Performance of Native Fluorescent *Pseudomonas* on *in vitro* Seed Germination and Seedling Vigour of *Sorghum bicolor* (L.) Moench

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Abstract

Plant Growth Promoting Rhizobacteria (PGPR) used in the production of biofertilizers is an effective and economic means to control plant diseases. They enhance seed emergence, plant growth, and yield. Rhizospheric soils of different crops like sunflower, chilli, cotton, groundnut, chickpea, sorghum etc., cultivated in Telangana region of Andhra Pradesh, India, were collected and processed for the isolation of *Pseudomonas fluorescens* (*Pf*) strains. A total of fifty four strains were identified as *Pf*, and growth promotion test was conducted, thirty three isolates showed potent growth promotion activity and the study exhibited a good picture of efficiency and interaction of *Pf* strains isolated. Maximum Vigour Index-I was shown by *Pf*2 whereas *Pf*8 strain recorded minimum.

1. Introduction

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield (Wu et al., 2005). The mechanisms by which PGPR promote plant growth are not fully understood, but are considered to include the ability to produce phytohormones against phytopathogenic microorganisms through the production of siderophores (Egambardiyeva, 2007; Sharoona et al., 2006) and the synthesis of antibiotics, enzymes and/or fungicidal compounds (Ahmad et al., 2006). Significant increase in growth and yield of agronomical important crops in response to inoculation with PGPR has been reported (Asghar et al., 2002; Biswas et al., 2000). *Pseudomonas* strains could affect seed germination and seedling growth (Shaukat et al., 2006). Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* were reported to increase root and shoot elongation in canola (Glick et al., 1997). Thus, it has been shown that *Pseudomonas* has the potential for agriculture exploitation and could be used as biofertilizers (Cakmakc et al., 2006). Fluorescent *Pseudomonas*, equipped with multiple mechanisms for biocontrol of phytopathogens and plant growth promotion, are being used widely (Banasco et al., 1998; Dileep et al., 1998; Pierson and Weller, 1994) as they produce a wide variety of antibiotics, chitinolytic enzymes, growth promoting hormones, siderophores, HCN and catalase. The use of PGPR has become a common practice in many regions of the world. Greater application of PGPR is possible in agriculture for biocontrol of plant pathogens and biofertilization (Siddiqui, 2006; Das et al., 2010; Lugtenberg and Kamilova, 2009; Saharan and Nehra, 2011). The bacterial strains isolated from *Lolium perenne* rhizosphere are capable of acting as plant growth promoting bacteria and show various plant growth promoting activities (Shoebitz et al., 2007).

Although the effects of these bacteria on growth and yields had been studied previously, but their effects on growth parameters were not evaluated simultaneously. The main objective of this investigation was to know the different *Pseudomonas fluorescens* strains to explore their effect on seed germination, growth parameters of sorghum seedlings under *in vitro* conditions.

2. Materials and Methods

2.1. Isolation of P. fluorescens (*Pf*)

Soil samples from the rhizosphere of different crops were collected during the year 2009, from different districts of

Telangana region of Andhra Pradesh, India. Thirty day old plants were uprooted and the roots with enclosed soil were placed in plastic bags and stored at 4º C. For the isolation of bacteria from the rhizospheric soil, 10 g of soil plant^{-1} was mixed with 90 mL of sterilized water. Serial dilution of the suspension (10^{-1}, 10^{-2}, 10^{-3}, $10^{-4,}$ 10^{-5} and 10^{-6}) was prepared; around 0.1 mL from each dilution spread on Kings 'B' media and incubated at 28ºC until the bacterial colonies were formed. After incubating for 24 h, the plates were detected under U.V. transilluminator by observing fluorescence effect in Kings 'B' medium and maintained for further use (Prasanna Reddy and Reddy, 2009).

2.2. Growth promotion test with sorghum seed

Plant growth promotion activity of *P. fluorescens* strains was tested with the seeds of sorghum, which was surface sterilized with 0.02% sodium hypochlorite for 2 min and rinsed thoroughly in sterile distilled water. For inoculation, seeds were coated with 1% CMC as an adhesive and rolled into the suspension of bacteria (10^8 cfu ml^{-1}). Germination test was carried out by the paper towel method. Ten seeds for each treatment with three replications in completely randomized design was done and incubated in growth chamber at 28ºC for 7 days. After incubation, the number of germinated seeds was counted. The root and shoot length of individual seedling was measured in order to determine the Vigour Index-I (Abdul Baki et al., 1973). The formula used for Vigor Index-I is as follows:

Vigor Index-I=(mean root length+mean shoot length)×% seed germination

2.3. Statistical analysis

Results are expressed as mean±standard error of mean (SEm). Statistical significance was determined using one way ANOVA followed by Tukey's test for multiple comparisons. Values with $p<0.05$ compared with control were considered statistically significant in all cases.

3. Results and Discussion

Table 1: *Pseudomonas fluorescens* isolated from different crops cultivated in Southern Telangana Zone of Andhra Pradesh, India

Isolate No.	Crop	Geographical location	Isolate No.	Crop	Geographical location
Pf1	Rice	Tudkurthy (Nagarkarnool)[a]	*Pf28*	Sun flower	Yadireddipally (Thandoor)[a]
Pf2	Rice	Tudkurthy (Nagarkarnool)[a]	*Pf29*	Sun flower	Tallapally (Telkapally)[a]
Pf3	Castor	Palem (Bijnepally)[a]	*Pf30*	Chilli	Tallapally (Telkapally)[a]
Pf4	Cotton	Palem (Bijnepally)[a]	*Pf31*	Cotton	Indrakol (Nagarkarnool)
Pf5	Chilli	Manganoor (Bijnepally)[a]	*Pf32*	Groundnut	Chennur (Gopalpet)[a]
Pf6	Groundnut	Elikacherla (Thimmajipet)[a]	*Pf33*	Groundnut	Nellikonduru[a]
Pf7	Rice	Lattupally (Bijnepally)[a]	*Pf34*	Groundnut	Sudhakal [a]
Pf8	Rice	Lattupally (Bijnepally)[a]	*Pf35*	Groundnut	Gundoor [a]
Pf9	Groundnut	Lattupally (Bijnepally)[a]	*Pf36*	Groundnut	Thadoor (Thadoor)[a]
Pf10	Redgram	Vasanthapuram (Bijnepally)[a]	*Pf37*	Chickpea	Manganoor (Bijnepally)[a]
Pf11	Castor	Lingasanipally (Bijnepally)[a]	*Pf38*	Sorghum	Kondapur (Narayanked)[b]
Pf12	Rice	Kottalgadda (Bijnepally)[a]	*Pf39*	Rice	Peddapendyala (Dharmasagar)[c]
Pf13	Sunflower	Lingasanipally (Bijnepally)[a]	*Pf40*	Sunflower	Chinnakarupumala (peddakothapally)[a]
Pf14	Rice	Kottalgadda (Bijnepally)[a]	*Pf41*	Maize	Peddapendyala (Dharmasagar)[c]
Pf15	Maize	Lingasanipally (Bijnepally)[a]	*Pf42*	Sugarcane	Kondapur (Narayanked)[b]
Pf16	Rice	Thimmajipet (Thimmajipet)[a]	*Pf43*	Sunflower	Kondapur (Narayanked)[b]
Pf17	Groundnut	Vattem (Bijnepally)[a]	*Pf44*	Rice	Wanaparthy (Wanaparthy)[a]
Pf18	Cotton	Edirepally (Thimmajipet)[a]	*Pf45*	Chickpea	Kondapur (Narayanked)[b]
Pf19	Rice	Kodkurthy (Bijnepally)[a]	*Pf46*	Maize	Chinnakarupumala(Peddakothapally)[a]
Pf20	Rice	Vattem (Bijnepally)[a]	*Pf47*	Chilli	Peddapendyala (Dharmasagar)[c]
Pf21	Rice	Edirepally (Thimmajipet)[a]	*Pf48*	Rice	Chinnakarupumala(Peddakothapally)[a]
Pf22	Castor	Nagarkurnool[a]	*Pf49*	Sorghum	Atchampet (Achampet) [a]
Pf23	Groundnut	Vattem (Bijnepally)[a]	*Pf50*	Sorghum	Palem (Bijnepally)[a]
Pf24	Redgram	Palem (Bijnepally)[a]	*Pf51*	Parthenium	Nagarkurnool (Nagarkurnool)[a]
Pf25	Chilli	Nadigadda[a]	*Pf52*	Parthenium	Palem (Bijnepally)[a]
Pf26	Maize	Thadoor (Thadoor)[a]	*Pf53*	Rice	Vanasthalipuram (Hyderabad)[d]
Pf27	Chilli	Karvanga (Telkapally)[a]	*Pf54*	Rice	Lingotam (Achampet)[a]

[a]Mahabubnagar District; [b]Medak District; [c]Warangal District; [d]Ranga Reddy District

A total of eighty three bacteria were isolated from the rhizospheric soil of different crops (Table 1). Among them, fifty four isolates were identified as *P. fluorescens* (*Pf*) and twenty nine as non-*Pf* bacteria using U.V. transilluminator, by observing fluorescence in Kings 'B' medium and showed gram-negative reaction.

Seed inoculation with *Pf* strains significantly enhanced seed germination and seedling vigor of sorghum seed. However, the rate of enhancement varied with different bacterial isolates. Thirty eight *Pf* isolates were found to increase the Vigor Index-I over the non-treated control (Table 2). The highest enhancement of vigor index (1395) and root length (7.4) was obtained from *Pf* 2, while lowest (150) by *Pf 54*. In case of shoot length *Pf* 19 showed maximum (4.4) whereas *Pf* 8 recorded minimum (0.38).

Bacterial inoculants are able to increase plant growth and germination rate, improve seedling emergence and protect plants from disease (Lugtenberg et al., 2002; Ashrafuzzaman et al., 2009). Gholami et al. (2009) reported that, inoculation of maize seed with PGPR increased the seed germination and seedling growth. Our studies revealed that under *in vitro* conditions, seed treatment with *Pf* improved seed germination and seedling vigor over the control (Raju et al., 1999; Niranjan et al., 2004; Niranjan et al., 2003; Das et al., 2010). These findings might be due to the augmented synthesis of hormones like gibberellins and auxins (Bharathi et al., 2004). As Agricultural practices become more sustainable, there is an increasing need for ecologically sound methods of disease control. Soil *Pseudomonads* possess a variety of promising properties which make them better biocontrol agents. Although the present study is just an initiative, it helps

Table 2: Effect of *P. fluorescens* inoculations on seed germination and other parameters of sorghum under *in vitro* conditions

Treatment	Shoot length	Root length	Vigour Index-I	Treatment	Shoot length	Root length	Vigour Index-I
Control	0.45±0.1	2.91±0.1	414±19.8	*Pf* 28	1.75±2.4	6.75±4.8	395±21.2
Pf 1	2.37±0.3	5.15±0.2	445±6.7	*Pf* 29	2.51±1.4	7.02±2.8	240±14.8
Pf 2	2.87±3.0	7.40±2.3	1395±7.0	*Pf* 30	2.87±0.5	6.45±1.1	379±15.5
Pf 3	0.92±0.6	7.31±0.5	824±12.7	*Pf* 31	1.0±1.4	3.55±3.7	422±23.3
Pf 4	1.27±1.3	4.90±1.3	802±4.9	*Pf* 32	1.15±1.6	3.99±5.5	592±16.4
Pf 5	1.91±1.5	5.82±0.8	655±7.0	*Pf* 33	1.50±1.0	5.43±2.3	437±24.0
Pf 6	1.72±1.4	6.47±3.0	904±7.5	*Pf* 34	0.75±0.6	3.78±0.8	297±3.6
Pf 7	0.67±0.0	4.75±0.0	434±2.8	*Pf* 35	2.37±0.5	6.08±0.8	483±35.3
Pf 8	0.38±0.1	6.66±1.9	847±4.9	*Pf* 36	0.78±0.3	3.12±0.9	350±14.4
Pf 9	0.85±0.8	5.62±1.1	623±4.5	*Pf* 37	2.83±0.4	4.51±0.9	504±6.2
Pf 10	1.02±0.8	7.07±2.3	823±13.4	*Pf* 38	2.93±0.2	5.93±2.4	275±7.0
Pf 11	1.16±1.3	5.20±0.9	676±8.2	*Pf* 39	3.22±0.8	3.75±0.3	520±13.8
Pf 12	2.21±0.0	4.10±0.0	515±6.6	*Pf* 40	3.83±1.1	7.24±0.8	645±6.9
Pf 13	3.17±0.2	6.25±1.7	322±16.9	*Pf* 41	3.33±0.4	4.15±0.1	293±10.6
Pf 14	1.15±0.1	6.91±1.2	717±4.2	*Pf* 42	2.33±0.0	6.86±1.1	589±9.7
Pf 15	2.05±0.7	6.25±1.1	479±10.6	*Pf* 43	0.72±0.9	4.75±1.0	406±11.3
Pf 16	2.18±0.4	6.25±0.4	660±14.1	*Pf* 44	1.46±1.2	4.31±3.2	523±18.2
Pf 17	0.97±0.0	5.91±0.6	576±8.2	*Pf* 45	2.57±0.7	5.53±1.0	525±21.2
Pf 18	1.87±0.1	6.50±2.2	268±3.5	*Pf* 46	1.51±0.8	4.38±1.1	715±20.8
Pf 19	4.66±0.4	5.62±0.2	793±18.1	*Pf* 47	2.53±0.7	4.75±1.7	425±21.2
Pf 20	3.66±0.9	5.91±0.1	820±9.4	*Pf* 48	2.03±0.7	6.03±0.0	305±7.0
Pf 21	0.68±0.1	4.17±0.6	412±3.1	*Pf* 49	1.57±0.0	1.51±2.7	404±8.3
Pf 22	3.47±0.3	6.18±0.3	758±11.0	*Pf* 50	2.66±1.8	4.07±0.0	320±0.1
Pf 23	0.52±0.3	3.11±1.1	358±15.9	*Pf* 51	2.87±0.5	4.33±0.2	615±7.0
Pf 24	0.4±0.2	5.84±3.4	873±17.6	*Pf* 52	1.68±0.4	5.12±1.2	431±15.2
Pf 25	3.0±0.0	5.41±0.8	460±14.0	*Pf* 53	1.75±1.1	5.0±0.0	584±23.3
Pf 26	2.15±0.6	5.53±0.7	842±31.1	*Pf* 54	0.58±0.6	4.25±2.5	150±13.8
Pf 27	2.15±0.9	5.22±1.3	444±25.4				

All values are mean±SEm of samples in triplicate. Vigour index-I values were significant at $p<0.05$ compared to control except *Pf* 21

in better understanding and utilization of *P. fluorescens* as a biofertilizer and biocontrol agent, finally it strengthens the vigour in the plant.

4. Conclusion

Our studies revealed that under *in vitro* conditions, seed treatment with *Pf* strains improved seed germination and seedling vigor over the control. Our results accounted, increased seed vigor over non treated control by *P. fluorescens*. These findings might be due to the augmented synthesis of hormones like gibberellins and auxins.

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