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Evaluation of non symbiotic nitrogen fixing bacterial influence on rhizobium nodulation behaviour in bacterial consortia

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Abstract
The present investigation was conducted in glass house of Department of Agricultural Microbiology, College of Agriculture, IGKV, Raipur, Chhattisgarh during *Rabi* season, 2016-17 comprising 9 treatments (treatments were different bacterial consortia and control without inoculate in bacteria and 3 replications in CRD with the objective to find out the effect of non rhizobial nitrogen fixer of bacterial consortia on rhizobium nodulation behaviour in chickpea. The results of nodule study under pot experiment revealed that the highest nodulation was recorded in with T5: chickpea with rhizobium +75%NPK i.e. 39.33 followed 38 plant^{-1} by T3: (Chickpea +consortia of *Rhizobium*, *Azotobacter*, *Azospirillum* +75%NPK) and least plant nodule was recorded as uninoculated of control with 12.33 plant^{-1} at 45 DAS. The highest fresh nodule weight was T5 (48.33 mg plant^{-1}) followed by T3 (45 mg plant^{-1}) and fresh weight of control was (18.33 mg plant^{-1}). Dry weight in T5 (22.67 mg plant^{-1}) followed by T3 (19.33 mg plant^{-1}) and dry weight of control was 6.33 (mg plant^{-1}). Further C1 also has maximum root length (27.00 cm) and root biomass (7.30g) as compared to other treatments. Overall results revealed that there is no significant reduction in nodulation by the presence of other non symbiotic nitrogen fixer. However, other plant growth was positively in presence of symbiotic and non symbiotic members in same microbial consortia.

Keywords: *Rhizobium*, *Azotobacter*, *Azospirillum*, Consortia.

Introduction

In order to make its cultivation sustainable and less dependent on chemical fertilizers, it is important to know how to use plant growth promoting rhizobacteria (PGPR) that can biologically fix nitrogen, solubilize phosphorus and induce some substances like plant hormones that could contribute to the improvement of plant growth. Rhizobacteria are a heterogeneous group of bacteria that can be found in the rhizosphere, at surfaces of the root and in association with roots, which can enhance the level or quality of plant growth directly and/or indirectly. A large number of bacterial genera including, *Azospirillum*, *Azotobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Klebsella*, *Pseudomonas*, and Serratia have beenreported as PGPR to enhance plant growth (Kumar *et al*. 2012, Gujral *et al*. 2013) [11, 5]. The plant growth promotion by PGPR involves either providing the growth promoting substances that are synthesized by the bacteria or facilitating the uptake of plant nutrients from the surrounding environment. Therefore, the identification, selection and application of suitable beneficial microorganisms can increase the options to deal with growing problems and can be also environmentally sound (Kilian *et al*., 2000, Woitke *et al*., 2004) [9, 25].

Further, mixed inoculant (microbial consortia) interacts synergistically with each other, increases plant growth and also protects them from phytopathogens (Maiyappan *et al*. 2010) [15]. It is also evident from the available literatures that microbial consortium of plant growth-promoting rhizobacteria improves the performance of plants growing in stressed soils (Panwar *et al*. 2014) [19]. Keeping in view fact, the present work is proposed to evaluate the effect of co-inoculation of non symbiotic PGPR and Rhizobia on growth and nodulation of chickpea.

Methods and Materials

Bacterial culture revival

The experiment was conducted in the glass house of Dept. of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh) during 2016-17 with chickpea crop. Rhizobacterial culture here procured in YEMA media, OKON'S media, JENSEN'S media. All isolates were obtained from department culture repository and maintained in YEMA media (for *Rhizobium*), OKON'S media (for *Azospirillium*), and JENSEN'S media (for *Azotobacter*). During this experiment four isolates were compared alone and in combination with the uninoculated control, the number of treatments was nine replicated three in completely randomized design. Chickpea (JG14) was taken as a test variety.

Glass house experiment

The medium used for growing chickpea crop was soil (Alfisol) which was well air dried and processed to good physical condition ideal for chickpea growth. This soil was filled in polythene bags @ 6 kg per bags. Soils was collected from a depth of 6 inches (15cm) from soil surface and thoroughly mixed and filled in each polyt *hene bag having*10 kg capacity. Two best consortia were selected from earlier studied by Nag 2016 [16], along with individual isolates. Uniform level of Nitrogen, Phosphorus and Potassium @ 20:40:20 kg/ha was applied as basal through urea, single super phosphate and murate of potash respectively*the* ***as per*** *(1 treatment decided (Table* Thus, the experiment comprising the following *nine* treatments with three replicated.

Seed treatment and showing

Healthy seeds of chickpea (JG 14) were taken for experimentation. Just before sowing, healthy seeds of chickpea were treated with Thiram @ 3 gm/kg of seed. All Plant Growth Promoting rhizobacteria culture inoculated separately to 25 ml nutrient broth in 50ml conical flask and incubated at 28±2^{0}C for72 hours. Equal volume of the broth cultures (OD_{600nm} : 0.4) here then used for the purpose of bacterization of seeds at the time of sowing.

About 24 hours before sowing, all the bags were irrigated by unsterilized water. Five holes (2 to 3 cm deep) per bags were made with the help of sterilized glass rod maintaining equal distance from hole to hole. Sowing of 5 seed/ pot was done on 13-12-2016 by placing one seed in each hole with the help of sterilized forcep. After germination a population of 3 plants per bags was maintained by thinning out the extra seedlings. Uniform irrigation to all pots was given as and when required. The nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) were applied @20:40:20 kg/ha by using urea, single super phosphate and murate of potash, respectively.

Nodulation study

Roots of uprooted plants were uprooted and washed carefully so that nodules were not damaged, then no. of nodules and their fresh weight, dry weight were recorded. After recording the fresh weight, the nodules were kept in small papers bags and were dried in hot air oven at 60^{0}C till their constant oven dry weight is obtained. The oven dried different nodule were grounded into powder through Wiley mill and used for N analysis. The nitrogen content in the plant samples was estimated by Micro – Kjeldhal method as described by Jackson (1973) using auto digestion and distillation system. The nitrogen concentrations of each component were multiplied with their respective biomass to obtain nitrogen uptakes.

Results and Discussion

The present investigation entitled "Effect of microbial consortia on nodulation behavior of chickpea under controlled conditions" was conducted at Department of Agricultural Microbiology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during the year 2016-17. The results obtained from these studies are depicted and discussed in this chapter.

Number of nodules

Nodulation of chickpea crop as affected by different soil inoculums as treatments at 45 DAS are presented in Table 3. Results indicated that the average number of nodules uninoculates plant was increased from 12.33 to 39.33, due to inoculation of seeds with promising native *Rhizobium* isolates at 45 DAS. The highest nodulations were found in plants raised from seeds inoculated with the chickpea containing T5 (with 39.33 no. of nodule plant^{-1}), followed by T3 (with 38.00 no. of nodule plant^{-1}). There were 12.33 nodules in most of the uninoculated control plants. Significantly higher nodulation was observed in T5 (39.33 avg. nodules plant^{-1}).

This observation found with PGPR was found significantly better for nodulations of chickpea (*Cicer arietinum L.*) over control in a pot and trial. *Rhizobium* strains differ in their ability to nodulate and in their capacity to fix nitrogen. Response differs due to different population levels of native and added strains of *Rhizobium* and due to the competitive ability of native *Rhizobium* with the added strain (Rupela and Dart 1980). Symbiotic nitrogen fixation by *Rhizobium* meets the N_2 requirement of the crop and leaves 40-108 kg/ha. in the soil (Subba Rao 1976) [13].

Nodule fresh weight

Results of nodules fresh weight are presented in Table 3 revealed that at 45 DAS fresh nodule weight significantly increased due to bacterization of plant (ranged 18.33 to 48.33 mg plant^{-1}). The fresh nodule weight increased significantly from 18.33 (mg plant^{-1}) of (control) to 48.33, 45.00, 42.00, 37.00, 34.00, 32.67, 32.33 and 30.00 (mg) due to inoculation of consortia (with T5, T3, T4, T8, T1, T7, T2, and T6), respectively. Fresh nodule weight was recorded 18.33 (mg plant^{-1}) as lowest and 48.33 (mg plant^{-1}) was highest among the inoculated plants.

Nodule dry weight

The result revealed that the dry weight of nodules increased from 6.33 (mg plant^{-1}) (control) to 22.67 (mg plant^{-1}). 19.33, 15.33, 14.00, 12.67, 10.00, and 9.33 (mg) per plant when chickpea seeds were grown with soil inoculums numbered treatment. (T5, T3, T4, T8, T1, T7, T2 respectively.

Maximum increase in dry weight of nodule was observed by isolate T5 (with 22.67 mg plant^{-1}), followed by T3 (with 19.33 mg plant^{-1}), followed by T4 (15.33 mg plant^{-1}), value of nodule dry weight was 6.33 (mg plant^{-1}) in case of uninoculated control while highest value of nodule dry weight, 22.67 (mg plant^{-1}) that was observed in seed inoculated with T3.

N content

Value of N- content in Nodule after 45 DAS in chickpea was increased from 1.14 percent as compared to the control to 5.77 percent. Highest N content was associated T5 (5.77) followed by T3 (5.56) while among inoculated minimum was found at T2, (2.83) respectively. Lowest value of N – nodule was (2.83) and highest was (5.77) by inoculated plant raised under green house mixed culture conditions.

Root length & Biomass accumulation

Data of root length recorded at 45 DAS crop growth presented in Table 4 At 45 DAS some of the microbial isolates alone and in combination found significant to enhance the root growth. However, rest of the microbial isolates alone and in combination found significant affected by applied PGPR at 45 DAS. Performance of treatments T3, T8, T7, T4, T5, T6, T1 and T2 were found well over controls respectively T9 (15.33 cm). Results of root biomass are presented in Table 4.5 revealed that at 45 DAS fresh root weight increased due to bacterization of plant. The fresh root biomass increased significantly from 4.12 (g) of (control) to 7.30, 6.80, 6.48, 6.38, 6.19, 5.94, 5.26, and 4.80 (g) due to inoculation with (T3, T8, T7, T4, T5, T6, T1, and T2). Among the treatments fresh root weight was recorded 4.80 (g) per plant in case of T2 where as lowest and 7.30g per plant was highest among the inoculated plants which was association with T3.

The dry weight of root increased from 0.74 g (control) to 2.71, 2.20, 2.09, 2.07, 2.03, 1.80, 1.39 and 1.37 g per plant when chickpea seeds were grown with treatment (T3, T8, T7, T4, T5, T6, T1, and T2) respectively. Maximum increase in dry weight was observed by isolate T3, followed by T8 (2.20g), followed by T7 (2.09 g), value of plant dry weight was 0.74 (g) per plant in case of uninoculated control while highest value of plant dry weight, 2.71 (g) per plant that was observed in seed inoculated with T3.

In the present investigation experiment was set to evaluate the effect of co-inoculation of other nitrogen fixer along with rhizobium in chickpea. Here we made consortia with rhizobium with three other isolates of *Azotobacter* and *Azospirillum* which were earlier characterized for their plant growth properties other than nitrogen fixation (Nag, 2015, Nag, 2016) [16]. The idea of inoculating legumes with rhizobia and other PGPR has long been pursued. There are reports of experiments conducted on chickpeas (Hamaoui *et al*. 2001) [7] and other legumes (Remans *et al*. 2008, Kumar and Chandra 2008, Lima *et al*. 2011) [12, 14]. Results indicated that inoculation alone and inoculation of Rhizobium with other nitrogen fixing bacteria increased most of growth indices. Dashadi *et al*. (2011) [2] also reported that co-inoculation of *Rhizobium* and *Azotobacter* increased most of growth parameter such as: number of nodule, nodulation, total nitrogen content, relative water content, root dry weight, mean day germination and day germination speed. Co-inoculation of rhizobia on seeds and *Azospirillum* resulted in outstanding increases in grain yield (Star *et al*. 2012, Hungria *et al*. 2013,) [23, 8]. Bai *et al*. (2003) reported that co-inoculation of *Bacillus* strains in soybean plants with *Bradyrhizobium japonicum* provided the largest increases in nodule number, nodule weight, shoot weight, root weight, total biomass, total nitrogen, and grain yield. Further, role of PGPRs co-inoculation greatly helped in increasing the root biomass and thus indirectly enhanced absorption of nutrients from surrounding environment. In present study combined inoculation of *Azotobacter* and *Azospirillum with Rhizobium* also increases root length and root biomass. These results are in the same line with Abdel-Wahab *et al*. (2008) [1], Sadaghiani *et al*. (2008) [22], Verma *et al*. (2010) [24].

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Table 1: Treatments for glass house experiment.

Treatment No.	Name of Isolate
T1	Chickpea+100%NPK
T2	Chickpea+75%NPK
T3	Chickpea +C1+75%NPK
T4	Chickpea +C2+75%NPK
T5	Chickpea+GmR8+75%NPK
T6	Chickpea+AZO137+75%NPK
T7	Chickpea+ASL3+75%NPK
T8	Chickpea+ASL4+75%NPK
T9	Control

100% NPK = Recommended Dose of Fertilizer (NPK) 20:40:20

Table 2: Details in this study of Bacterial Consortia used in present study

Consortia	Bacterial Members	PGPR properties of consortia
C1	GmR8(*Rhizobium*)+AZO137 (*Azotobacter*)+ASL3 (*Azospirillum*)+ASL4 (*Azospirillum*)	BNF, siderophore production, IAA, temperature, pH, salt tolerance
C2	GmR8+AZO137+ASL4	BNF, siderophore production, IAA, Temperature, pH, salt tolerance

Table 3: Nodulation study of chickpea in Pot Experiment

Tr. No.	Name of Isolates	Average Nodules at 45 DAS			
		No. of Nodules (plant^{-1})	Weight of Fresh Nodules (mg plant^{-1})	Weight of Dry Nodules (mg plant^{-1})	% of Nitrogen in nodule at 45 DAS
T1	Chickpea+100%NPK	29.67	34.00	10.00	3.70
T2	Chickpea+75%NPK	27.00	32.33	9.33	3.40
T3	Chickpea +C1+75%NPK	38.00	45.00	19.33	5.56
T4	Chickpea+C2+75%NPK	35.43	42.00	15.33	4.53
T5	Chickpea+GmR8+75%NPK	39.33	48.33	22.67	5.77
T6	Chickpea+AZO137+75%NPK	27.00	30.00	12.67	3.48
T7	Chickpea+ASL3+75%NPK	28.67	32.67	9.33	3.75
T8	Chickpea+ASL4+75%NPK	30.17	37.00	14.00	2.83
T9	Control	12.33	18.33	6.33	1.14
	SE (m)	1.22	1.44	0.77	0.09
	CD	3.64	4.29	3.72	0.52

Table 4: Effect of Rhizosphere bacterial consortia on performance of chickpea. On Root length, biomass fresh weight and dry weight at 45 DAS

Tr. No.	Name of Isolates	Avg. Root length (cm)	Biomass fresh weight (g plant-1) at 45 DAS	Biomass dry weight (g plant-1) at 45 DAS
T1	Chickpea+100%NPK	19.33	5.26	1.39
T2	Chickpea+75%NPK	17.00	4.80	1.06
T3	Chickpea +C1+75%NPK	27.00	7.30	2.71
T4	Chickpea+C2+75%NPK	24.00	6.38	2.07
T5	Chickpea+GmR8+75%NPK	23.67	6.19	2.03
T6	Chickpea+AZO137+75%NPK	23.40	5.94	1.80
T7	Chickpea+ASL3+75%NPK	24.39	6.48	2.09
T8	Chickpea+ASL4+75%NPK	24.72	6.80	2.20
T9	Control	15.33	4.12	0.74
	SE (m)	0.97	0.51	0.35
	CD	2.89	1.53	1.06

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