Influence of seed biopriming with different isolates of *Pseudomonas fluorescens* on the growth of paddy

Ajay Tomer[1], Ramji Singh, Durga Prasad[2] and Saurabh Kumar Singh[3]

ABSTRACT

Many strains of the *Pseudomonas fluorescens* have been characterized as plant growth promoting rhizobacteria (PGPR) and they enhance the growth and yield of economical important paddy crops. The effect of bio-priming on paddy paddy seed growth was assessed at Crop Research Centre S.V.B.P.A.&T Meerut India by studying the effect of fluorescent Pseudomonas strains for their growth stimulatory effect on paddy plants in Randomized Block Design (RBD) in pot conditions. A total of nineteen isolates of the rhizobacteria *Pseudomonas fluorescens* were selected from the fields of paddy, wheat, mustard, chilli, sorghum and pearlmillet and these strains were used as bio-priming agents for paddy paddy seed. The highest increase in shoot dry weight was seen in the isolate *Pseudomonas fluorescens* SVP 12 followed by SVP 11 and SVP 13 with 93.33, 86.67 and 80 per cent enhancement in shoot dry weight. All isolates of *P. fluorecens*, when applied as seed bio-priming of paddy paddy seed, had some stimulatory effect on growth and physiological parameters of treated plants indicating that such strains could be use for enhancing the yield and quality of paddy paddy crop.

Keywords: Biopriming, PGPR, Root growth, Shoot growth

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INTRODUCTION

Agriculture in modern era largely rely on the use of agrochemicals for managing the plant diseases and to enhance crop productivity (Oerke, 2006). Indiscriminate use of chemicals has created serious threats to human health and environment and have resulted in an enhanced curiosity in bio-agent application as an effective way of reducing the application of agrochemicals. Several naturally occurring soil bio-control agents had shown better capability to alienate field crop pathogens and the application of few useful fungi and bacteria for crop protection is being considered as a feasible replacement of chemical pesticides (Correa and Soria, 2010; O'Callaghan, 2016).

Rhizobacteriae are bioagents which multiply in rhizosphere of crop plants and play a crucial role in growth and development of plants (Kloepper *et al*., 1980; Lugtenberg *et al*., 2009; Ahemad and Kibret, 2014). Significant increases in growth and yield of agronomically important crops in response to inoculation with PGPR have been reported. In this context, *Azospirillum*, *Pseudomonas* and *Azotobacter* isolates influence seed emergence and seedling growth. It has been shown that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculation. Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* could increase root and shoot elongation in wheat (Chakraborty *et al*., 2013; Kumar *et al*., 2014; Islam *et al*., 2014). Incubation of crop plants with *Azospirillum* might induce several characteristics, which enhanced plant biomass, nutrient uptake, tissue N content, plant length, leaf size root and shoot length of cereals (Mirza *et al*., 2006; Nadeem *et al*., 2013; Doni *et al*., 2013). So it is considered that the capability of *Rhizobacteria, Azospirillum* and *Pseudomonas* can be exploited for field utilization as natural biofertilizers for promoting growth and development of plants and this also would help the management of plant diseases also reported earlier. Accordingly, this work was to study the influence of seed bio-priming with the rhizobacteria, *Pseudomonas fluorescens* on seedling growth and development of paddy paddy plant under the pot conditions.

MATERIALS AND METHODS

A pot experiment was conducted to assess the effect of biopriming on paddy seed growth at S.V.B.P.A. and T. University, Meerut, Uttar Pradesh. Nineteen bacterial isolates of *P. fluorescens* as listed in table 1 were used for bio-priming. Paddy seeds (variety PB 1121) were decontaminated with 0.02% bleaching agent for 2 min, and gently washed with purified sterile water and coated with jaggery as stickers for attachment of bacterial colony (10^8 cfu ml^{-1}) on upper surface of paddy seed. Seeds were shown in plastic pots with 15cm breadth having capacity for retention of 2 kg amount of soil.

Table 1. *Pseudomonas fluorescens* isolates for the present study and the sources of isolation

Isolates	Source crop
Pseudomonas fluorescens (SVP1), (SVP2)(SVP3)	Paddy
Pseudomonas fluorescens (SVP4), (SVP5), (SVP6), (SVP7)	Wheat
Pseudomonas fluorescens (SVP8), (SVP9), (SVP10)	Mustard
Pseudomonas fluorescens (SVP11)	Chilli
Pseudomonas fluorescens (SVP12)	Chilli
Pseudomonas fluorescens (SVP13)	Chilli
Pseudomonas fluorescens (SVP14)	Chilli
Pseudomonas fluorescens (SVP15)	Sorghum
Pseudomonas fluorescens (SVP16)	Sorghum
Pseudomonas fluorescens (SVP17)	Sorghum
Pseudomonas fluorescens (SVP18)	Pearl millet
Pseudomonas fluorescens (SVP19)	Pearl millet
Check Control	

All 19 isolates sequenced in 60 plastic pots *i.e.*, 3 depicts with 20 pots per replication and two seeds were grown in each plastic pot. These seedlings were removed at 21st day after the sowing of seeds and rinsed with tap water. The data were recorded on following parameters.

Length of root and shoot

Root and shoot length were measured with the help of a meter scale of three randomly selected plants from each pot in each replication. Average length was used for statistical analysis. Percentage of increase in length of root and were calculated by the methods as follow (Wood and Roper, 2000):

Fresh weight of root and shoot

Paddy plants from treated as well as check pots were softly uprooted from the respective pots. Root and shoot were separated from collar region. Fresh weights were measured by taking weight on electronic balance up to three decimal digits. Percent increase in fresh weight of root and shoot were calculated by using following formulas (Wood and Roper, 2000).

Dry weight of root and shoot

Root as well as shoot of the selected plants after 21 days of sowing were cut to seperate from collar region, dried to the stage of moisture free in a hot air oven at 75^0 C for 48 hrs (till attaining constant mass), weight recorded on electronic balance and expressed in gram. Percent increase in dry weight of root and shoot were calculated by the methods as follows (Wood and Roper, 2000).

RESULTS AND DISCUSSION

Length of root and shoot

Out of all nineteen, few isolates resulted in excellent enhancement (Table 2), isolate of *P. fluorescens* SVP 12 and SVP 11 could be regarded as best performing isolates for enhancement of root length as they resulted in 104.99 and 103.23 per cent increase of root length respectively followed by isolate, SVP 17 which resulted in 92.38 per cent increase, SVP 19 with 82.11 per cent increase and SVP 14 with 70.09 per cent increase.

In case of shoot length, the isolate SVP 12 was best performing isolate with 104.45 per cent increase in shoot height followed by isolate SVP 6 which resulted in 98.68 per cent increase in shoot height. The third best performing isolate was SVP 19 with 94.56 per cent increase. The isolate SVP 17 and SVP 9 were fourth and fifth in order for enhancement of shoot height, respectively. All these isolates resulted in significantly different height of paddy shoot. The minimum increases in shoot height were recorded due to isolate SVP 11 followed by SVP 15 which resulted in 27.02

Table 2. Effect of seed biopriming of paddy with different isolates of *Pseudomonas fluorescens* on root and shoot length of paddy paddy plants

Name of Isolates	Root length (cm) Mean	SE Value	Percent Increase	Shoot length (cm) Mean	SE Value	Percent Increase
Pseudomonas fluorescens (SVP1)	14.00	0.2	23.17	32.00	0.57	58.15
Pseudomonas fluorescens (SVP2)	18.83	0.23	65.69	34.33	1.2	69.69
Pseudomonas fluorescens (SVP3)	17.00	0.45	49.56	29.30	0.05	44.81
Pseudomonas fluorescens (SVP 4)	14.13	0.17	24.34	29.03	0.12	43.49
Pseudomonas fluorescens (SVP 5)	17.23	0.17	51.61	33.23	0.08	64.25
Pseudomonas fluorescens (SVP 6)	18.30	0.23	61.00	40.20	0.05	98.68
Pseudomonas fluorescens (SVP 7)	17.73	0.2	56.01	35.30	0.05	74.46
Pseudomonas fluorescens (SVP 8)	18.40	0.05	61.88	33.00	0.05	63.10
Pseudomonas fluorescens (SVP 9)	18.13	0.12	59.53	38.13	0.08	88.47
Pseudomonas fluorescens (SVP 10)	17.17	0.14	51.03	36.23	0.03	79.08
Pseudomonas fluorescens (SVP 11)	23.10	0.15	103.23	25.70	0.05	27.02
Pseudomonas fluorescens (SVP 12)	23.30	0.05	104.99	41.37	0.08	104.45
Pseudomonas fluorescens (SVP 13)	14.27	0.08	25.51	33.37	0.08	64.91
Pseudomonas fluorescens (SVP 14)	19.33	0.08	70.09	32.80	0.05	62.11
Pseudomonas fluorescens (SVP 15)	15.80	0.05	39.00	27.17	0.08	34.27
Pseudomonas fluorescens (SVP 16)	16.93	0.08	48.97	34.33	0.08	69.69
Pseudomonas fluorescens (SVP 17)	21.87	0.08	92.38	38.30	0.15	89.29
Pseudomonas fluorescens (SVP 18)	16.90	0.11	48.68	35.33	0.08	74.63
Pseudomonas fluorescens (SVP 19)	20.70	0.11	82.11	39.37	0.08	94.56
Control	11.37	0.08	0.00	20.23333	0.03	0.00
CD at 5%	0.867			2.769		

and 34.27 percent increase respectively. The shoot height of untreated paddy plants was minimum (20.23 cm) among the all treatments. Thus it is clear that all these isolates of *P. fluorecens* had some stimulatory effect on plant growth.

Correlative development of seed quality characteristics through rhizobacteria have been noticed earlier in pearl millet (Niranjan *et al*., 2004) and sunflower (Moeinzadeh *et al*., 2010). Increase in growth of chickpea by inoculation of *P. fluorescence* was also reported by Rokhzadi *et al*. (2008).

Fresh weight of root and shoot

As in case of root and shoot height, similar trend was observed in case of enhancement of fresh weight of paddy plants due to seed bio-priming with *P. fluorescens* (table 3). The isolates SVP-5 were found to be most effective for enhancing the root fresh weight with 95.69 percent increase. The isolate SVP 12 and SVP 1 were almost equally effective for enhancing the root fresh weight and second in order with significantly different level 92.28 and 90.63 of root fresh weight. The isolate SVP 11 was also effective and at fourth position with 80.78 percent enhancement, followed by isolate SVP 3 with 68.37 percent enhancement.

The isolate SVP 7 was least effective with nearly 4.19 percent increase in root fresh weight. It is worthwhile to mention that untreated paddy plants resulted only 0.22g weight of fresh root.

In case of shoot fresh weight (Table 3), the isolate SVP 11 was found to be most effective with 87.54 per cent increase of shoot fresh weight followed by SVP 10 with 82.28 per cent increase and also the SVP 1 with 80.53 per cent increase. The isolate SVP 3 and SVP 14 were next in order with 68.06 and 51.31 per cent enhancement of shoot fresh weight. The isolate used in the treatment T8, T16 and T18 were least effective for enhancing the fresh weight of shoot with only 5.55, 3.99 and 2.63 per cent enhancement. The paddy seed without bio-priming with *P. fluorescens* resulted in 0 .34 g fresh weight of shoot. The paddy seed without bio-priming with *P. fluorescens* resulted lowest weight of fresh shoot. These results on root and shoot weight also in accordance with the findings of Santoro *et al.* (2016). They also reported improved shoot fresh weight in *Menthe piperita* with the inoculation of Fluorescent Pseudomonas Strains.

Table 3. Effect of seed biopriming of paddy with different isolates of *Pseudomonas fluorescens* on root and shoot fresh weight of paddy plants.

Name of Isolates	Fresh weight of root (g) (Mean)	S.E.	Percent Increase	Fresh weight of shoot (g) Mean	S.E.	Percent Increase
Pseudomonas fluorescens (SVP1)	0.410	0.06	90.698	0.618	0.001	80.53
Pseudomonas fluorescens (SVP2)	0.242	0.01	12.713	0.392	0.001	14.51
Pseudomonas fluorescens (SVP3)	0.362	0.01	68.372	0.575	0.001	68.06
Pseudomonas fluorescens (SVP 4)	0.267	0.01	24.186	0.485	0.001	41.67
Pseudomonas fluorescens (SVP 5)	0.421	0.01	95.659	0.429	0.001	25.32
Pseudomonas fluorescens (SVP 6)	0.253	0.01	17.674	0.421	0.001	22.98
Pseudomonas fluorescens (SVP 7)	0.243	0.01	12.868	0.433	0.001	26.49
Pseudomonas fluorescens (SVP 8)	0.256	0.01	19.070	0.361	0.001	5.55
Pseudomonas fluorescens (SVP 9)	0.296	0.01	37.674	0.458	0.001	33.79
Pseudomonas fluorescens (SVP 10)	0.368	0.01	71.163	0.624	0.001	82.28
Pseudomonas fluorescens (SVP 11)	0.389	0.01	80.775	0.642	0.001	87.54
Pseudomonas fluorescens (SVP 12)	0.413	0.01	92.248	0.458	0.001	33.69
Pseudomonas fluorescens (SVP 13)	0.243	0.01	12.868	0.415	0.001	21.13
Pseudomonas fluorescens (SVP 14)	0.264	0.01	22.791	0.518	0.001	51.31
Pseudomonas fluorescens (SVP 15)	0.299	0.01	39.070	0.416	0.001	21.52
Pseudomonas fluorescens (SVP 16)	0.245	0.01	13.953	0.356	0.001	3.99
Pseudomonas fluorescens (SVP 17)	0.224	0.01	4.186	0.381	0.001	11.30
Pseudomonas fluorescens (SVP 18)	0.306	0.01	42.326	0.351	0.001	2.63
Pseudomonas fluorescens (SVP 19)	0.290	0.01	34.884	0.413	0.001	20.74
Control	0.215	0.01	0	0.342	0.001	0.00
CD at 5%	0.004			0.002		

Dry weight of root and shoot

The data regarding dry weight of root and shoot presented in table 4 indicates that in case of enhancement of root dry weight, the isolate SVP 12 and SVP 8 were most effective with 111.10 per cent and 100 percent increase in root dry weight, followed by isolate SVP 6 and SVP 4 with 88.9 per cent increase in the root dry weight, followed by isolate SVP 11 and SVP 1 were found to be equally effective with 77.80 per cent increase in root dry weight followed by isolate SVP 13 with 67.7 per cent increase. The isolate SVP 19, SVP 14 and SVP 2 were least effective with 22% percent increase in root dry weight, paddy seed without bio-priming resulted in only .09g weight of dry root.

Maximum increase in shoot dry weight was seen in the isolate *Pseudomonas fluorescens* SVP 12 followed by SVP 11 and SVP 13 with 93.33, 86.67 and 80 per cent enhancement in shoot dry weight respectively. The isolates SVP 10 and SVP 5 were fourth and fifth in this order with 70.33 and 60 per cent increase in shoot dry weight. The isolate SVP 4 and SVP 6 were ineffective with no enhancement in dry weight of shoot, followed by the isolate SVP 2 and SVP 8 with 6.67 percent increase, paddy paddy seed without biopriming resulted in only 0.15g dry weight of shoot. Similarly, Sindhu *et al*. (2002) also recorded higher gains in plant dry weight ratios in chickpea treated with *Pseudomonas* strains (MRS13, CRS55b and CRS68). Co-inoculation of soybean with *Bradyrhizobium japonicum* USDA 110 and *Pseudomonas putida* TSAU 1 strains significantly increased the root dry weight (18%), shoot dry weight (45%) and total dry weight (43%) of plants also been reported by Jabborova *et al*. (2018).

Thus this study observed that application of *P. fluorescens* as seed bio-priming agent is effective in enhancing the root and shoot growth and overall crop yield and the findings of present investigation are quite in conformity with the findings of other workers which have been mentioned above. All 19 isolates of *P. fluorecens* as seed biopriming of paddy showed some stimulatory effect on plant growth. Inoculation of Pseudomonas also improved fresh and dry weight of root and shoot. These effects thereby could help in significant increases in yield of paddy. This technique may further help in industrialization of bio-agent production.

Table 4. Effect of seed biopriming of paddy with different isolates of *Pseudomonas fluorescens* on root and shoot dry weight of paddy plants.

Name of Isolates	Dry weight root (g) Mean	S.E.	Percent Increase	Dry weight of shoot (g) Mean	S.E.	Percent Increase
Pseudomonas fluorescens (SVP1)	0.160	0.006	77.8	0.230	0.006	53.33
Pseudomonas fluorescens (SVP2)	0.110	0.006	22.2	0.160	0	6.67
Pseudomonas fluorescens (SVP3)	0.130	0.006	44.4	0.180	0.006	20.00
Pseudomonas fluorescens (SVP 4)	0.170	0.006	88.9	0.150	0.006	0.00
Pseudomonas fluorescens (SVP 5)	0.110	0.006	22.2	0.240	0.006	60.00
Pseudomonas fluorescens (SVP 6)	0.170	0.006	88.9	0.150	0.006	0.00
Pseudomonas fluorescens (SVP 7)	0.130	0.006	44.4	0.180	0	20.00
Pseudomonas fluorescens (SVP 8)	0.180	0.006	100.0	0.160	0	6.67
Pseudomonas fluorescens (SVP 9)	0.110	0.006	22.2	0.220	0.006	46.67
Pseudomonas fluorescens (SVP 10)	0.130	0.006	44.4	0.260	0.006	73.33
Pseudomonas fluorescens (SVP 11)	0.160	0.006	77.8	0.280	0.006	86.67
Pseudomonas fluorescens (SVP 12)	0.190	0.006	111.1	0.290	0	93.33
Pseudomonas fluorescens (SVP 13)	0.150	0.006	66.7	0.270	0	80.00
Pseudomonas fluorescens (SVP 14)	0.110	0.006	22.2	0.180	0.006	20.00
Pseudomonas fluorescens (SVP 15)	0.117	0.009	29.6	0.170	0	13.33
Pseudomonas fluorescens (SVP 16)	0.120	0	33.3	0.233	0	55.55
Pseudomonas fluorescens (SVP 17)	0.100	0	11.1	0.182	0	21.33
Pseudomonas fluorescens (SVP 18)	0.120	0	33.3	0.175	0	16.67
Pseudomonas fluorescens (SVP 19)	0.110	0	22.2	0.170	0	13.33
Control	0.090	0	0.0	0.150	0	0.00
CD at 5%	0.015			0.011		

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Ajay Tomer[1*], Ramji Singh, Durga Prasad[2] and Saurabh Kumar Singh[3]

Department of Plant Pathology, Sardar Vallabhbhi Patel University of Agriculture & Technology, Meerut-250 110, India

[1]Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara-144 411, India

[2]Department of Plant Pathology, Buat Banda UP, India.,

[3]Department of Horticulture, Janta Vedic College, Baraut-250 611, India.

***Corresponding author:**

E-mail: ajaytomer1489@gmail.com

https://orcid.org/0000-0003-0743-