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Studies on the inoculation effect of PGPR on the enhancement of growth and yield parameters in rainfed groundnut (*Arachis hypogaea* L.)

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Abstract

The application effect of different formulations of *Bradyrhizobium* and *Paenibacillus* cells, viz., single strain inoculation, co-inoculation and co-aggregates application together with 75% recommended N and P level on the enhancement of growth and yield parameters of rainfed groundnut was studied under *in-vitro* conditions. It was observed that the application of each formulation of *Bradyrhizobium* and *Paenibacillus* cells augmented the growth and yield parameters of rainfed groundnut to a higher level when compared to uninoculated control. However, the application of *Bradyrhizobium* and *Paenibacillus* cells, as, natural co-aggregates, exhibited the highest performance followed by co-inoculation and single strain inoculation of PGPR cells. It was concluded the application of PGPR cells viz., *Bradyrhizobium* and *Paenibacillus* as Interbacterial co-aggregates, together with 75% recommended N and P level augmented the growth and yield parameters of rainfed groundnut to the highest level when compared to control (100% recommended N and P level without bio inoculation) and thus a saving of 25% recommended N and P level is possible due to co-aggregates formulation of PGPR cells.

Keywords: DNA isolation, Genotyping, Salting out, Rapid, Non-enzymatic, organic solvents, human blood.

Introduction

Groundnut or peanut (*Arachis hypogaea* L.) is an important oilseed crop called as wonder nut and poor men's cashew nut. Being a valuable source of all the nutrients, groundnut plays a vital role in providing an inexpensive source of high quality dietary protein (26%) edible oil content in the diets (45-50%). It is one of the most important sources of food and cash crop of our country. (Naik *et al.*, 2014) [15]. Groundnut is exposed to various biotic and abiotic stresses, i.e., most sensitive to moisture stress and it is susceptible to a host of diseases and pests which limits the productivity along with factors like declining soil fertility; poor nutrition etc., Among major nutrients, Phosphorus is an essential nutrient for crop growth and yield. Although, legumes can fix their own nitrogen, they often need phosphorus and potassium for better nodulation and good seed formation (Asiedu *et al.*, 2000) [2].

Now-a-days, rainfed groundnut production management strategies mainly focus on chemical amelioration, including, the use of chemical fertilizers and pesticides which are too expensive and also leads to several environmental hazards. In this context, plant growth promotion by free living, beneficial soil microorganisms, as a biological approach, might be an alternative strategy to overcome the biological and environmental hazards posed by the persistent use of synthetic chemicals (Gunnell *et al.*, 2007; Leach and Mumford, 2008) [7, 14]. Moreover, the biological approach has a great potential in supplying 'N' and 'P' nutrition which eventually lead to sustainable production of groundnut grown rainfed conditions. The bacterial genera viz., *Bradyrhizobium* and *Paenibacillus* are commonly encountered from the rhizosphere and nodule of legume plants. Being a leguminous plant, groundnut interacts with slow growing rhizobia belonging to the genus *Bradyrhizobium* to form root nodules (Zhang *et al.*, 2012) [21]. The interaction of groundnut plants with *Bradyrhizobium* strains have been discussed elaborately Agah *et al.*, (2013). *Paenibacillus polymyxa* (Haggag, 2007) [10], a common soil bacterium which possess a wide range of activities, including, plant growth promotion and bio dissolution of plant nutrients, including, phosphorus in the rhizosphere of Many crop plants (Kloepper *et al.*, 2004; Timmusk *et al* 1999) [11, 18].

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Agricultural bio inoculant formulation plays a crucial role in the potential success of the bio inoculants. In the recent years, several new agricultural bio inoculant formulations have been proposed of which the EPS mediated Interbacterial co-aggregates seems to be a promising one for the production of multipurpose agricultural bio inoculant with multiple benefits (Neyra *et al.*, 1999) [17]. However, there were no earlier reports regarding the development and use of Interbacterial co-aggregates in groundnut crop, available. Hence, the present research work has been undertaken with an aim to exploit the positive role of interbacterial co-aggregates, comprising the genera of *Bradyrhizobium* and *Paenibacillus polymyxa*, on plant growth stimulation, nitrogen fixation and phosphate solubilization in rainfed groundnut cv.VRL -2.

Materials and Method

Bacterial strains

The efficient isolates of *Bradyrhizobium arachidis* (B-5) and *Paenibacillus polymyxa* (P-5), isolated from the rhizosphere of Groundnut grown at Kambainallur, Dharmapuri district, Tamil Nadu state, India were used in the present study. The *Bradyrhizobium* and *Paenibacillus* cells were maintained in Yeast extract mannitol agar and Nutrient glucose agar slants, respectively, and incubated at $28 \pm 2^\circ\text{C}$, with monthly transfer.

Preparation of inoculum

Bradyrhizobium arachidis (B-5) and *Paenibacillus polymyxa* (P-5) isolates were grown in Yeast extract mannitol broth and Nutrient glucose broth, respectively, in shaking bath at $28 \pm 2^\circ\text{C}$ for 24 hr. Then the media were centrifuged separately, at $5000 \times g$ for 10 min to harvest the log phase cells and the pellets were washed three times with 0.1M phosphate buffer (pH 6.8), individually. Finally the cells of *Bradyrhizobium arachidis* and *Paenibacillus polymyxa* were resuspended, separately, in the same buffer at a cell concentration of 1×10^7 CFU/mL by measuring OD at 420 nm for *Bradyrhizobium arachidis* and 540 nm for *Paenibacillus polymyxa* and used as inoculum.

Preparation of *Bradyrhizobium* and *Paenibacillus* interbacterial co-aggregates

The co-aggregation of *Bradyrhizobium* and *Paenibacillus* isolates were prepared in Co- Ag buffer, as described by Grimaudo and Nesbitt (1997). One ml aliquot of each PGPR cells *viz.*, *Bradyrhizobium* (B-5) and *Paenibacillus* (P-5) were mixed together in 10 ml CO-Ag buffer. The mixtures were vortexed for 10 s, shaken on a rotary platform shaker for 3 min and left undisturbed at room temperature for 24 h. All Co-Ag

reactions were performed in triplicate and uninoculated buffer served as control.

Pot culture experiment

The effect of different formulations of *Bradyrhizobium* and *Paenibacillus* cells *viz.*, single strain inoculation, co-inoculation and co-aggregates application on the enhancement of growth and yield in rainfed groundnut was studied under pot culture condition. The study was conducted during (Nov, 2014 to Feb, 2015) with rainfed groundnut cv. VRL-2 at the polyhouse of Department of Microbiology, Faculty of agriculture, Annamalai University, Annamalai Nagar, India. Rectangular cement pots with $18 \times 12 \times 12$ " size were filled with 45 kg of field soil, flooded with water for two days and brought into fine puddle condition. The rainfed groundnut seeds were soaked for 30 min in the different formulations of PGPR cells *viz.* *Bradyrhizobium arachidis* (B-5) cells alone and *Paenibacillus polymyxa* (P-5) cells alone, co-inoculation of *Bradyrhizobium arachidis* (B-5) and *Paenibacillus polymyxa* (P-5) and co-aggregates of *Bradyrhizobium arachidis* (B-5) and *Paenibacillus polymyxa* (P-5) so as to get a final population of 1×10^7 cells per seed. The experimental studies were performed in a randomized block design with three replications and the following were the treatments, 1) control + 100% N and P, 2) *Bradyrhizobium arachidis* (B-5) alone + 75% N and P, 3) *Paenibacillus polymyxa* (P-5) alone + 75% N and P, 4) *Bradyrhizobium arachidis* (B-5) and *Paenibacillus polymyxa* (P-5) Co-inoculation + 75% N and P and 5) *Bradyrhizobium arachidis* (B-5) and *Paenibacillus polymyxa* (P-5) co-aggregates + 75% N and P, application.

A fertilizer schedule of 25: 50: 75 (100% NPK ha) was followed for control plots while all other treatments followed 75% recommended dose of 'N' and 'P'. The entire dose of P_2O_5 and K_2O has been applied basally as super phosphate and murate of potash and hand weeding on 30th DAS. Proper irrigation schedules done as per the recommendations. Five representative samples of plant hills in each pot were pegmarked for periodical observation.

The plant height, root and shoot dry weight, nitrogen and phosphorus content (Bremner, 1960; Jackson, 1973) [13] were recorded on 60 th DAS and yield parameters on pod Wight, seed weight and haulm yield of rainfed groundnut was recorded during the time of harvest.

Statistical analysis: The experimental results were statistically analyzed in randomized block design (RBD) and in Duncan s multiple range test (DMRT) as per the procedure described by Gomez and Gomez (1984).

Results and Discussion

Table 1: Effect of different formulations of PGPR cells on the enhancement of growth and yield parameters in rainfed groundnut (*Arachis hypogaea* L.)

Treatment	Sampling time after 60 th DAS ^{a,b}					
	Plant eight (cm)	% over control	Root dry weight (g/plant)	% over control	Shoot dry weight (g/plant)	% over control
Control*	26.67 \pm 0.3	-	0.78 \pm 0.3	-	1.75 \pm 0.6	-
<i>Bradyrhizobium</i> alone***	32.26 \pm 0.5	20.9	0.97 \pm 0.2	24.3	2.01 \pm 0.5	14.8
<i>Paenibacillus</i> alone***	29.36 \pm 0.2	10.08	1.07 \pm 0.4	37.1	1.94 \pm 0.7	10.8
<i>Bradyrhizobium</i> + <i>Paenibacillus</i> Co-I****	38.76 \pm 0.1	45.3	1.25 \pm 0.7	60.2	2.02 \pm 0.1	28.5
<i>Bradyrhizobium</i> + <i>Paenibacillus</i> Co-A*****	47.62 \pm 0.4	78.5	1.35 \pm 0.1	73.0	2.42 \pm 0.4	38.2
LSD(P=0.05)	0.91		0.016		0.07	

Table 2: Effect of different formulations of PGPR cells on the enhancement of growth and yield parameters in rainfed groundnut (*Arachis hypogaea* L.)

Treatment	Sampling time after 60 th DAS ^{a,b}					
	Total nitrogen content (in per cent)	% over control	Phosphorus content (in per cent)	% over control	Nodule dry weight (mg/plant)	% over control
Control*	1.20 ± 0.4	-	0.64 ± 0.4	-	8.64 ± 0.4	-
<i>Bradyrhizobium</i> alone***	2.36 ± 0.5	96.65	0.87 ± 0.5	35.9	10.76 ± 0.5	-
<i>Paenibacillus</i> alone***	2.90 ± 0.1	141.6	0.73 ± 0.1	14.0	9.62 ± 0.1	24.5
<i>Bradyrhizobium</i> + <i>Paenibacillus</i> Co-I****	2.01 ± 0.3	67.5	0.89 ± 0.3	39.0	13.76 ± 0.3	11.3
<i>Bradyrhizobium</i> + <i>Paenibacillus</i> Co-A*****	2.64 ± 0.3	120	1.03 ± 0.3	60.9	15.18 ± 0.3	59.25
LSD(P=0.05)	0.91		0.016		0.07	75.6

Table 3: Effect of different formulations of PGPR cells on the enhancement of growth parameters in rainfed groundnut (*Arachis hypogaea* L.)

Treatment	Sampling time after 60 th DAS ^{a,b}					
	Pod weight	% over control	Seed weight	% over control	Haulm yield (kg/ha)	% over control
Control*	5.23 ± 0.20	-	6.21 ± 0.50	-	2947	-
<i>Bradyrhizobium</i> alone***	6.21 ± 0.50	10.1	7.64 ± 0.86	23.0	3536	19.9
<i>Paenibacillus</i> alone***	5.61 ± 0.41	7.2	6.83 ± 0.64	9.9	3175	7.7
<i>Bradyrhizobium</i> + <i>Paenibacillus</i> Co-I****	7.82 ± 0.32	49.5	10.4 ± 0.98	67.4	3924	33.1
<i>Bradyrhizobium</i> + <i>Paenibacillus</i> Co-A*****	9.1 ± 0.50	73.9	11.3 ± 0.33	81.9	4826	63.7
LSD(P=0.05)	0.46		0.44		0.23	

A: Average of three replication ± SD

B: Values followed by different letters are significantly differed at 5% level according to student's t test

* - At 100 % recommended level N and P without bioinoculation

** - At 75 % recommended level N and P.

+ - Individual application of *Bradyrhizobium* (BM-5), *Paenibacillus* (PB-5) isolate at 1×10^7 CFU mL⁻¹ inoculum level.

++ - Coinoculation of *Bradyrhizobium* (BM-5), *Paenibacillus* (PB-5) isolate at 1×10^7 CFU mL⁻¹ inoculum level.

+++ - Natural coaggregation of *Bradyrhizobium* (BM-5), *Paenibacillus* (PB-5) isolate at 1×10^7 CFU mL⁻¹ inoculum level.

DAS – Days after sowing

The co-aggregates application of *Bradyrhizobium arachidis* and *P. polymyxa* recorded the maximum plant height, 47.62 cm, root dry weight, 1.35 g/plant, shoot dry weight 2.42 g/plant (Table 1), nitrogen content 2.64 per cent, phosphorus content 1.03 per cent, Nodule dry weight (Table 2) on 60th DAS respectively when compared to other formulations and the highest value of pod weight (9.1), seed weight (11.3) and haulm yield (4826 kg/ha) (Table 3), when compared to other formulations. The effect of *Paenibacillus* inoculation on the enhancement of growth and yield parameters of groundnut has already been reported by many researchers (Guemouri-Athmani *et al.*, 2000; Von der Weid *et al.*, 2000) [9, 19]. The positive effect of *Pseudomonas* and *Bacillus* co-inoculation has already been reported by El Komy *et al.* (2004) [5] in wheat. Neyra *et al.* (1999) [17], reported the positive effect of *Azospirillum* and *Rhizobium* cofloc on the enhancement of growth and yield in common bean. Many studies indicated that a level of 10 to 50 per cent N and P could be saved due to the bio-inoculation of PGPR (Acharya *et al.*, 1999; Gunarto *et al.*, 1999) [1, 6]. Dinakar (2010) [4] reported the augmentation of growth and yield of upland rice due to the application of 'Intergeneric co-aggregates' of different *Azospirillum* strains. In the present study, the co-aggregates application of *Bradyrhizobium arachidis* and *P. polymyxa* increased the growth and yield in groundnut to a higher level when compared to other formulations. However, there were no earlier reports regarding the beneficial effect of Interbacterial microbial co-aggregates application on growth stimulation not available for discussion. This is the first comprehensive report regarding the beneficial effect of *Bradyrhizobium arachidis* and *P. polymyxa* cells, as

Co-aggregates, on the enhancement of growth parameters in groundnut cv. VRL-2

It was observed that all the formulations of *Bradyrhizobium arachidis* (BM-5) and *Paenibacillus polymyxa* (PB-5) cells *viz.*, single strain inoculation, co-inoculation and co-aggregates application on the enhancement of growth and yield parameters *viz.*, plant height, root and shoot dry weight, nitrogen and phosphorus content and Pod and Seed weight and Haulm yield was studied under pot culture condition (Table 1-3). The application of the different formulations of *Bradyrhizobium arachidis* (BM-5) and *Paenibacillus polymyxa* (PB-5) cells was found to augment the growth and yield parameters of groundnut cv VRL-2 when compared to control (100% N and P level without any bioinoculation). Among the different formulations, the application of *Bradyrhizobium arachidis* (BM-5) and *Paenibacillus polymyxa* (PB-5) cells, as co-aggregates, improved growth and yield parameters of groundnut cv VRL-2 to a higher level, followed by co-inoculation of *Bradyrhizobium arachidis* (BM-5) and *Paenibacillus polymyxa* (PB-5), *Bradyrhizobium arachidis* (BM-5) alone and *Paenibacillus polymyxa* (PB-5) alone treatment. Interestingly, the application of Interbacterial co-aggregates, comprising of *Bradyrhizobium* and *P. polymyxa* cells together with 75% recommended N and P level could augment the growth and yield parameters of groundnut to a higher level when compared to groundnut crop grown in 100% recommended N and P level without any bioinoculation and thus a saving of 25% recommended N and P fertilizers could be achieved.

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