, Mg, P and, especially, K are also present in chickpea seeds. Chickpea is a good source of important vitamins such as riboflavin, niacin, thiamin, folate and the vitamin A precursor β-carotene.

The primary limitation of crop production in semi-arid regions is the lack of available moisture and nutrients resulting in lower crop yield unless the nutrient status is enhanced by a regular or substantial addition of nutrients. Among the primary nutrients, phosphorus deficiency is probably the major limiting factor to the yield of chickpea in soils, particularly in tropical region having high phosphorus fixing capacity. Amelioration of phosphorus deficiency by application of costly phosphorus fertilizer is not a viable option to many resource poor farmers of these regions. Several attempts were made to evolve technology for substituting or

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supplementing costly phosphatic fertilizer using micro-organisms capable of solubilizing the native and applied phosphorus. The use of phosphate solubilizing bacteria (PSB) and endophytic fungi viz.Vesicular Arbuscular Mycorrhizae (VAM) are common inhabitant of roots of several plants and can improve drought tolerance, nodulation, root growth and yield by increasing nutrient uptake through increases in the exploration of larger soil volume by mobilizing nutrient sources or by excretion of chelating compounds or ectoenzymes.

MATERIALS AND METHODS

Description of Study Area

Field experiments were conducted during winter (*rabi*) seasons at the research farm of Indian Agricultural Research Institute, New Delhi, situated at a latitude of 28^{0}40´N and longitude of 77^{0}12´E, altitude of 228.6 meters above the mean sea level (Arabian sea). The mean annual rainfall of Delhi 650 mm and more than 80% generally occurs during the south-west monsoon season (July–September). In the first year the precipitation was 41.14 mm during the growth period but during the second year only 26.61 mm rainfall was received. Climatologically Delhi attains a semi-dry sub-tropical climate with extremes hot dry summer and cold winter. The initial fertility status of the experimental ûeld was 251 kg ha^{-1} alkaline permanganate oxidizable nitrogen (N), 9.9 kg ha^{-1} available phosphorus (P) 269 kg ha^{-1} 1 N ammonium acetate exchangeable potassium (K) and 0.42% organic carbon.

Experimental Treatments and Design

The experiment was laid out in a balanced confounded asymmetrical factorial design with three replications in which four levels of biofertilizers (no inoculation, *Pseudomonas striata, Glomus fasiculatum* and *Pseudomonas striata + Glomus fasiculatum*), three levels of phosphorus (0, 30 and 60 kg P_2O_5 ha^{-1}) and two mode of phosphorus application (dry placement and aqua placement) with 20,000 litres of water, 4x2^2 in 8 plots per block augmented by 4 treatments in each of the blocks. Therefore, two blocks per replication and 12 plots per block.

Land preparation

The experimental field was ploughed by a tractor drawn disc harrow followed bydouble discing and planking to provide a good tilth.

Mode of fertilizer application

The variable doses of phosphorus (30 and 60 kg P_2O_5 ha^{-1}) in the form of single super phosphate were placed as dry and aqua (variable phosphorus dose + 20,000 l water ha^{-1}) were applied at the time of sowing with the help of manually operated hand plough having two tubes, one for sowing seed and another for placement of fertilizer.

Seed inoculation

The seed was inoculated properly with the culture of obtained from the Division of Microbiology, IARI, New Delhi. Ten per cent cold gur solution was prepared by boiling and then cooled. The inoculated seed was air dried in shade before used for sowing in the field.

Application of VAM in the field

Three VAM spores per seed were applied in the field. Based on the total number of seeds, the total number of VAM spores were calculated. The pure VAM culture that contained 1800 spores per g of culture was obtained from the Division of Microbiology, IARI, New Delhi. The pure VAM culture was mixed thoroughly with sand and applied with the seed through metallic tube attached to handle of the plough.

Number of nodules and dry weight of root plant^{-1}

Root of chickpea was exposed at 75 days after sowing through excavation method (Weaver, 1926). Roots of five plants were collected from sampling row. After washing the roots in fresh water, nodules were separated from roots and counted. Roots were dried inn oven at 65^{0}C for 48 hours and their weight was recorded.

Proline analysis

Plant samples were collected at 135 days after sowing for proline estimation in the fully opened fresh leaf by the method of Chinard (1952).

Grain yield

At the time of maturity the net plots (leaving two border rows on each side and four plants from each side of the length) were harvested and sun-dried for three days in the ûeld and then the total biomass yield was recorded. After threshing, cleaning and drying the grain yield was recorded at 14% moisture.

RESULTS AND DISCUSSION

Number of nodules plant^{-1}

Result showed that inoculation of biofertilizers significantly increased the number of nodules plant^{-1} in the experiments (Table1). The percentage increase in nodule numbers plant^{-1} with *Pseudomonas striata*, *Glomus fasiculatum* and *Pseudomonas striata + Glomus fasiculatum* over no inoculation was 23.4, 38.3 and 73.4 in the first year and 24.5, 44.2 and 85.2 in the second year, respectively. Dual inoculation of *Pseudomonas striata + Glomus fasiculatum* recorded the maximum number of nodules plant^{-1}. This findings is in conformity with the findings reported by Gaur (1990). Increase in nodule number due to *Pseudomonas striata* is not only because of its increased supply of native soil phosphorus to higher plants but also its direct effect on nodule bacteria. Increased nodulation in Chickpea with PSB (*Pseudomonas striata*) inoculation has also been observed by Tiwari *et al,* (1988) and Jain *et al,* (1999). VAM (*Glomus fasiculatum*) inoculation also markedly improved nodulation mainly by providing the high phosphorus requirements for the nitrogen fixation process (Bagyaraj and Verma, 1995). Application of phosphorus significantly increased the number of nodules plant^{-1} in the experiments (Table1). The percentage increase with 30 and 60 kg P_2O_5 ha^{-1} over no phosphorus was 16.8 and 34.6 in the first year and 22.8 and 40.0 in the second year, respectively. The number of nodules plant^{-1} has been enhanced due to phosphorus application. This is quite logical because phosphorus nutrition development of nodule bacteria. Better nodulation in chickpea with adequate supply of phosphorus has been reported by Parihar and Tripathi (1989) and McDermott and Triplett (2000).

Dry weight of root plant^{-1}

Data pertaining to dry weight of roots plant^{-1} at 75 DAS as presented in Table1. Data revealed that biofertilizer inoculation significantly influenced the dry weight of roots in both the years. Dual inoculation of PSB+VAM recorded higher dry weight of roots followed by VAM and PSB and no inoculation. The percentage increase with PSB, VAM and PSB+VAM as compared to no inoculation was respectively 9.7, 23.9 and 30.6 in the first year and 5.8, 16.0 and 31.3 in the second year respectively. Combined inoculation of PSB+VAM stimulated increase in root dry weight. It corroborated the finding of Piccini and Azcon (1987). This indicates a strong synergistic effect between PSB and VAM. This significant stimulation of root dry weight by PSB

Table 1 : Effect of *Pseudomonas striata* and *Glomus fasiculatum*, phosphorus and mode of phosphorus placement to number of nodules and root dry weight of Chickpea

Treatments	Number of nodules plant^{-1}		Dry weight of roots (mg plant^{-1})	
	First year	Second year	First year	Second year
Biofertilizers				
No inoculation	9.4	6.1	374	363
Pseudomonas striata	11.6	7.6	410	384
Glomus fasiculatum	13.0	8.8	463	421
Pseudomonas striata + Glomus fasiculatum	16.3	11.3	488	477
SEm(±)	0.28	0.21	8.5	6.3
CD (0.05)	0.80	0.60	24.3	17.9
P levels (kg P_2O_5 ha^{-1})				
0	10.7	7.0	360	365
30	12.5	8.6	459	424
60	14.4	9.8	482	444
SEm(±)	0.24	0.17	7.4	5.4
CD (0.05)	0.70	0.50	21.0	15.5
Mode of P application				
Dry placement	12.2	8.1	427	408
Aqua placement	12.9	8.8	440	415
SEm(±)	0.21	0.14	6.0	4.5
CD (0.05)	0.60	0.40	NS	NS

Table 2 : Effect of *Pseudomonas striata* and *Glomus fasiculatum*, phosphorus and mode of phosphorus placement to proline content and grain yield of Chickpea

Treatments	Proline content (µg g^{-1} of fresh leaf) at 135 DAS		Grain yield (kg ha^{-1})		
	First year	Second year	First year	Second year	Pooled
Biofertilizers					
No inoculation	218	346	2316	1805	2060
Pseudomonas striata	195	302	2494	1971	2233
Glomus fasiculatum	179	248	2566	2039	2303
Pseudomonas striata + Glomus fasiculatum	164	209	2728	2089	2408
SEm(±)	1.45	1.84	40	39	26
CD (0.05)	4.12	5.24	114	112	76
P levels (kg P_2O_5 ha^{-1})					
0	200	290	2361	1863	2112
30	186	275	2578	2021	2300
60	180	264	2635	2043	2341
SEm(±)	1.25	1.60	34	34	23
CD (0.05)	3.57	4.54	98	97	66
Mode of P application					
Dry placement	191	281	2452	1938	2195
Aqua placement	187	272	2600	2014	2307
SEm(±)	1.03	1.30	28	27	19
CD (0.05)	2.92	3.71	80	NS	54

may be the result of increased availability of insoluble phosphate in soil as applied phosphorus. This increase in root biomass was also reported by Mukherjee and Rai (2000).

Additionally, stimulation of root dry weight by VAM may be the result of altered source-sink relationship as well as effect of nodule weight. Application of phosphorus signiûcantly increased dry weight of roots $plant^{-1}$ in both the years. The percentage increase with 30 and 60 kg P_2O_5 over no phosphorus was 27.6 and 33.9 in the first year and 16.1 and 21.4 in the second year, respectively. Application of phosphorus significantly increased root dry weight This might be attributed to better proliferation of roots and increased nodulation due to phosphorus availability. It has been reported that phosphorus play a beneûcial role in legume growth by promoting extensive root development and nodulation (Sharma and Yadav, 1976). Aqua placement recorded more dry weight as compared with dry placement in both the years. The per cent increase with aqua placement over dry placement was 3.0 and 1.7 in the ûrst and second year, respectively.

Aqua placement of phosphorus recorded higher root biomass. This might be due to better availability of phosphorus in early stage of root development and helped to increase higher root dry weight in later stage of crop.

Proline content

Data on proline content in leaf was significantly inûuenced by biofertilizer inoculation, levels and mode of phosphorus application are presented in table 2. Maximum proline accumulation was observed in uninoculated plants at all the stages of crop growth as compared to PSB ,VAM and PSB+VAM in both the years. Minimum proline content was observed with PSB +VAM as compared to PSB and VAM inoculated separately. VAM inoculated plants have had lower concentration proline and it is an indication of greater drought resistance i.e. less injury. Dual inoculation of PSB+VAM recorded minimum proline content. This indicated a strong synergistic effect between PSB and VAM. Maximum proline content was observed in control plant while minimum proline accumulation was noticed with 60 kg P_2O_5 followed by 30 kg P_2O_5 ha^{-1} in both the years. Aqua placement of phosphorus accumulated less amount of proline as compared to dry placement of phosphorus. Aqua placement of phosphorus recorded less amount of proline which may be due to higher leaf moisture content.

Grain yield (kg ha^{-1})

Results showed that inoculation with biofertilizers showed significant effect on grain yield as compare to no inoculation in both the years as well as in the pooled data (Table 2). The pooled data showed that PSB+VAM recorded higher grain yield than VAM and PSB inoculation. The percentage increase with PSB, VAM and PSB+VAM as compared to no inoculation was 7.68,10.79 and 17.78 in first year and 9.19,12.96 and 16.23 in the second year and 8.40, 11.80 and 16.89 in pooled data, respectively.

The higher grain yield due to biofertilizer inoculation might be due to increase in nodulated roots, higher amount of root as well as less moisture stress. The PSB is known to produce vitamins (Baya *et al*, 1981) and IAA and GA like growth substances (Sattar and Gaur, 1987). These growth factors in combination with better nutritional condition due to increase in availability of phosphorus in soil might have played a signiûcant role in increasing the grain yield of chickpea. On the other hand, VAM helped in supply of essential nutrients and water to plants resulting in better growth that led to increase in grain yield. The dual inoculation recorded higher grain yield apparently arising from a synergistic effect between PSB and VAM. The increase in grain yield by PSB and VAM has been reported by several workers (Tiwari *et al*, 1988, Yadav and Shrivastava, 1997; Jain *et al*,1999; Mukherjee and Rai, 2000 and Meshram *et al.*, 2000). Phosphorus application increased in grain yield up to with 60 kg $P_2 0_5$ ha^{-1}. The percentage increase with 30 and 60 kg $P_2 0_5$ ha^{-1} over no phosphorus application was 9.19 and 11.60 in the first year, 8.48 and 9.66 in the second year and 8.90 and 10.8 in pooled data, respectively. The improvement in yield with increased supply of phosphorus might be due to profuse nodulation leading to increased nitrogen fixation which in turn had positive effect on photosynthetic approaches resulting in higher grain yield. Almost similar observations were reported by many workers (Sarkar *et al*, 1995; Saraf *et al*, 1997; Jain *et al*, 1999; Mukherjee and Rai, 2000). Application of phosphorus through aqua fertilizer significantly increased the grain yield in the first year and in the pooled data. In the first year the precipitation was 41.14 mm during the growth period but during the second year only 26.61 mm rainfall was received. Temperature rise was high at grain filling stage in the Second year. Higher grain yield in the first year was thus due to favourable weather in terms precipitation and temperature.

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