

USE OF *PSEUDOMONAS AERUGINOSA* WITH RHIZOBIA IN THE CONTROL OF ROOT ROT DISEASE OF MASHBEAN (*VIGNA MUNGO* (L.) HEPPER)

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Abstract

Use of *Pseudomonas aeruginosa* with or without *Badyrhizobium* sp., significantly reduced *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani* infection on mashbean (*Vigna mungo* (L.) Hepper) under green house conditions. *P. aeruginosa* also enhanced plant growth and showed greater nodulation in mashbean plants.

Introduction

Mashbean (*Vigna mungo* (L.) Hepper) is an important pulse crop cultivated over an area of 57,400 ha., producing an yield of 2,84,000 tons in Pakistan (Anon., 1997). Of the various disease causing organisms, the root knot nematode (*Meloidogyne* spp.) and root infecting fungi viz., *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Kühn and *Fusarium* spp., produce serious losses in mashbean crop (Ehteshamul-Haque, 1994; Ghaffar, 1995). Several genera of bacteria have the ability of promoting plant growth and are termed as PGPR, the Plant Growth Promoting Rhizobacteria (Kloepper *et al.*, 1989). Similarly many strains of rhizobacteria specially the fluorescent Pseudomonads and Bacilli are reported to suppress disease and enhance plant-growth and yield when applied as seed inoculants (Podile & Prakash, 1996; Buysens *et al.*, 1996). Of the *Pseudomonas* species, *P. aeruginosa*, a plant growth promoting rhizobacterium (Hofte *et al.*, 1991) have been found as an effective biocontrol agent of root rot pathogens (Izhar *et al.*, 1995). Similarly rhizobia, the root nodulating bacteria, are also known to control soilborne root infecting fungi (Ehteshamul-Haque & Ghaffar, 1993; Siddiqui *et al.*, 1998). Experiments were therefore carried out to evaluate the potential of co-inoculation of *Pseudomonas aeruginosa* (Shroeter) Migula with Bradyrhizobia in the control of root rot disease caused by *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani* on mashbean.

Materials and Methods

Five day old cultures of *P. aeruginosa* maintained on King's B medium and Bradyrhizobia multiplied on Yeast Extract Mannitol Agar medium were used. Seeds of mashbean after surface sterilization with 1% $\text{Ca}(\text{OCl})_2$ for three minutes were rinsed thoroughly with sterile distilled water. Surface disinfested seeds were treated with cultures of *Bradyrhizobium* sp., Karachi University Culture Collection - 823 mungbean isolate (cfu $3.8 \times 10^9 \text{ ml}^{-1}$), KUCC-819 mashbean isolate (cfu $2.9 \times 10^9 \text{ ml}^{-1}$) and *Bradyr-*

Table 1. Use of *Pseudomonas aeruginosa* with or without rhizobia in the control of soilborne root infecting fungi on mashbean.

Treatments	<i>M. phaseolina</i>	<i>F. solania</i> Infection %	<i>R. solani</i>
Control	72.91	58.33	69.75
<i>P. aeruginosa</i> (Pa-3)	24.99	16.66	45.83
<i>P. aeruginosa</i> (Pa-5)	35.41	8.33	70.83
<i>P. aeruginosa</i> (Pa-7)	25.00	0.00	31.25
<i>Bradyrhizobium</i> sp., (KUCC-823)	37.50	14.58	50.00
<i>Bradyrhizobium</i> sp., (KUCC-819)	35.41	20.83	49.99
<i>B. japonicum</i>	25.00	0.00	62.50
Pa-3 + KUCC-823	27.00	0.00	52.08
Pa-3 + KUCC-819	22.91	6.25	58.33
Pa-3 + KUCC-569	25.00	6.25	60.41
Pa-5 + KUCC-823	31.25	0.00	68.75
pa-5 + KUCC-819	25.00	0.00	68.75
Pa-5 + KUCC-569	0.00	14.58	45.83
Pa-7 + KUCC-823	0.00	6.25	45.83
Pa-7 + KUCC-819	18.75	12.50	50.00
Pa-7 + KUCC-569	6.25	18.75	50.00
L.S.D. <0.05 = 7.0	Treatment = 16.30,		Pathogen

hizobium japonicum KUCC-569 soybean isolate (cfu 2.5×10^9 ml⁻¹) using 1% gum arabic as sticker. After treatment each seed was found to contain KUCC-823, cfu 2.0×10^6 ; KUCC-819, cfu 2.2×10^6 and KUCC-569, cfu 2.2×10^6 . Six seeds were sown in 8cm diam., plastic pot each containing 350 gm sandy loam soil, pH 8.1. Before sowing the seeds, water cell suspension of *P. aeruginosa* strains Pa-3 (cfu 3.5×10^8 ml⁻¹), Pa-5 (cfu 3.3×10^8 ml⁻¹) and Pa-7 (cfu 1.5×10^8 ml⁻¹) were drenched in each pot @ 25 ml / pot. A set of pots inoculated with different strains of *P. aeruginosa* and or rhizobia were also kept for comparison. Pots without bacterial inoculum served as control. The soil had a natural infestation of 4-11 sclerotia of *M. phaseolina* g⁻¹ of soil as determined by wet seiving and dilution technique (Sheikh & Ghaffar, 1975), 7.5% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and 2500 cfu g⁻¹ of soil of a mixed population of *Fusarium* spp., as assessed by soil dilution technique (Nash & Snyder, 1962). There were 4 replicates of each treatment and the pots were randomized on a screen house bench of Soilborne Disease Research Laboratory, Department of Botany, University of Karachi where soil was kept at 50% W.H.C. (Keen & Raczowski, 1921). After germination only 4 seedlings were kept in each pot.

Plants were uprooted after 6 week growth and plant growth parameters such as plant height, root length, fresh weight of shoot and root and number of nodules per plant were recorded. To determine the incidence of fungi, roots were surface sterilized with 1% $\text{Ca}(\text{OCl})_2$ and after washing in running tap water, 5 one cm long root pieces from each plant were plated onto PDA plates containing penicillin (100,000 units/L.) and streptomycin (0.2g / L.). After incubation for 5 days at 28°C incidence of root infecting fungi were recorded. Data were analysed and subjected to Factorial ANOVA (FANOVA) followed by Least Significance Differences (LSD) according to Gomez & Gomez (1984).

Results

Combined use of *P. aeruginosa* strain Pa-5 with *B. japonicum* and strain Pa-7 with *Bradyrhizobium* sp., KUCC-823 showed complete control of *M. phaseolina* infection. Root infection by *F. solani* was completely prevented by strain Pa-7 or *B. japonicum* used alone or strain Pa-3 used with KUCC-823 or strain Pa-5 used with KUCC-823 or KUCC-819. Infection of *R. solani* was significantly controlled where *P. aeruginosa* strain Pa-3 or Pa-5 and *Bradyrhizobium* sp., KUCC-823 or KUC-819 were used separately, strain Pa-3 used with KUCC-823, strain Pa-5 used with *B. japonicum* and Pa-5 used with either *Bradyrhizobium* sp., or *B. japonicum*. *P. aeruginosa* enhanced biocontrol effects of rhizobia in the control of *M. phaseolina*, *F. solani* and *R. solani* infection (Table 1).

Use of *P. aeruginosa* alone or in-combination with rhizobia increased all the growth parameters. Maximum plant height was observed in treatment where strain Pa-3 was used with KUCC-823. *P. aeruginosa* strain (Pa-7) used alone showed highest fresh weight of shoot. Strain Pa-5 used alone exhibited greater root length whereas strain Pa-7 showed highest fresh weight of root. Strain Pa-3 used in-combination with KUCC-819 showed maximum number of nodules per plant. *P. aeruginosa* used alone showed better nodulation as compared to its use with Bradyrhizobia (Table 2).

Discussion

Microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents since rhizosphere provides front line defence for roots against attack by pathogens (Weller, 1998). In the present study, use of *P. aeruginosa* (PGPR) with or without rhizobia significantly controlled root infecting fungi viz., *M. phaseolina*, *F. solani* and *R. solani* on mashbean. *P. aeruginosa* has been reported to reduce growth of *M. phaseolina*, *R. solani*, *Sclerotium rolfsii* and *F. solani* (Podile *et al.*, 1988; Izhar *et al.*, 1995). Antifungal activity of *P. aeruginosa* is attributed to the production of certain antibiotics (Levy *et al.*, 1992) and siderophores (De Mayer *et al.*, 1997; Buysens *et al.*, 1996). Similarly use of rhizobia for the control of root infecting fungi on okra, sunflower, soybean and mungbean has also been reported (Ehteshamul-Haque & Ghaffar, 1993). Rhizobia besides fixation of atmospheric nitrogen are also known to produce toxic metabolites (Chakraborty & Purkayastha, 1984) which have inhibitory effect on soil borne pathogens.

Table 2. Use of *Pseudomonas aeruginosa* with or without rhizobia on growth of mashbean (Values in parenthesis represent a percentage (+ve) increase or (-ve) decrease over control.)

Treatments	Plant height (cm)	Shoot weight (gm)	Root length (cm)	Root weight (gm)	No. of nodules per plant
Control	13.99	0.52	14.04	0.35	9.41
<i>P. aeruginosa</i> (Pa-3)	18.76 (+34.09)	0.85 (+63.46)	22.08 (+57.26)	0.62(+77.14)	16.12(+71.30)
<i>P. aeruginosa</i> (Pa-5)	18.31 (+30.87)	0.74 (+42.30)	21.68 (+54.41)	0.60 (+71.42)	23.37 (+148.35)
<i>P. aeruginosa</i> (Pa-7)	19.62 (+40.24)	0.89 (+71.15)	19.95 (+42.09)	0.65 (+85.71)	19.47 (+106.09)
<i>Bradyrhizobium</i> sp., (KUCC-823)	18.62 (+33.09)	0.73 (+40.38)	19.00 (+35.32)	0.47 (+34.28)	15.37 (+63.33)
<i>Bradyrhizobium</i> sp., (KUCC-819)	18.81 (+34.45)	0.83 (+59.61)	19.18 (+36.60)	0.41 (+17.41)	21.75 (+131.13)
<i>B. japonicum</i> (KUCC-569)	19.37 (+38.45)	0.88 (+69.23)	16.75 (+19.30)	0.47 (+34.28)	17.50 (+85.97)
Pa-3 + KUCC-823	19.93 (+42.45)	0.78 (+50.00)	17.00 (+21.08)	0.50 (+42.85)	14.93 (+58.66)
Pa-3 + KUCC-819	18.93 (+35.31)	0.88 (+69.23)	16.37 (+16.59)	0.47 (+34.28)	22.43 (+138.36)
Pa-3 + KUCC-569	18.93 (+35.31)	0.86 (+65.31)	16.43 (+17.02)	0.48 (+37.14)	15.43 (+63.97)
Pa-5 + KUCC-823	18.75 (+34.02)	0.78 (+50.00)	15.31 (+9.04)	0.37 (+05.71)	12.87 (+36.76)
Pa-5 + KUCC-819	18.12 (+29.52)	0.85 (+63.46)	17.04 (+21.36)	0.42 (+20.00)	20.08 (+113.39)
Pa-5 + KUCC-569	17.85 (+27.59)	0.79 (+51.92)	14.31 (+01.92)	0.43 (+22.85)	13.04 (+38.57)
Pa-7 + KUCC-823	16.81 (+20.15)	0.58 (+11.53)	15.25 (+08.61)	0.43 (+22.85)	09.68 (+02.84)
Pa-7 + KUCC-819	15.97 (+14.15)	0.66 (+26.92)	16.16 (+15.09)	0.54 (+54.28)	14.10 (+49.84)
Pa-7 + KUCC-569	18.87 (+34.88)	0.72 (+38.46)	15.25 (+08.61)	0.46 (+31.42)	09.31 (-01.06)
L.S.D. p<0.05	2.0	0.18	3.7	0.14	5.87

In the present study PGPR and rhizobia used alone or in-combination showed better plant growth as compared to untreated control. Both PGPR and rhizobia besides production of antimicrobial compounds are also known to release plant growth regulators (Hussain & Vancura, 1970; Sheng, 1993; Evensen & Blavins, 1984) which play an important role in plant growth enhancement. In the present study use of PGPR showed similar effects on plant growth as that of rhizobia. PGPR showed similar nodulation as that caused by rhizobia. It is interesting to note that a combined use of *P. aeruginosa* with Bradyrhizobia showed greater nodulation on chickpea as compared to their separate use (Izhar *et al.*, 1995). Presumably inoculated strains of rhizobia were less rhizospheric competent in the presence of PGPR as compared to indigenous rhizobia and their population might have declined soon after inoculation thus producing lesser number of nodules on roots. Increased number of nodules in PGPR inoculated plants may presumably be due to the presence of *P. aeruginosa* in the rhizosphere and its toxin may increase the susceptibility of roots to rhizobial infection (Bolton *et al.*, 1990). Indigenous rhizobia remain unaffected as also *Agrobacterium tumefaciens*, a close relative of rhizobia with better results where more than one biocontrol agent were used (Jordan *et al.*, 1984). Successful nodulation and nitrogen fixation by legumes inoculated with rhizobia also depends on strain's competitiveness, its ability to survive in soil as well as its compatibility with the host (Ham, 1980; Dowling & Broughton, 1986).

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