Differential response of sesame under influence of indigenous and non-indigenous rhizosphere competent fluorescent pseudomonads

Fluorescent pseudomonads are known to produce a wide variety of secondary metabolites such as fluorescent pigments[1], siderophore[2], antibiotics[3], enzymes[4], phytohormones[5], 1-aminocyclopropane-3-carboxylic acid (ACC) deaminase[6], cyanide[7] and solubilize some minerals[8] that directly or indirectly[9] influence plant growth. These pseudomonads are a heterogenous group of bacteria almost aggressively colonizing a plethora of legumes and non-legumes[10,11]. The ability to colonize a particular root system depends on a host of biotic and abiotic factors which include root exudates, seed leechates and ability of these groups of bacteria to be attracted towards them. These are also reported to be endophytic in nature promoting the overall growth of the plant. Plant pathogenic pseudomonads do exist but this group is basically an enigma found in every rhizosphere. Still the ability to survive in a rhizosphere varies greatly within species in the genus. The present study was undertaken with an objective to evaluate the ability of *Pseudomonas aeruginosa* strains originally isolated from rhizosphere of various unrelated crops, to colonize sesame rhizosphere and provide benefits to sesame growth. Plant growth promoting strains of *P. aeruginosa* GRC1$^{rif+ tet+}$ (ref. 12), an isolate of potato rhizosphere known to aggressively colonize *Brassica campestris*[13] and *Arachis hypogea*[14]; *P. aeruginosa* PS2^{str+} (ref. 15), an isolate from groundnut rhizosphere; *P. aeruginosa* PSII^{neo+} (ref. 16), an isolate from sunflower rhizosphere; *P. aeruginosa* LES4^{tet+} (ref. 17), an isolate from tomato rhizosphere; *P. aeruginosa* PRS4^{gen+} an isolate from velvet bean rhizosphere were compared with *P. aeruginosa* PSI5$^{azio+ kan+}$, an isolate from sesame rhizosphere, for root colonization and early growth parameters of sesame. The antibiotic resistance property of these strains was utilized for determination of rhizospheric competence, and it is denoted as superscript plus sign, with antibiotic initials. Plant growth promoting activities of PSI5 were evaluated and quantified[18] whereas all other pseudomonads have been screened for their plant growth promoting activities earlier[12–17].

Sesame seeds bacterized[19] with individual exponential phase washed cells of different pseudomonads in seven treatments with a separate control were sown in field experiments, conducted in Haridwar, Uttarakhand, India during July–December 2007 and 2008. Bacterized and non-bacterized seeds were sown in sandy loam soil (71% sand, 15% silt and 14% clay, 0.035% total organic matter, pH 7.4, water holding capacity 35%). A uniform plant population was maintained with an intra-row spacing of 15 cm with triplicate arrangement in a randomized block design (RBD). Field was normally irrigated as and when required without adding any fertilizer. Root colonization in different treatments was determined using standard assay. Plants were uprooted and 1 g of root adhering soil particles was serially diluted in sterile normal saline. Suitable dilution of suspensions was spotted 10 µl each in triplicate on nutrient agar media (NAM) plates amended with respective antibiotics. After incubation (24 h, 28 ± 1°C), total population of pseudomonads was enumerated. NAM plates without any antibiotic were used to determine the total indigenous aerobic culturable soil bacteria (IAB). Vegetative parameters such as germination percentage (15 DAS (days after sowing)), seedling fresh weight (SFW), seeding dry weight (SDW), shoot length, root length and seed yield per plant (120 DAS) were recorded at the time of harvesting. Data were analysed statistically using analysis of variance (ANOVA) and least significant difference (LSD).

P. aeruginosa PSI5$^{azio+ kan+}$ enhanced SFW and SDW, shoot and root length as compared to control and other treatments but was statistically non-significant over non-indigenous pseudomonads. Ability of all pseudomonads to increase growth can be attributed to their multiple plant growth promoting activities (Table 1). Germination across all treatments varied and was maximum in treatment VI (*P. aeruginosa* PSI5$^{azio+ kan+}$). SFW enhanced by *P. aeruginosa* PSI5$^{azio+ kan+}$ was statistically similar to *P. aeruginosa* LES4^{tet+} (ref. 19) with 116% and 115% increment over control. Such a strong increase might be correlated with production of indole-3-acetic acid (IAA) and ACC deaminase. Enhanced germination thus achieved confers considerable benefits on plant growth which are carried over to the total yield of fully mature plants as in our case. Root and shoot lengths increased by 28% and 31% as compared to control by the use of indigenous *Pseudomonas* while among non-indigenous pseudomonads, maximum increase in root and shoot lengths was 27% and 28% which was statistically significant over control but comparable to indigenous strain *P. aeruginosa* PSI5$^{azio+ kan+}$. Seed yield per plant showed an overall increase of 80% over control when allochthonous *Pseudomonas* was used while among autochthonous pseudomonads, maximum increase over control was by treatment I (*P. aeruginosa* GRC$_1^{rif+ tet+}$) with a statistical significant increase of 62%. Total yield of sesame increased by 11% in treatment VI (*P. aeruginosa* PSI5$^{azio+ kan+}$) over treatment I (*P. aeruginosa* GRC1$^{rif+ tet+}$) followed by other pseudomonads.

The results of root colonization clearly present the difference in using indigenous and non-indigenous strains, as in both years the average population of *P. aeruginosa* PSI5$^{azio+ kan+}$ was higher compared to the treatment by other isolates. Further, a population homoeostasis was recorded for IAB in all treatments. This reflects that the treatment of pseudomonads did not have any inhibitory effect on the population of indigenous bacteria in sesame rhizosphere. Increased colonization of plant growth promoting rhizobacteria (PGPR) resulted in enhanced plant growth and the same was observed in our study, which is in accordance with earlier reports[10,11]. Recently, a mixture of microbes or a consortia[20] has been advocated for engineering rhizosphere for increased plant growth and disease control[21]. Here, the results indicate that a crop is influenced by use of single inoculants and there can be marked variation in growth and yield of a

Table 1. Plant growth promoting attributes of fluorescent pseudomonads and their effect on plant growth parameters of sesame

Treatments		PGP attributes					Antagonism		Plant growth parameters					
Isolate	Rhizospheric origin	IAA (μg/ml)	ACC deaminase	P (μg of P/ml)	S (U/ml/h)	HCN (OD at 625 nm)	1	2	G (%)	RDW (g)	SDW (g)	RL (cm)	SL (cm)	G/P
P. aeruginosa GRC1$^{rif+ tet+}$	Potato	+ (31)	–	+ (67)	+ (29)	–	+ (55)	–	78.8**	16.2**	43.2**	26.2*	170.3**	42.6**
P. aeruginosa PS2^{str+}	Groundnut	+ (36)	–	+ (55)	+ (17)	+ (0.09)	+ (61)	–	76.3**	13.5**	40.7**	24.7*	168.9**	38.8**
P. aeruginosa PSII^{neo+}	Sunflower	+ (30)	–	+ (71)	+ (12)	+ (0.05)	–	–	77.9**	14.1**	42.5**	25.6*	173.8**	39.2**
P. aeruginosa LES4^{tet+}	Tomato	+ (42)	–	+ (75)	+ (22)	–	+ (72)	+ (68)	80.3**	14.7**	44.3**	26.2*	180.7**	41.3**
P. aeruginosa PRS4^{gen+}	Velvet bean	+ (40)	–	+ (68)	+ (15)	–	+ (78)	–	79.1**	12.4**	39.8**	22.8*	165.3**	40.8**
P. aeruginosa PSI5$^{azi+ kan+}$	Sesame	+ (41)	+	+ (76)	+ (19)	–	+ (70)	+ (63.3)	83.5**	17.3**	45.6**	27.1*	185.9**	47.3**
Control		na	na	na	na	na	na	na	61	10.1**	18.6**	21.6	141.3	26.3

PGP attributes are mean of three independent experiments. Field data is a mean of two year trials. Values are mean of 15 randomly selected plants. *Significant at $P > 0.01$ level of ANOVA; **Significant at 0.01 level of LSD compared to control. G, Germination; RDW, Root dry weight; SDW, Seedling dry weight; RL, Root length; SL, Shoot length; G/P, Seed yield per plant; +, Attribute positive; –, Attribute negative; IAA, Indoleacetic acid; P, Phosphate solubilization; Sid, Siderophore; ACC, 1-aminocyclopropane-3-carboxylic acid; 1, *Macrophomina phaseolina*; 2, *Fusarium oxysporum*; %, Pathogen inhibition percentage (control – treatment/control × 100).

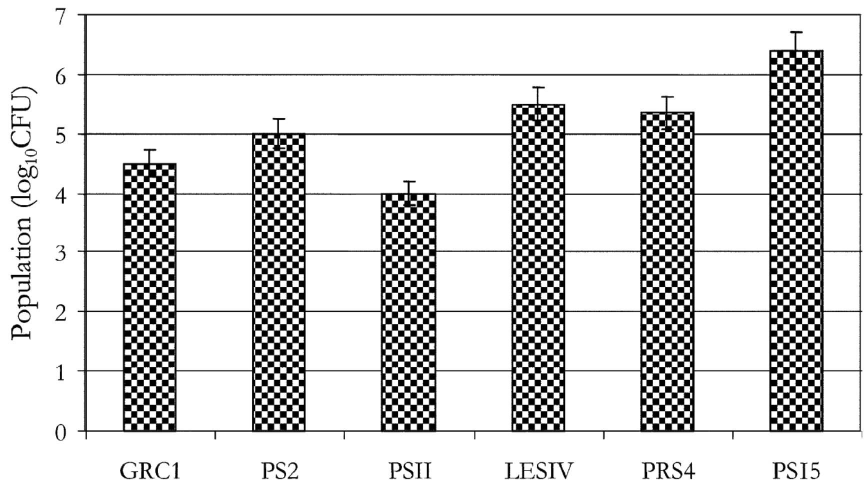

Figure 1. Root colonizing ability of various isolates in sesame rhizosphere. Bars represent standard error of mean.

crop when local rhizosphere is the source of the beneficial strain over a non-local strain. Effective root colonization is a prerequisite attribute needed for the success of PGPR in plant growth and yield promotion as achieved in our study. Thus the successful colonization by fluorescent pseudomonads in sesame rhizosphere promotes growth and also proves the efficacy of indigenous microflora over non-indigenous microflora. These isolates may be applied as bioinoculants for sustainable and environment friendly cultivation of sesame.

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ABHINAV AERON[1]
PIYUSH PANDEY[2]
D. K. MAHESHWARI[1,*]

[1]*Department of Botany and Microbiology, Gurukul Kangri University, Hardwar 249 404, India*
[2]*Department of Microbiology, S.B.S.P.G. Institute of Biomedical Sciences and Research, Balawala, Dehradun 248 161, India*
**For correspondence.*
e-mail: maheshwaridk@gmail.com