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Evaluation of Different Strains of *Pseudomonas fluorescens* for the Biocontrol of Fusarium Wilt of Chickpea

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Abstract: Evaluation of different strains of ***Pseudomonas fluorescent*** for the biological control of chickpea wilt shows that among 36 rhizobacterial isolates, Isolate 1, Isolate 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 and 36, during ***in vitro*** studies, the isolate No. 3, 6, 12, 16, 18, 21, 22, 23, 27, 29 and 33 shows antagonistic activity during inhibition of ***Fusarium oxysporum f.sp. ciceri*** by rhizobacterial isolates, whereas by using water culture in test tubes the isolate No. 1, 2, 4, 5 and 9 show antagonistic activity by reducing wilt incidence by 91.67, 94.33, 94.37, 96.00 and 96.00% decrease over control, respectively. In try experiment isolate No. 1, 2, 4, 5 and 9 proved to be the antagonistic ones.

Key words: *Pseudomonas fluorscens*, biological control, chickpea wilt, *Fasarium oxysporum f.sp. ciceri*

Introduction

Free living saprophytic rhizosphere bacteria have been investigated for their effect on growth and yield of crops throughout the 20th century. The term plant growth promoting rhizobacteria (PGPR) was coined for the subset of total rhizoshere bacteria colonizing plant roots upon inoculation which have positive effects on plant growth (Kloepper and Schroth, 1978).

Among these rhizobacteria strains of ***Pseudomonas fluorescens*** have been studied extensively as biocontrol agents of plant disease (Campbell, 1989; Hebber ***et al.***, 1992; Knudsen and Spurr, 1987 and Weller, 1988). Some strains particularly effective for controlling several soil-borne pathogens (Bull ***et al.***, 1991; Kwok, 1987; MCLoughlin ***et al.***, 1992 and Weller, 1988). Because these bacteria posses many desirable attributes, their potential for use in disease control strategies is substantial (Compbell, 1989).

Biological control of ***Fusarium*** wilts as for other soil-borne diseases is often inconsistent (Weller, 1988). There are different factors, which are responsible for the inconsistent performance, among these are lacks of correlation between ***in vitro*** and ***in vivo*** performance of biocontrol agents.

So the rhizobacterial isolates were applied to control ***Fusarium oxysporum*** sp. ***Ciceri*** first by ***in vitro*** inhibition, where there is direct contact of pathogen and antagonistic bacteria.

Moreover in water culture the host was incroporated and again the bacteria were evaluated and lastly the plants were grown in trays having soil, which somewhat reflect the field conditions. So the rhizobacterial isolates were evaluated under different conditions for the determination of the bacteria, which remain antagonistic throughout the screening series and can be utilized further.

Materials and Methods

A number of thirty-six isolates of rhizobacteria were isolated from the chickpea rhizosphere soil, by using the glucose peptone medium by dilution plate techniques (Wollum, 1982). These isolates were screened on the basis of biocontrol activity against the *Fusarium oxysproum* f. Sp. *ciceri* under different conditions. The detail of each experiment is given below.

***In vitro* inhibition of *Fusarium osysporum* f.sp.*ciceri* (F.o.c.) by rhizobacterial isolates**

Rhizobacterial isolates were tested for their ability to inhibit *F.o.c in vitro,* on agar plates as described by Weller and Cook (1986) and Wong and Baker (1984). The pathogen F.o.c. was transferred to petridishes of 9 cm dia. Containing fresh PDA to produce fungal mycelium plugs. Each bacterial isolates was streaked at opposite ends of GPAM agar plates near the edge and incubated at 27±1°C for 48 h. An agar plug (5 mm dia.) containing fungal mycelium, taken from the radiating edge of a culture grown on PDA was place in the center of each plate. Plates were incubated for about five days more or until the leading edge of the fungus reached the edge of the plate. The size of the zone of inhibition of fungal growth around each bacterial strain was used as a measure of the ability of that strain to inhibit F.o.c. and was scored as previouly described by Weller and Cook (1986).

−= no zone of inhibition and the fungus overgrew the bacterial colony
+= a distinct zone of inhibition less that 6 mm
++= a distinct zone of inhibition of 6-10 mm
+++= a distinct zone of inhibition greater than 10 mm

Each treatment was replicated six times and entire experiment was performed thrice

Selection of rhizobacterial isolates for biological control of *Fusarium oxysporum* f. sp. *ciceri* (F.o.c.) by using water culture in test tubes

Selection of rhizobacterial isolates was done by a modified method (Nene ***et al.***, 1981) Fifteen seeds were surface sterilized in 2.5% sodium hypochlorited for 5 min. seeds of cultivar AUG-480 were placed in sterilized riverbed sand in 15 cm pots @ 1 kg per pot.

Twenty ml of aqueous suspension of F.o.c. 6.5×10^5 cfu ml^{-1} inoculum was poured in to each sterilized 150×15 mm glass tube. Ten day old (from sowing) seedlings were removed from sand, the

root system was washed in running water and then rinsed in sterilized distilled water. One seedling was transplanted into each tube and held in vertical position by a cotton plub. Sterilized distilled water was added after every 2 days to make up the loss and equal amounts of test rhizobacterial strains were added. For each line a non inoculated seedling (without strain) was kept as check. The pathogen inoculated check usually wilts with in 7-10 days, while the other showed different behaviour depending upon the antagonistic ability of strains. Data were recorded about disease incidence. The healthy seedlings remained green up to 3 weeks.

Selection of rhizobacterial isolates for the biocontrol of *Fusarium oxysporum* f. sp. (F.o.c.) using infested soil (field soil) in plastic trays

Bacterial strains were selected by usnig infested field soil in plastic trays of (36x28x8 cm^3), each tray containing 5 kg (3500 cfu g^{-1}) of soil. Chickpea seeds were sown at a distance of 6 cm both from row to row and plant to plant. Thus total of 20 seeds were sown. The seeds were already treated with different bacterial isolates as explained below

Broth cultures were grown for 7 days on GPAM so as to contain 10^7 to 10^8 cuf ml^{-1}. They were then transferred to sterilized plastic tubes containing sterilized peat and slurry was prepared with 10% sugar solution. For the inoculation of 300 g seeds 40 ml broth (calculated on the basis of seed size) culture and 20 g sterilized peat was used. Control was treated with similar slurry without rhizobacterial cultures. Treated seeds were dried for 24 h in the air under shade. Three trays were used for screening (one as control) for each isolate. Data were recorded on the basis of percent disease incident.

Results

Screening of rhizobacterial isolates against *Fusarium oxysporum* f. sp. *ciceri* by *in vitro* Inhibition, water culture and in trays

The screening results showed that the rhizobacterial isolates number 3, 6, 12, 16, 18, 21, 22, 23, 27, 29 and 30 showed great antagonistic activity against ***Fusarium oxysporum*** f. sp. ***ciceri*** during ***in vitro*** inhibition of rhizobacterial isolates while other shows variable response (Table 1). In case of rhizobacterial isolates for the biocontrol of F.o.c. by using water culture in test, the isolates number 1, 2, 4, 5 and 9 showed greatest antagonistic activity by reducing 91.67, 94.33, 94.67, 96.00 and 96.00% decrease over control, respectively other isolates showed variable results (Table 2). In trays experiment the isolate number 1, 2, 4, 5 and 9 proved to be the antagonistic ones (Table 3), other isolates in case of ***in vitro*** inhibition water culture and pot experiment showed different antagonistic abilities. But these five isolates i.e. number 1, 2, 4, 5 and 9 were remained antagonistic only in water culture and tray experiment. These 36 isolates were screened by using these series of screening techniques because there is no correlation between ***in vitro*** inhibition of the pathogen with bacterial with bacterial isolates and sisease suppression

in the field (Fravel, 1988). Moreover, the biocontrol capacity that microorganism express ***in vitro*** may not be effectively expressed under soil condition (Burr ***et al.***, 1978). The tray experiment reflects the field conditions to some extent, so these five isolates (i.e. isolates number 1, 2, 4, 5 and 9) were utilized for further studies alone and also in various combination.

Discussion

Rhizobacteria isolates from the chickpea rhizosphere were investigated for their biocontrol activity. The rhizobacteria isolated and when evaluated for growth promotion against the same hosts are effective rather to use those on other crops (Javed and Arshad, 1997). These 36 rhizobacteria were named as Isolate-1, Isolate-2 ..... Isolate-36. These isolates were screened first by ***in vitro*** inhibition of the ***Fusarium oxysporum*** f. sp. ***ciceri*** by rhizobacteria. These 36 isolates were screened by using these series of screening techniques because there is no correlation between ***in vitro*** inhibition of the pathogen with bacterial isolates and disease suppression in the field (Fravel, 1988). Moreover, the biocontrol capacity that microorganisms express ***in vitro*** may not be effectively expressed under soil condition (Burr ***et al.***, 1978).

The screening results show that eleven isolates showed great antagonistic activity against the pathogen. Khot ***et al.*** (1996) isolated rhizobacteria from the rhizosphere of chickpea plants. Out of 36 isolates, five inhibited the growth of *F. **Oxysporum*** f. sp. ***ciceri in vitro*** plate assays for fungal antagonism. Similarly Myatt ***et al.*** (1992) isolated 1000 bacteria from chickpea rhizosphere and evaluated for their potential as biological control agents of ***Phytophthora meagasperma*** f. sp. ***Medicaginis*** root rot of chickpea ***in vitro***. Following ***in vitro*** plate assays for fungal antagonism, out of 1000 bacteria 31 isolates showed antagonistic ability against the pathogens.

Pierson and Weller (1994) evaluated 80 strains of fluorescent ***pseudomonas*** during ***in vitro*** inhibition of ***Gaeumannomyces graminis var. tritici***, eleven isolates showed antagonistic activity against the pathogen. Weller and Cook (1993) evaluated 60 isolates of rhizobacteria, 24 isolates during ***in vitro*** inhibition of *G.g. var. tritic*. Harris ***et al.*** (1994) screened 5000 bacterial isolates by ***in vitro*** inhibition against ***Rhizoctonia solani*** in seedlings of ***Celosia argentea***, 91 isolates showed the antagonistic activity against the pathogen.

It is recognized that ***in vitro*** assay for antagonistic potential has inherent limitations. Although many studies have utilized the agar plate assay method to determine biocontrol potential (Broadbent ***et al.***, 1971 and Weller ***et al.***, 1985) but (Hagedorn ***et al.***, 1989; Fravel 1988; William and Asher, 1996) showed that no correlation existed between inhibition of pathogen on agar and disease control in plants. There appears to be agreement that the agar inhibition assay is not ideal however, many researchers use combinations of methods and appreciate the limitations of the agar assay. Antibiosis can be an important mechanism in biocontrol and the production of antibiotics and siderophores can be detected ***in vitro*** assay (Weller ***et al.***, 1985 and Thomashow ***et al.***, 1990).

Table 1: ***In vitro*** inhibition of ***Fusarium oxysporum*** f. sp. ***ciceri*** by rhizobacterial isolates

Isolates	Zone of inhibition (mm)
1	-
2	-
3	+++
4	-
5	-
6	+++
7	+
8	-
9	-
10	+
11	-
12	+++
13	-
14	++
15	-
16	+++
17	-
18	+++
19	+
20	+
21	+++
22	+++
23	+++
24	+
25	++
26	++
27	+++
28	+
29	+++
30	-
31	-
32	-
33	+++
34	+
35	+
36	-
Control	Full by F.o.c.

– No zone of inhibition and the fungus overgrew the bacterial colony

Table 2: Selection of rhizobacterial isolates for the biological control F.o.c. by using water culture in test tubes

Isolates	% Wilt incidence original order
1	8.330r
2	4.000t
3	22.330i
4	5.333s
5	4.000t
6	24.330fg
7	12.330q
8	25.670de
9	5.667s
10	23.330ghi
11	20.670j
12	23.670gh
13	22.670hi
14	13.330pg
15	26.330d
16	17.330n
17	18.670lm
18	15.330o
19	13.670p
20	17.330n
21	25.000ef
22	28.670c
23	24.330fg
24	33.670b
25	14.330op
26	18.000mn
27	17.330n
28	19.330kl
29	23.670gh
30	22.670hi
31	20.330jk
32	22.670hi
33	25.670de
34	26.330d
35	22.330i
36	19.670jkl
Control	100.000a

* Means sharing a common letter do not differ significantly at 5% level of significance

Table 3: Selection of rhizobacterial isolates for the biological control of F.o.c. by using tray method

Isolates	% wilt incidence
1	67.00n
2	56.00q
3	72.33klm
4	58.67p
5	52.33r
6	73.00jkl
7	78.33bcd
8	78.67bc
9	62.33o
10	75.33ghi
11	76.00efgh
12	78.33bcd
13	70.67m
14	75.67fghi
15	71.33lm
16	74.33hijk
17	73.67ijk
18	78.67bc
19	72.33klm
20	74.67hij
21	75.67fghi
22	77.67bcdef
23	72.67jklm
24	78.00bcde
25	76.33defgh
26	77.00cdefg
27	75.33ghi
28	73.00jkl
29	76.33defgh
30	79.67ab
31	72.67jklm
32	75.33ghi
33	77.00cdefg
34	76.00efgh
35	74.67hij
36	78.33bcd
Control	81.00a

Means sharing a common letter do not differ significantly at 5% leverl of significance

Moreover results obtained by Iswandi (Bossier ***et al.***, 1988 and Iswandi, 1986) showed that the ***in vitro*** inhibitory effect of the ***Pseudomonas*** strain 7NSK2 on the growth of various phytopathogenic fundi was at least partly due to pyoverdin produciton.

As application of diferent species of ***Pseudomonas*** originally isolated from the rhizosphere of plant to plant culture media such as nutrient solution reduced root and stem rot in both laboratory and green house conditions (Paulitz ***et al.***, 1992; Rankin, 1992; Zhou and Paulitz, 1993). Root exudates which contain many organic and inorganic substances, are important in the establishment and maintanance of populations of rhizosphere microorganisms (Barber and Martin, 1976; Rovira, 1969; Schroth and Cook, 1964). The same 36 isolates were screened in hydroponic culture, because these substrates have a lower microbial content than soil, reducing the competition for establishment of biocontrol agents and allowing biocontrol agents
with a low competitive ability to become established. These substrates are in control environment. Five isolates reported to be antagonistic. But under field conditions there is much variability and hence are various factors, which affect the effeicacy of biocontrol agents. Burr ***et al.*** (1978) reported that biocontrol capacity that microorganisms express ***in vitro*** may not be effectively expressed under soil condition so these 36 isolates further screened in tray experiment as done by (Harris ***et al.***, 1994; Weller and Cook, 1983; Myatt ***et al.***, 1992) which some what reflected the field conditions again five isolates 1, 2, 4, 5 and 9 were selected for further research.

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