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GROWTH PROMOTING POTENTIAL OF NEW BACTERIAL ISOLATES SINGLY AND IN COMBINATION ON *TRIGONELLA* SPS.

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Sixteen strains of Rhizobacteria belonging to *Pseudomonas, Azotobacter, Azospirillum* and *Rhizobium* were isolated and studied for their plant growth promoting potential singly and in different combinations on *Trigonella foenum graecum*. Coinoculations have resulted in increase in lateral roots and leghaemoglobin content of nodules. The SR-2 treatment containing *Rhizobium sps., Bacillus sps.* and *Pseudomonas sps.* seemed to be the best possible combination studied in comparison to other combinations. The concentration of soluble phosphorus also increased significantly upto 14.5 ppm (maximum) in the SR-2 treatment. Stimulation of vegetative growth parameters like root length, shoot length and number of leaves was found to be maximum in the SR-1 treatment followed closely by SR-8 treatment. The role of *Azotobacter* and *Azospirillum* as combined biofertilizer was not found suitable. Another interesting observation was that the *Rhizobium* sps. (all four isolates) showed good nodulation and maximum availability of phosphorus in the soil and at the same time acted as a buffering agent for maintaining pH of the soil. However, the combination of 4 sps of *Rhizobium*, 5 sps of *Pseudomonas* and Bacillus each showed the maximum vegetative growth and lateral root formation. All the results were statistically analysed and values were found to be significant at $P< 0.005$.

Keywords: Coinoculation; Leghaemoglobin; Nodulation; Phosphorus solubilization; Plant Growth Promoting Rhizobacteria.

Introduction

There is currently considerable interest in rhizosphere organisms that improve the establishment of crop plants. Different plant microbe interactions especially colonization by a variety of microbes in and around roots may results in symbiotic, associative, neutralistic or parasitic relations within the plant depending upon the type of microorganisms, soil nutrient status, plant defence system and soil environment[1]. With the more recent interest in replacing chemical fertilizers and pesticides with bacterial inoculants, considerable efforts have been directed towards laboratory and green house studies aimed at developing a better understanding of the functioning of these organisms[2]. Plant Growth Promoting Rhizobacteria (PGPR) have potential capability to enhance either directly or indirectly the plant growth and productivity. Interactions between these PGPR's with *Rhizobium* may be antagonistic or synergistic and the beneficial effects of such interactions could be exploited for economic gain[3]. On co-inoculation with symbiotic bacteria, rhizospheric bacteria may increase nodulation through a variety of mechanisms. Sayyed *et al.*[4] have observed that treatment of wheat seeds with fluorescent *Pseudomonas* have resulted in increase in yield. PGPR's have been known to induce phytoalexin production by plant, creating antibiosis in rhizosphere or siderophore production for out competing pathogens[5].Some PGPR function by facilitating the formation of longer roots and enhancing the survival of seedlings[6]. However, the reports are scanty on the use of mixed culture inoculants. Therefore, attempt is being made in this study to examine the feasibility of using two or more type of PGPR's in combinations for co-inoculation and study their behaviour in field conditions using *Trigonella* sps as host.

Materials and Methods

Plant growth promoting rhizobacteria: 16 strains of rhizobacteria belonging to *Pseudomonas, Bacillus, Azotobacter, Azospirillum*, and *Rhizobium* were used from this laboratory and isolated from the rhizosphere and rhizoplane of legume/cereal fields. The PGPR potential was already established in our earlier studies in laboratory conditions[7].

Seeds and chemicals: All chemicals used in the present study were of AR grade from Hi-media, Bombay (India). Seeds of fenugreek (*Trigonella* sp.) were obtained from Gujarat Cooperative Marketing Society Ltd. (GUJCOMASOL, India) for sowing in pots.

Plant growth under non sterile conditions: For detecting the co-inoculation response under non sterile soil conditions, unsterilized farm soil was used. For this experiment 21 cm diameter earthen pots were used. They

were filled with approximately 8 kg of farm soil. The soil was saturated with water before sowing.

Mature healthy seeds of *Trigonella* were selected and surface sterilized using acidic alcohol (Ethanol : Sulphuric acid, 7:3 v/v). The alcohol was finally decanted and seeds were washed thoroughly with five to six changes of distilled water [8].

These surface sterilized seeds were inoculated either with each isolate of *Rhizobium* alone or with a mixed culture of either or all *Bacillus* , *Pseudomonas*, *Azotobacter* and *Azospirillum* (Table 1) in a total of 9 combinations. The ratio used was 1:1, 1ml of *Rhizobium* each having the density of $37x10^5$ per ml. This was used to treat every 10 seeds and the culture was allowed to be absorbed by the seeds for 30 mins. Control treatment did not include any bacteria. Pots were kept in an open greenhouse and supplied with tap water as and when required. Simultaneously, a set of uninoculated control was maintained.

Table 1. Various combination of Rhizobia and Rhizobacteria.

Treatment	Strain Combinations
SR-1	*Rhizobium*
SR-2	*Rhizobium +Bacillus +Pseudomonas*
SR-3	*Rhizobium +Bacillus +Azotobacter*
SR-4	*Rhizobium +Bacillus +Azospirillum*
SR-5	*Rhizobium +Pseudomonas*
SR-6	*Rhizobium +Pseudomonas +Azotobacter*
SR-7	*Rhizobium +Pseudomonas +Azospirillum*
SR-8	*Rhizobium +Azotobacter +Azospirillum*
SR-9	*Rhizobium +Bacillus +Pseudomonas +Azotobacter +Azospirillum*

Rhizobium: 4 species isolated from *Trigonella*
Bacillus: 5 species isolated from *Trigonella* and *Vigna*
Pseudomonas: 5 species isolated from *Trigonella* and *Vigna*
Azotobacter: Azotobacter chroococum
Azospirillum: Azospirillum brasilense

All sets were replicated five times and statistical analysis of data carried out using ANOVA and comparisons of means were performed by the LSD test at $P<=0.05$ [8].

Phosphorus estimation: Soluble phosphorus present in the soil was extracted by the method of Jackson[9] and the concentration of phosphorus present in the soil was estimated by chlorostannus reduced molybdophosphoric acid blue method. Estimation was again performed 45 days after seed germination[10].

Evaluation of biofertilizer effect: Measurement of plant growth parameter was done after 45 days of seed germination. Plants were carefully uprooted and washed under running tap water. Length, fresh weight and dry weight of root and shoot, number of flowers; number, diameter and size of leaves; number, size and colour of nodules, presence of lateral roots etc, was recorded[8].

Leghaemoglobin estimation in nodules: In 0.5 g of fresh nodule tissue Drabkins solution was added for the estimation of the leghaemoglobin content. Absorbance of the supernatant was measured at 540 nm using Spectronic20. This was then correlated with the standard of Cyanomethenoglobin[11].

Results and Discussion

The observation on the effect of co-inoculation with different Rhizobacteria with *Rhizobium* showed an increase in number and size of nodules in all inoculated plants in comparison to uninoculated control. Co-inoculation also resulted in increase in lateral roots, leghaemoglobin content and stimulated vegetative growth of plants. Among the other combinations tested the SR -2 treatment containing *Bacillus* and *Pseudomonas* showed a significant increase in fresh weight , dry weight , nodule number and size along with maximum root proliferation (Table 2). Presence of *Bacillus* along with *Rhizobium* SR-2, SR-3, SR-4 treatment has shown an increase in number of nodules formed. However maximum number of nodules were found in single *Rhizobium* composite strain SR-1,which was 2.33 times higher in comparison to that of SR-8 treatment (Table 3). The size of nodule was maximum in SR-1 treatment which was approximately double than SR-5 combination. The SR-2 treatment showed consistent good results, resulting in a 1.2 times increases of shoot and root length in comparison to SR-1 (Table 2). The size and number of nodules were also almost at par in comparison with SR-1 treatment (Table 3). Stimulation in plant biomass, shoot nitrogen and nodulation was also observed by Parmar and Dadarwal[8] using different strains of *Pseudomonas* along with *Rhizobium*. A significant increase in phosphorus availability at pH 7.1 was also maximum by using SR-1 treatment (Table 4). However, all treatments showed a significant increase in phosphorus availability in comparison to uninoculated control. Similar pattern of phosphorus solubilizations has been observed by Vidhyasekaran *et al.*[12] in groundnut sps. Patel and Dave[13] have reported that among bacteria the most efficient phosphate solubilizers belong to the genera *Bacillus* and *Pseudomonas*, which is also observed in the present study. In our earlier studies[14] the combination of *Bacillus* and *Azotobacter* showed a solubilization of 116 ppm in 100 ml of Pikovskaya medium, 2.2 times higher than single culture isolates in laboratory conditions. In addition *Rhizobium* was also found to show a high concentration of soluble phosphorus after 45 days of application. The leghaemoglobin content of nodules was found to be maximum in case of SR-1 closely followed by the SR-2 treatment, which may also correlated with number and size of nodules (Table 4). Combination of PGPR's (*Bacillus, Saccharomyces, Klebsiella, Bradyrhizobium sps.*) resulted in increased number of nodules, maximum nodule weight,

Table 2. Effect of different treatments on vegetative growth and lateral root formation of *Trigonella plant.*

Treatment	Root length (cms.)	Shoot length (cms.)	Leaf number	Lateral roots
Control	15.8(2.3)	20.3(3.3)	16.3(1.33)	+
SR-1	21.9(2.45)	29.16(2.52)	28.4(4.22)	++
SR-2	26.1(2.81)	32.3(3.01)	25.1(3.90)	++++
SR-3	32.4(4.13)	35.4(2.88)	25.0(2.75)	+++
SR-4	30.8(3.25)	32.4(3.72)	24.89(2.89)	+++
SR-5	25.3(3.13)	42.3(2.91)	26.3(4.18)	++
SR-6	23.4(2.55)	42.7(4.13)	25.8(3.83)	+++
SR-7	21.9(3.15)	33.5(3.33)	24.5(2.51)	+++
SR-8	22.3(4.15)	35.5(4.50)	26.3(3.11)	+++
SR-9	25.5(4.32)	28.5(3.55)	25.4(3.75)	+++

*Value mean of five replicates, Standard deviations are shown in parentheses.
*Value were significant at $P<0.05$.

Table 3. Effect of different treatments on nodulation and leghaemoglobin content of *Trigonella* plant.

Treatment	Number of nodules/Plant	Size of nodule (mm)	Colour of nodules	Leghaemoglobin content (mg/ 500mg)
Control	15.6(4.15)	1.8(0.95)	White	0.065(0.0005)
SR-1	51.4(6.50)	3.8(1.11)	Red	1.9(0.07)
SR-2	45.2(8.16)	3.8(1.15)	Red	1.8(0.09)
SR-3	43.8(3.41)	3.2(0.91)	Red	1.2(0.07)
SR-4	42.2(4.85)	2.1(1.21)	Pink	1.2(0.06)
SR-5	42.4(5.61)	1.8(0.82)	Pink	1.1(0.08)
SR-6	31.0(4.65)	2.9(0.75)	Red	1.7(0.07)
SR-7	34.0(3.09)	2.7(0.61)	Red	1.45(0.06)
SR-8	22.0(2.59)	2.3(0.95)	Pink	0.085(0.006)
SR-9	29.2(3.71)	2.6(1.05)	Pink	0.085(0.007)

*Value mean of five replicates, Standard deviations are shown in parentheses.
*Value were significant at $P<0.05$.

Table 4. Effect of different treatments on phosphorus content and pH of field soil.

Treatment	Initial pH (0 days)	Final pH (45 days)	Concentration of soluble P (ppm) (0 days)	Concentration of soluble P (ppm) (45 days)
Control	7.0	7.0	2.3	5.1(1.33)
SR-1	7.0	7.1	2.3	14.5(2.59)
SR-2	7.0	7.1	2.3	9.0(2.91)
SR-3	7.0	7.0	2.3	6.0(1.51)
SR-4	7.0	7.0	2.3	5.6(1.65)
SR-5	7.0	8.5	2.3	14.0(5.01)
SR-6	7.0	8.0	2.3	8.0(1.71)
SR-7	7.0	8.0	2.3	6.5(2.51)
SR-8	7.0	8.5	2.3	6.0(0.91)
SR-9	7.0	8.0	2.3	6.0(0.95)

*Value mean of five replicates, Standard deviations are shown in parentheses.
*Value were significant at $P<0.05$.

nitrogenase and yield in cowpea[15]. Similarly, Srivasan *et al.*[16] has concluded that co-inoculation of *Rhizobium etli* with *Bacillus* enhance nodulation. Further, it was also observed that root hair proliferation and enhanced nodulation can be seen on co-inoculation of *Rhizobium-Bacillus*[17]. This is corroboration with our observation that nodulation, and lateral root proliferation is maximum in SR-2 treatment (Table 2). On the other hand, Plazinski and Rolfe[18] have concluded that co-inoculation between *Rhizobium, Azospirillum* leads to increased lateral root formation but decline in nodulation. However, multistrain inoculants always perform either as good or better than single strain inoculants in their study conducted in chickpea. The use of *Azospirillum* as a co-inoculant with *Rhizobium* has been cited to lead in plant growth stimulation[18]. Promotion of root length has been studied as one of the major markers by which beneficial effect of plant growth promoting bacteria is studied[19]. Promotion of root length in canola and mung bean plants was studied using 1-cyclopropenylmethyl butyl ether with the conclusion that root elongation is due to ethylene inhibitors present in certain rhizospheric bacteria[19]. Glick and Pasternak[20] have suggested that *Azospirillum* strains capable of enhancing mineral uptake, by organic acid secretions which may be one of the reasons for enhanced phosphorus and nitrogen content in comparison to uninoculated control. We have not found this treatment to give extraordinary results. According to the data presented in this study, the SR-2 treatment containing *Rhizobium, Bacillus* and *Pseudomonas* seems to be the best possible combination which has led to increased plant growth, nodulation, leghaemoglobin content and phosphorus availability in the soil.

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