Some Pathogenic Properties of *Rhizoctonia solani* to Sugar Beet Seedlings

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Abstract

Rhizoctonia solani causes various diseases in many crops world wide, it also causes losses in sugar beet grown in Poland and the Netherlands. In this research three isolates of *R. solani* was tested, two AG5 and one AG4. They differed in pathogenic activity in field and laboratory tests. The AG5 isolates were weak pathogenic but caused the decrease of cotyledons and first pair of leaves dimension. The AG4 isolate caused totally pre-emergence damping-off and its activity was the result of mycelium colonization ability and fungus metabolites activity.

Keywords: *Rhizoctonia solani;* sugar beet; plant dimension decrease; pathogenicity; culture filtrate

INTRODUCTION

Rhizoctonia solani survives as a saprotrophyte in soils, starts attacking host tissues when the conditions are favorable. *R. solani* isolated in Minnesota from sugar beet seedlings belonged to various anastomosis groups, but predominated AG4 and AG5 (WINDELS & NABBEN 1989; WINDELS *et al*. 1997). The isolates from AG4 are reported as strong pathogenic to sugar beet seedlings, but some of them are mildly pathogenic or not pathogenic (PAPAVIZAS *et al*. 1975; WINDELS & NABBEN 1989). *R. solani* AG5 are reported as not pathogenic as AG4 but able to significantly reduce stands (WINDELS & NABBEN 1989). One of the possible way of *Rhizoctonia solani* activity is toxins production (SHERWOOD & LINDBERG 1962; FRANK & FRANCIS 1976), although the "mycelial" way of action is also possible (DODMAN *et al*. 1968). The aim of this work was to research the pathogenicity of three isolates of *R. solani* (two AG5 and one AG4) isolated from sugar beet seedlings in Poland. Because of AG4 isolate occurred strongly pathogenic its way of activity was determined.

MATERIALS AND METHODS

In this research three isolates of *R. solani* were used: 101 5D, 2L4 and 9a 1C. All were isolated from diseased sugar beet seedlings in south-west Poland.

Anastomosis groups were determined by pairings with known testers from IRS laboratory and by pectic enzyme patterns (SCHNEIDER *et al*. 1997).

In a field experiment, 18 seeds of Polish cultivar Janus of sugar beet were sown to the microplots randomly infested with three isolates of *R. solani*: 101 5D, 9a, 2L4, control was not infested. Soil was fallow land. Microplots were delimited using buckets (diam. 0.35 m) without bottom, to each plot 145–150 cm^3 of inoculum was added. Inocula were prepared in corn-sand medium by GARRET (1970) method, in this test 2-week old *Rhizoctonias* were used. Each combination of this test was three times repeated. After the emergence, seedlings were observed every several days. Of each plant with damping-off symptoms fungi were isolated by conventional methods. Also condition of seedlings was estimated by measuring the length of cotyledons and first pair of true leaves.

In the laboratory tests the pots with sterilized soil (about 180 cm^3) were amended with 40 cm^3 of 2-week old corn-sand *R. solani* inoculum, in controls sterilized sand was added. In each pot ten pregerminated, healthy seeds were sown and covered with a soil layer.

Because of this that isolate 2L4 was strongly pathogenic was used in laboratory tests. For the isolate 2L4 measurement of its mode of action was

done: first the test of the germination sugar beet seeds in the presence of inoculum 2L4 *R. solani* isolate using differentiated liquid media. Near seeds inocula of the fungus were placed (four inocula 5 mm diam. cutted from two weeks old *R. solani*/ten seeds). Media used were: liquid Czapek Dox medium, soil solution (1 kg of soil + 1 dm^3 of tap water) and tap water. As the control not infested combination with tap water was used. The germination and seedlings condition were estimated.

To check the possibility of toxic properties of fungi metabolites this isolate was grown in liquid Czapek Dox medium. After a month medium was drained off and used in the next experiment as a culture filtrate. In this test seeds were surface sterilized in 3% H_2O_2 and dried. Part of them was pregerminated in sterilized distilled water. The bottoms of the Petri dishes (100 mm diam.) were lined with blotting paper, than 5 cm^3 of filtrates were added to each Petri dish, in controls an equal amount of distilled water was added. Controls were prepared both with seeds and tentatively germinated seeds. To each dish ten seeds or ten seeds with sprouts were placed. After three and five days the health condition of seedlings was estimated as well as the percentage of sprouts dry mass. All laboratory experiments were three times replicated.

Statistical calculation was made by the variance method. Significant differences between averages were measured by Duncan's multiple range test. Data followed by the same letter are statistically not different in tables' columns ($P = 0.05$).

RESULTS

Both anastomosis reactions and pectic enzyme patterns resulted that 101 5D and 9a 1C were AG5 and 2L4 isolate was AG4.

Field experiment shown that *R. solani* 2L4 was strongly pathogenic and caused pre-emergence damping-off, no seedling was obtained in microplots amended with this fungus (Figure 1). In combinations with two other fungi the emergence was worse and a bit slower than in the control (Figure 1), it was mostly because of the emergence lacking. One diseased seedling from both plots inoculated with AG5 *R. solani* and from the control four ones were taken. From them fungi were isolated: on seedling from 9a 1C were *Alternaria alternata* and *Fusarium sambucinum*, on seedling from 101 5D were *R. solani* and *F. oxysporum*, and on four control seedlings were *R. solani* (4 times), *F. sambucinum*, *Gliocladium virens*, *Pythium* sp. (each for one time).

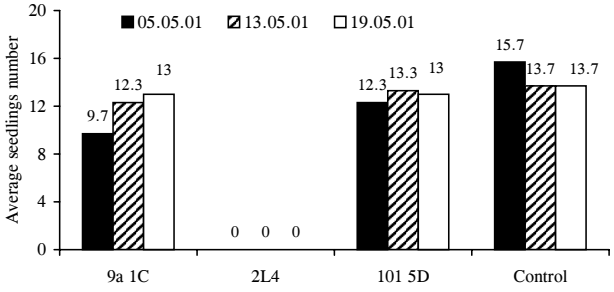

Figure 1. The influence of three *R. solani* isolates on the field emergence of sugar beet

During the field observations it was visible that seedlings from *R. solani* infested plots were smaller, the average dimension of cotyledons and first pair of leaves were decreased comparing to the control (Table 1).

The laboratory tests showed also weak pathogenic activity of AG5 isolates (average 9 seedlings per pot) and 2L4 isolate was also strongly pathogenic causing totally pre-emergence damping-off. The responsibility for this way of pathogenic activity was a result of mycelial seeds colonization and fungus metabolites activity (Tables 2 and 3). The metabolites of 2L4 isolate caused the increase of dry seedlings mass (Table 3). They allowed to some seedlings to emergence but immediately after that sprouts were damaged, they were about a half smaller than control sprouts. Around seeds treated by filtrates darker halos were visible than in the control.

Mycelium of the isolate 2L4 was more active in the variant with soil + tap water and in Czapek Dox, but in this last variant the seeds emergence was very slow and weak. In case of the variant soil + tap water the emergence was not different than in the control, they were rotted and closely coated by *R. solani* 2L4 mycelium. Mycelium coated also seedlings in other infected combinations.

Table 1. Influence of three *R. solani* isolates on the dimension of cotyledons and first pair of true leaves of sugar beet

Isolate	Average length (cm)	
	of cotyledons	of true leaves
9a 1C	3.52 a	3.41 a
101 5D	4.18 b	4.33 b
2L4	no emergence	
Control	5.13 c	6.08 c

*Data indexed by the same letter are not statistically significantly different ($P = 0.05$) in columns

Table 2. Influence of 2L4 isolate of *R. solani* on the germination of sugar beet seeds in different liquid media

	Medium	Germination (%)	Health of sprouts
Infested	Tap water	95.0 b	100% rotted
	Liquid Czapek Dox	7.5 a	100% rotted
	Soil + tap water	80.0 b	100% rotted
Control (tap water) not infested		87.5 b	3.75% rotted

*Data indexed by the same letter are not statistically significantly different ($P = 0.05$) in columns

Table 3. Influence of culture filtrates *R. solani* 2L4 on sugar beet seeds and seedlings

	Seed germination	Pregerminated seeds (%)	
		injured sprouts surface	sprouts dry mass
2L4	36.7 b	85.0 b	9.3 b
Control	66.7 a	5.0 a	6.0 a

*Data indexed by the same letter are not statistically significantly different ($P = 0.05$) in columns

DISCUSSION

The field results of *R. solani* AG5 pathogenicity corresponded to this obtained by WINDELS *et al.* (1997) and WINDELS and NABBEN (1989). In literature frequently are reported AG4 strains strongly pathogenic causing completely pre-emergence damping-off (PAPAVIZAS *et al.* 1975). In this research the totally pre-emergence damping-off of AG4 isolate was obtained in field and in laboratory tests. AG5 strains although were very mildly pathogenic, they caused significant reduction of leaves and cotyledons dimension, this way of action was not reported since now, only inhibition of root growth as well as stimulation caused by some weakly virulent isolates were reported (IACOBELLIS & DEVAY 1987). In some cases toxins also could decrease and increase the root and foliage portion (FRANK & FRANCIS 1976). It is possible that the weakly virulent *Rhizoctonia solani* AG5 can influence of the yield by decreasing the plant dimension.

The AG4 isolate produced strongly pathogenic toxins present in the culture filtrate. Treating seeds or seedlings with culture filtrates always caused the root rot, in some cases not all seedling's surface was rotted during a few day observation. The way of action of filtrates increasing sprouts percent dry weight, it means that the water content was less that in the control, this leads to greater susceptibility of plant (RUBIN & ARCICHOWSKA 1971). Tests showed that in the case of AG4 isolate there is a simultaneous action of hyphal tips and toxic metabolites, although the previous effect of toxins is possible.

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