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DIFFERENTIATION OF *RHIZOCTONIA* SPP. BASED ON THEIR ANTIGENIC PROPERTIES

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Abstract: Antigenic properties and serological relationship was investigated in binucleate and multinucleate *Rhizoctonia* spp. isolates from strawberries, soybean, alfalfa and potato plants from Serbia, from Spain, anastomosis group testers and in strawberry roots inoculated with binucleate *Rhizoctonia* AG A and AG I. Two polyclonal antisera, unabsorbed and cross absorbed, were used in dot-immunobinding assay for these investigations. Antisera were produced against mycelial antigens of two isolates, which belong to different anastomosis groups (AG) of binucleate *Rhizoctonia* - AG A and AG I. Both unabsorbed antisera reacted positively with all tested *Rhizoctonia* spp. isolates, and the reaction was absent with control isolates (*Pythium* sp., *Agaricus* sp. and *Fusarium* sp.). The results prove a close serological relationship among *Rhizoctonia* spp. isolates, and diversity between *Rhizoctonia* spp. and isolates from different taxonomic groups. Also, both unabsorbed antisera reacted with higher intensity with closely related antigens (belonging to the same AG) than with ones from another AG of binucleate *Rhizoctonia* or *R. solani* (multinucleate *Rhizoctonia*). After cross absorption specificity of the antisera was enhanced, especially with the antiserum raised against mycelial proteins of binucleate *Rhizoctonia* AG I. This antiserum reacted positively only with antigens from the same AG, after cross absorption with antigens from AG A of binucleate *Rhizoctonia* and from *R. solani* AG 2-2. It proved to be specific to AG I of binucleate *Rhizoctonia,* and able to differentiate isolates of this AG from others. In this way the serological homology among isolates of one AG was proven, and also the diversity among isolates which belong to different AGs of binucleate *Rhizoctonia* as well as isolates of *R. solani*.

Key words: *Rhizoctonia* spp., binucleate *Rhizoctonia, Rhizoctonia solani,* anastomosis groups, serological identification, serological relationship, dot-immunobinding assay.

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Introduction

Species of *Rhizoctonia* are significant soilborne plant pathogens which cause worldwide important diseases on most of world`s important crop plants, such as: cereals, cotton, sugarbeet, potato, vegetables, field crops, turfgrasses, ornamentals, fruit trees and forest trees. Economic importance, as well as the knowledge of *Rhizoctonia* spp. is growing rapidly but in spite of that there is still some lack of understanding in systematic relationships and genetic variations within and among the complex. The lack of fossil record, absence of the teleomorph, phenotypic variation and pathological diversity are the reasons why *Rhizoctonia* taxonomy is progressing slowly. Identification and differentiation of the species belonging to *Rhizoctonia* complex is difficult if one is using only conventional criteria. The present system of the identification of *Rhizoctonia* spp. isolates which belong to *Thanatephorus* sp. or *Ceratobasidium* sp. (phylum *Basidiomycota)* is based on morphology, pathology, and the ability of their hyphae to anastomose (Ogoshi et al., 1979: Ogoshi, 1987; Burpee et al., 1980; Sneh et al., 1996). To date, 12 anastomosis groups (AG) have been recognized (designated as AG 1 - AG 11 and AG BI) and also 12 subgroups (designated as AG 1-IA, 1-IB, 1-IC, 2-1, 2-IIIB, 2-2IV, 4HG-1, 4HG-II, 6HG-1, 6GV, 9TP and 9TX) in *R. solani,* and there are 22 AGs belonging to binucleate *Rhizoctonia* (Sneh et al., 1991; Ogoshi et al, 1991; Sneh et al., 1996). The concept of AGs and subgroups represents a very successful method for the classification of *Rhizoctonia* spp. but it provides little insight into the genetic diversity of these fungi (Jabaji - Hare, 1996). This is the reason why, in recent years, a lot of attention has been given to new approaches among which serology takes an important place. This paper examines the possibilities of differentiating antigenic properties as well as serological relationship within the *Rhizoctonia* complex using dot-immunobinding assay.

Materials and Methods

Fungal isolates, plant material.- Following isolates were investigated: from strawberries, designated as 4 and P6, belonging to binucleate *Rhizoctonia* AG I (Vico, 1992; 1994a; 1994b), and isolates 4a, 4c, MLE, MČA, M2, SS1 and SS2 belonging to binucleate *Rhizoctonia* AG A (Vico, 1992; 1994a; 1994b: 1997), from soybean, isolate 9, *Rhizoctonia solani* AG 1 (Vico, 1997), from alfalfa, isolate LRh-II, *Rhizoctonia solani* AG 2-2 (Vico, 1997) and from potato, isolate RhKsp, *Rhizoctonia solani* AG 2-2 (Vico, 1997). These isolates originating from different hosts were collected in several localities in Serbia. Identification was based on their morphology, pathogenicity, ecological and cultural characteristics, but also on number of nuclei per cell and hyphal anastomosis (Vico, 1992; 1994a; 1994b; 1997).

Rhizoctonia spp. isolates designated as Rh-L and Rh-R, *Rhizoctonia solani* AG 3 (Vico, 1997), originate from Spain and were supplied by dr Maria Muntanola - Cvetković (Faculty of Biology, University of Belgrade), and also tester isolates of binucleate *Rhizoctonia* AG I and AG A (Ogoshi et al., 1983) were included in the study.

Besides *Rhizoctonia* spp., as control, isolates ŠAM (*Agaricus* sp.,), Pn (P*ythium* sp. from peppermint plants), L-Fus (*Fusarium oxysporum* f.sp. *medicaginis*, from alfalfa) were included in tests.

Tests were performed also with strawberry plants cv. Senga Sengana and hybrid 3/2/88/R, healthy and inoculated with isolate MLE (binucleate *Rhizoctonia* AG A) and isolate 4 (binucleate *Rhizoctonia* AG I).

Antigen preparation.- Antigens used for the production of antisera were prepared according to Adams and Butler (1979) from the mycelia of isolates MLE (binucleate *Rhizoctonia* AG A) and 4 (binucleate *Rhizoctonia* AG I). Protein concentration was determined according to Lowry et al. (1951).

Antigens used for serological studies and those used for cross absorption of the antisera were prepared in Tris-HCl (Adams and Butler, 1979), and in PBS (1g of frozen mycelium homogenized in 4 ml of PBS, centrifuged 1 hour at 1500 g).

Antigens from strawberry plants were prepared by homogenizing 0.1g of roots in 1ml PBS or TBS-T alone or with the addition of the following antioxidants: 0.01 M DIECA with 0.01 M EDTA and 0,1 M ascorbic acid; or 0.01 M DIECA with 0.01 M EDTA and 0.2% β-mercaptoethanol; or 1% Na_2SO_3; or 10% Na_2SO_3 ; or 1% tioglicolic acid; or 0.1 M ascorbic acid with 0.2 ml dihlormethane. The suitability of the antioxidants was checked in tubes (when homogenized samples, after 10 minutes centrifugation at 2000 g, were incubated 3 hours at room temperature and than the color changes were assessed) and after a test on nitrocellulose membrane was performed. Suspension of carpophores of isolate ŠAM and mycelium of isolate L-Fus were used as control. Color changes were assessed after the reaction was developed.

Production of antisera.- Polyclonal antisera raised against mycelial proteins of isolate 4 (binucleate *Rhizoctonia* AG I) and isolate MLE (binucleate *Rhizoctonia* AG A) were produced by immunizing two rabbits. For the first one, during 10 days, 5.8 mg/ml of protein (mycelium of isolate 4), and for the second one 20.9 mg/ml of protein (mycelium of isolate MLE) was injected intramuscularly. After 4 days the booster injection was given, and the blood was taken 10 days later. Antisera raised against the mycelial proteins of isolate 4 was designated as As 1, and antisera raised against the mycelial protein of isolate MLE was designated as As 2. The titer of antibody was determined using agglutination test, Quachterlony agar double - diffusion test and dot-immunobinding assay.

Preparation of antisera for serological tests.- Unabsorbed and cross absorbed antisera As 1 and As 2 were used in serological investigations. Cross absorption

was done by mixing the antigen with antisera in 1:2 ratio, for every step of absorption, according to Adams and Butler (1979).

For the investigations of antigenic properties of *Rhizoctonia* spp. using dot-immunobinding assay the following cross-absorbed antisera were used: As 1+MLE (As 1 cross-absorbed with antigen- isolate MLE), As 2+4 (As 2 cross-absorbed with antigen 4), As 1+MLE+MLE (As 1+MLE cross absorbed with antigen MLE, second step), As 2+4+4 (As 2+4 cross absorbed with antigen 4, second step), As1+RhKsp (As 1 cross absorbed with antigen RhKsp), As 2+RhKsp (As 2 cross absorbed with antigen RhKsp), and As 1+RhKsp+MLE (As 1+RhKsp cross-absorbed in the second step with antigen MLE).

Serological methods.- Agglutination test was used to determine the level of antibody in As 1 and As 2 according to Tošić and Šutić (1977). The first check to determine agglutination was done after 30-50 minutes, but the results were more obvious after a period of 3-4 hours. During that time probes were kept in humid chambers at 4^{o}C.

Quachterlony agar double-diffusion test was used for the assessment of antibody titer in As1 and As 2. The test was done using Ion agar according to Tošić and Šutić (1977) in 1% concentration. 40 g/l of poly-ethilen glicol (PEG, 6000 mol wt.) was added according to Adams and Butler (1979) to enhance the visibility of precipitation lines, and for the same reason the gels were stained in 0.05% Comassie blue in 7% acetic acid and 50% methanol. Destaining was done in 10% methanol and 7.5% acetic acid.

Dot-immunobinding assay was used for determination of antibody level in As 1 and As 2 and for the investigation of the serological relationship of different *Rhizoctonia* spp. isolates. The assay was done according to the method shown in catalog No 170-6545 Bio-Rad Laboratories, Richmond CA, USA, as described by O 'Donnell et al. (1982), modified by Shukla et al. (1989). Antisera dilution 1:100 and 1:1000 were used when titer of antibody was assessed, the dilution 1:500 was used for unabsorbed and dilution 1:50, or 1:25 was used for absorbed antisera during the studies of serological relationship. Goat-anti-rabbit antisera was used in 1:2500 dilution. The reaction was developed for about 3 minutes until lilac-blue color appeared on the paper which was a sign of a positive reaction.

Results and Discussion

Antibody titer.- Two antisera were produced during the investigations: As 1 and As 2. The titer of antibody in As 1 determined by agglutination test is 1/8, and of the As 2 is 1/32. Using agar double diffusion test titer of both As 1 and As 2 was ½, while it was much higher when determined by dot-immunobinding assay when it was 1/1000 for both antisera.

Suitability of buffers and antioxidants for use in dot-immunobinding assay.- Buffers and antioxidants used for antigen preparation from mycelium and

strawberry roots have shown different efficiency. Results of these investigations are shown in Table 1 and 2.

T a b . 1. - Influence of antigen preparation on the intensity of serological reaction

	Antigen[a]											
Antisera	P6 [T]	4	4a	4c [T]	MLE [T]	MČA	Pn [T]	M2	SS1	SS2	9[T]	L-Rh-II
As 1 (1:100) [k]	+++	++++	+++	++	+++	+++	-	+++	+++	++	±	++++
As 2 (1:100)	++[*]	++	+++	±	+++	+++	-	+++	++	+++	++	++

[a]Antigens 4 and P6 are binucleate *Rhizoctonia* AG I; 4a, 4c, MLE, MČA, M2, SS1, SS2 are binucleate *Rhizoctonia* AG A; Pn is *Pythium* sp.; 9 = *R. solani* AG 1; L-Rh-II= *R. solani* AG 2;

[T] Antigens prepared in 0,1M Tris-HCl, others were prepared in 0,01M PBS.

[k]Antiserum As 1 is raised against antigen 4 (binucleate *Rhizoctonia* AG I) and As 2 is raised against antigen MLE (binucleate *Rhizoctonia* AG A), (antisera concentration is shown in brackets).

*Here - responds to negative reaction, ± = very weak positive reaction, + = weak positive reaction, ++ = moderate positive reaction, +++ = strong reaction and ++++ = very strong reaction, respectively.

Reaction intensity depended on the serological relationships of the isolates but also on the buffer used. Antigens prepared in PBS have shown better reactivity than the ones prepared in Tris-HCl buffer.

T a b . 2. - Influence of the antioxidants on color changes of root extracts from strawberry and mushroom extract (*Agaricus* sp.) .

	In tubes						
Extract from	Color change after the use of antioxidants						
	1[*]	2	3	4	5	6	7
Healthy root	+++[**]	+	+	+++	++	-	++
Inoculated root	+++	+	+	+++	++	-	++
Mushroom	+++	++	++	+	++	-	-
	On nitrocellulose paper[a]						
Extract from	Color change after the use of antioxidants						
	1	2	3	4	5	6	7
Healthy root	+	+	±	±	+	-	±
Inoculated root[b]	+	+	+	++	++	++	+
Mushroom	++	-	±	±	±	-	+
Mycelium of isol. 4	+++	/	/	/	/	/	/
Mycelium of L-Fus	-	/	/	/	/	/	/

*Antioxidant 1=0.01M PBS, 2=0.01M DIECA with 0.01M EDTA and 0.1M ascorbic acid in TBS-T, 3=0,01M DIECA with 0,01M EDTA and 0.2% β-mercaptoethanol in PBS, 4=1% Na_2SO_3, 5= 10% Na_2SO_3, 6=1% tioglicolic acid and 7= 0.1M ascorbic acid with 0.2 ml dihlormethane.

**Here - responds to no changes in color, + = barely detectable change of color, ++ = detectable change of color, +++ = very intensive color change and / = not investigated, respectively.

[a] Membrane was developed with As 1 (antigen isolate 4).

[b] Strawberry root inoculated with isolate 4.

Root samples prepared in 1% tioglicol acid and mushroom extracts prepared in 1% tioglicol acid and 0.1M ascorbic acid with 0.2 ml dihlormethane have shown no color changes after 3 hours of incubation in tubes (Table 2.). Also, after the test on nitrocellulose membrane there was no false reactions with antisera used. This means that the most suitable way of sample preparation is with 1% tioglicol acid. Mushroom extracts can also be prepared in 0.01M DIECA, 0.01M EDTA and 0.1M ascorbic acid in TBS-T (Table 2.).

Serological reactions.-Antisera As 1 and As 2, which were produced in the course of investigations when used in dot-immunobinding assay, reacted with homologus but also with other antigens. Enhancement of the antisera and the clasification of serological relationships, that is specific antigenic properties were examined after cross absorbing the antisera. Summary of the results of the serological investigations is presented in Table 3.

Both antisera As 1 and As 2 have shown positive reaction with all *Rhizoctonia* spp. tested, and also with the samples of strawberry roots infected with binucleate *Rhizoctonia* AG A and AG I. The reaction was absent with antigens from other genera (*Agaricus* sp., *Pythium* sp. and *Fusarium* sp.), and with healthy root samples (Table 3. column 1 and 6). Among antigens from the same AG and the homologous antisera the reaction was of the highest intensity. As 1 (raised against antigen 4, binucleate *Rhizoctonia* AG I) had the most intensive reaction with isolates 4 and P6, which both belong to the same AG, as well as with the tester strain for AG I. The reaction was less intensive with isolates members of AG A of binucleate *Rhizoctonia* and isolates of *R. solani.* Antisera As 2 (raised against antigen MLE, binucleate *Rhizoctonia* AG A) behaved similarly as As 1 with his homologous and closely related antigens. The reaction was more intensive with isolates belonging to AG A (4a, 4c, MČA, M2, SS1, SS2 and tester isolate AG A), and less intensive with isolates from binucleate *Rhizoctonia* AG I, as well as *R. solani* isolates. As 1 reacted positively with root extract from strawberry cv. Senga sengana inoculated with isolate MLE and strawberry hybrid 3/2/86/88/R infected with both isolates MLE and 4. There was no reaction of this antiserum with strawberry roots of cv. Senga sengana inoculated with isolate 4. On the contrary, As 2 reacted with extracts from roots of cv. Senga sengana inoculated with both isolates, and the reaction was absent with strawberry hybrid 3/2/86/88/R.

The results obtained in the reactions with unabsorbed antisera show that *Rhizoctonia* spp. isolates are serologically related, and they differ from *Agaricus* sp. with whom they are taxonomically close (all belong to division *Basidiomycota),* from *Fusarium* sp. and *Pythium* spp. with whom they are taxonomically distant. Using dot-immunobinding assay both antisera As 1 and As 2 recognize all *Rhizoctonia* spp. isolates tested, and the way to differentiate AG is the intensity of the reaction. With homologus AG both antisera show the most intensive reaction, while the reaction is weaker with isolates of another AG of binucleate *Rhizoctonia* and isolates of *R. solani* (multinucleate *Rhizoctonia*).

Also both antisera can recognise infected strawberry roots- the reaction is positive. This means that both antisera can be used to detect *Rhizoctonia* spp. in strawberry roots.

Tab. 3. - Serological reactions of *Rhizoctonia* spp. isolates from different hosts, *Rhizoctonia* spp. tester isolates, root samples, healthy and inoculated with isolates of binucleate *Rhizoctonia*, and isolates *Agaricus* sp., *Pythium* sp. i *Fusarium* sp. with unabsorbed and cross absorbed antisera As 1 and As 2.

	Antiserum								
a **Antigen**	As 1	As 1 +MLE	As 1 + MLE+MLE	As 1 +RhKsp	As 1 +RhKs p+MLE	As 2	As 2 + 4	As 2 +4+4	As 2 +RhKsp
	1	2	3	4	5	6	7	8	9
4	++++*	+++	+++	+++	+++	++	-	-	±
P6	++++	+++	+++	++	±	++	±	±	+
4a	+++	++	/	/	/	+++	+	/	/
4c	++	++	/	/	/	+++	+	/	/
MLE	+++	++	+	++	-	+++	+	+	++
MČA	++	++	/	/	/	+++	+	/	/
M2	+++	++	++	++	-	+++	+	+	+++
SS1	++	+	/	/	/	+++	+	/	/
SS2	++	+	/	/	/	+++	+	/	/
AG I	+++	++	/	/	/	++	±	/	/
AG A	++	+	/	/	/	++	+	/	/
9	++	++	±	-	-	++	±	±	+
L-Rh-II	++	+	±	-	-	+	±	±	+
RhKsp	++	+	+	-	-	+	±	±	-
Rh-L	+	±	±	-	-	+	±	-	-
Rh-R	+	±	/	/	/	+	±	/	/
ŠAM	-	-	/	/	/	-	-	/	/
Pn	-	-	-	-	-	-	-	-	-
L-Fus	-	-	/	/	/	-	-	/	/
K/zk	-	-	-	-	-	-	-	-	-
SS/4	-	-	-	-	-	±	-	-	-
SS/MLE	±	±	±	-	-	+	-	-	-
H/4	±	-	/	/	/	-	-	/	/
H/MLE	±	-	/	/	/	-	-	/	/

[a] Isolates 4 and P6= binucleate *Rhizoctonia* AG I, isolates 4a, 4c, MLE, MČA, M2, SS1 i SS2 =binucleate *Rhizoctonia* AG A, AG A and AG I = tester isolates, 9= *R. solani* AG 1, L-Rh-II and RhKsp =*R. solani* AG 2-2, Rh-L and Rh-R =*R. solani* AG 3, isolate ŠAM =*Agaricus* sp., Pn=*Pythium* sp.; L-Fus=*Fusarium oxysporum* f. sp. *medicaginis*, K/zk =healthy strawberry root, SS/4 =strawberry root cv. Senga sengana, inoculated with isolate 4, SS/MLE=strawberry root cv. Senga sengana, inoculated with isolate MLE, H/4=root of strawberry hybrid 3/2/86/88/R inoculated with isolate 4 and H/MLE=root of strawberry hybrid 3/2/86/88/R inoculated with isolate MLE.

*Here, - stands for no reaction, ± = very weak positive reaction, + = weak positive reaction, ++ = moderate positive reaction, +++ = strong reaction, ++++ = very strong reaction and / = not investigated, respectively.

After cross absorption the specificity of the antisera was enhanced. After absorption of the antisera As 1 with antigen MLE, in one and two steps (As 1 +MLE and As 1 + MLE + MLE) (Table 3, column 2 and 3) the reaction with antigens 4 and P6 (AG I) was very strong, but it was also present with members of the other AG (AG A) of binucleate *Rhizoctonia* and isolates of *R. solani.* After absorption with isolate RhKsp (*R. solani)* antisera As 1 reacted positively with isolates of binucleate *Rhizoctonia* while the reaction was absent with isolates of *R. solani* (Table 3, column 4). The result proves serological similarity between *Rhizoctonia solani* isolates, and among the isolates of binucleate *Rhizoctonia* spp. Also serological similarity inside the group of either binucleate or multinucleate *Rhizoctonia* spp. is greater than between the groups. Antiserum cross absorbed in the second step with antigen MLE (As 1+RhKsp+MLE) showed high specificity in reacting only with isolates of AG I to which it was raised against (Table 3, column 5). The results obtained show that it is possible to serologically differentiate isolates from different AGs of binucleate *Rhizoctonia*. Specificity of the antisera was enhanced with every step of cross- absorption and every step eliminated the positive reaction of the whole corresponding AG.

Antisera As 1 +RhKsp can be used to differentiate binucleate *Rhizoctonia* from *R. solani*, and the antisera As 1 +RhKsp+MLE can be used to differentiate AG of binucleate *Rhizoctonia.*

Cross absorbed antisera As 2+4 and As 2+4+4 reacted with strong intensity with homologous antigens (AG A), while the reaction was less intensive with isolates from AG I (P6) of binucleate *Rhizoctonia,* as well as with *R. solani* isolates (9, L-Rh-II and RhKSp) (Table 3, column 7 and 8). Reactions with these antisera show that isolate P6 (binucleate *Rhizoctonia* AG I) is serologically more closely related to isolates of AG A, than isolate 4. On the other hand antisera As 2+RhKsp reacted with stronger intensity with antigens from AG A than from AG I (P6,4), AG 1 (9), AG 2-2 (L-Rh-II)(Table 3, column 9) which showed that isolates 9 and L-Rh-II (*R. solani* AG 1 and AG 2-2) are serologically closer related with isolates of AG A than AG I of binucleate *Rhizoctonia.* Isolate RhKSp (*R. solani* AG 2-2) is serologically closer to AG I of binucleate *Rhizoctonia* spp. Serologically, the most distant from binucleate *Rhizoctonia* are isolates Rh-L and Rh-R (*R. solani* AG 3). This part of investigations shows that antisera As 2+4 and As 2+4+4 differentiate isolates from AG A and AG I of binucleate *Rhizoctonia*, as well as from *R. solani,* based on reaction intensity, and can be used for this purpose.

Serological investigations using both polyclonal and monoclonal antisera raised against mycelial proteins, secreted proteins or lecitins have been used to immunologically differentiate isolates of different AG of *R. solani.* First polyclonal antisera against mycelial proteins of isolates which belong to six AG *R. solani* (AG 1, 2-1, 2-2, 3, 4 and 5) were developed by Adams and Butler

(1979). These antisera distinguished all but the subgroups 2-1 and 2-2 when used in agar double diffusion test. More recently, Mathiew and Broker (1991) serologically differentiated AG 8 from AG 2-1, 3 and 4 with an antisera prepared against secreted proteins of *R. solani* AG 8 isolates. Results of these authors have shown that there are also differences within AG. Although achieving these results polyclonal antisera are still of little use because of their cross-reactivity with several AGs (Jabaji-Hare, 1996). In the attempts to solve this problem monoclonal antisera have shown more specificity. Thorton et al. (1994) produced an antisera specific for *R. solani* AG 4, but Dusunceli and Fox (1992) produced a monoclonal antisera which had a positive reaction with a range of AGs of *R. solani* (AG 1, 2-1,2-2, 3, 4, 5, 6 and 8).

The results of our investigations have shown that cross absorbtion of polyclonal antisera is a reliable and less difficult way of distinguishing among isolates of *Rhizoctonia* spp.

Conclusion

Based on the results obtained in our investigation with polyclonal antisera we can conclude that *Rhizoctonia* spp. differ in their antigenic properties from other taxonomically more or less related genera. When antisera raised against mycelial proteins of binucleate *Rhizoctonia* AG I and AG A are used in dot-immunobinding assay, these antisera react with homologous antigens and related *Rhizoctonia* spp. This means that *Rhizoctonia* spp. are serologically related, but they can easily be differentiated after cross absorption of the antisera which shows the antigenic diversity of these isolates. Also, cross absorption of the antiserum enhances the specificity of an antiserum and when cross absorbed antisera are used in dot-immunobinding assay AG of an unknown *Rhizoctonia* spp. isolate can be identified.

REFERENCES

1. Adams, G. C. Jr. and Butler, E. E. (1979): Serological relationship among anastomosis groups of *Rhizoctonia solani.* Phytopathology 69: 629-633.
2. Burpee, L. L., Sanders, P. L. Cole, H. Jr. and Sherwood, R.T. (1980): Pathogenicity of *Ceratobasidium cornigerum* and related fungi representing four anastomosis groups. Phytopathology 70: 843-846.
3. Dusunceli, F. and Fox R., T., V. (1992): Accuracy of methods for estimating the size of *Thanatephorus cucumeris* populations in soil. Soil Use and Management 8: 21-26.
4. Jabaji-Hare, S. (1996): Biochemical methods. In: *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Ed by Sneh, B., Jabaji-Hare, S., Neate, S. and G. Dijst. Kluwer Academic Publishers, Dordrecht, Boston, London. P.p. 65-71.

5. Lowry, O.H., Rosebrough, N. J., Farr, A. L. and Randall, J.K. (1951): Protein measurement with folin-phenol reagent. J. Biol. Chem. 193: 265-275.
6. Mathiew, J.S. and Broker, J. D. (1991): The isolation and characterization of polyclonal and monoclonal antibodies to anastomosis group 8 of *Rhizoctonia solani.* Plant Pathol. 40: 67-77
7. O'Donnell, I.J., Shukla, D.D. and Gough, K.H. (1982): Electro-blot radio immunosorbent of virus infected plant sap - a powerful technique for detecting plant viruses. J. Virol. Meth. 4: 19-26.
8. Ogoshi**,** A. (1987): Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. Ann. Rev. Phytopathol. 25: 125-143.
9. Ogoshi, A., Cook, R.J. and Bassett, E.N. (1991): *Rhizoctonia* species and anastomosis groups causing root rot of wheat and barley in the Pacific Northwest. Phytopathology 81: 784-788.
10. Ogoshi, A., Oniki, M., Araki, T. and Ui, T. (1983): Studies on the anastomosis groups of binucleate *Rhizoctonia* and their perfect states. J. Fac. Agr. Hokkaido Univ. 61:244-260.
11. Ogoshi, A., Oniki, M., Sakai, R. and Ui, T. (1979): Anastomosis grouping among isolates of binucleate *Rhizoctonia.* Trans. Mycol. Soc. Japan 20: 33-39.
12. Shukla, D. D., Jilka, J., Tošić, M. and Ford, R. E. (1989): A novel approach to the serology of potyviruses involving affinity purified polyclonal antibodies directed towards virus-specific N-termini of coat proteins. J. Gen. Virol. 70: 13-23.
13. Sneh, B., Burpee, L.L. and Ogoshi, A. (1991): Identification of *Rhizoctonia* species. APS Press, St. Paul, Minn. USA.
14. Sneh, B., Jabaji-Hare, S., Neate, S. and G. Dijst (1996): *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Kluwer Academic Publishers, Dordrecht, Boston, London.
15. Thorton, C. R., Dewey, F. M. and Gilligan, C. A. (1994): Development of monoclonal antibody based immunological assays for the detection of live propagules of *Rhizoctonia solani* in the soil. In Modern assays for Plant Pathogenic Fungi: Identification, Detection and quantification. CAB International Oxford, UK. Ed by Schots, A., Dewey, F. M. and Oliver, R. P.p.29-35.
16. Tošić, M. and Šutić, D. (1977): Viroze Bilja - praktikum (Plant viroses - practicum). Fac. of Agriculture, Beograd-Zemun.
17. Vico, I. (1992): Etiološka proučavanja truleži izdanka i korena jagode (Etiological investigation of crown and root rot of strawberries). MSc. Thesis, Fac. of Agriculture, Belgrade – Zemun.
18. Vico, I. (1994a): Black crown and root rot of strawberries in Yugoslavia. Zaštita bilja 45: 53-60.
19. Vico, I. (1994b): Investigation of anastomosis groups of Binucleate *Rhizoctonia* spp. isolated from strawberries. Phytopathol. Medit. 33: 165-167.
20. Vico, I. (1997): Prilog taksonomiji fitopatogenih gljiva roda *Rhizoctonia* DC ex Fr. (Contribution to the studies of the taxonomy of plant pathogenic *Rhizoctonia* DC ex Fr.). Ph.D. Thesis. Fac. of Agriculture, Belgrade – Zemun.

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RAZLIKOVANJE *RHIZOCTONIA* SPP. NA OSNOVU ANTIGENIH OSOBINA

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R e z i m e

Antigene osobine i serološki medjuodnosi ispitivani su kod dvojedarnih i višejedarnih *Rhizoctonia* spp., izolovanih iz jagode, soje, lucerke i krompira prikupljenih na teritoriji Srbije, izolata iz Španije, standard izolata anastomoznih grupa i izolata prisutnih u zaraženom korenu jagode. Ispitivanja su obavljena dot-blot metodom primenom dva poliklonalna antiseruma, pre i posle unakrsne apsorpcije. Antiserumi su proizvedeni na miceliju dva izolata, pripadnika različitih anastomoznih grupa (AG) dvojedarnih *Rhizoctonia* spp. - AG A i AG I. Neapsorbovani antiserumi pozitivno su reagovali sa svim ispitivanim izolatima *Rhizoctonia* spp.,dok su reakcije izostale sa kontrolnim izolatima (*Pythium* sp., *Agaricus* sp. i *Fusarium* sp.), što dokazuje serološku srodnost izmedju izolata *Rhizoctonia* spp., kao i serološku različitost *Rhizoctonia* spp. od izolata drugih taksonomskih grupa. Osim toga, reakcija oba neapsorbovana antiseruma bila je intenzivnija sa srodnim izolatima (pripadnicima iste AG), nego sa izolatima druge AG dvojedarnih *Rhizoctonia* ili *R. solani* (višejedarna *Rhizoctonia*), što govori o većoj serološkoj srodnosti u okviru jedne AG. Unakrsnom apsorpcijom antiseruma srodnost izolata iz iste grupe je i potvrdjena. Specifičnost antiseruma na ovaj način se povećava, što je naročito izraženo kod antiseruma proizvedenog na proteine micelije dvojedarne *Rhizoctonia* AG I. Ovaj antiserum je, nakon apsorpcije antigenom iz AG A i *R. solani* AG-2-2, pozitivno reagovao samo sa homologim antigenima (izolati iz AG I), odnosno ovaj antiserum može razlikovati izolate AG I dvojedarnih *Rhizoctonia* od ostalih *Rhizoctonia* spp. Na ovaj način potvrdjena je serološka srodnost izmedju izolata iste AG, i serološke razlike u odnosu na pripadnike drugih AG kako dvojedarnih *Rhizoctonia* spp. tako i *R. solani.*

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