INTERACTION BETWEEN ROOT-NODULES BACTERIA AND DAMPING-OFF FUNGI OF SOYBEAN.

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التفاعل بين بكتريا العقد الجذرية وفطريات الذبول الطرى لبادرات فول الصويا

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ملخص البحث

أدت بكتريا العقد الجذرية لفول الصويا (ريزوبيم جابونيكم) وكذلك راشح مزرعتها الى تثبيط نمو الفطريات سكليروشيوم رولفزياى ، وريزوكتونيا سولانى ، وماكروفومينا فاسيولينا . وقد وجد أن راشح مزرعة البكتريا العقدية غير سام لكل أصناف فول الصويا المختبرة سواء فى المعمل أو تحت ظروف الصوبه ، بل على العكس أدى الراشح الى تشجيع انبات البذور فى المعمل وكذلك ظهور البادرات فوق سطح التربة تحت ظروف الصوبة ، وقد ازداد معدل انبات البذور وكذلك معدل ظهور البادرات فوق سطح التربة بازدياد تركيز راشح البكتريا .

وقد وجد أن بكتريا العقد الجذرية لفول الصويا لها المقدرة على تقليل موت بادرات فول الصويا المتسبب عن سكليروشيوم رولفزياى ، وريزوكتونيا سولانى ، وماكروفومينا فاسيولينا . من ناحية أخرى أدت الفطريات المختبرة سواء كانت منفردة أو مجتمعة الى تقليل معدل تكوين العقد الجذرية عند اجتماعها مع بكتريا العقد الجذرية بالمقارنة بمعدل تكوين العقد الناتج عن البكتريا منفردة ، وقد كان الفطر سكليروشيوم رولفزياى أكثر الفطريات تأثيرا على تكوين العقد الجذرية ثم الفطر ريزوكتونيا سولانى فى حين كان الفطر ماكروفومينا فاسيولينا أقلها تأثيرا .

وقد حدث أقل معدل لتكوين العقد الجذرية عند العدوى بالثلاث فطريات معا ، ثم العدوى المزدوجة بالفطرين سكليروشيوم رولفزياى وريزوكتونيا سولانى .

ABSTRACT

In vitro experiments the soybean root-nodule bacterium (Rhizobium japonicum) and its cell free culture filtrate inhibited the growth of Sclerotium rolfsii, Rhizoctonia solani and Macrophomina phaseolina in paired liquid culture significantly. R. japonicum culture filtrate was not phytotoxic to all three soybean cultivars tested. It promoted either seed germination in the laboratory or seedling emergence in the greenhouse. The seed germination and the emergence rate were increased at higher concentration of the R. japonicum culture filtrate.

Soybean root-nodule bacterium (R. japonicum) are able to reduce soybean damping-off caused by S. rolfsii, R.solani and M. phaseolina. Root-nodulation decreased when the tested fungi-seperately or in combination-were combined in inoculation with R. japonicum, as compared with R. japonicum alone. S. rolfsii was most effective in reducing nodulation, followed by R. solani, and finally by M. phaseolina. The lowest number of nodules per plant was found when the three pathogens were combined, followed by a double inoculation by S. rolfsii plus R. solani.

INTRODUCTION

The interaction existed between soil-borne plant pathogenic fungi and root nodules bacteria drew the attention of several investigators. Drapeau et al. (1973) tested the antifungal activity of three different Rhizobium isolates against some fungi. Some of the tested fungi are potential phytopathogens to legumes. In several cases, they observed inhitition zones. Similar results were obtained with the cell-free extract of the media on which Rhizobium had been grown. They pointed out that different strains of Rhizobium showed different degrees of activity towards Colletotrichum destructivum.

Chou and Schmitthenner (1974) stated that dry weight of soybean plants infected with Phytophthora megasperma var. sojae alone was lower than that of plants inoculated with P. megasperma var. sojae, Rhizobium japonicum and/or Endogone mosseae. They also found that soybean infected by P. megasperma var. sojae (race 3) developed fewer root nodules than those infected by Pythium ultimum or P. megasperma var. sojae (race 1) or the control.

Orellana et al. (1976) reported that Rhizoctonia solani significantly reduced nodule weight of Lee and Kent soybean inoculated with R. japonicum and grown in a N-free sand nutrient substrate as compared with plants grown with R. japonicum alone. For Lee soybeans, 63% decrease in fixed nitrogen per plant due to the fungus was demonstrated. In Rhizobium inoculated soybeans grown in the presence of R. solani, nodule weight and total nitrogen content per plant were also reduced.

Orellana and Worley (1976) observed cell dysfunction in young root nodules of soybeans grown in the presence of R. solani. They suggested that this dysfunction may be due to the toxic fungal metabolites diffusing throughout the nodule. Such dysfunction would interfere with nitrogenase and symbiotic N_2 fixation activities and, where the nitrogen supply is suboptimal, would reduce soybean yield.

Tu (1978) reported that R. japonicum significantly reduced root rot of soybean caused by Phytophthora megasperma in the greenhouse. In vitro, when R. japonicum was added to growing mycelia of P. megasperma, the bacteria colonized growing hyphal tips. He suggested that, by covering hyphal tips, Rhizobia might prvent contact between P. megasperma and host root tissue, and thereby reduce the chance or P. megasperma infection.

Purkayastha et al. (1981) studied the interaction between M. phaseolina and R. japonicum in vivo and in vitro. They found that pre-inoculation of soybean plants with R. japonicum significantly reduced severity of charcoal rot disease caused by M. phaseolina.

When R. japonicum was grown in liquid culture with M. phaseolina, the growth of M. phaseolina was markedly inhibited.

Orellana and Mandava (1983) stated that phytotoxic compounds (m-hydroxiphenylacetic and m-methoxiphenylacetic acid) of R. solani are involved in nodule impairment and reduced N_2-fixation in soybean.

Shatla et al. (1983) studied the interaction between R. solani and R. japonicum in their influence on soybean plants. They reported that the percentage of pre- and post-emergence damping-off of soybean caused by R. solani decreased significantly when R. solani was combined with R. japonicum, as compared with R. solani alone. They also pointed out that the total number and size of nodules per plant decreased when the two organisms were combined together as compared with R. japonicum alone.

Chakraborty and Purkayastha (1984) reported that bacterization of soybean seeds or roots with R. japonicum significantly reduced charcoal rot caused by M. phaseolina. R. japonicum inhibited the growth of M. phaseolina when the two organisms were grown together in yeast extract maintol broth medium as compared with the growth of M. phaseolina when it was grown alone. Replacement of nutrient medium by culture filtrate of R. japonicum significantly reduced mycelial growth of M. phaseolina. Culture filtrate of R. japonicum yielded a toxic substance which was identified as rhizobiotoxin. This compound was also detected in the roots of soybean inoculated with either R. japonicum alone or in combination of R. japonicum and M. phaseolina. Dosage response curves with rhizobiotoxin showed it to be antifungal.

The aim of this work is to add further informations on the interaction between root-nodules bacterium (Rhizobium japonicum) and damping-off fungi (Sclerotium rolfsii, Rhizoctonia solani and Macrophomina phaseolina) of soybean.

MATERIALS AND METHODS

1. Effect of *R. japonicum* on the growth of *S. rolfsii*, *R. solani* and *M. phaseolina* in mixed culture:

Rhizobium japonicum was cultured in 250 ml flasks containing 50 ml yeast extract mannitol medium (YEM) and incubated at 28°C for 48 hours. Each tested fungus was grown first in culture plates, each containing 15 ml of PDA medium and incubated for 3 days at 30°C. One agar block, 6 mm in diameter, with a mycelial mat of each tested fungus was transferred to 250 ml flasks containing 50 ml YEM and incubated at 30°C. Both fungus and bacterium also were grown together in YEM solution. One agar block from the edge of a fungal colony and 0.5 ml of bacterial suspension were used as inocula for each flask (50 ml YEM solution/250 ml flask) and incubated at 30°C. Four flasks were used for each particular treatment as replicates. After 4, 8, 12 and 16 days of inoculation, the mycelia were collected, dried at 60°C for 96 h, and weighed.

2. Effect of *R. japonicum* culture filtrate on:

a) The growth of *S. rolfsii*, *R. solani* and *M. phaseolina*:

R. japonicum was grown in yeast extract mannitol broth for 6 days at 28°C. Finally the bacteria were removed by centrifugation (8,500 rpm for 20 min. according to Chakraborty and Purkayastha, 1984). One agar block, 6 mm in diameter, from the advancing zone of the mycelial mat of each tested fungus was transferred to 250 ml flasks containign 50 ml YEM and incubated at 30°C for 7 days. At the end of this period the flasks of each fungus were divided to three groups. In the first group, the mycelia were collected, dried at 60°C for 96 h, and weighed. In the second group, the solution was replaced aseptically by 50 ml of *R. japonicum* culture filtrate and incubated for another three days at 30°C. The third group remained as it was, and was incubated also for another three days at 30°C. Four flasks were used for each particular treatment. Finally, mycelia were collected, dried at 60°C for 96 h, and weighed.

b) The germination of S. rolfsii sclerotia in the laboratory:

R. japonicum culture filtrate was prepared and considered as 100% concentration. Different concentrations (0, 10 ... 80 and 90%) were prepared using sterilized water, S. rolfsii sclerotia were obtained by growing the fungus on PDA plates and incubation at 30°C for 3 weeks. The sclerotia were soaked in each concentration for three hours, then 25 sclerotia were transferred aseptically to Petri dishes containing 15 ml PDA and incubated at 30°C. Four Petri dishes were used for each particular treatment as replicates. The experiment was observed daily and the germinated sclerotia were counted 4 days after inoculation.

c) The germination of soybean seeds (phytotoxicity test):

The phytotoxicity of R. japonicum culture filtrate to soybean seeds was tested in the laboratory as well as in the greenhouse.

Three soybean cultivars namely; Calland (bad seed quality), Colombus (moderate seed quality) and Williams (good seed quality) were used in this experiment. R. japonicum culture filtrate was prepared. Soybean seeds were soaked in R. japonicum culture filtrate with different concentrations (0, 10, ... 90 and 100%) for 30 min., then 15 seeds were transferred to each Petri dish containing 3 filter papers saturated with the desired concentration of R. japonicum culture filtrate. Six plates were used for each particular treatment as replicates. The plates were incubated at room temperature (25 ± 2°C) and observed daily. After three days germination data were recorded.

In the greenhouse test, 20 soybean seeds (after soaking in R. japonicum culture filtrate for 30 min.) were sown in each plastic pot (20 cm in diameter) and replicated six times for each particular treatment. The number of plants in each pot was recorded 3 weeks after sowing, and percentages of emergence were calculated.

3. **Interaction between Rhizobium japonicum; and S. rolfsii, R. solani and M. phaseolina - separately and in combination under greenhouse conditions:**

Calland, the most susceptible cultivar to S. rolfsii, R.solani and M. phasolina, was used for this purpose. Inocula of S. rolfsii, R. solani and M. phaseolina (the most virulent isolate of each fungus) were prepared. Inocula of each fungus alone or in all possible combinations were mixed thoroughly with the soil at the rate of 2% of soil weight 7 days before sowing. Non-infested soil was used as control. The pots of each treatment were divided to two groups, the first group was inoculated with R. japonicum as nitragin granules inoculum (The nitragin Co. Milwaukee, Wisconsin 53209 U.S.A.) at the rate of 1 g per pot (20 cm in diameter) at the day of sowing, while the second group was not inoculated. 20 soybean seeds were sown in each pot. Pre- and postemergence damping-off data, as well as the number of survived plants were recorded.

For estimating the effect of the tested fungi on soybean nodulation, the plants in the first group were removed carefully from the pots after irrigation, then the roots were washed. Number and size of nodules per plant were recorded 5 weeks after sowing.

RESULTS

1. Effect of R. japonicum on the growth of S. rolfsii, R. solani and M. phaseolina in mixed culture:

Results in Table (1) show that R. japonicum inhibited the growth of S. rolfsii, R. solani and M. phaseolina in culture significantly.

Table (1): Effect of R. japonicum on the growth of S. rolfsii, R. solani and M. phaseolina in mixed culture in yeast extract mannitol broth.

Fungus	R. japonicum	Average dry weight of mycelia (mg) 4 days	8 days	12 days	16 days
S. rolfsii	-	22.3	45.9	63.7	37.4
	+	8.8	18.1	23.0	13.8
R. solani	-	95.1	196.8	392.5	162.7
	+	30.5	89.7	201.5	98.9
M. phaseolina	-	84.4	185.2	380.1	141.3
	+	29.2	77.6	196.0	82.5
L.S.D. 5%		3.2	3.0	3.6	2.8
1%		4.5	4.4	5.2	3.4

2. Effect of R. japonicum culture filtrate on:

a) The growth of S. rolfsii, R. solani and M. phaseolina:

To prove that the growth reduction of the tested fungi in mixed culture with R. japonicum was not due to competition for or deficiency of nutrients, the bacterial culture filtrate was bio-assayed by the replacement method.

The results in Table (2) indicate that the cell-free culture filtrate of R. japonicum significantly inhibited the growth of S. rolfsii, R. solani and M. phaseolina.

Table (2): Effect of R. japonicum culture filtrate on the growth of S. rolfsii, R. solani and M. phaseolina in yeast extract mannitol broth.

Fungus	Variant	Average dry weight of mycelia (mg)		
		Initial*	Final**	Difference in growth
S.rolfsii	Medium	41.5	77.2	35.7
	R. japonicum culture filtrate	41.5	45.9	4.4
R.solani	Medium	193.5	372.7	179.2
	R. japonicum culture filtrate	193.5	214.2	20.7
M.phaseolina	Medium	181.3	362.2	180.9
	R. japonicum culture filtrate	181.3	197.7	16.4
L.S.D. 5%			4.1	
1%			5.5	

* 7 days after inoculation.

** 3 days after replacement of the medium with R. japoncium culture filtrate.

b) The germination of S. rolfsii sclerotia in the laboratory:

The results revealed that cell-free culture filtrate of R. japonicum significantly reduced the germination of S. rolfsii sclerotia in vitro (Table 3). The inhibitory rate decreased with decreasing concentration of R. japoncium culture filtrate. In undiluted culture filtrate only 28% of the sclerotia germinated, as compared to 100% in the control.

Table (3): Effect of R. japonicum culture filtrate on germination of S. rolfsii sclerotia in vitro.

Concentration of R. japonicum culture filtrate	% of germinated sclerotia
0 (Control)	100
10	100
20	92
30	84
40	75
50	61
60	55
70	44
80	33
90	30
100	28
L.S.D. 5%	4.2
1%	5.9

c) The germination of soybean seeds (phytotoxicity test):

Data presented in Tables (4) and (5) show that differently concentrated R. japonicum culture filtrates were not phytotoxic to all three soybean cultivars tested either in the laboratory or under greenhouse conditions. On the contrary, cell-free culture filtrate of R. japonicum increased either the seed germination in the laboratory or seedling emergence in the greenhouse for all three soybean cultivars tested. Percentage of seed germination in the laboratory and emergence in the greenhouse were increased at higher concentration of R. japonicum culture filtrate for all soybean cultivars tested.

Table (4): Phytotoxicity test of differently concentrated R. japonicum culture filtrate in the laboratory.

Concentration of R. japonicum culture filtrate	% of germinated seeds		
	Calland	Colombus	Williams
0	72.2	83.3	88.9
10	72.2	83.3	88.9
20	73.3	84:5	88.9
30	73.3	84.5	90.0
40	75.6	85.6	91.1
50	75.6	85.6	91.1
60	76.7	86.7	92.2
70	80.0	86.7	94.4
80	82.2	88.9	96.7
90	84.5	91.1	96.7
100	85.6	91.1	97.8
L.S.D. 5%	3.0	3.3	3.5
1%	3.9	4.1	4.3

Table (5): Phytotoxicity test of differently concentrated R. japonicum culture filtrate in the greenhouse.

Concentration of R. japonicum culture filtrate	% of emerged seeds		
	Calland	Colombus	Williams
0	68.8	82.5	87.5
10	68.8	82.5	87.5
20	71.3	83.8	88.8
30	72.5	85.0	90.0
40	72.5	85.0	90.0
50	75.0	85.0	91.3
60	75.0	86.3	91.3
70	76.3	87.5	92.5
80	80.0	88.8	93.8
90	82.5	90.0	93.8
100	83.8	90.0	95.0
L.S.D. 5%	3.5	3.9	4.4
1%	4.4	4.7	5.5

3. Interaction between R. japonicum and the pathogens-separately and in combination-under greenhouse conditions:

a) Damping-off disease:

Data presented in Table (6) indicate that percentages of pre- and post-emergence damping-off were significantly decreased when the tested fungi-separately or in all possible combinations - were combined with R. japonicum, as compared with variants not inoculated with R. japonicum. The percentages of survived plants were increased in case of inoculation with R. japonicum. It could be concluded that

Table (6) Effect of R. japonicum on damping-off disease caused by S. rolfsii, R. solani and M. phaseolina, separately and in combinations, under greenhouse conditions.

Treatment		non-inoculated with R. japonicum			inoculated with R. japonicum		
		pre-emergence damping-off %	post-emergence damping-off %	healthy surviving plants %	pre-emergence damping-off %	post-emergence damping-off %	healthy surviving plants
1. S. rolfsii		70.1	5.3	24.6	61.3	3.6	35.1
2. R. solani		49.1	7.0	43.9	38.6	3.4	58.0
3. M. phaseolina		19.4	3.5	77.1	15.8	0.0	84.2
4. S. rolfsii + R. solani		86.0	8.8	5.2	82.5	3.3	14.2
5. S. rolfsii + M. phaseolina		78.9	7.0	14.1	73.7	3.4	22.9
6. R. solani + M. phaseolina		57.9	8.7	33.0	49.2	3.4	47.4
7. S. rolfsii + R. solani + M. phaseolina		91.3	8.7	0.0	89.5	3.4	7.1
8. Control		0.0	0.0	100.0	0.0	0.0	100.0
L.S.D. for inoculation with R. japonicum	5%	1.4	1.4	1.7			
	1%	1.9	1.9	2.2			
for fungi combination	5%	3.0	3.0	3.5			
	1%	4.0	3.9	4.7			

soybean root-nodule bacteria have the ability to reduce the damping-off disease caused by S. rolfsii, R. solani and M. phaseolina.

b) Nodulation of soybean:

Results in Table (7) revealed that the total numbers of nodules per plant were decreased when the fungi separately or in combination were combined in inoculation with R. japonicum as compared with R. japonicum alone. S. rolfsii was the most effective fungus tested for reducing soybean nodulation (3.1 nodules/plant), followed by R. solani (5.5 nodules/plant), while M. phaseolina was the least effective one (8.2 nodules/plant) compared with R. japonicum alone (12.7 nodules/plant). The least number of nodules per plant (1.0 nodule/plant) occurred when the three fungi tested were combined together, followed by S. rolfsii plus S. solani (1.5 nodules/plant).

Table (7): Effect of S. rolfsii, R. solani and M. phaseolina, separately and in combinations, on the nodulation of soybean under greenhouse conditions.

Treatment		Average No. of nodules/plant		Size of nodules		Total No.of nod./plant
		Main root	lateral roots	Large	Small	
1.S.rolfsii	R. japonicum	1.0	2.1	0.0	3.1	3.1
2.R. solani	R. japonicum	2.0	3.5	2.2	3.3	5.5
3.M.phaseolina	R. japonicum	4.0	4.2	2.0	6.2	8.2
4.S.rolfsii + R.solani	R. japonicum	0.5	1.0	0.0	1.5	1.5
5.S.rolfsii + M.phaseolina	R. japonicum	1.0	1.2	0.0	2.2	2.2
6.R.solani + M.phaseolina	R. japonicum	1.5	3.2	0.7	4.0	4.7
7.S.rolfsii + R.solani + M.phaseolina	R. japonicum	0.3	0.7	0.0	1.0	1.0
8.R.japonicum		4.4	8.3	5.3	7.4	12.7
L.S.D. 5%						1.3
1%						1.8

DISCUSSION

Significant inhibition in the in vitro growth rate of S. rolfsii, R. solani or M. phaseolina was found when each fungus was grown in a liquid mixed culture with the soybean root nodule bacterium (Rhizobium japonicum). Similar results have been recorded by Purkayastha et al. (1981). Cell-free culture filtrate of R. japonicum also significantly inhibited the growth of S. rolfsii, R. solani or M. phaseolina. Owens et al. (1968) proved that rhizobiotoxin-isolated from R. japonicum-inhibited the growth of Salmonella typhimurium. Chakraborty and Purkayastha (1984) found that rhizobiotoxin isolated from R. japonicum markedly inhibited the growth of M. phaseolina.

Cell-free culture filtrate of R. japonicum at different concentration inhibited the germination of S. rolfsii sclerotia, however, it stimulated seed germination and emergence of soybean plants. The inhibition rate of sclerotial germination decreased by decreasing concentration of the culture filtrate. Chakraborty and Purkayastha (1984) showed that the inhibition of M. phaseolina growth increased by increasing the concentration of rhizobiotoxin.

R. japonicum decreased the soybean damping-off rate and increased percentage of survived plants when it was combined in inoculation with S. rolfsii, R. solani and M. phaseolina, separately or in all possible combinations, as compared with variants not inoculated with R. japonicum This might be due to the antagonistic effect of R. japonicum on the pathogenic fungi. Chakraborty and Purkayastha (1984) concluded that rhizobiotoxin produced by R. japonicum plays an important role in the reduction of charcoal rot disease of soybean caused by M. phaseolina. In the present study, R. japonicum as well as its cell-free culture filtrate significantly reduced the growth rate of S. rolfsii, R. solani and M. phaseolina. Moreover, germination of S. rolfsii sclerotia was also reduced by culture filtrate. This result supports the findings of Chakraborty and Purkayastha (1984). Our observations are in agreement with those obtained by Chou and Schmitthenner (1974), Orellana et al. (1976), Tu (1978), Purkayastha et al. (1981), Shatla et al. (1983) and Chakraborty and Purkayastha (1984).

Nodulation of soybeans by R. japonicum was reduced significantly by root infection with S. rolfsii, R. solani or M. phaseolina, compared with R. japonicum infection alone. S. rolfsii was the most effective fungus tested for reducing soybean nodulation, followed by R. solani, while M. phaseolina was the least effective one. The least number of nodules per plant occurred when the three tested fungi were combined together, followed by S. rolfsii combined with R. solani, whereas S. rlfsii combined with M. phaseolina and R. solani combined with M. phaseolina were less effective in reducing soybean nodulation. This may be due to the toxic effect of fungal metabolities on R. japonicum. Orellana and Worley, (1976) suggested that cell dysfunction in young nodules of soybean grown in the presence of R. solani may be due to the toxic fungal metabolities diffusing throughout the nodule. They added that such dysfunction would interfere with nitrogenase and symbiotic N_2- fixation activities, then the nitrogen supply becoming suboptimal, hence soybean yield becoming reduced. Orellana and Mandava (1983) reported that phytotoxic compounds (m-hydroxiphenylacetic and m-methoxiphenylacetic acid) of R. solani are involved in nodule impairment and reduced N_2-fixation in soybean. The results in the present study are similar to those of Chou and Schmitthenner (1974), Orellana and Worley (1976), Orellama et al. (1976), Shatla et al. (1983) and Zambolim and Schenck (1984).

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