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In the present investigation twenty four isolates of Fluorescent *pseudomonas* were evaluated with pigeonpea cv. Rajeevlochan for their plant growth promotion activity. Seeds treated with isolate P-233 produced maximum total root length (326.65 cm), total surface area (21.76 cm^2), total root volume (0.116 cm^3) and maximum number of tips (3448.00) as compared to control. Efficacy of different isolates of Pseudomonas for pigeonpea plants varied to induce shoot and root length of pigeonpea plant ranging from 11.4 cm to 17.8 cm and 15.4 cm to 24.4 cm, respectively. Maximum shoot length (17.8 cm) and root length (24.4 cm) were recorded when seeds were treated with P233 as compared to control. Isolates P11 showed highest average fresh shoot weight (0.42g) and dry shoot weight (0.188g). While isolate P143 performed with highest average root fresh weight (0.274g) and dry root weight (0.04g). Plants treated with P233 produced maximum average number of nodulation (20.6) as compared to control.

Key words: Disease, disinfection, inoculum, pathogen, PGPR, rhizobacteria, solarisation, wilt

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is the fifth prominent pulse crop in the world and second most important pulse crop after chickpea in India (Patel and Patel, 2012). It represents about 5% of world legume production and more than 60% is being produced in India. India accounts for more than 72 per cent and 61 per cent of world's area and production respectively (Tiwari, 2012). Among the diseases, vascular wilt caused by *Fusarium oxysporum* f. sp. *udum* is the most important soil borne disease of pigeonpea (Pande *et al.* 2013). The disease may cause 67 per cent when it occurs at pod maturity stage and 100 per cent at pre-pod stage.

The continuous cultivation of crop for 2-3 years in the same field leads to more wilting and loss of plant due to wilt disease (Telangre *et. al*. 2016). The pathogen is both seed and soil borne in nature hence management of the disease is very difficult(Mahesh *et al.* 2010). Soil solarization/disinfection, crop rotation and mixed cropping are the best ways to eliminate soil-borne pathogens but their use especially solarization/disinfection is costly and not applicable in large area and crop rotation and mixed cropping do not give rapid control (Niranjana *et. al*. 2009). Seed treatment with synthetic fungicides such as Carbendazim, Benlet, and Thiram considerably reduces wilt incidence in pigeonpea. However, their possible threat for development of chemical resistance, by fungal pathogens and non-target side effects on other plant pathogens and beneficial microorganisms (Choure and Dubey, 2012). The use of resistant varieties is the most effective alternative approach to controlling wilt disease but development of resistant varieties is a tedious and time consuming procedure and their breakdown in the face of high pathogenic variability in the pathogen population, the usefulness of many resistant cultivars is restricted to only a few years (Niranjana *et al.* 2009). Fluorescent *pseudomonas* is such a plant growth promoting rhizobacteria that has both the attributes i.e. to protect the plant against pathogenic micro organisms and to promote plant growth (Siddiqui and Shakeel, 2009). *Pseudomonas* spp. are the most extensively studied plant growth-promoting rhizobacteria (PGPR), and are known to protect the plant from

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many deleterious soil and foliar plant pathogenic microorganisms (Bhattacharjee and Dey, 2014). The mechanisms involved in Fluorescent pseudomonas mediated plant growth promotion and disease suppression are ability to produce various compounds including phytohormones, siderophores, antibiotics, chitinase, â-1,3 glucanase, and hydrogen cyanide (HCN), Phosphate solubilization and induced systemic resistance against a broad diversity of pathogens (Podile and Kishore, 2006; Niranjana *et al*. 2009 ; Siddiqui and Shakeel, 2009; Choure and Dubey, 2012). Hence, an experiment was designed to evaluate the plant growth promotion activity of Fluorescent *Pseudomonas* on pigeonpea.

MATERIALS AND METHODS

Root system morphology of pigeonpea varieties following bipartite interaction with Fluorescent Pseudomonas

The cultures of Fluorescent Pseudomonas were inoculated in 100 ml conical flask containing 25 ml King's B broth and incubated at 28±1°C for 48 hrs. For seed soaking inoculum of all the 24 isolates were diluted with 35 ml of sterilized distilled water. Twenty ml of the diluted bacterial suspension was dispensed in Tarsons tube. Five seeds of pigeonpea were soaked in the diluted suspension for 3 hours. Seeds were planted in pots containing soil mixed with sand and compost in the ratio of 3:1:1. The plants were uprooted after 15 days of sowing. While uprooting utmost care was taken to avoid root damage. Plants were then washed with tap water, stretched on fixed clean transparent surface followed by measurement of shoot length and root length. Apart from their ability to alleviate abiotic stress, the 24 isolates of Fluorescent pseudomonas were tested for their plant growth promoting activity in pigeonpea.

Study of Root Characterization

Root systems are responsible for the capture of below-ground resources such as nutrients and water. As such, they are thought to play a central role in the yield establishment of crop plants. The availability of a given resource for the plant can be seen as the integration of soil and roots bio-physical constraints. Therefore, detailed datasets containing root system architecture, root placement and soil resource dynamics are required to improve our understanding of resource capture by plant roots. The roots of *Pseudomonas* treated pigeonpea plants were used for root scanning which gave the detailed information about all root parameters. The root scanning was done by using root scanner Epson Perfection V700/ V750 3.81 version and WinRhizo Reg 2009 software. The data was recorded automatically in the computer for different root parameters including root length, average root diameter, root volume, number of tips, forks, surface area etc. Following procedure was used for root scanning:

Acquiring Washed Roots

The first step is acquiring washed roots. This can be the most difficult and laborious step in the experiment if plants are grown in a solid medium. The roots were washed with tap water two times to remove soil completely and the roots were preserved in 25% spirit in Tarson tubes for root scanning. The procedure was conducted cautiously to prevent supplementary root damage and losses.

Preparing roots for scanning

Roots are floated in water in acrylic trays on the scanner. This allows the roots to be arranged to reduce overlap and crossing of roots. Plastic forceps were used as tools. This is a delicate work; good lighting and steady hands are helpful.

Scanning roots

For best results, WinRhizo is used with an approved scanner, which allows the roots to be lit from above and below while being scanned. This is an important feature (called "Dual Scan" in Regent's documentation), which reduces shadows on the root image. Positioning System allows the trays to be consistently placed, thus obviating the need to preview each scan. Optimum scanning resolution depends on the type of samples. Lower resolution can speed up scanning significantly, especially if the samples require the use of large trays. Root length analyses are carried out with grayscale images; saving images in grayscale reduces the image file size substantially.

Threshold parameters

Analysis results can be sensitive to the threshold parameters used. WinRhizo can automatically set

these, but one can also manually tweak them from time to time.

Analizing scanned images

To analyze the image, selects the region(s) of interest and it is analyzed. When scanned images are analyzed, the software uses thresholding to determine what is root and what is not root (each pixel is classified as either root or not root based on its grayscale value; this is why shadows in images are problematic). Portions of the image can be excluded from analysis if necessary, and there are basic editing tools if minor image editing is required.

PGPR activity of Fluorescent *pseudomonas* with pigeonpea

In the present investigation twenty four isolates of Fluorescent *pseudomonas* were used to evaluate the plant growth promotion activity. Pigeonpea seeds (Rajeevlochan) were treated with different isolates of Fluorescent *pseudomonas*. Treated seeds were sown in pots containing soil. From different treatment combinations seedlings were harvested at fifteen days after sowing and observations were recorded for shoot length, root length, shoot fresh and dry weight, root fresh and dry weight and nodulation.

RESULTS AND DISCUSSION

Root system morphology of pigeonpea varieties following bipartite interaction with Fluorescent pseudomonas

Root systems are responsible for the capture of below-ground resources such as nutrients and water. As such, they are thought to be playing a central role in the yield establishment of crop plants. Image analyses systems provide a quick determination of various root morphological parameters. In the present investigation, roots of pigeonpea (*Cajanus cajan*) treated with different isolates of Fluorescent pseudomonas were screened for study of morphological characteristics. Root characters were assessed to evaluate the efficacy of different *Pseudomonas* sp. to promote plant growth. Observations were recorded 15 days after sowing. The roots of Fluorescent *pseudomonas* treated plants were used for root scanning which gave the detailed information about all root parameters which included total length, total surface area, average diameter, root volume, tips and fork. Images analysed and root measurements with Epson perfection v700/ v750 3.81 version scanner with WinRhizo Reg software are presented in Table 1. The root pieces were neatly spread on the tray, a potential effect of overlapping on final estimations was excluded. Spreading roots in water instead of directly placing on the scanner surface is preferred in regular root studies. Plant roots are complicated three dimensional objects and they are problematical for geometrical evaluation in two dimensional planes. WinRHIZO has been established as an appropriate choice for the root morphological evaluation (Bauhus and Messier, 1999; Bouma *et al*. 2000).

The observations of the roots of pigeonpea for total length, total surface areas, average diameter, root volume, tips and fork ranged from 135.65 cm to 326.65cm, 10.14 cm^2 to 21.76 cm^2, 0.20mm to 0.26mm, 0.057cm^3 to 0.116 cm^3, 1227.33 to 3448 and 957 to 2761.33 respectively. Seeds treated with isolate P-233 produced maximum total root length (326.65 cm), total surface area (21.76 cm^2), total root volume (0.116 cm^3) and maximum number of tips (3448.00) as compared to control (Fig. 1a, b). Kloepper *et al*. (1986) and Khalid *et al*. (2004) suggested that the reason for such increase was attributed to synthesis of phytohormones by PGPRs at various stages, resulting in increased root growth followed by enhanced absorption of essential nutrients. Rolfe *et al*. (1997) and Canbolat *et al*. (2006) reported that generally, application with PGPR effects development of lateral roots) and subsequently increases in root proliferation and nutrient uptake. Root length of the Pseudomonas inoculated seedlings in present study are greater than in bacterial untreated plants. The PGPR inoculation may effectively increase the surface area of roots. Increase in root growth may be responsible for shoot growth, which further makes the plant absorb nutrients effectively.

Efficacy of Fluorescent pseudomonas isolates for plant growth promoting activity in pigeonpea

In the present investigation twenty four isolates of Fluorescent *pseudomonas* were screened for the plant growth promotion on pigeonpea, *Cajanus cajan* (Table 2). For evaluating the PGPR activity, Fluorescent *pseudomonas* treated seeds derived

Table 1: Efficacy of different isolates of Fluorescent *pseudomonas* to induce root characters in pigeonpea

Treatment	Total length (cm)	Total surface area (cm^2)	Average diameter (mm)	Root volume (cm^3)	No. of tips	No. of forks
Control	181.88±20.76	12.01±0.91	0.21±0.010	0.063±0.004	2,064.66	1,768.333
P5	237.92± 30.88	15.25±1.83	0.20±0.003	0.078±0.009	2,785.33	1,999.667
P11	253.39±4.24	16.93±0.47	0.21±0.003	0.090±0.004	2,228.00	1,617.333
P67	208.40±23.02	14.72±1.96	0.21±0.009	0.072±0.018	2,359.33	1,466.000
P99	180.39±16.93	13.21±0.81	0.23±0.010	0.077±0.003	1,281.66	986.000
P126	226.51±30.49	15.63±2.37	0.22±0.018	0.087±0.017	1,813.66	1,437.667
P72	180.76±16.01	13.02±1.02	0.23±0.007	0.075±0.006	1,808.00	1,487.333
P216	190.51±32.44	12.44±1.62	0.21±0.013	0.065±0.007	2,349.00	1,672.667
P205	197.45±24.08	12.61±1.69	0.20±0.006	0.064±0.010	1,925.33	1,263.667
P161	144.08±13.70	10.14±0.64	0.22±0.010	0.057±0.004	1,227.33	1,027.000
P176	190.07±12.87	13.45±0.78	0.22±0.006	0.076±0.005	1,800.66	1,411.000
P76	180.79±16.09	11.69±0.63	0.20±0.009	0.060±0.002	1,902.00	1,712.333
P248	158.82±11.67	11.13±0.48	0.22±0.008	0.062±0.002	1,572.33	1,136.000
P141	158.97±16.43	12.88±0.14	0.26±0.028	0.085±0.009	1,533.66	1,029.667
P129	216.57±12.43	16.65±0.72	0.24±0.004	0.102±0.003	1,675.33	1,648.667
P151	135.50±11.62	11.19±0.79	0.26±0.007	0.074±0.005	1,410.00	957.000
P201	204.46±26.30	16.36±1.58	0.25±0.022	0.106±0.013	1,748.00	1,932.000
P143	254.28±32.11	16.61±1.48	0.21±0.010	0.087±0.005	2,004.66	1,818.000
P85	185.01±21.48	13.96±1.61	0.24±0.012	0.084±0.011	1,718.66	1,345.000
P179	194.61±34.79	14.24±3.01	0.23±0.008	0.083±0.022	1,841.00	1,783.000
P247	208.90±29.28	13.97±1.64	0.21±0.012	0.076±0.011	2,586.66	1,356.333
P124	232.86±18.33	17.81±1.85	0.24±0.008	0.109±0.014	2,182.66	1,805.000
P233	326.65±55.33	21.76±4.55	0.21±0.011	0.116±0.030	3,448.00	2,579.000
P6	204.95±33.36	14.04±1.90	0.22±0.020	0.078±0.014	2,539.33	2,761.333
P167	234.78±6.91	15.23±1.07	0.20±0.009	0.079±0.009	2,242.66	1,480.333

*values after ± represents standard deviation of mean of three replications **Treatment = Seed treatment with isolates of Fluorescent *pseudomonas*.

Table 2: Efficacy of Fluorescent *pseudomonas* isolates for plant growth promoting activity in pigeonpea

Treatment	Avg. shoot length (cm)	Avg. root length (cm)	Avg. shoot fresh weight (g)	Avg. shoot dry weight (g)	Avg. root fresh weight (g)	Avg. root dry weight (g)	Nodulation
Control	14	19.4	0.38	0.162	0.168	0.027	11
P5	17	22.7	0.39	0.164	0.132	0.035	14.2
P11	17.2	20.6	0.42	0.188	0.212	0.036	14.8
P67	12	17	0.336	0.172	0.213	0.033	17.3
P99	16.2	21.4	0.35	0.166	0.232	0.03	10.6
P126	14.8	21	0.38	0.142	0.204	0.023	11.8
P72	17	21.6	0.31	0.154	0.104	0.023	13.2
P216	12	19.8	0.37	0.108	0.165	0.026	6
P205	14.4	15.4	0.284	0.132	0.1554	0.027	9.6
P161	13.4	20.8	0.34	0.14	0.172	0.027	11
P176	13.6	22.5	0.292	0.154	0.093	0.028	8.6
P76	12.8	17	0.37	0.148	0.117	0.023	10.8
P248	12.2	20.2	0.29	0.138	0.174	0.035	10.2
P141	14.4	20.8	0.33	0.162	0.163	0.036	13.8
P129	13.6	22.4	0.34	0.144	0.131	0.032	11.2
P151	13.8	22.2	0.35	0.156	0.169	0.032	10
P201	15.6	20.8	0.33	0.172	0.196	0.033	12.8
P143	13	22	0.3	0.144	0.274	0.04	11.4
P85	11.4	20.6	0.32	0.118	0.226	0.028	9.2
P179	13	21	0.34	0.148	0.178	0.028	12
P247	11.8	21.6	0.31	0.148	0.197	0.031	13
P124	16.6	21	0.38	0.176	0.267	0.038	19
P233	17.8	24.4	0.39	0.171	0.273	0.027	20.6
P6	12.8	22	0.37	0.132	0.128	0.03	9.4
P167	13.8	19.6	0.3	0.162	0.195	0.037	9.6

* Each set of data are average of five replications

Table 3: Isolates of Fluorescent *pseudomonas* with highest PGP activity on pigeonpea

Avg. shoot length (cm)	Avg. root length (cm)	Avg. shoot fresh weight (g)	Avg. shoot dry weight (g)	Avg. root fresh weight (g)	Avg. root dry weight (g)	Nodulation
P233	P233	P11	P11	P143	P143	P233
P11	P5	P5, P233	P124	P233	P167	P124

15 days old pigeonpea plants were measured for root length, shoot length, fresh weight of shoot, dry weight of shoot, fresh weight of root, dry weight of root and nodulation.

Efficacy of different isolates of *Pseudomonas* for pigeonpea plants varied to induce shoot and root length ranging from 11.4 cm to 17.8 cm and 15.4

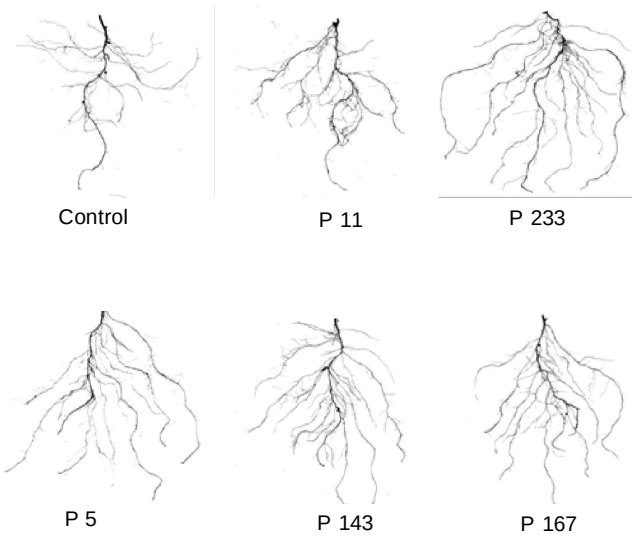

Fig. 1 (A): Root scanning to determine differences in different root parameters in untreated control and between the roots derived from pigeonpea plants developed after seed treatment with different isolates of Fluorescent *pseudomonas*

cm to 24.4 cm, respectively. Maximum shoot length (17.8 cm) and root length (24.4 cm) were recorded when seeds were treated with P233 as compared to control. Isolates P11 showed highest average fresh shoot weight (0.42g) and dry shoot weight (0.188g). While isolate P143 performed with highest average root fresh weight (0.274g) and dry root weight (0.04g). Plants treated with P233 produced maximum average number of nodulation (20.6) as compared to control (Table 3).

Some landmarks along the way include the discoveries that these bacteria frequently increase plant growth, productivity and disease control properties in different crop plants (Dileep Kumar and Bezbaruah, 1997). Dwivedi and Johri (2003) rec-

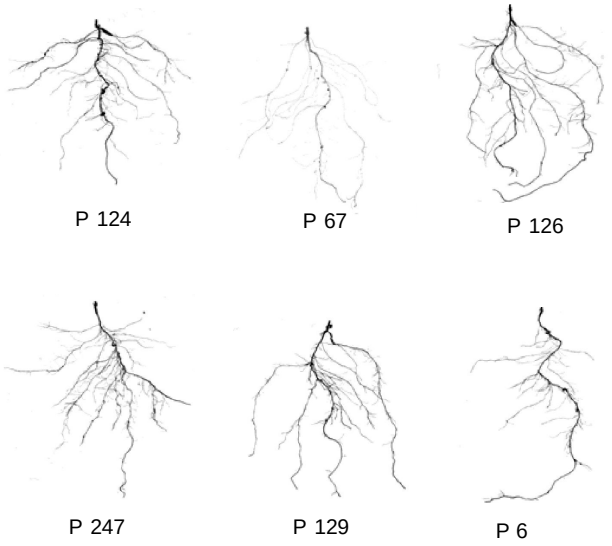

Fig. 1 (B): Root scanning to determine differences in different root parameters in untreated control and between the roots derived from pigeonpea plants developed after seed treatment with different isolates of Fluorescent *pseudomonas*

ognized that secondary metabolites produced with antibiotic activity in different strains of Fluorescent pseudomonads have a major factor in suppression of root pathogens. Pigeonpea seeds treated with Fluorescent pseudomonas (@10 g/kg seed) significantly increase the plant height, fresh weight and dry weight with reduced wilt incidence and increased crop yield (Telangre *et al*. 2013). Rani *et al.* (2012) isolated several strain of Fluorescens pseudomonas and characterized the PGPR from the rhizosphere soil of pigeonpea and found significantly enhanced growth and crop yield. The PGPR are known to participate in many important ecosystem processes, such as the biological control of plant pathogens, nutrient cycling, nitrogen fixation and/or seedling growth (Persello-Cartieaux *et al.* 2003).

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