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Full Length Research Paper

Analysis of *Rhizoctonia solani* isolates associated with sugar beet crown and root rot from Serbia

Vera Stojšin[1], Dragana Budakov[1]*, Barry Jacobsen[2], Ferenc Bagi[1], Eva Grimme[3] and Oliver Neher[3]

[1]Faculty of Agriculture, Department of Environmental and Plant Protection, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia.
[2]Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59715 MT, USA.
[3]University of Idaho, Twin Falls Research and Extension Centre, CSI Evergreen Building 315 Falls Avenue, East Twin Falls, 83301 ID, USA.

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***Rhizoctonia solani* is one of the most important sugar beet pathogens worldwide with anastomosis groups (AGs) 2-2 and 4 as the most pathogenic strains on sugar beet. AG 2-2 (intraspecific groups IIIB and IV) can cause both root and crown rot and damping-off, while AG-4 is typically associated only with seedlings damping-off. A total of 20 isolates of *Rhizoctonia* spp. from sugar beet roots, showing characteristic crown and root rot symptoms, were collected from 4 localities in Serbia. Regarding colony morphology and cultural characteristics, they were divided into 2 groups, which corresponded to their pathogenic, anastomosis and molecular traits. Sequence analysis proved that the first group of isolates were closely related (sequence homology 100%) to AG-4, subgroup HG II, whereas the second group was determined to belong to AG 2-2 IIIB (sequence homology 99%). These two groups differed in range of hosts and in disease intensity on sugar beet, bean and soybean plants. This is the first detailed report on *R. solani* anastomosis groups that cause sugar beet crown and root rot in Serbia.**

Key words: *Rhizoctonia solani*, sugar beet, anastomosis groups, pathogenicity, ITS rDNA.

INTRODUCTION

Plant pathogenic fungus *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) is a polyphagous species which is distributed in soils worldwide (Harveson, 2003). Its heterogenic population causes symptoms on a wide range of hosts (Sneh et al., 1991). *Rhizoctonia* is typically a sterile fungal genus, characterized by division into binucleate and multinucleate groups. *R. solani* is multinucleate and based on hyphal anastomosis between isolates divided into 13 anastomosis groups which may be additionally divided into intraspecific groups (Carling, 1996; Carling et al., 2002; Guillemaut et al., 2003; Sharon et al., 2006).

In the USA, more than 24% of acres planted to sugar beet have economic damage from this pathogen, while in Europe only 5 to 10% of the planted area is considered to have economic losses (Jacobsen, 2006). Similar information for Rhizoctonia crown and root rot in Serbia is not available (Jasnić et al., 2006). However, *R. solani* was isolated from 0 to 18.2% of sugar beets showing typical crown and root rot symptoms during the period from 2000 to 2005 (Stojšin et al., 2006). This fungus causes damping-off, root and crown root, as well as foliar blight. According to Windels and Nabben (1989), *R. solani* AGs -1, -2-2, -4 and -5 can cause damping-off of sugar beet. Additionally, AG-3 and AG-5 were isolated from sugar beet with symptoms of dark discoloration on petiole basis (Windels et al., 1997). The causal agent of Rhizoctonia crown and root rot of sugar beet is typically characterized as belonging to AG 2-2 with individual isolates being placed into intraspecific groups IIIB and IV.

In this study, a detailed description of cultural characteristics, anastomosis group and pathogenicity of *R. solani* isolates from sugar beet root with a typical crown

Corresponding author: E-mail: dragana.budakov@gmail.com. Tel: +381(0)691655691. Fax: +381(0)21450616.

and root rot symptoms, which originate from Serbia, was given.

MATERIALS AND METHODS

Isolates

Isolates used in this study were collected during vegetative period in 2005 from five localities (Belgrade, Veliki Radinci, Kuzmin, Golubinci and Srpski Miletić) in Vojvodina Province, Serbia. Isolations were done on WA, after which isolates were hyphal tipped, transferred to slant PDA and kept on 4°C until further investigation. Additionally, two well-characterized *R. solani* isolates from the USA: R9 (AG 2-2 IV) and NBR1 (AG 2-2 IIIB) were also included.

Morphological characteristics

Tested isolates were examined for macroscopic characteristics typical of *R. solani*, such as development and change of mycelial colour and sclerotia formation. Isolates were incubated on PDA in an incubator at 25 to 26°C in the dark for 10 days. Microscopic characteristics such as mycelial appearance, branching and septation of hyphae and existence of multinucleate cells were also determined (Bandoni, 1971).

Cultural characteristics

Growth and the appearance of mycelia, sclerotia and teleomorph formation were examined on PDA at eight different temperatures (5, 10, 15, 20, 25, 30, 35 and 38°C). Minimal, maximal and optimum temperatures for growth were determined. Fungal growth and development were observed for 10 days.

Pathogenicity tests

Pathogenicity of tested *R. solani* isolates was evaluated by artificial inoculations, on 9 week old sugar beet plants of susceptible cultivar Delta. Inoculum was produced on sterile barley seeds, which were inoculated with *R. solani* isolates (Gaskill, 1968). Sterile barley seeds inoculated with sterile media was used as control. Plants were inoculated using method that was set up by Windels and Nabben (1989). The soil was scraped away from the sugar beet root to the depth of 2 to 2.5 cm with a sterilized spatula, and 2.5 cm^3 of barley grain inoculum was placed in the hole in contact with taproot. Experiment was performed in a greenhouse with 25 to 26°C temperature and daily irrigation. Wilting and decay of leaves as well as necrosis of petal basis was observed 2, 3 and 4 weeks after inoculation. Scale from 1 to 5 was used for decay assessment, where 1 = healthy plants, 2 = 1 leaf with petiole base necrosis, 3 = 2 to 4 leaves with petiole base necrosis, 4 = more than 4 leaves with petiole base necrosis, and 5 = completely decayed plant. Root rot was evaluated 4 weeks after inoculation, when all plants were removed from pots. Root disease index scale from 1 to 5 was used, where 1 = no symptoms, 2 = less than 25% root rot, 3 = 25 to 50% root rot, 4 = 50 to 75% root rot and 5 = 75 to 100%.

Moreover, based on knowledge on host range of anastomosis groups associated with sugar beet and experience about which species are rotated with sugar beet in Serbian conditions, 5 field crops were selected to determine pathogenicity of *R. solani* isolates. Those were: Wheat (*Triticum vulgare*)-cultivar Balerina, maize (*Zea mays*)-cultivar NS 640, soybean (*Glycine max*)-cultivar Balkan, bean (*Phaseolus vulgaris*)-cultivar 20 and potato (*Solanum tuberosum*)-cultivar. Marlene Inoculum was produced on sterile barley grains (Gaskill, 1968). Inoculation was performed during planting. Plants were grown under controlled temperature conditions (25 to 26°C) and were watered daily. Pathogenicity evaluation was performed after 2 weeks. The root disease index was based on visual rating scale from 0 to 5, where 0 = healthy plants, 1 = sporadic necrotic spots on the lower part of the stem, 2 = fusion of the necrotic spots without forming of lesions, 3 = necrotic lesions, 4 = necrotic lesion ringed lower part of the stem, 5 = completely decayed plants. Furthermore, in order to fulfil Koch's postulates, re-isolations were done from plant tissues with characteristic *R. solani* symptoms and pure cultures were tested for features typical of *R. solani*.

Hyphal anastomosis reaction

Anastomosis tests were performed using the method described by Liu and Sinclair (1991). Prior to microscopic examination samples were dyed with cotton blue in dilute lactophenol (Parmeter et al., 1969). Previously characterized isolates from the USA: R9 (AG 2-2 IV) and NBR1 (AG 2-2 IIIB) were used as standards and representatives of anastomosis group most pathogenic on sugar beet. Hyphae were examined on a compound microscope at 400 ×, while the absence of septum was confirmed at 1000 ×. Examination of the reaction between hyphae was categorized according to Carling (1996).

Molecular characteristics

DNA was extracted from 100 mg ground fungal tissue using the Fungal DNA Kit (Omega Bio-Tek Inc., Lilburn, GA, USA) and following the protocol recommended by the manufacturer. Internal transcribed spacer region of ribosomal DNA was amplified using ITS 1F and ITS 4 set of primers that were primarily described by White et al. (1990). The PCR reaction was performed in 50 µL total volume consisting of 25 µL of PCR Master Mix (Promega Corporation, Madison, WI, USA), 5 µL of $MgCl_2$, 2 µL of each primer (concentration 10 pmol μL^{-1}), 2 µL of DNA template and 14 µL of PCR Grade water. The amplification was performed in PCR thermal cycler (Whatman Biometra, Goettingen, Germany). The cycle parameters were: An initial denaturation (95°C, 2 min), 38 cycles of denaturation (95°C, 1 min), annealing (55°C, 45 s) and extension (72°C, 1 min). Final extension was at 72°C for 5 min.

DNA sequencing and data analysis

After the amplification of the ITS region of the rDNA, each product was purified using the QIAquick PCR Purification Kit and protocol (Qiagen Inc., Valencia, CA, USA). Purified rDNA was sequenced in DNA Sequencing Facility at University of California, Berkeley.

Analysis of ITS sequences was performed using on-line software CLUSTALW. Sequence data base of National Centre for Biotechnology Information-NCBI) – GenBank, which was entered via web page www.ncbi.nlm.nih.gov, was used for information on *R. solani* isolates (Table 2).

RESULTS

Morphological characteristics

A total of 22 isolates from sugar beet roots with typical symptoms of Rhizoctonia root rot was isolated (Table 1).

Table 1. Optimum growth temperature and root rot disease index of *Rhizoctonia solani* isolates used in this study.

Rhizoctonia spp. isolate code	Locality	Optimum growth temperature (°C)	Root disease index			
			Maize	Sugar beet[a]	Bean[b]	Soybean[b]
RhBG 1	Belgrade	25 - 30	-	4.10	-	-
RhBG 2	Belgrade	25 - 30	-	4.40	-	-
RhBG 3	Belgrade	25 - 30	-	4.00	-	-
RhBG 4	Belgrade	25 - 30	-	4.50	-	-
RhBG 5	Belgrade	25 - 30	-	4.40	-	-
RhVR 1	Veliki Radinci	25 - 30	-	2.90	-	-
RhVR 2	Veliki Radinci	25 - 30	-	3.00	-	-
RhVR 3	Veliki Radinci	25 - 30	-	3.05	-	-
RhVRT 1	Veliki Radinci	25	2.20	5.00	1.95	2.45
RhVRT 2	Veliki Radinci	25	-	4.90	-	-
RhVRT 3	Veliki Radinci	25	-	4.95	-	-
RhVRT 4	Veliki Radinci	25	-	4.80	-	-
RhKZ 1	Kuzmin	25 - 30	-	2.65	-	-
RhKZ 2	Kuzmin	25 - 30	0.00	2.50	1.15	2.80
RhKZ 3	Kuzmin	25 - 30	-	2.60	-	-
RhKZ 4	Kuzmin	25 - 30	-	2.60	-	-
RhGL 1	Golubinci	25 - 30	-	3.00	-	-
RhGL 2	Golubinci	25 - 30	-	3.20	-	-
RhGL3	Golubinci	25 - 30	-	3.25	-	-
RhGL 4	Golubinci	25 - 30	-	3.15	-	-
R9	Montana, USA	25	-	4.85	-	-
NBR1	Montana, USA	25	-	4.70	-	-

[a] Percent of sugar beet root discoloration due to infection by *Rhizoctonia solani* based on the root disease index scale from 1 to 5, where 1 = no symptoms, 2 = less than 25% root rot, 3 = 25 to 50% root rot, 4 = 50 to 75% root rot and 5 = 75 to 100% root rot. Values calculated on ratings of 20 plants per isolate. [b] Disease index scale based on visual description of symptoms from 0 to 5, where 0 = healthy plants, 1 = sporadic necrotic spots on the lower part of the stem, 2 = fusion of the necrotic spots without lesions, 3 = necrotic lesions, 4 = necrotic lesion ringed lower part of the stem, 5 = completely decayed plants. Values calculated on ratings of 20 plants per isolate.

Two isolates (SM6 and SM7 from Srpski Miletić) were proven to be binucleate and therefore, excluded from further research. All other isolates (RhBG1, RhBG2, RhBG3, RhBG4, RhBG5, RhVR1, RhVRT2, RhVR3, RhKZ1, RHKZ2, RhKZ3, RhKZ4, RhGL1, RhGL2, RhGL3, RhGL4, RhVRT1, RhVRT2, RhVRT3 and RhVRT4) showed typical features of *R. solani* complex including brown pigmentation of hyphae, branching near distal septum, constriction of hyphae and formation of septum short distance from the place of branching, the presence of dolipore septa and multinuclear cells in young vegetative hyphae (Parmeter and Whitney, 1970).

Cultural characteristics

None of the tested isolates grew on 5, 35 and 38°C. Regarding cultural characteristics, isolates were divided into 2 groups. First group included isolates RhVRT1, RhVRT2, RhVRT3, RhVRT4, R9 and NBR1, which showed slower growth in average on all temperature values in comparison with the second group of isolates. Their optimum temperature was 30°C and they did not show any activity on 10°C. The second group of isolates (RhBG1, RhBG2, RhBG3, RhBG4, RhBG5, RhVR1, RhVRT2, RhVR3, RhKZ1, RHKZ2, RhKZ3, RhKZ4, RhGL1, RhGL2, RhGL3 and RhGL4) had temperature optimum between 25 and 30°C and they grew on 10°C. Concentric zonation was more evident on lower temperatures (10 and 15°C), especially with the second group of isolates. Sclerotia started to form after 4 to 5 days and after 8 to 10 days on temperatures of 20, 25 and 30°C and 10 and 15°C, respectively. The second group of isolates on lower temperatures produced abundant small, white sclerotia on the surface of the agar. In addition, teleomorph formation (basidia and basidiospores) did not occur during 10 day period on neither of tested isolates.

Pathogenicity tests

At the end of all pathogenicity experiments, re-isolations were done from all examined plant species with typical

Table 2. *Rhizoctonia solani* sequences recovered from the GenBank (National Center for Biotechnology Information – NCBI) and sequence comparison with Serbian isolates RhKZ, RhBG, RhGL, RhVR and RhVRT1 using Clustal W.

AG and subset	Host and geographic origin	GenBank accession number	Sequence similarity				
			RhKZ1 (%)	RhBG4 (%)	RhGL3 (%)	RhVR3 (%)	Rh VRT1 (%)
AG 1-IA	*Oryza sativa*, Japan	AB000017	94	94	94	94	93
AG 1-IB	*Beta vulgaris*, Japan	AB000038	91	91	91	91	94
AG 1-IC	*Beta vulgaris*, Japan	AB122142	93	93	93	93	93
AG 2-1	*Solanum tuberosum*, USA	AB000026	94	94	94	94	94
AG 2-2IIIB	*Beta vulgaris*, USA	AB054857	92	92	92	92	99
AG 2-2 IV	*Beta vulgaris*, USA	AB054859	93	93	93	93	97
AG 2-3	*Glycine max*, Japan	AB054870	94	94	94	94	92
AG 3	*Beta vulgaris*, USA	AB019006	93	93	93	93	90
AG 3PT	*Solanum tuberosum*, USA	AB019013	93	93	93	93	93
AG 3TB	*Nicotiana tabacum*, USA	AB000001	95	95	95	95	91
AG 4 HGI	*Beta vulgaris*, Japan	AB000028	96	96	96	96	91
AG 4 HGII	*Beta vulgaris*, Japan	AB000033	100	100	100	100	93
AG 4 HGIII	*Beta vulgaris*, USA	AF354075	97	97	97	97	91
AG 5	*Beta vulgaris*, Japan	AF153777	92	92	92	92	92
AG 6	*Pterostylis acuminata*, Australia	AF153784	94	94	94	94	92
AG 6GV	Soil, Japan	AF354101	95	95	95	95	94
AG 6HGI	Soil, Japan	DQ301740	93	93	93	93	91
AG 7	Soil, Japan	AB000003	95	95	95	95	94
AG 8	*Triticum aestivum*, Australia	AB000011	95	95	95	95	90
AG 8ZGI-1	Soil, Australia	AF153795	95	95	95	95	93
AG 8ZGI-2	Soil, Australia	AF153797	95	95	95	95	90
AG 8ZGI-3	*Hordeum vulgare*, Australia	AF354068	95	95	95	95	93
AG 8ZGI-4	*Hordeum vulgare*, Scotland	AF354066	91	91	91	91	84
AG 9	*Solanum tuberosum*, USA	AF354109	94	94	94	94	91
AG 9TX	*Solanum tuberosum*, USA	AB000037	94	94	94	94	91
AG 9TP	*Solanum tuberosum*, USA	AB000046	94	94	94	94	91
AG 10	*Hordeum vulgare*, Australia	AF354071	90	90	90	90	92
AG11	*Glycine max*, USA	AF354114	92	92	92	92	92
AG12	*Pterostylis acuminata*, Australia	AF153803	93	93	93	93	94
AG BI	Soil, Japan	AB000044	94	94	94	94	92

symptoms. *R. solani* was successfully re-isolated to confirm Koch's postulates. The results of the research showed that all examined isolates proved to be virulent on sugar beet. Determination of the area under the disease progress curve (AUDPC) according to Wolf and Veerret (2002) was used to detect differences in pathogenicity among tested isolates (Figure 1). The fastest sugar beet decay caused isolates RhVRT1, RhVRT2, RhVRT3, RhVRT4, R9, NBR1, RhBG1, RhBG2, RhBG3, RhBG4 and RhBG5, while the slowest disease development was determined on plants inoculated with isolates RhVR1, RhVRT2, RhVR3, RhKZ1, RHKZ2, RhKZ3, RhKZ4, RhGL1, RhGL2, RhGL3 and RhGL4. Root rot index varied from 2.5 (RhKZ1 and RhKZ2) to 5 (RhVRT1).

More also, isolates with the lowest and the highest pathogenicity on sugar beet were taken for further research and pathogenicity test on maize, wheat, bean, soybean and potato. None of the tested isolates caused symptoms on wheat and potato. Symptoms were detected on maize, bean and soybean plants with reddish-brown lesions which formed on the root and lower part of the stem. On bean plants, isolate RhVRT1 was more virulent than RhKZ2, while on soybean plants isolate RhKZ2 was more aggressive than RhVRT1 (Table 1). On maize, however, only isolate RhVRT1 proved to be pathogenic.

Anastomosis group reaction

Based on anastomosis group reaction, all tested isolates were also divided into two groups. Isolates RhBG1, RhBG2, RhBG3, RhBG4, RhBG5, RhVR1, RhVRT2, RhVR3, RhKZ1, RHKZ2, RhKZ3, RhKZ4, RhGL1, RhGL2, RhGL3 and RhGL4 did not anastomose with

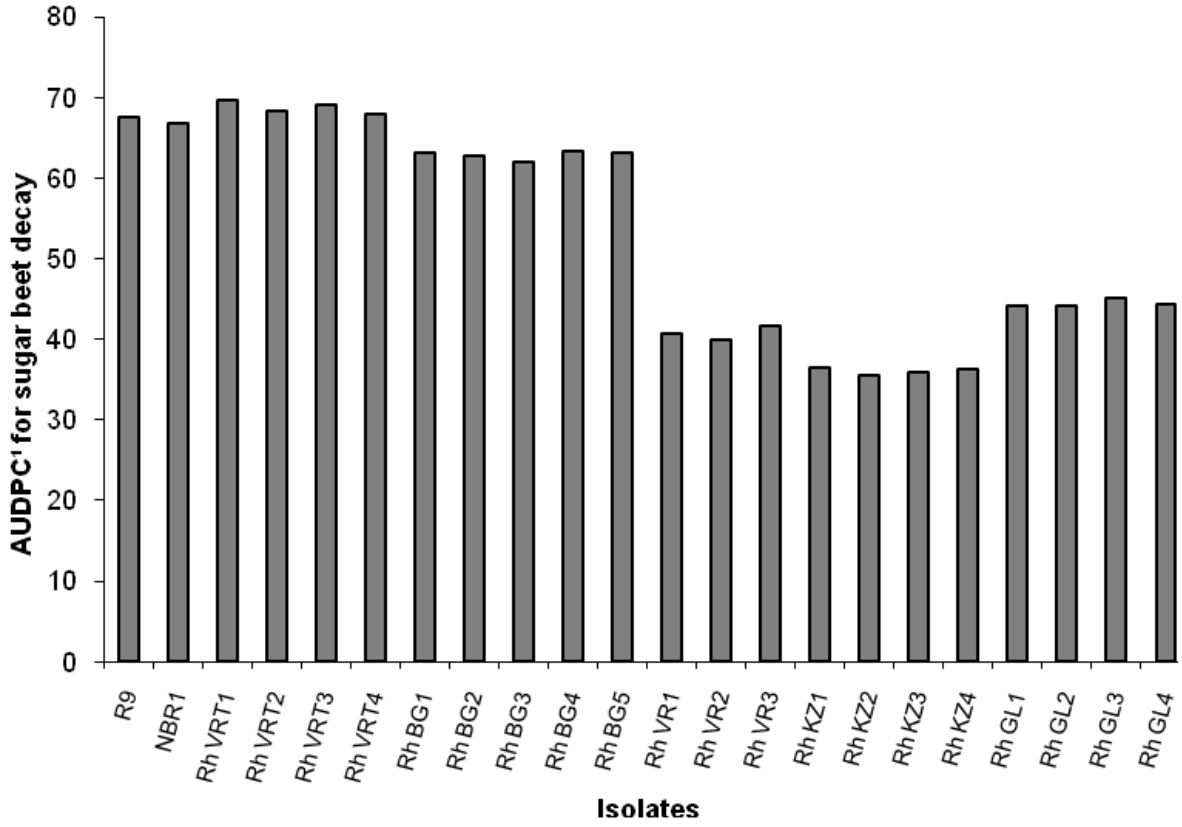

Figure 1. Sugar beet decay in period of 4 weeks after inoculation, based on weekly data. ¹AUDPC = Area under disease progress curve calculated based on three individual ratings using the formula $\Sigma (Y_{i-1}+Y_i)/2 * (t_i - t_{i-1})$.

standard isolates R9 (AG 2-2 IV) and NBR1 (AG 2-2 IIIB) from the USA, but since they anastomosed with each other it was concluded that isolates belong to the same anastomosis group. The second group consisted of isolates RhVRT1, RhVRT2, RhVRT3 and RhVRT4, which anastomosed perfectly with isolate R9 (C3), while with isolate NBR1, hyphal fusion with adjacent cell dying was observed (C2). Based on this, it was concluded that isolates RhVRT1-RhVRT4 belong to the group 2-2.

Molecular characteristics of isolates

Based on morphological, cultural, pathogenicity tests and anastomosis group determination, all isolates showed distinctive characteristics and were divided into two groups. The first group consisted of isolates RhVRT1, RhVRT2, RhVRT3 and RhVRT4 - all from Veliki Radinci, while the second group included other isolates from the same locality (RhVT1, RhVT2 and RhVR3), then isolates from Belgrade (RhBG1, RhBG2, RhBG3, RhBG4 and RhBG5), Golubinci (RhGL1, RhGL2, RhGL3 and RhGL4) and Kuzmin (RhKZ1, RhKZ2, RhKZ3 and RhKZ4). For rDNA sequencing, representatives from each group and locality were chosen. Within each locality, isolates with the highest pathogenicity on sugar beet root were molecularly characterized. Isolates RhVRT1, RhVR3, RhKZ1, RhGL3 and RhBG4 showed that the size of the ITS region varied from 713 to 716 base pairs. Sequences were compared between themselves and with 30 isolates (CLUSTALW), randomly chosen representatives of *R. solani* AGs-1 to 12, whose sequences were downloaded from GenBank (Table 2).

Based on cross-comparison, tested isolates were further divided into two groups. The first group consisted of isolates RhKZ1, RhBG4, RhGL3 and RhVR3, whose sequences were completely identical (100%) in cross-comparison. Similarity of these sequences with randomly chosen representatives from AGs 1-12 (CLYSTAL W) showed that homology ranged from 90 to 100% (Table 2). ITS sequences of tested isolates were 100% compatible with the representatives of the AG-4 HG II. Isolate RhVRT1 had the highest sequence similarity with isolate AB054857 that belong to AG 2-2 IIIB (99%).

DISCUSSION

This is the first detailed report representing the characteristics of *R. solani* on sugar beet in Serbia with regard to its morphological and cultural features, pathogenicity as well as anastomosis group composition. Diseases

caused by *R. solani* from soybean, potato and alfalfa in Serbia were studied by Vico (1997) and isolates from soybean were identified as AG 1-IA and from alfalfa and potato as AG 2-2IV. Further, the occurrence of this fungus in Serbia was associated with ornamental plants, such as chrysanthemum (Vico et al., 2005). *R. solani* has been reported to cause Rhizoctonia root and crown rot on Serbian sugar beet fields from the year of 2000. Since then, it was sporadically present in up to 18.2% of the isolations from the roots with root and crown rot symptoms (Stojšin et al., 2006). However, it is considered that *Rhizoctonia* spp. is about to increase its significance in forthcoming years. This might occur due to climatic changes and global increase of temperature which will inevitably bring introduction of irrigation in sugar beet growing practice in Serbia. All these factors in combination with constantly present inoculum in the soil will certainly cause the raise of disease intensity (Budakov, 2008).

Integrated research of morphological, pathogenic and molecular characteristics serves for the determination of groups and subgroups in *R. solani.* The importance of correct determination of anastomosis groups within *R. solani* complex is very important because of different virulence levels present at different anastomosis groups (Carling et al., 2002). Every description of *R. solani* isolates on the level of colony morphology is recommended to be carried out together with the incubating on different temperatures (Liu and Sinclair, 1991). Faster growth of AG 4 in comparison with AG-2 on the same temperature regime was previously described (Bandy et al., 1984; Ogoshi, 1972). It has been determined that isolates belonging to AG 2-2 have optimal growth on the temperature of 30°C, while on the lower temperatures (10 and 15°C) concentric zonation is more expressed (Liu and Sinclair, 1991). Differences in optimal temperature for the growth of isolates belonging to anastomosis group 2-2 and 4 have been spotted by Tomaso-Peterson and Trevthan (2007) and according to them AG 4 has a lower temperature optimum (25 °C) than AG 2-2 (27 °C).

The results of this research showed that isolates RhBG1-RhBG5, RhVR1-RhVR3, RhKZ1-RhKZ4 and RhGL1-RhGL4 (AG 4 HGII) have temperature optimum between 25 and 30°C, while the optimum for the isolates RhVRT1-RhVRT4, R9 and NBR1 was 30°C. In this research, it has been determined that classification of the isolates on the basis of the required temperature and also on the growth intensity in certain temperature conditions, clearly shows the presence of differences between isolates from anastomosis group 2-2 and 4. Accurate determination of anastomosis groups in *R. solani* is extremely important. However, methods based on hyphal anastomosis are usually inaccurate, and often prone to subjective ranking. Additionally, they have been proven to be unreliable when it comes to subset identification (Carling et al., 2002). One of the most important techniques for characterizing groups and subgroups of *R. solani* is determination of virulence because members of the same group often share same pathogenicity level and host range (Sneh et al., 1991). As for morphological and cultural characteristics, it has been reported that *R. solani* isolates from turf grasses show enough diversity in colony colour and sclerotia formation that they can be preliminary assigned to a certain anastomosis group (Zhang and Dernoeden, 1995). In these studies, isolates of medium pathogenicity on sugar beet demonstrated more plasticity and faster growth on range of temperatures than the one with the highest pathogenicity.

Strausbaugh et al. (2011) also reported that the majority of tested *R. solani* isolates from sugar beet grown in Intermountain West region in the USA were from AG 2-2 IIIB and AG-4. Genetic heterogeneity between and within anastomosis groups was evaluated by Fenille et al. (2003) using sequence analysis of the ITS region of the ribosomal DNA. Comparison of the ITS region is significant not only for the determination of anastomosis groups, but these sequences are also useful for verifying subsets. Polymorphism between AGs was revealed in ITS1 and ITS2 sequences of ribosomal DNA, while 5.8s rDNA sequence is completely conservative across all AGs (Kuninaga et al., 1997). In this research, 80% of examined isolates were determined to belong to AG 4 HGII, with ITS sequence homology with referent AG 4 HGII isolates of 100%. In general, AG 4 isolates are known to be pathogenic on wide variety of hosts (Kuninaga et al., 1997), including sugar beet (Windels and Nabben, 1989). AG 4 HG-II is pathogenic on soybean seedlings causing damping-off and hypocotyl rot (Fenille et al., 2000), on turf grass (Hsiang, 2000), on coffee seedlings (Kuramae et al., 2000) and on sugar beet (Sneh et al., 1991; Gonzalez et al., 2006). Other isolates (RhVRT1-RhVRT4) were determined as AG 2-2 IIIB and described as the most pathogenic on sugar beet. While *R. solani* AG 2-2 IIIB and IV can both cause damping – off and crown and root rot of sugar beet and can attack *Phaseolus* sp. and soybean, they differ in that AG 2 – 2 IIIB can attack wheat, maize, rice and matt rush whereas AG 2 – 2 IV does not attack wheat or maize (Jacobsen, 2006).

This research showed the importance of identifying the correct AG of *R. solani* associated with crown and root rot of sugar beet since the host range of AG 2-2 IIIB and IV are very much different than that of AG 4 (Sneh et al., 1991; Windels and Nabben, 1989). These differences can affect the recommended crop rotations used to partially control the crown and root rot disease of sugar beet.

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