Indian J. Agric. Res., 35(1): 31-35, 2001

RHIZOBACTERIA FROM FIELD GROWN MUNGBEAN : PLANT GROWTH PROMOTING POTENTIAL

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ABSTRACT

Rhizobacteria isolated from mungbean roots were screened for their plant growth promotory effects on mungbean under aseptic pot culture conditions. The effect varied from stunted growth to elongation of root and shoot to neutral response. Out of 121 isolates tested, application of 16 isolates resulted in better growth of mungbean plants throughout the 30 days experiment, causing an improvement of plant growth by 62% to 106% relative to the control (judged from dry mass). Of these, 3 were classified as *Pseudomonas* sp., 9 as *Bacillus* sp. and 4 as *Enterobacter* sp.

INTRODUCTION

Currently research is being focussed on to altering the native microflora of plant roots to achieve either increased plant growth (Suslow and Schroth, 1982) or biological control of soil borne diseases (Kloepper *et al.*, 1980 a; Weller and Cook, 1983). When proper bacterial strain is used, plant roots are extensively colonized by the introduced strain, which suggests a close bacteria-plant association that allows for beneficial plant growth or disease protection. Such bacterial inoculants are known as plant growth promoting rhizobacteria (PGPR). Many rhizobacteria, especially strains of fluorescent pseudomonads and bacilli isolated from root surfaces or rhizosphere soils are reported to enhance plant growth/yield and simultaneously suppress disease when applied as seed inoculants (Dileep *et al.*, 1998).

We isolated a number of rhizobacteria from rhizotic zones of mungbean and documented the dominant bacterial classes in the rhizosphere of mungbean. In the present study, we examine the efficacy of these rhizobacterial isolates to promote plant growth in mungbean (*Vigna radiata* L. Wiltzek).

MATERIAL AND METHODS

Rhizobacteria which were isolated from rhizosphere, rhizoplane and endorhizosphere of field - grown mungbean were tested on mungbean cv. Pusa 105 for their effect on plant growth under sterile growth room conditions. Soil and sand used for experiment were sterilized by autoclaving for 4h each day, for three consecutive days. The plastic cups were sterilized by swabbing with 70% ethanol. Seeds were surface sterilized with 1% chloramine - T solution for 5 min and then washed repeatedly with six changes of sterile water. Surface sterilized seeds were then imbibed in sterile water for 4 h. Four seeds were sown in sterilized plastic cups filled with 200 g of sterile soil-sand mixture (4:1).

Each of the rhizobacterial isolate was grown to log-phase in nutrient broth and

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optical density was adjusted to 10^8 cells/. ml. One ml of each isolate was added over the sown seeds using sterilized pipettes and covered with soil-sand mixture. Treatments were replicated four times and two plants per cup were maintained. The cups were arranged in a completely randomized block design. Uninoculated controls were maintained.

The plants were watered regularly with sterilized water and harvested after 35 days of sowing. Root and shoot lengths and their fresh weight were recorded. The dry weights of shoot and root were recorded after drying the samples in an oven at 80°C till constant weights were obtained.

The data were statistically analyzed by doing the analysis of variance (ANOVA) for individual characters on the basis of mean values (Panse and Sukhatme, 1961).

RESULTS AND DISCUSSION

All the rhizobacterial isolates were screened for their effect on root and shoot growth of mungbean cv. Pusa 105 under sterile pot culture conditions. The effect was noted 30 days after sowing. The effect varied from stunted growth to elongation of root and shoot to neutral response.

Out of 121 isolates tested, 74 isolates increased the root length, 2 isolates showed neutral response whereas others showed root length less than control. The effect on root elongation was significant with three of the isolates after 30 days of growth and the per cent increase in root length over control was more than 50% (Table 1). Such enhancing effect was observed in case of isolates X3, NG-ER-6 and EG-RS-5.

The effect on shoot growth was variable. Out of 121 isolates, 105 increased the shoot length whereas in other cases, the shoot length was less than control (Table 1). Isolates W5, X6, Y4, Y9, NG-ER-7, EB-RS-3, EB-RS-4, EB-RS-7, EB-RS-8, EB-RS-10, EG-RS-2, PF I, PF II and PF VI showed significant effect on shoot length after 30 days of growth; per cent increase in shoot length over control was 20% or more.

Similar effects were observed on fresh and dry root and shoot weight. Isolates D4, D7, D8, NG-ER-4, NG-ER-9, EG-RS-5 and EB-RS-8 caused an increase of 50% or more in fresh root weight over control whereas isolates A5, B4, D4, D8, Y4, Y14, EB-RS-3, EG-RS-3, EG-RS-4, EG-RS-5 and EB-RS-10 resulted in 50% or more increase in dry root weight as compared with the controls (Table 1).

Table 1. Effect of inoculation of rhizobacterial isolates on plant growth of mungbean under sterile soil conditions (Average of 12 replications ; n = 121)

Per cent decrease/ increase over control (%)	Root length	Shoot length	Fresh root wt.	Dry root wt.	Fresh shoot wt.	Dry shoot wt.
< - 40	0*	0	3	1	1	0
-40 to -30	0	0	2	1	4	0
-30 to -20	0	1	3	3	4	0
-20 to -10	9	3	7	9	12	0
-10 to 0	36	12	18	20	53	0
0	2	0	3	8	0	0
0 to 10	33	47	24	23	27	11
10 to 20	17	45	18	22	13	15
20 to 30	8	12	10	14	4	24
30 to 40	9	1	16	5	2	21
40 to 50	4	0	10	7	0	8
50 to 60	1	0	4	4	0	26
>60	2	0	3	4	1	16

n = Total number of rhizobacterial isolates

* = Number of bacterial isolates responding to a particular parameter.

Table 2. Details of selected rhizobacteria which increased biomass of mungbean by more than 60%

Bacteria	Isolate No.	Dry wt. (mg)	Increase in dry wt. (%)
Bacillus sp.	X4	251 (96)[a]	62
Bacillus sp	Y2	283 (128)	82
Bacillus sp.	Z2	253 (98)	63
Bacillus sp	NG-ER-5	282 (127)	82
Bacillus sp.	NG-ER-7	275 (120)	77
Enterobacter sp.	EB-RS-1	320 (165)	106
Enterobacter sp.	EB-RS-4	318 (163)	105
Bacillus sp.	EB-RS-2	268 (113)	73
Bacillus sp.	EG-RS-3	251 (96)	62
Bacillus sp.	EG-RS-4	295 (140)	90
Bacillus sp.	EG-RS-5	265 (110)	71
Enterobacter sp.	EG-ER-2	255 (100)	65
Enterobacter sp.	KG-ER-1	255 (100)	65
Pseudomonas sp.	PF I	290 (135)	87
Pseudomonas sp.	PF II	280 (125)	81
Pseudomonas sp.	PF IV	295 (140)	90
Uninoculated control		155	
LSD (p=0.01)		20.93	

a= Values in parentheses are the difference between the men of treatments and the mean of control.

Isolate A9 caused a 268% increase in fresh shoot weight over control. However, the increase in dry shoot weight was only 16% over the control. The fresh weight of mungbean shoots treated with isolates NG-ER-5, NG-ER-7, EB-RS-4 and PF V was higher by 25% or more than that of control plants; the corresponding increase in dry shoot weight over control was 82%, 77%, 105% and 58%, respectively. Three isolates EG-RS-2, EG-RS-4 and PF I showed 20% or more increase in fresh shoot weight over the control with corresponding increase in dry shoot weight as 73%, 90% and 87%, respectively (Table 2).

Sixteen isolates viz., X4, Y2, Z2, EB-RS-1, EB-RS-4, NG-ER-5, NG-ER-7, EG-ER-2, KG-ER-1, EG-RS-2, EG-RS-3, EG-RS-4, EG-RS-5, PF I, PF II and PF IV gave highest biomass production (Table 2). The percentage increase obtained in the biomass of mungbean with these isolates when compared to uninoculated control was more than 60%.

Rhizobacteria which caused inhibition of plant growth and showed lesser root and shoot fresh and dry weights than corresponding uninoculated controls were considered as deleterious rhizobacteria (DRB). The observations of this study are consistent with those of Alstrom (1987) who reported that two rhizosphere fluorescent pseudomonads S596 and S628, suppressed growth in young plants of *Phaseolus vulgaris* (cv. Bonita) and *Lactuca sativa* (cv. Montana) under non-sterile and sterile conditions, respectively. The reason for phytotoxic reaction in the present study could be due to liberation of potentially toxic volatiles such as hydrogen cyanide (HCN), ethylene, ammonia, etc. However, Suslow (1982) speculated that "rhizosphere bacteria that may be deleterious under axenic conditions may actually be beneficial

to plant growth in natural systems". Thus the effect, either inhibitory or promotory may not always be absolute but is dependent on growing conditions and plant species.

Out of the 121 rhizobacterial isolates tested, 105 increased the shoot length whereas only 74 increased the root length over uninoculated control. Savithiry and Gnanamanickam (1987) also observed that bacterized plants were 25.74% taller and appeared greener than the non-bacterized plants. Vrany and Fiker (1984) also reported that inoculation of potato tubers with isolates of *Pseudomonas fluorescens - putida* bacteria, caused a 111% increase in stem length and 122% increase in plant mass. The growth response of roots was even more intensive, reaching 166% in contrast to our observations where shoot growth promotion was more intensive than that of roots. The root and shoot elongation could be ascribed to production of auxins (IAA) and gibberellins, respectively. Studies with strains of *Pseudomonas fluorescens* producing high or low amount of IAA have indicated such effects (Scott, 1972; Loper and Schroth, 1986 ; Dubeikovsky *et al.*, 1993).

Rhizobacterial isolates mostly belonging to the genera *Pseudomonas, Bacillus* and *Enterobacter*, were found to increase fresh and dry root weight by over 50% over uninoculated control. Similar effects of these isolates were seen on shoot fresh and dry weight causing an improvement of plant growth by 62% to 106% relative to the control (judged from dry mass). Van Peer and Schippers (1988) also documented increases in root and shoot fresh weight for tomato, cucumber, and potato as a result of bacterization with *Pseudomonas* strains whereas Ousley *et al.* (1993) demonstrated that biomass of four different *Trichoderma* strains WT, T35, 20 and 47 when added to nonsterilized potting compost at 1% (w/w), promoted shoot fresh weight of lettuce (*Lactuca sativa* L.). Similar results were obtained in field trials by Burr *et al.* (1978), Kloepper *et al.* (1980b), Geels *et al.* (1986) and Lalande *et al.* (1989).

Generally, bacterization of mungbean plants by selected isolates of rhizobacteria was seen to bring about an improved growth. Since the experiment was conducted under sterile conditions, it can be concluded that the action of rhizobacteria on plants does not involve the effect of antagonistic properties of pseudomonads and other microorganisms and that the principal influence may be the effect of growth regulators/promotors.

However, the basis for the variation in effects on plant growth by different rhizobacterial isolates, obtained in the present study, is unknown but may relate to soil type, soil fertility level, moisture content and light conditions. Light conditions are known to affect the metabolic functions of the plants which are reflected in root exudation (Katznelson and Shirley, 1965; Van Wuurde and Tonneyck, 1978) and thus in colonization of plant roots by microorganisms and in their metabolism. Ambient growth conditions also influence the effect of hormones responsible for root and shoot elongation and growth.

ACKNOWLEDGEMENT

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