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Effect of plant growth-promoting rhizobacterial composite culture on the growth of chickpea seedlings

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In the present study, 20 soil and plant samples from 13 villages of Raipur, Durg, and Balod District of Chhattisgarh (India) were collected from chickpea fields. From these samples, a total of 86 isolates including 16 *Rhizobium*, 40 *Azotobacter*, 29 *Azosprillum*, and one PSB were obtained on selective culture media. All the isolates were screened for their plant growth-promoting traits. Three (GmR8, ASL3 & ASL4) out of 86 were finally selected for further studies. One *Azotobacter* isolate, i.e., Azo137, was selected from the departmental culture collection. Finally, four isolates including GmR8 (*Rhizobium*), ASL3, ASL4 (*Azospirillum*), and Azo137 were selected for composite culture formulations. GmR and ASL4 were siderophore-producing isolates, whereas ASL3 and Azo137 were IAA producer along with their ability to fix nitrogen. Five composite cultures were prepared randomly and tested for effect on the growth of chickpea (the seedling test and the pot experiment). Among all the composite culture groups, C2 (GmR8, Azo137, ASL4) significantly increased the root (10.84 cm) and shoot (8.10 cm) length, whereas biomass (3.60 g) was the highest in the case of C1 (GmR8, Azo137, ASL3, ASL4) of seedlings as compared to the control (6.80 cm, 2.60 cm, and 3.30 g, respectively). Overall, the study revealed a better performance of composite or mixed culture over individual bacteria.

Keywords: rhizobacteria, consortia, chickpea, agriculture

INTRODUCTION

Chickpea is a very important pulse crop which contains 21.1% protein, 61.5% carbohydrates, and 4.5% fat. According to the FAO data, in 2016, world production of chickpeas was 12.1 million tonnes. (FAOSTAT, 2017). About 65% of the global area with 68% of global chickpea production is contributed by India (Reddy and Mishra, 2010). The production is still not adequate to meet the domestic demand due to its low productivity (850 kg/ha). The major causes of low productivity of chickpea in India are low yield potential and susceptibility of improved present-day cultivars to various biotic and abiotic stresses (Gowda et al., 2011).

A large array of bacteria including species of *Arthrobacter*, *Alcaligenes*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, and *Serratia* have been reported as plant growth-promoting rhizobacteria (PGPR)

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to enhance plant growth (Kloepper et al., 1988; Okon, Labandera-Gonzalez, 1994; Glick et al., 1995). Van Loon et al. (1997) critically reviewed the reasons for poor performance of agricultural bioinoculants in natural environments and in the plant rhizosphere after which he suggested that instead of using a single strain for a single trait, it was better to use a microbial consortium having multifarious uses. The present work is the initial step in the development of a microbial consortium for enhancing the growth of the chickpea.

MATERIALS AND METHODS

Collection of soil samples and soil characteristics

Twenty soil samples were collected from 13 villages in District Raipur, Durg, and Balod districts of Chhattishgarh (Table 1). Representative samples of surface soil with intact plants were collected from a chickpea growing farmer's field. These samples were properly tagged, sealed, and stored in a refrigerator for further study. Physico-chemical properties like, determination of soil pH, organic carbon, and dehydrogenase activity were determined by using previously described methods (Walkley, Black 1934; Klein et al., 1971).

Isolation of plant growth-promoting rhizobacteria (PGPR)

Isolation of plant growth-promoting bacteria was done by the serial dilution method followed by plating on Yeast Extract Mannitol Agar (YEMA) for (Rhizobium), *Azospirillum* agar, Azotobacter agar, and Pikovskaya's agar (for PSB), and incubated at their respective temperatures.

Chrome azurol sulfonate assay for screning for siderophore production

Chrome Azurol Sulfonate (CAS) dye measuring 60.5 mg was dissolved in 50 ml DI water

Table 1. **Details of soil and plant samples collected from different locations in Chhattisgarh**

No.	Village name	Soil sample No.	pH	Organic carbon, %	DHA (ugTPF/h/g)
1	Achoti	1	8.51	0.18	16
		2	7.09	0.345	24
2	Amethi	1	6.99	0.67	26
		2	6.43	0.88	22
3	Malpuri khurd	1	8.04	0.43	36
		2	7.81	0.315	20
4	Ahiwara	1	7.17	0.165	14
		2	5.90	0.99	14
5	Sandi	1	8.13	0.345	22
		2	8.18	0.78	37
6	Khamtarai	1	7.92	0.58	41
		2	8.04	0.46	75
		3	8.28	0.84	30
7	Hingna	1	8.09	0.33	40
8	Meduka (Pendra road)	1	5.47	0.43	44
9	Latabod (Balod)	1	5.86	0.96	30
10	Sankara (Balod)	1	5.94	1.215	108
11	Khuteri (Balod)	1	7.68	0.705	18
12	Sankari(Balod)	1	5.65	0.90	11
13	Limora (Balod)	1	7.36	2.11	60

and mixed with 10 ml iron (III) solution (1 mM $FeCl_3 \cdot 6H_2O$ and 10 mM HCl). Under stirring the solution was added to 72.9 mg HDTMA (Hexadecyltrimethylammonium bromide) dissolved in 40 ml DI water. The resultant dark blue solution was autoclaved. A mixture of 750 ml DI water, 100 ml 10X MM9 salts (60 g/L Na_2HPO_4, 30 g/L KH_2PO_4, 5 g/L NaCl, 10 g/L NH_4Cl, 2 ml of 1 M $MgSO_4$, 20 ml of 20% glucose and 100 µl of 1 M $CaCl_2$), 15 g agar and 0.1 M 10.29 g of Tris-HCl was made with pH of the solution 6.8. After cooling to 50°C, 30 ml of Tryptone as carbon source was added. Finally, the dye solution was added along the glass wall with enough agitation to achieve mixing without foaming (Schwyn, Neilands, 1987; Krey, 2008). Culture was directly spotted on CAS agar plates and incubated for 48 h at 28°C and examined for growth and production of orange halos surrounding the colonies (Krey, 2008).

Formulation of composite cultures

Four rhizobacteria isolates were finally selected based on their PGPR properties and compared alone and in combination with the chickpea seedlings germinated by paper towel method. Chickpea seeds were surface-sterilized with 1% sodium hypochlorite for 5 min and washed five times with sterilized distilled water. Seeds were soaked with inoculum (10^8–10^9 colony forming units (CFU)) for 5 min, and dried in air. After bacterization, 50 seeds were placed in wet germination paper (three layers), covered with polythene, and incubated in incubator at 25°C for five days. Seeds soaked in distilled water were treated as control. Three replications for each treatment were maintained. The total number of treatments for this experiment was ten, which are summarized in Table 2.

Seedling growth parameters

The root length, shoot length, and fresh and dry weight were recorded for each seedling and the total nitrogen content in the seedlings was estimated by Micro-Kjeldahl method as described by Jackson (1973) using auto digestion and distillation system. Available nitrogen was determined by alkaline $KMnO_4$ method of Subbiah and Asija (1956).

RESULTS AND DISCUSSION

Out of 20 plant samples, only 16 had good nodules. From these 16 samples, *Rhizobium* was

Table 2. **Details of seedling treatment along with PGP properties of different PGPRs used in this study**

Treatment	Details	PGPR	PGPR properties
T1	C1	GmR8+AZO137+ASL3+ASL4	BNF, siderophore production, IAA, temperature, pH, salt tolerance
T2	C2	GmR8+AZO137+ASL4	BNF, siderophore production, IAA, temperature, pH, salt tolerance,
T3	C3	GmR8+ASL3	BNF, siderophore production, IAA
T4	C4	AZO137+ASL3+ASL4	IAA, temperature, pH, salt tolerance, BNF, siderophore production
T5	C5	AZO137+ASL3	IAA, temperature, pH, salt tolerance, BNF, siderophore production
T6	GmR8	*Rhizobium* isolate (from this study)	BNF, siderophore production
T7	Azo137	*Azotobacter* (selected from previous study Nag 2015)	BNF, IAA, temperature, pH, salt tolerance
T8	ASL3	*Azosprillum* (from this study)	BNF, IAA
T9	ASL4	*Azosprillum*(from this study)	BNF, siderophore production
T10	Control		

successfully isolated on YEMA plates. These isolates were then further screened for their ability of producing siderophores and IAA. Out of 16 samples screened, only one isolate (GmR8) was found to possess the ability of producing siderophores on a CAS agar plate. A total of 86 isolates were then tested for their ability to produce IAA and siderophore. Out of 86 isolates, one *Rhizobium* isolate (GmR8) and one *Azospirillum* (ASL4) were siderophore-producing whereas ASL3 (*Azospirillum*) was IAA-producing (Fig. 1).

Formulation of the composite culture

Four bacterial isolates were finally selected for further study, based on their plant growth-promoting ability. One out of four, Azo137, was selected from a previous study conducted in the department of Agricultural Microbiology, College of Agriculture, Raipur (Nag, 2015). Five different compositions were formulated randomly by using these four isolates (Table 2).

Effect of composite cultures on chickpea seedlings

The observations of seedling treatment were recorded after five days of germination. Growth parameters in incubated conditions showed significant difference as compared to that of the control (Fig. 2). The data presented in Table 3 indicated that the increase in the shoot length of the seedlings was in the range of 2.60 to 8.10 cm. The shoot length of the seedlings observed were 7.65, 8.10, 4.14, 6.76, 6.71, 6.35, 4.72, 4.41, 3.26, and 2.60 cm from T1, T2, T3, T4, T4, T5, T6, T7, T8, T9 and T10, respectively. Also, this study revealed that the biggest shoot length was found to be 8.10 cm, followed by 7.65 cm on T2 and T1, respectively, which was significantly bigger than the control, i.e., 2.60 cm (T10). Similarly, the variation in the root length was found to be between 6.80 and 10.84 cm out of which the biggest was recorded in T2. There was a significant increase in fresh weight of seedling from 9.23 to 13.93 g. The highest being 13.93 mg was followed by 13.53 mg from treatment T1 and T3, respectively among all the treatments. Further, the highest dry weight of seedling per seedling was found to be 3.93 g which is an increase of about 0.60 g from the lowest, that of the control.

There is a clear indication from the experimental data that there was a noteworthy jump in nitrogen content (seedling nitrogen content) from 11.32% in the control to 17.66% in T1 due to seed inoculation. The lowest value among the inoculated treatments remains at 15.66% in T8. The overall result revealed that the composite culture C1, i.e., (GmR8+Azo137+ASL3+ASL4) remarkably increased the root and shoot length along with biomass of seedlings as compared to that of the control. There was no significant variation observed in the pot experiment except nodulation among all the parameters studied.

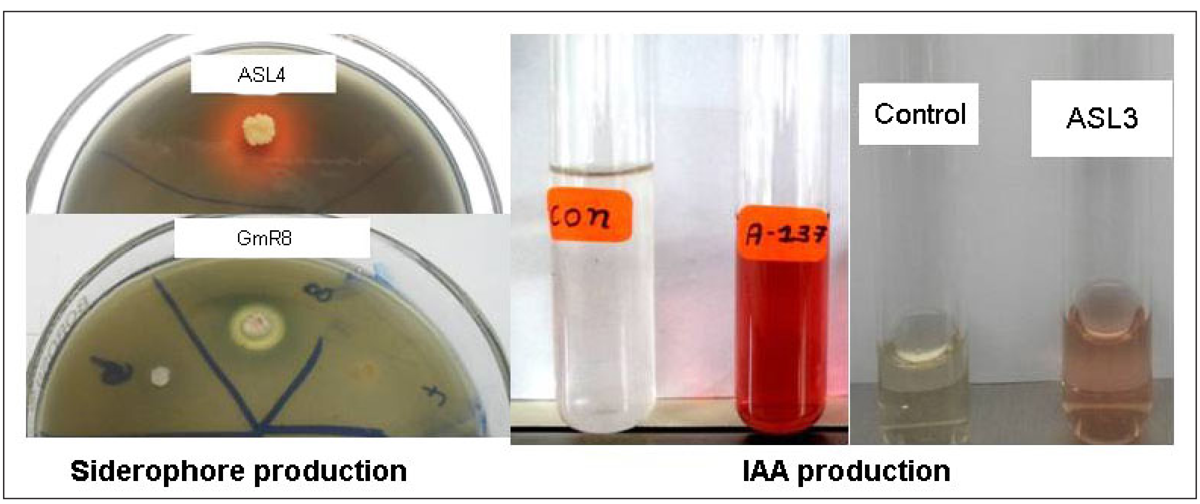

Fig. 1. Bacterial isolates selected for the study

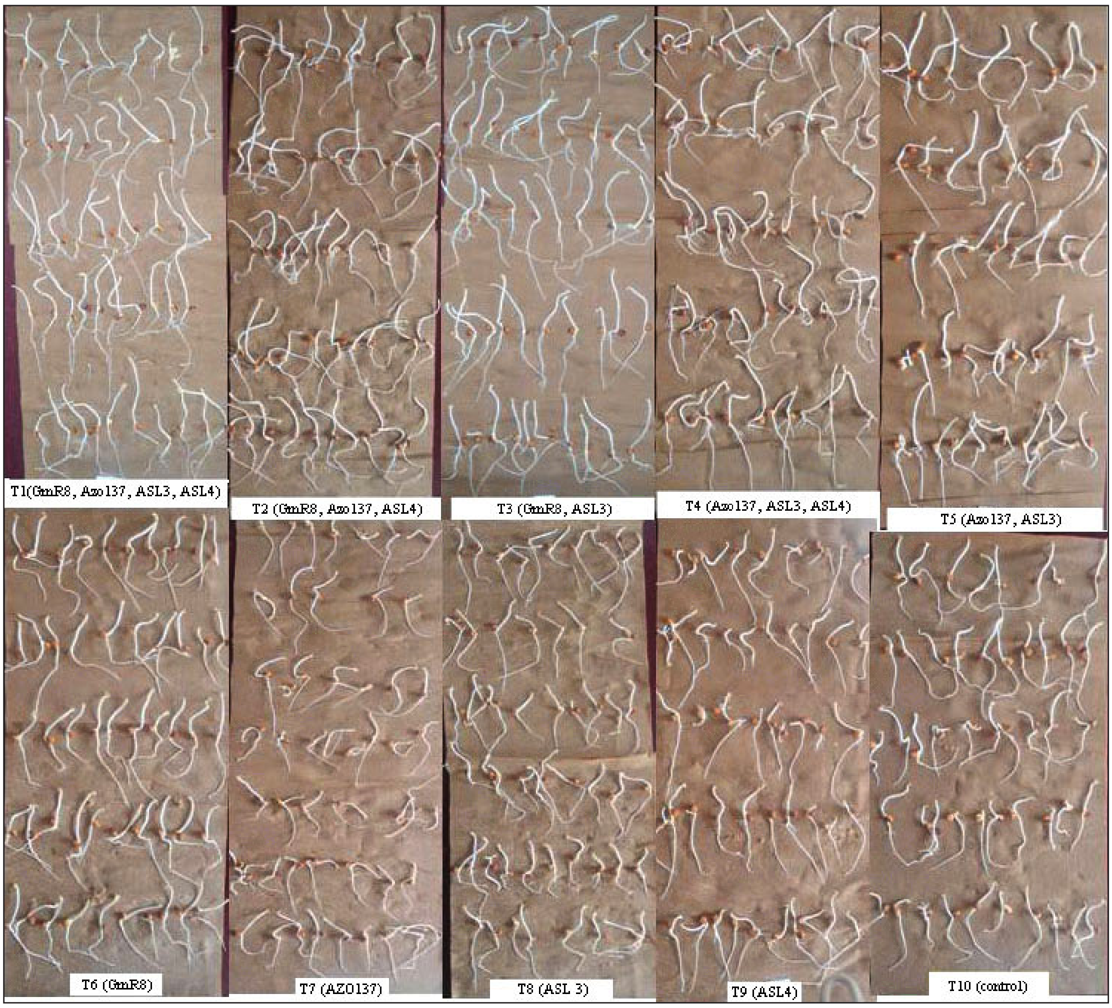

Fig. 2. Effect of composite culture on chickpea seedlings

Table 3. **Effect of the multipurpose composite culture on the growth of chickpea seedlings. The chickpea seedlings were germinated for five days and the following different parameters were observed**

Treatments		Seedling nitrogen content, %	Fresh weight of seedling, g	Dry weight of seedling, g	Shoot length of seedling, cm	Root length of seedling, cm
T1	C1	17.66	13.93	3.93	7.65	9.93
T2	C2	15.99	11.50	3.60	8.10	10.84
T3	C3	16.99	13.53	3.80	4.14	7.40
T4	C4	16.66	13.10	3.63	6.76	9.25
T5	C5	15.99	12.60	3.53	6.71	9.35
T6	GmR8	16.09	11.57	3.57	6.35	7.63
T7	AZO137	17.59	10.83	3.40	4.72	7.84
T8	ASL3	15.66	12.50	3.43	4.41	7.66
T9	ASL4	16.36	9.90	3.63	3.26	7.45
T10	Control	11.32	9.23	3.30	2.60	6.80
S Em		0.515	0.515	0.186	0.3090	0.4240
CD		1.543	1.543	N/A	0.9260	1.2690

However, nodulation in the treatment associated with C1 composite group was also found to be good.

PGPR are the invisible entity behind the visible physical growth of plants. A wide range of applicability starting from enhancing the nutrient uptake and production growth regulators in plants to siderophore production and serving as a biocontrol agent are the few beneficial effects of PGPR worth mentioning (Vejan et al., 2016). Considerable amount of studies have been conducted on the effect of plant growth-promoting rhizobacteria on the growth of chickpeas by a number of researchers (Karnwal, Kumar, 2012; Yadav et al., 2010; Dasgupta et al., 2015). However, very little progresses on the application of composite culture of PGPR on chickpea growth has been highlighted. One such work on composite culture was carried out by Wani et al. (2007) by inoculating *Mesorhizobium ciceri* with *Azotobacter chroococcum* and *Bacillus* sp. and, indeed, the results were very encouraging. A threefold increase in the seed yield was observed, followed by an increase in the seed protein level. Also an enhancement in pod and straw yield was noted by Qureshi et al. (2009) by co-inoculating *Mesorhizobium ciceri* and *Bacilus megaterium* in chickpea. Similar results of the use of composite cultures of PGPR on plant growth were also seen in several other crops like maize (Agbodjato et al., 2016), wheat (Khan, Zaidi, 2007), common bean (Korir et al., 2017), and green gram (Gupta et al., 2003).

Several scientific researchers are in tune with the view that a composition of many PGPRs always gives a better result as compared to individuals (Martins et al., 2004; Ladwal et al., 2012; Chandra, Pareek, 2015) and this case there was no exception. Growth parameters like nitrogen content, fresh and dry weight, shoot length as well as root length were much higher in C1 than in T6, T7, T8, and T9, which were composed of single inoculants. However, it must also be kept in mind that compatibility among different microbial genera and strains must be a positive and synergistic one (Tilak et al., 2006). As per the data obtained from the above experiment, C1 culture stands ahead of other composite cultures, i.e., C2, C3, C4, and C5 with respect to most of the growth parameters studied. Thus, this highly signifies the importance of suitable compatibility among different microbial strains that is to be strictly maintained. Finally, after analyzing the experimental data, we could arrive at a conclusion that the use of a composite culture of PGPR in chickpeas could pave the way for a multifaceted effect on plant growth.

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RIZOBIJŲ KULTŪRŲ POVEIKIS SĖJAMOJO AVINŽIRNIO DAIGŲ AUGIMUI

Santrauka

Šio tyrimo metu iš 13 kaimų (Raipur, Durg ir Balod rajonai, Indija) buvo surinkti sėjamojo avinžirnio laukų dirvožemio ir augalų mėginiai. Iš visų selektyvios kultūros terpės mėginių atrinkti 86 izoliatai, iš kurių 16 buvo *Rhizobium*, 40 *Azotobacter*, 29 *Azosprillum* ir vienas PSB. Patikrinta, kokias augimo savybes skatino visi izoliatai. Trys (GmR8, ASL3 ir ASL4) iš 86 izoliatų buvo atrinkti tolesniam tyrimui. Vienas *Azotobacter* izoliatas (Azo137) buvo parinktas iš Žemės ūkio mikrobiologijos departamento kultūrų kolekcijos. GmR8 ir ASL4 izoliatai gamino sideroforą, o ASL3 ir Azo137 buvo IAA gamintojai ir gebėjo fiksuoti azotą. Iš visų sudėtinių kultūrų grupių C2 (GmR8, Azo137, ASL4) gerokai padidino šaknies (10,84 cm) ir ūglio (8,10 cm) ilgį, didesne biomase (3,60 g) pasižymėjo C1 (GmR8, Azo137, ASL3, ASL4) sėklos, palyginti su kontroline grupe (atitinkamai 6,80 cm, 2,60 cm ir 3,30 g).

Raktažodžiai: rizobijos, konsorciumas, sėjamasis avinžirnis, žemdirbystė