Journal of Food Legumes 28(1): 45-49, 2015

Relative field responsiveness and dependency of chickpea to combined inoculation of *Mesorhizobium Ciceri*, arbuscular mycorrhizal fungi, phosphobacteria and phosphorus under inceptisol

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(Received : November 12, 2014 ; Accepted : February 20, 2015)

ABSTRACT

The relative field responsiveness of chickpea due to the synergistic interaction of *Mesorhizobium ciceri*, phosphobacteria (PSB-*Bacillus megaterium*), arbuscular-mycorrhizal fungi (AMF-*Glomus* sp.) and phosphorus and its relative field dependency to the bioinoculats and phosphorus were studied in slightly alkaline inceptisol condition. The combined inoculation of *Mesorhizobium ciceri*, PSB, AMF exhibits highest nodule number, AMF colonization and biomass production in chickpea under slightly alkaline inceptisol condition. The bioinoculants either PSB or AM fungi enhanced the plant P uptake and AM fungi exhibits relatively better P responsiveness and recorded maximum Relative Field Inoculation Dependency (RFID) value (based on P uptake) of 42.34 percent at 40 Kg P_2O_5 ha^{-1} application under slightly alkaline inceptisol condition. The combined inoculation of *Mesorhizobium ciceri*, PSB, AMF and phosphorus at 40 Kg P_2O_5 ha^{-1} exhibits highest Relative Field Inoculation Responsiveness (RFIR) value of 21.66 per cent (based on yield response). From the view point of nodulation, dry matter production, root colonization, Relative Field Inoculation Dependency (RFID), Relative Field Inoculation Responsiveness (RFIR) and yield enhancement, inoculating *Mesorhizobium ciceri*, phosphobacteria (PSB - *Bacillus megeterium*) and AM fungi (AMF - *Glomussp*) in the presence of P (40 Kg P_2O_5 ha^{-1}) to be considered as balanced combination for sustainable chickpea production in the slightly alkaline inceptisol conditions.

Keywords: Arbuscular Mycorrhizal Fungi, Chickpea, *Mesorhizobium ciceri,* Phosphobacteria, Relative Field Inoculation Responsiveness and dependency

Chickpea (*Cicerarietinum* L.) holds a unique position among pulse crops due to its seed protein content and wide adaptability in ecologically diverse environments. It plays a significant role in farming systems as a substitute for fallow in cereal rotations, where it contributes to the sustainability of production. Leguminous crops form two types of symbiotic association with microorganisms and in which rhizobia involved in atmospheric nitrogen fixation and AM fungi concerned with the uptake of P.Mycorrhizal infection has particular value for legumes because nodulation and symbiotic nitrogen fixation by rhizobia require an adequate phosphorus supply and restricted root system of the crops leads to poor competition for soil phosphorus (Carling *et al.,* 1978). A good number of literatures say that rhizobia inoculation works favourably in respect to nodulation and biological nitrogen fixation in chickpea (Solaiman *et al.,* 2006, Zaidi *et al.,* 2003). Many workers have reported enhancement of phosphate uptake and growth of leguminous plants by vesicular arbuscularmycorrhizal fungi (Izagirre 2000, Guriqbal *et al.,* 2001). Phosphate Solubilizing Microorganisms (PSM) interacts well with arbuscularmycorrhizal fungi in phosphorus deficient soils (Poi *et al.,* 1989). Moreover, the PSM survive longer around mycorrhizal rootscompared with non-mycorrhizal roots and act synergistically with the arbuscularmycorrhizal fungus, leading to increased plant growth (Singh, 1990). Rhizobium and Phosphobacteria when inoculated together colonized the rhizosphere and enhanced the growth of chickpea by providing them with nitrogen and phosphate,respectively (Gull *et al.,* 2004). Simultaneous dual inoculation of arbuscularmycorrhizal fungi and PSM has been shown to stimulate plant growth more than inoculation of either microorganism alone in certain situations when the soil is phosphorus-deficient (Piccini and Azcon, 1987). Combined inoculation of Rhizobium and application of PSM and arbuscularmycorrhizal fungus or phosphorusgave the greatest yield and hadvariable effects on nodulation in mungbean (Zaidi *et al.,* 2004), lentil and field pea (Guriqbal *et al.,* 2001) and chickpea (Poi *et al.,* 1989; Subba Rao *et al.,* 1986). It is evident from the earlier studies that a positive interaction exists between root colonization, phosphorus uptake and growthpromotion, as observed by a few researchers (Zaidi *et al.,* 2003; Zaidi and Khan, 2005) and the field response of chickpea to the combined inoculation effect of the microorganisms and phosphorus under inceptisol conditions are scanty. Keeping these facts in mind, the present experiment was carried out to assess the response of the inoculation in presence and absence of phosphorus/bioinoculants on growth, productivity and nutrient uptake in chickpea cv. DCP 92-3

MATERIALS AND METHODS

A field experiment was conducted at Indian Institute of Pulses Research, New Research Farm, Kanpur, Uttar Pradesh, India. The soil was sandy loam having the organic carbon 0.3%, pH - 8.4, CEC - 0.56 dsm^{-1}, available P (Bray) - 11 to 15 kg ha^{-1} , N - 80 to190 kg ha^{-1}, K-110 to170 kg ha^{-1}, S -15 to 20 kg ha^{-1}, Zn - 0.11 to 0.31 ppm, Fe - 4 to 6 ppm. The field experiment was designed in factorial randomized block design. Phosphorus applied in three levels viz., 0, 40 and 80 kg P_2O_5 ha^{-1}. There were five treatment combinationsviz., T_1-Uninoculated; T_2-*Mesorhizobium ciceri*; T_3-*Mesorhizobium ciceri* + PSB; T_4-*Mesorhizobium ciceri* + AM fungi and T_5-*Mesorhizobium ciceri* + PSB + AM fungi. Vermiculate based maize rootbitsinoculum of AM fungi (Glomus sp.) was used at the rate of 100g per m2 area. AMFinoculum mixed with sand and was applied in sowing furrows.Similarly PSB (Bacillus megeterium) having the cell load of 2.3×10^9 $cfug^{-1}$ also applied at the rate of 2kg $ha^{-1}$by mixing with sand and applied in the sowing furrows selectively for the treatments. *Mesorhizobiumciceri* inoculants containing 4.5×10^9cfu g^{-1}mixed with seeds with the help of rice gruel and seed treated. After sowing, the furrows were closed with soil andcompacted mechanically. Plants were allowed to grow. Weeding and irrigation were done as per crop requirement. With the minimum disturbance of roots, the plants were carefully uprooted at 50 percent flowering so that no nodules were left in the soil. The roots were washed with water. Nodules from the roots separated and the number of nodules and weight were recorded. The plants and roots were first air dried and then oven dried at 65°C for 72 hours.The phosphorus content of the plant samples, on dry weight basis was determined by ammonium metavanadate method (Jackson, 1973). Functional feeder roots segments were stained for assessing AMF colonization and the per cent AMF colonization was estimated (Phillips and Hayman, 1970) and Relative field inoculation responsiveness (Baon *et al.,* 1993) and dependency (Plenchette *et al.,* 1983) were also calculated adopting the formulas as proposed below

Relative Field Inoculation Responsiveness (%) = (Yield with Inoculation – Yield without Inoculation) / (Yield without Inoculation) × 100

Relative Field Inoculation Dependency (%) = (P uptake with Inoculation – P uptake without Inoculation) / (P uptake with Inoculation) × 100

Note: Above equations has been used for yield, biomass and P uptake vice versa to calculate responsiveness and dependency at particular level of fertility.

RESULTS AND DISCUSSION

Nodulation, AMF colonization

Significantly greater number of nodules per plant in chickpea inoculated with *Mesorhizobium ciceri* as compared to the control was recorded.In general, there was a significant increase in root nodulation in the presence of PSB and AM fungi over the control (Table 1.). The maximum number of nodules might be attributed to greater availability of phosphorus, which was crucial for nodulation (Hayman, 1983). Similar evidence on the effect of P in N_2 fixation in the French bean (Saber *et al.,* 2005) was reported earlier.The number of nodules produced on legume plants isgenerally used an index for assessing the N_2 fixingefficiency of nodule bacteria. However, this does notreflect the true efficiency of particular rhizobial strainssince manually counted nodules may also include nodulesproduced by the indigenous populations.

The better nodulation in the case of composite inoculation at the flowering stage (Table 1)appeared to be a result of the favourable effects of PSM in making more P soluble and available to the plants, which consequently promoted root development. In the present study, a positive relationshipbetween the plant biomass and nodule numbers and P contents of chickpea plants further suggested the involvement of P in the establishment of an effective *Mesorhizobium ciceri* - chickpea symbiosis, which consequently increased the biological N_2 fixation, and yield of chickpea plants.

Table 1. Dependency of combined bioinoculation and phosphorus fertilization on nodulation and AMF colonization, phosphorus uptake and relative Inoculation Dependency in chickpea *cv.* DCP 92-3(at 50%flowering stage)

Particulars	Nodule number (No. $plant^{-1}$)				AMF colonization (Percentage)				P uptake per plant (mg $plant^{-1}$)				Relative Field Inoculation Dependency* (RFID) (in Percent) based on P uptake			
P levels (kg ha^{-1})	0	40	80	Mean*	0	40	80	Mean*	0	40	80	Mean*	0	40	80	Mean
T1	8.7	8.7	8.3	8.6	42.0	46.0	38.0	42.0	5.00	4.48	5.69	5.10	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T2	10.6	12.0	8.7	10.4	46.0	48.0	40.0	44.7	6.04	5.29	5.96	5.79	17.22 (20.80)	15.31 (18.08)	4.53 (4.75)	11.92 (13.53)
T3	11.0	11.5	9.2	10.6	46.0	48.0	42.0	45.3	6.32	7.60	8.14	7.35	20.89 (26.40)	41.05 (69.64)	30.10 (43.06)	30.61 (44.12)
T4	10.6	11.5	10.1	10.7	52.0	58.0	48.0	52.7	6.51	7.77	8.43	7.56	23.20 (30.20)	42.34 (73.44)	32.50 (48.15)	32.54 (48.24)
T5	10.6	11.5	11.5	11.2	54.0	62.0	50.0	55.3	6.90	7.45	8.54	7.63	27.54 (38.00)	39.87 (66.29)	33.37 (50.09)	33.16 (49.61)
S.Ed(±)	0.18	0.25	0.22	-	0.47	0.94	0.17	-	0.25	0.33	0.29	-	-	-	-	-
C.D. (P=0.05)	0.38	0.54	0.49	-	1.02	2.05	2.50	-	0.55	0.71	0.61	-	-	-	-	-

- Calculated values;Values in the parenthesis are Relative Field Inoculation Responsiveness(RFIR) (in Percent) based on P uptake

Microscopic examination of stained roots showed a high percentage of mycorrhizal colonization in roots of all chickpea plants treated with AMF. Root colonization by AMF also increased significantly in plants inoculated with PSB and *Mesorhizobium* cicerialong with P fertilization; the best combination was T5 - *Mesorhizobium ciceri* + PSB + AM fungi and 40 kg P_2O_5 per ha (Table 1.). Solaiman *et al.* (2012) reported the highest root colonization in AMF inoculation in combination with Rhizobium along with P fertilizer in all the studied chickpea varieties.Xie *et al.* (1995) suggested that certain flavonoids induced in the signal exchange cascade during rhizobial symbiosis formation do have a stimulatory effect on the establishment of the AM symbiosis. Long (2001) and Oldroyd *et al.* (2005) declared that Nod factors or lipo-chito-oligosaccharide signalling molecules are central to the initial establishment of the legume rhizobial symbiosis and could effect mycorrhization.

Phosphorus uptake and relative inoculation dependency

Our data indicated that P uptake increased in plants inoculated solely with PSB or AM fungi and at dual inoculation with both microorganisms (Table 1.).Among the treatments the inoculation of PSB/AM fungi either individually or in combinations recorded significantly higher P uptake. The highest P uptake was observed in plants subjected to combined inoculation with *Mesorhizobium ciceri* + PSB + AMF with 80 kg $P_2O_5ha^{-1}$. Among the inoculation treatments,the combined inoculation of *Mesorhizobium ciceri* + PSB + AMF showedthe greatest positive effect on P uptake and received RFID values of 33.16 % over the control. The bioinoculants either PSB or AM fungi enhanced the plant P uptake and AM fungi exhibits relatively better P responsiveness and dependency (based on P uptake) in all the levels of phosphorus application (0,40 and 80 Kg P_2O_5 ha^{-1}) and recorded maximum RFIDvalue of 42.34 percent at 40 Kg P_2O_5 ha^{-1} applicationunder slightly alkaline inceptisol condition.

Plant growth correlated positively with the total P concentration measured in tissues. (Lynch *et al.,* 1991) Similarly, P absorption ability was reported to be strongly connected with dry matter production (Lynch *et al.,* 1991). The fact that plant growth and nutrientuptake increased in the presence of AM fungi suggested astrong synergistic relationship between root colonization,P uptake and growth promotion. In agreement with thesefindings, Zaidi *et al.* (2003) observed that in low P soilsplant growth and nutrient uptake in chickpea weregreater after inoculation with tripartite culture of *Mesorhizobium,* PSB and *G fasciculatum* than afterinoculation with each organism alone. Because the phosphate made available by PSB acting on sparingly soluble P sources may not reach the root surface due to limited diffusion, it was proposed that if the solubilised phosphate were taken up by an AMF mycelium, this synergistic microbial interaction should improve P supply to the plant (Barea *et al.,* 2005).

Inoculation of phosphate-solubilizing bacteria (PSB) enhanced nodulation and N_2-fixation (^{15}N) byalfalfa plants, in parallel with an increase in the P content of plant tissues (Toro *et al.,* 1998). It is therefore thought that an improvement in P nutrition of the plant resulting from thepresence of PSB was responsible for increased nodulation and N_2-fixation, as it is well-known that these processes are P-dependent (Barea *et. al.,* 2005).

The differences in relative mycorrhizal dependency between crop species or even cultivars are also related to other plant factors such as root structure, plant growth rate and microorganism in the rhizosphere which could affect the demand for P.AMF are known to be effective in increasing nutrient uptake, particularly phosphorus, and biomass accumulation of many crops in soils low in phosphorus (Turk *et al.,* 2006) or soils that fix phosphorus due to a high concentration of calcium and high pH values. Lisette *et al.* (2003) reported that co-inoculation with rhizobia and compatible AMF could dramatically enhance pea growth, plant N and P content.Therefore with right combination of chemical fertilizer and biological fertilizer we can achieve to the expected yield, with lower danger for environment. The result of the present study reveals the importance of the application of biological fertilizer in low external input and sustainable agriculture.

Plant biomass, yield and Relative Field Inoculation Responsiveness

The effect of different treatments on plant biomass differed significantly. The inoculation effects of *Mesorhizobium ciceri,* PSB and AMfungieither singly or in combinationson chickpeawere variable (Table 2.). The singleinoculation of *Mesorhizobium ciceri* not differed significantly toincrease the total biomass accumulation in chickpea at vegetative and flowering stage of the crop compared to the control. Among the treatments, co - inoculation of *Mesorhizobium ciceri* + AMFsignificantly enhancedthe biomass accumulation at vegetativestageand *Mesorhizobium ciceri* + PSB combinations in flowering stagecompared to the control.The combined inoculation of *Mesorhizobium ciceri* + PSB + AMF and 40 kg P_2O_5 per ha augmented the total dry weight of chickpea plants by 37.74 % and 36.36 % RFIR values at vegetative and flowering stage respectively, compared to the control, and was superior to all the other treatments.These results are in concurrence with Solaiman *et al.* (2006) who reported that dual inoculation of *Rhizobiumand* AMF in the presence of P performed best in chickpea. Specific compatibilities between AM fungi and *Rhizobium* have been reported in pea plants (Xavier and Germida, 2003).

The positive effects of the inoculation of AMF on plant growth are generally attributed to the improved uptake

Table 2. Field Responsiveness of combined bioinoculation and phosphorus fertilization on plant biomass in chickpea *cv.* DCP 92-3

Stage of crop	Plant biomass (g plant^{-1}) at Vegetative Stage				Relative Field Inoculation Responsiveness - RFIR* (in Per cent) at Vegetative Stage				Plant biomass (g plant^{-1}) at 50%Flowering stage				Relative Field Inoculation Responsiveness - RFIR* (in Per cent) at 50%Flowering stage			
P levels (kg ha^{-1})	0	40	80	Mean*	0	40	80	Mean*	0	40	80	Mean*	0	40	80	Mean*
T1	0.47	0.61	0.53	0.54	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	3.09	2.86	3.29	3.08	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T2	0.50	0.62	0.57	0.56	6.38 (6.00)	1.64 (1.61)	7.55 (7.02)	3.70 (3.57)	3.18	3.02	3.41	3.21	2.91 (2.83)	5.59 (5.30)	3.65 (3.52)	4.22 (4.05)
T3	0.49	0.68	0.63	0.6	4.26 (4.08)	11.48 (10.29)	18.87 (15.87)	11.11 (10.00)	3.27	3.80	4.00	3.69	5.83 (5.50)	32.87 (24.74)	21.58 (17.75)	19.81 (16.53)
T4	0.49	0.77	0.7	0.65	4.26 (4.08)	26.23 (20.78)	32.08 (24.29)	20.37 (16.92)	3.29	3.68	3.87	3.61	6.47 (6.08)	28.67 (22.28)	17.63 (14.99)	17.21 (14.68)
T5	0.55	0.84	0.73	0.73	17.02 (14.55)	37.70 (27.38)	37.74 (27.40)	35.19 (26.03)	3.33	3.90	3.88	3.70	7.77 (7.21)	36.36 (26.67)	17.93 (15.21)	20.13 (16.76)
S.Ed(±)	0.026	0.030	0.033	-	-	-	-	-	0.054	0.059	0.064	-	-	-	-	-
C.D. (P=0.05)	0.055	0.065	0.070	-	-	-	-	-	0.117	0.128	0.139	-	-	-	-	-

- Calculated values; Values in the parenthesis are Relative Field Inoculation Dependency (RFID) (in Percent) based on biomass

of nutrients of low mobility, especially phosphorus (Weber *et al.,* 1993, Marschner and Dell, 1994).Piccini and Azcon (1987)reported significant increase in grain yields in alfalfa plants due to combined inoculation of phosphate solubilizing bacteria and vesicular-arbuscularmycorrhizal fungi on the utilization of rockphosphate.

Above ground biomass at harvest and seed yield in general increased significantly with all the treatments withrespectto the control (Table 3.). Increase in Seed yield due to inoculation of *Mesorhizobium ciceri* alone received the Seed yield - RFIR value of 5.08 per cent only. Seed yield - RFIR increased even further to 15.32 % when *Mesorhizobium ciceri* + PSB + AMF were combinely inoculated. The Seed yield - RFIR efficiency was more pronounced when it was applied in combination with 40 kg P_2O_5 per ha and recorded the highest RFIR value of 21.66 % (based on enhanced seed yield). Such successful establishment of combined inoculants has been reported by many other workers (Bansal, 2009; Khanna and Sharma, 2011, Suneja *et al.,* 2007) and reported as potential inputs forimproving crop yields in a number of leguminous crops.

In conclusion, this study revealed that the chickpea dependence and its field inoculation responsiveness especially to the inoculation of *Mesorhizobium ciceri,* Phosphobacteria (PSB-*Bacillus megaterium*), ArbuscularMycorrhizal Fungi (AMF-*Glomus* sp.) and phosphorus; the findings of the present study clearly define the synergistic effect of combined inoculation and which to be a beneficial strategy for improving symbiotic efficiency, plant growth and yield ofchickpea. Thus, in nutrient deficient semi aridinceptisol type soils, the inoculation of chickpea with a combination of PSB and mycorrhizal fungi mayhelp the plants to obtain P from native insoluble P sources/applied fixed fertilizer P and would be beneficial for a sustainable nutrient managementand reduction on chemical fertilizers especially in developing countries like India. It can also play a role inmaking organic farming sustainable and cost effective. However, their

Table 3. Field Responsiveness of combined bioinoculation and phosphorus fertilization on biomass and seed yield in chickpea *cv.* DCP 92-3

Particulars	Biomass (at harvest) (tonnes ha^{-1})				Seed Yield (tonnes ha^{-1})				Relative Field Inoculation Responsiveness* (RFIR) (in Per cent) based on Yield			
P levels	0	40	80	Mean*	0	40	80	Mean*	0	40	80	Mean*
T1	1.773	2.387	2.587	2.249	0.971	1.219	1.413	1.201	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T2	2.213	2.507	2.600	2.440	1.077	1.221	1.488	1.262	10.92 (9.84)	0.16 (1.77)	5.31 (5.04)	5.08 (5.33)
T3	2.427	2.560	2.773	2.587	1.08	1.272	1.571	1.308	11.23 (10.09)	4.35 (4.17)	11.18 (10.06)	8.91 (8.18)
T4	2.387	2.773	2.827	2.662	1.091	1.475	1.52	1.362	12.36 (11.00)	21.00 (17.36)	7.57 (7.04)	13.41 (11.82)
T5	2.640	2.640	2.907	2.729	1.131	1.483	1.541	1.385	16.48 (14.15)	21.66 (17.80)	9.06 (8.31)	15.32 (13.29)
S.Ed(±)	0.059	0.041	0.045	-	0.016	0.024	0.032	-	-	-	-	-
C.D. (P=0.05)	0.127	0.095	0.087	-	0.034	0.052	0.070	-	-	-	-	-

- Calculated values; Values in the parenthesis are Relative Field Inoculation Dependency (RFID) (in Percent) based on Yield

application needs to be further evaluated under different agro climatic conditions in the field.

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