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Effect of plant growth promoting rhizobacteria on seed germination and plant growth on beans

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Abstract

A Total number of 24 rhizobacterial strains were isolated from different crop soils with the aim to find out some potential biocontrol agents against bean wilt. The five selected isolates were subjected to biochemical test *viz.* starch hydrolysis, catalase test, oxidase test, arginine hydrolysis, gelatin liquefaction, H_2S production, oxygen requirement test, indole production, urease test, nitrate reduction test and methyl red test for identification. The five rhizobacterial isolates and fungicide (carbendazim) were used for application of seed treatment of beans for 12 and 1 h to study their effect on seed germination. Seed treatment for 12h with *Stenotrophomonas maltophilia* gave maximum seed germination (86.67%). All selected PGPR isolates enhanced plant growth parameter such as shoot length (34.40 cm) and root depth (26.22 cm).

Keywords: Seed germination, plant growth, beans (*Phaseolus vulgaris*)

Introduction

Beans as a vegetable it is highly nutritious, being rich in vitamin A and C and has been associated to several health benefits like reduction of cholesterol level (Rosa *et al*., 1998) [31], and coronary heart diseases (Anderson *et al*., 1999; Bazzano *et al*., 2001) [3, 8], favorable effects against cancer (Hangen and Bennink, 2002) [15], decrease of diabetes and obesity (Geil and Anderson, 1994) [13], high antioxidant capacity (Heimler *et al*., 2005) [16], antimutagenic (Azevedo *et al*., 2003) [6] and antiproliferative effects (Aparicio-Fernández *et al*., 2006) [4].

An array of microorganisms inhabits the rhizosphere. Among these certain strains of fluorescent pseudomonads have received special attention because of their potential to function as biological agents for the management of soil-borne pathogenic and oomyceteous fungi that attack plant roots and cause considerable damage to the crop worldwide (Landa *et al*., 2002; De Souza *et al*., 2003; Ramette *et al*., 2003; Weller, 2007) [20, 12, 30, 36]. Plant growth promoting rhizobacteria (PGPR) comprise a group of soil and rhizosphere free-living bacteria that colonize roots in a competitive environment, thereby exert a beneficial effect on plant growth (Kloepper, 2003; Bakker *et al*., 2007) [17, 7]. PGPRs have been tested both under *in vitro* as well as *in vivo* conditions for induced systemic resistance (ISR) against fungal pathogens in various crops such as beans, carnation, cucumber, radish, tobacco, tomato and *Arabidopsis* (Chen, *et al*., 1995; Park and Kloepper, 2000) [11, 27]. However, *Pseudomonas* and *Bacillus* spp together with *Streptomyces* spp constitute the most prominent bacterial population found in rhizosphere of numerous crop plants. These PGPR act as biocontrol agents though production of antibiotics, triggering induced local or systemic resistance, or preventing the deleterious effects of xenobiotics by degradation or by acting as rhizoremediators (Glick *et al*., 2007; Van Loon, 2007: Aseri *et al*., 2008) [14, 35, 5].

Materials and Methods

To assess growth promoting potential of Plant Growth Promoting Rhizobacteria (PGPR) isolated from the rhizosphere of different crops and their biological control efficacy against *Fusarium oxysporum* f.sp. *phaseoli*, a soil borne pathogen, causing wilt of bean crop.

Identification of selected rhizobacterial isolates

The rhizobacterial isolates were identified based upon their Biochemical characteristics.

Biochemical characterisation

The selected rhizobacterial isolates were subjected to biochemical tests by employing the standard procedures in order to characterize the isolates. Different biochemical tests were performed such as Starch hydrolysis, Catalase test, Oxidase test, Arginine hydrolysis, Gelatin liquefaction, Hydrogen sulfide test, Oxygen requirement test, Indole production, Urease test, Nitrate reduction test, Methyl Red Test.

Germination test

Selected bacterial isolates like *Pseudomonas fluorescens*, *Bacillus cereus*-1, *Bacillus cereus*-2, *Stenotrophomonas*

maltophilia, *Pseudomonas* sp. and Carbendazim were used for germination test. Seeds of beans were surface sterilized with 0.02% Sodium hypochlorite for 2 minutes and rinsed thoroughly in sterile distilled water. For inoculation, two sets of seeds in case of each PGPR strain were coated with 1% CMC as an adhesive and rolled into the suspension of bacteria (10^8 cfu ml^{-1}) for one and 12 hours, respectively. Ten seeds for each treatment with six replications were taken and germination tests were carried out by incubating for 7 days at 28 °C. After seven days the number of strongly germinated seeds having length of radicles more than the half of the seed length was counted.

Evaluation of PGPR strains for disease management and growth promoting properties under field condition

A field experiment was conducted to access the biocontrol and growth promoting potential of selected bacterial isolates, I-1 (*Pseudomonas fluorescens*), I-4 (*Bacillus cereus*-1), I-5 (*Stenotrophomonas maltophilia*), I-20 (*Bacillus cereus*-2) and I-21 (*Pseudomonas* sp).

Seed treatment for 1 hour and 12 hour

The selected PGPR isolates *viz.*, I-1, I-4, I-5, I-20 and I-21 were grown on nutrient agar medium for 48 hours and harvested with sterile distilled water and adjusted to the concentration of 1×10^8 cfu/ml for use as seed treatment for 1 hour and 12 hour on bean seed. Healthy seeds of French Bean variety Shalimar French Beans1 were surface sterilized with 0.1 per cent $HgCl_2$ prior to seed treatment.The seeds were subsequently washed three times to remove excess of $HgCl_2$ (mercuric chloride). The surface sterilized beans seeds were divided into two lots. One lot was given seed treatment of selected PGPR isolates for one hour and the other for 12 hours. The seeds were soaked in five selected PGPR suspension (1×10^8 cfu) separately for 1 hour and then placed on blotting paper and dried under shade. In another case seed were submerged in respective PGPR suspensions overnight (12 hr) and then dried in shade.

In case of standard check, seeds were moistened and treated with carbendazim @ 2 g/kg, and shade dried before sowing. Sterile distilled water was used in case of control.

Field experiment

The seeds thus treated or bioprimed were sown in a field trial of CRBD with three replications. One lot of seed without any treatment was sown in control plots whereas, seed treated with carbendazim @ 2 g/kg were also sown as check.

Observations recorded

1. Shoot length
2. Root depth

Results and Discussion

Soil-borne plant diseases including wilts and root rots are major biotic production constrains in most pulse crops including beans. In view of the increasing concerns about use of chemical pesticides, there is a growing research effort for identification of biological control measures involving native rhizosphere microbiome. Therefore there is a need to isolate and characterise rhizosphere microbiome to identify effective strains that can be upscaled as biocontrol agents.

Biochemical characterisation of rhizobacterial isolates

Out of the 24 rhizobacterial isolates tested *in vitro* for their biocontrol activity potential against *F. oxysporum* f.sp *phaseoli* through dual culture technique, The five most effective rhizobacterial isolates were subjected to different biochemical test (Table-1).

The selected rhizobacterial isolates (I-1, I-4, I-5, I-20 and I-21) were tested for their biochemical characteristics. The four isolates (I-4, I-5, I-20 and I-21) proved positive for starch hydrolysis, whereas, isolate I-1 proved negative for the same test. Isolate I-1, I-4, I-20 and I-21 proved positive for catalase test but isolate I-5 proved negative. Isolates I-1, I-4 and I-20 were strongly positive for oxidase test and isolates I-5 and I-21 were late positive for the same test. Only isolate I-1 was positive for arginine hydrolysis test and rest of the four isolates (I-4, I-5, I-20 and I-21) were negative. Isolates I-1, I-4 and I-20 were negative for gelatine liquefaction as compared to I-5 and I-21 that gave positive result for the same test. Isolates I-1, I-5 and I-21 were positive for H_2S test and isolates I-4 and I-20 were negative. There is no colour change for the isolate (I-1, I-4, I-5, I-20 and I-21) in the tube sealed with Vaseline, demonstrating that the bacteria were aerobic. Isolate I-21 proved positive for indole production but isolates I-1, I-4, I-5 and I-20 proved negative for the same test. In case of the urease test, all isolates except isolate I-20 proved negative. All the isolates showed positive for the nitrate reduction test whileas negative in methyl red test. These morphological and biochemical characters of all the five bacterial isolates were referred to "Bergey's Manual of Systamic Bacteriology" (Krieg and Holt 1984: MacFaddin, 2000) [18,]. The isolate I-1, I-4, I-5, I-20 and I-21 showed resemblance with *Pseudomonas fluorescens*, *Bacillus* sp, *Stenotrophomonas* sp., *Bacillus* sp., and *Pseudomonas* sp. Respectively. These and similar findings have also been corroborated by Nathan *et al.*, (2011), [23] Meera and Balabaskar, (2012) [21], Ambawade and Pathade, (2015) [2].

Effect of Plant Growth Promoting Rhizobacteria on germination of beans seed

Selected potential biocontrol isolates like *Pseudomonas fluorescens*, *Bacillus cereus* 1, *Stenotrophomonas maltophilia*, *Bacillus cereus* 2, *Pseudomonas* sp, and the fungicide Carbendazim were used for seed treatment of beans (Table-2). Maximum seed germination was recorded in case of seed treatment for 12 hours with *Stenotrophomonas maltophilia* (86.67%) followed by seed treatment for 12 hours with *Pseudomonas fluorescens* (83.33%). Alavi *et al.* (2013) [1] twice germination rate in rape seeds inoculated with a mutant strain of *Stenotrophomonas maltophilia*. The seed germination and plant growth promotionhas been attributed to higher level of spermidine synthase combined withhighly active spermidine export proteins regulated by *S. maltophilia*. The PGPR-induced increase in seed emergence, early germination and/or growth may be due to increased synthesis of auxins and gibberellins that trigger the activity of specific enzymes, such as amylase (Bharathi *et al*., 2004: Ambawade and Pathade, 2015) [9, 2]. These findings are in line with that of Raju, *et al.* (1999) [29], Niranjan, *et al*. (2003a) [25], Niranjan,

et al. (2004) [26], Shaukat, *et al*. (2006a) [33], Shaukat, *et al*. (2006b) [34].

Effect of Plant growth promoting Rhizobacteria on shoot length of beans *in vivo*

Maximum shoot length was recorded (Table-3) in case of seed treatment for 12 hours with *Stenotrophomonas maltophilia* (35.1 cm), and for 1 hour with the same organism (32.93 cm) and seed treatment for 12 hours with *Pseudomonas fluorescens* (32.43 cm). During the year 2017 seed treatment for 12 hours with *Stenotrophomonas maltophilia* (33.7 cm) recorded highest shoot length, followed by seed treatment for 12 hours with *Pseudomonas fluorescens* (29.6 cm). Pooled data revealed that all the treatment significantly enhanced shoot length. Maximum shoot length was recorded in Seed treatment for 12 hours with *Stenotrophomonas maltophilia* (34.4 cm), seed treatment for 12 hours with *Pseudomonas fluorescens* (31.02 cm). Similar results were also found by Naz and Bano (2012) [24] in *Stenotrophomonas maltophilia* vis-a-vis seedlings of *Zea mays* under normal and induced salt stress conditions. The PGPR *Stenotrophomonas maltophilia* perpetrate high phosphate solubilization and acid phosphatase activity both qualitatively and quantitatively that leads to significant growth promotion in plants (Kumar and Audipudi 2015) [19]. Many workers have propounded that *Pseudomonas putida* and *Pseudomonas aeruginosa* enhance shoot length followed by *Bacillus subtilis*, *Paenibacillus polymyxa* and *Bacillus boronophillus* in many crops. The plant growth enhanced because of root colonization of plant growth promoting rhizoabcteria which exudate plant hormones (IAA), phosphorus and ammonia. (Persello-carticauset *et al*- 2003: Yadav *et al*. 2010: Bhattacharya and Jha 2012) [28, 37, 10]

Effect of Plant growth promoting Rhizobacteria on root depth of beans *in vivo*

The seed treatment for 12 hours with *Stenotrophomonas maltophilia* (25.5 cm) recorded the maximum root depth followed by seed treatment for 12 hours with *Pseudomonas fluorescens* (22.27), seed treatment for 1 hour with *Pseudomonas fluorescens* (22.23 cm) (Table-4). During the year 2017 maximum root depth was recorded in the seed treatment for 12 hours by with *Stenotrophomonas maltophilia* (26.93 cm). Pooled data revealed that the seed treatment for 12 hours by *Stenotrophomonas maltophilia* (26.22 cm) showed maximum root depth, followed by seed treatment for 12 hours with *Pseudomonas fluorescens* (22.82cm). The increase in root length might be also due to ability of *P. fluorescens* to inhibit the growth of *F. oxysporum* in rhizosphere or due to siderophore production by microbes involved. Moreover, a better root system (increased root length) in seeds treated with *P. fluorescens* tolerate or escape root infections, thereby facilitate an active absorption of nutrients to promote plant growth and health (M'Piga *et al*.,1997: Sayyed *et al*. 2005, Yadav *et al*. 2010, Naz and Bano, 2012) [22, 32, 37, 24].

Table 1: Biochemical characters of the rhizobacterial isolates

Treatments	Isolates				
	I-1	I-4	I-5	I-20	I-21
Starch hydrolysis	-	+	+	+	+
Catalase test	+	+	-	+	+
Oxidase test	+	+	L+	+	L+
Arginine hydrolysis	+	-	-	-	-
Gelatin liquefaction	-	-	+	-	+
H_2S (Hydrogen sulfide) test	+	-	+	-	+
Oxygen requirement test	+	+	+	+	+
Indole production	-	-	-	-	+
Urease test	-	-	-	+	-
Nitrate reduction test	+	+	+	+	+
Methyl red test	-	-	-	-	-

L+: Late +ve

Table 2: Effect of PGPR *in vitro* seed treatment for 12 hour and 1 hour on germination of bean cv. Shalimar French Bean-1

Treatment Duration	Treatment	Germination (%)
12 h	*Pseudomonas fluorescens*	83.33 (9.18)
	Bacillus cereus 1	70 (8.42)
	Stenotrophomonas maltophilia	86.67 (9.36)
	Bacillus cereus 2	71.67 (8.52)
	Pseudomonas sp.	73.33 (8.62)
1 h	*Pseudomonas fluorescens*	75 (8.71)
	Bacillus cereus 1	76.67 (8.81)
	Stenotrophomonas maltophilia	78.33 (8.90)
	Bacillus cereus 2	76.67 (8.80)
	Pseudomonas sp.	68.33 (8.32)
Fungicide	Carbendazim	51.83 (7.27)
Control		53.33 (7.36)
C.D. ($p \leq 0.05$)		0.44

Data is mean of six replications

Values in parenthesis are square root transformed values

Table 3: Effect of PGPR on shoot length of French beans

Treatment Duration	*Treatment*	Shoot length (cm)		
		2016	2017	Pooled
12 h	*Pseudomonas fluorescens*	32.43	29.60	31.02
	Bacillus cereus 1	26.40	26.60	26.50
	Stenotrophomonas maltophilia	35.10	33.70	34.40
	Bacillus cereus 2	28.70	24.80	26.75
	Pseudomonas sp.	28.43	27.70	28.07
1h	*Pseudomonas fluorescens*	30.70	28.60	29.65
	Bacillus cereus 1	28.73	25.37	27.05
	Stenotrophomonas maltophilia	32.93	29.03	30.98
	Bacillus cereus 2	28.73	24.50	26.62
	Pseudomonas sp.	25.63	25.67	25.65
Fungicide	*Carbendazim*	27.84	22.63	25.24
Control	*Distilled water*	18.27	21.90	20.09
C.D. ($p \leq 0.05$)		3.05	3.26	2.06

Table 4: Effect of PGPR on root depth of French beans

Treatment Duration	Treatment	Root depth (cm)		
		2016	2017	pooled
12h	*Pseudomonas fluorescens*	22.27	23.37	22.82
	Bacillus cereus 1	20.03	19.43	19.73
	Stenotrophomonas maltophilia	25.50	26.93	26.22
	Bacillus cereus 2	19.27	18.47	18.87
	Pseudomonas sp.	22.03	20.37	21.20
1h	*Pseudomonas fluorescens*	22.23	21.20	21.72
	Bacillus cereus 1	14.73	15.47	15.10
	Stenotrophomonas maltophilia	22.07	22.67	22.37
	Bacillus cereus 2	17.60	18.10	17.85
	Pseudomonas sp.	19.33	18.30	18.82
Fungicide	Carbendazim	20.97	16.20	18.59
Control	Distilled Water	11.97	12.00	11.99
C.D. ($p \leq 0.05$)		4.18	2.53	2.57

Conclusion

Bean plants delineating symptoms of wilt/root rot were found associated with *Fusarium oxysporum* (Schlecht.) f.sp. *phaseoli* Kendrick and Synder. 24 rhizobacterial isolates were isolated and identified from the field of different crops whose biochemical cheristics were observed. Based on biochemical characterization, I-4 and I-20 were closely related to *Bacillus cereus*, I-5 to *Stenotrophomonas maltophilia*, and that of isolate I-21 with *Pseudomonas* sp. In seed treatment and biopriming *Stenotrophomonas maltophilia* (st) showed the best plant growth promotional ability. It recorded the highest seed germination, shoot length, root depth.

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