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Effect of plant growth promoting rhizobacteria on black scurf disease of potato and their ability to promote growth

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ABSTRACT: **Plant growth-promoting rhizobacteria strains; B4 (*Becillus cereus*), B5 (*Bacillus subtilis*), M1 (*Enterobacter cloacae*) and AZ10 (*Azotobacter* sp.) were tested for their ability to biologically control black scurf disease in potato incited by *Rhizobacterium solani* (Kuhn (teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk). Based on their ability to control development of black scurf disease and enhancement of potato yield, the strain B5 was recorded the best in inhibiting growth of *R. solani* in dual culture by 94.22 per cent and enhanced per plant tuber yield by 68.98 per cent in comparison to control. The results of *in vitro* studies indicate effectiveness of all the four PGPR strains in controlling black scurf disease of potato and can be used as potential biocontrol agents of *R. solani* in field conditions.**

KEY WORDS: Biological control, black scurf, PGPR, *Rhizoctonia solani*

INTRODUCTION

Production of quality disease-free potato is the most important factor in improving financial status of the farmers of this country. Black scurf disease of potato caused by *Rhizoctonia solani* (Kuhn (teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk) is an economically important disease in all the potato growing areas in India. The fungus produces brown to black lesions on the stem or below soil surface and black sclerotia on progeny potato (Dutt, 1979; Wicks *et al.*, 1996). Infected seed tubers and soil born inocula form the source of infection of black scurf or stem canker in a field crop of potato. Different methods have been used to control *R. solani* of which the most used are cultural practices, solarization, fungicides and biological control. The last method has been tried by many researchers during the last many years (Kloepper, 1993; Manoranjitham *et al.*, 1999; Mondal *et al.*; 2000) and is based on the reduction of inoculum potential and its pathogenic activity by use of plant growth promoting rhizobacteria (PGPR). The present research work had been aimed at testing the potential of PGPR strains in enhancing potato growth parameters *in vivo* and level of biocontrol against black scurf disease of potato *in vitro*.

MATERIALS AND METHODS

Bacterial cultural

The antagonistic PGPR strains used in this study were originally isolated from rhizosphere soil of diseased potato plants because microbial biomass is found to be much greater in diseased

soil than in conducive soils. For collecting samples, one-month-old potato plants were uprooted and the soil clinging to roots was dislodged by washing the roots in 200 ml of sterile distilled water. The soil samples were then serially diluted and a loopful of the diluted suspension was streaked on to a Petri-plate containing nutrient dextrose agar (NDA) and Jensen's (1954) agar media. Plants were then incubated for 48 hours 30° C. After incubation, a well-isolated colony was picked-up and restreaked onto fresh media plates in order to maintain purity of the bacterial cultures. The purified PGPR cultures were stored for future use in 5 ml. sterilized distilled water contained in a screwcapped tubes at 4° C (Schisler and Slininger, 1994)

***In- vitro* assay**

In vitro Petri-plate assays were conducted by dual culture method of Montealegre *et al.* (2003) at the bacteriology laboratory of Central Potato Research Institute Campus (CPRIC), Modipuram, Meerut (U. P.) during 2003. All the four PGPRs namely, *Bacillus cereus* strain B4, *Bacillus subtilis* strain B5, *Enterobacter cloacae* strain M1 and *Azotobacter* sp. strain AZ10 were tested for their *in vitro* ability to inhibit growth of *R. solani*. The growth of two-day-old cultures of *R. solani*. was placed in the cente of a Petri-plate containing potato dextrose agar (PDA) medium. A circular line was made with a 6 cm diameter Petri-plate, dipped in a suspension of bioantagonistic bacteria (10^9cfu./ml) was placed surrounding fungal inoculum. Plates were cultured for 72 hours at 22° C and growth diameter of *R. Solani* (pathogen) was measured and compared with control growth where bacterial suspension was replaced by sterile distilled water. Each antagonistic treatment was repeated four times and results were expressed as mean of inhibition for statistical analysis.

Field experiment

Two field experiments were conducted both in early (September 2002- November 2002) and main (October 2003-January 2004) potato seasons at CPRIC, Modipuram, Meerut (U. P.) to assess the antagonistic efficacy of PGPR strains on improvement in plant growth of potato. Treatments were arranged in a randomized block design and replicated four times. Experiment was planted in field soil having sandy loam texture, pH value 7.2 and EC 25 MS. Seed potato were treated for 20 minutes with 10^6 cfu/ml suspension of 48 hour old cultures of strains B4, B5, M1 and AZ10 (Sunaina *et al.*, 1997). In total, there were five treatments including control. Observations per plant were recorded on number of stems, tuber yield, root length, shoot length and root volume. Data were analyzed using ANOVA and significant differences were established at P=0.05 using M stat C stastistical software.

RESULTS AND DISCUSSION

All the selected PGPR strains showed antagonistic properties with little variation against *R. solani* (Table I). In dual culture experiment, the PGPR strain B5 was found extremely effective in inhibiting mycelial growth of *R. solani* and recorded 94.22 per cent reduction in growth over control. Strain B5 was next followed by another strain B4, which recorded 83.33 per cent reduction in radial growth over control. The other two antagonistic strains M 1 and AZ 10 were found to be moderately effective and recorded per cent reduction in growth of *R. solani* by 43.61 and 60.83, respectively over control (Table 2). In case of antagonistic activity of Bacillus strains B 4 and B 5, a change in mycelial colour from white to dark brown was observed where antagonistic bacterial growth interacted with *R. solani* mycelium. Microscopic examination of this zone revealed inhibitory effect of PGPR strain B 4 and B 5 on *R. solani* mycelium, which can be correlated due to cytoplasm leakage by cell lysis enzyme. Similar results have been cited by Alippi and Monarco (1994) who observed hyphal deformation of *R. solani* and *Pythium ultimum* as a result of treatment with *B. subtilis* strain which secreted a volatile metabolite with fungicide properties. In case of interaction of PGPR strains AZ 10 and M 1 with *R. solani*, no physical contact between antagonistic strains and pathogenic mycelium was observed. Both these PGPR strains formed a clear zone around *R. solani* growth, which can be due to secretion of antibiotics by antagonistic strains. This type of activity has also

Table 1. PGPR strains used in biological control of black scurf disease of potato incited by *R. solani*

PGPR Strain	Origin	Location	Identifying agency
Bacillus subtilis B 5	Potato rhizosphere	Bhowali, Uttaranchal	IMI, England
Bacillus cereus B 4	Potato rhizosphere	Bhowali, Uttaranchal	IMI, England
Enterobacter cloacae M1	Potato rhizosphere	Modipuram, Meerut (U.P.)	IMI, England
Azotobacter sp. AZ 10	Tomato rhizosphere	Pabalikhas, Meerut (U.P.)	Awaited

Table 2. Growth of PGPR strains and *R. solani* in dual culture on PDA

Treatment	Colony diameter of *R. solani* (mm)		Per cent reduction over control
	PGPR strains	*R. solani*	
Bacillus subtilis B5	84.00	5.20	94.22
Bacillus cereus B4	75.00	15.00	83.33
Enterobacter cloacae M1	39.00	50.75	43.61
Azotobacter sp. AZ10	54.00	35.25	60.83
Control (*R. solani*)	90.00	90.00	0.00
SEM ±	0.50	2.27	
CD (P=0.05)	1.54	7.00	

been detected and reported by Silva *et al.* (2001) as a result of treatment of *R. solani* either with *B. subtilis* or *B. lentimorbus*.

The PGPR stains that were found most effective in controlling black scurf disease of potato under laboratory conditions were found equally effective in improving potato plant growth when tested under field conditions. Results of PGPR inoculation of potato tubers before planting in field revealed superiority of these strains over control in improving growth parameters of potato plant. Significant differences were observed only in PGPR strain B5 treatment which recorded enhanced growth in plant root accompanied by excessive branching and number of root tips. Maximum per plant tuber yield of 666 g corresponded with highest value of root volume of 32 CC in case of PGPR strains B 5 treatment. This treatment was closely followed by PGPR strain B 4 where per plant yield of 534 g and root volume of 29CC were recorded. The remaining two treatment of strain M1 and AZ 10 were even though superior to control but did not show significant differences when compared with each other (Table 3).

The results suggest that a large and heavily branched root system observed in potato plants arising from PGPR treated strains leads to improved uptake of water and nutrients. The increase in water and nutrient uptake by excessive root branching of PGPR treated plants has ultimately revealed its effect in enhancing per plant yields to a significant level by *Bacillus* strains. The results suggest that the strains *B. cereus* B 4, *B. subtilis* B 5, *E. cloacae* M 1 and *Azotobacter* sp. AZ 10 have an excellent potential to be used as biocontrol agents of *R. solani* in potato at the field level.

Table 3. Efficacy of PGPR strains in improving growth parameters of potato plants

Treatment	Average value per plant				
	No. of stems	Wt. of tubers (g)	Root length (cm)	Shoot length (cm)	Root volume (cc)
Bacillus subtilis B 5	3.60	666.00	34.26	64.44	32.00
Bacillus cereus B 4	3.20	534.00	29.78	59.58	29.00
Enterobacter cloacae M 1	4.20	380.00	29.26	54.96	27.00
Azotobacter sp. AZ 10	4.40	469.00	27.20	53.18	27.00
Control (Water)	3.40	392.00	23.64	52.20	23.00
SEM±	0.84	74.26	2.37	2.85	3.17
CD (P=0.05)	2.50	222.65	7.13	8.55	9.50

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