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Original scientific paper

Conventional and real-time PCR assays for detection and identification of *Rhizoctonia solani* AG-2-2, the causal agent of root rot of sugar beet

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SUMMARY

Soil-borne fungi belonging to the genus *Rhizoctonia* are considered to be among the most destructive sugar beet pathogens. Although multinucleate *R. solani* AG-2-2 is frequently detected as the main causal agent of root rot of sugar beet worldwide, several binucleate (AG-A, AG-E and AG-K) and multinucleate *Rhizoctonia* (*R. solani* AG-4, AG-5 and AG-8) have also been included in the disease complex. Due to their soil-borne nature and wide host range, the management of Rhizoctonia root rot of sugar beet is highly demanding. Identification of *Rhizoctonia* AG associated with root rot of sugar beet is the essential first step in determining a successful disease management strategy. In this paper we report a highly specific and sensitive real-time PCR protocol for detection of *R. solani* AG-2-2 which showed a high level of specificity after testing against 10 different anastomosis groups and subgroups, including AG-2-1 as the most closely related. Moreover, a similar conventional PCR assay showed the same specificity but proved to be at least a 100 times less sensitive. Future research will include further testing and adaptation of this protocol for direct detection and quantification of *R. solani* AG-2-2 in different substrates, including plant tissue and soil samples.

Keywords: *Rhizoctonia solani* AG-2-2; sugar beet; morphology; molecular identification; specific detection, real-time PCR

INTRODUCTION

Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is an important crop in European regions with moderate climate where it is grown for its high sugar content (Wibberg et al., 2016). As a crop with high monetary return (Wolf & Verreet, 2002), sugar beet was in 2016 cultivated on over 1.7 million ha in the EU (http:/fao.org/faostat). In Serbia, sugar beet is also considered to be a valuable and profitable crop. In 2017 it was grown on almost 50,000 ha (www.stat.gov.rs). Root diseases of sugar beet, most frequently caused by *Rhizoctonia solani, Macrophomina phaeseolina, Fusarium oxysporu*m, *Pythium* spp., *Phytophthora* spp., and other agents, are an important constraint to profitable production worldwide, causing yield reductions of up to 50% (Jacobsen, 2006).

In Europe and the USA, the most important sugar beet root diseases are caused by worldwide distributed, soil-borne *Rhizoctonia* spp., subdivided into numerous anastomosis groups and subgroups (Ogoshi, 1976). Several multinucleate and binucleate *Rhizoctonia* have been described as sugar beet pathogens. In some sugar beet growing regions, binucleate AG-A, AG-E and AG-K (Strausbaugh et al., 2011; Miles et al., 2013), as well as multinucleate *R. solani* AG-4, AG-5 and AG-8 have been involved in the Rhizoctonia root rot disease complex (Olaya & Abawi, 1994; Strausbaugh et al., 2011), while multinucleate *R. solani* AG-2-2 is frequently detected as the main causal agent (Kiewnick et al., 2001; Jacobsen, 2006; Bolton et al., 2010, Abbas et al., 2014). Symptoms related to *Rhizoctonia* infections of sugar beet mainly include seedling damping-off and root rot with a characteristic presence of circular, necrotic lesions covering large portions of root (Harveson et al., 2009), as well as common above-ground symptoms of wilting and leaf chlorosis. Long-term persistence of *R. solani* is secured over many years via mycelium and sclerotia in crop debris. Under favorable conditions, sclerotia germinate to infect sugar beet roots (Harveson et al., 2009; Kiewnick et al., 2001; Jacobsen, 2006).

Managing Rhizoctonia root rot of sugar beet is difficult due to the soil-borne nature and wide host range of the pathogen (Allen et al., 1985; Kiewnick et al., 2001). Considering the known differences among AGs regarding host range and fungicide sensitivity, reliable and fast detection and identification of *Rhizoctonia* AG associated with root rot of sugar beet is the essential first step in developing a successful disease management strategy (Buhre et al., 2009; Amaradasa et al., 2014; Lakshman et al., 2016). Determination of AG composition of *Rhizoctonia* spp. by morphology-based identification or by isolation, followed by sequencing and phylogenetic analyses, is labor-intensive and time-consuming. Protocols for specific detection based on conventional (end point) PCR are available for several AGs, such as *R. solani* AG-1, AG-2 (Matsumoto, 2002; Salazar et al., 2000) and AG-3 (Lees et al., 2002). Real-time PCR based on specific detection is also available for several *Rhizoctonia* AGs, such as *R. solani* AG-1-IA (Sayler & Yang, 2007), AG-3 (Lees et al., 2002), AG-2-1 (Sturrock et al., 2015), AG-2-2 (Abbas et al., 2014), and AG-8, AG-10, AG-I, and genotypes of *R. oryzae* (Okubara et al., 2008).

In Serbia, root rot is one of the most important diseases of sugar beet. Until 2000, *Fusarium* spp. and *Macrophomina phaesolina* had been considered the most prevalent pathogens of sugar beet in Serbia (Marić et al., 1970). Since then, *R. solani* has been reported to be widely distributed (Stojšin et al., 2006), causing quantitative and qualitative reductions in yield of sugar beet (Vico et al., 2006). First data on molecular characterization confirmed the presence of *R. solani* AG 4-HGII (Stojšin et al., 2007) and *R. solani* AG 2-2 (Stojšin et al., 2011). As population diversity of *Rhizoctonia* spp. in any particular region may change due to different factors (Fenille et al., 2002; Hua et al. 2014), the main objectives of this study were to: (i) characterize *Rhizoctonia* isolates infecting sugar beet in Serbia based on morphological features and AG pairing; (ii) identify isolates by sequencing their ITS region of rDNA (ITS1, 5.8S rDNA and ITS2 regions), and (iii) develop a specific and sensitive conventional and real time PCR protocols for detection and identification of *R. solani* which is pathogenic to sugar beet in Serbia.

MATERIAL AND METHODS

Fungal isolation

Isolates of *Rhizoctonia* were obtained from symptomatic sugar beet plants collected in the localities of Vašica and Adaševci (Srem District, Serbia) during 2015. Up to 10 randomly distributed samples with symptoms were collected per each locality, then stored at 5°C and processed within 24-48 h. Tissue fragments from the border between necrotic and healthy root tissue were thoroughly washed with tap water for 2 h, surface sterilized for 2-3 min with 50% commercial bleach (2 % sodium hypochlorite), placed on potato dextrose agar (PDA; 200 g potato, 20 g dextrose, 17 g agar and 1 liter of distilled H_2O) and incubated at 24°C

for 3-5 days. *Rhizoctonia*-like colonies were transferred onto fresh PDA and water agar (WA, 17 g agar and 1 liter of distilled H_2O) in order to obtain hyphal tip isolates. The isolates were maintained on PDA slants at 4°C in the Fungal Collection of the Department of Phytopathology, Institute of Phytomedicine, University of Belgrade - Faculty of Agriculture.

Morphological identification and AG determination

Morphological characterization of *Rhizoctonia* isolates was based on colony appearance and growth rate, as well as hyphal branching pattern and number of nuclei present in young hyphae. Colony appearance was assessed in 7-day old cultures grown on PDA at 24°C in darkness, while growth rate was determined by measuring two perpendicular colony diameters in five cultures per isolate, and calculating an arithmetic average for each isolate. For the growth rate comparison, Student's t-test at 5% and 1% levels were performed (Sokal & Rohlf, 1995). Hyphal branching pattern was observed directly using a compound microscope Olympus CX41. The number of nuclei within hyphal cells was determined using a modified clean slide technique (Kronland & Stranghellini, 1988) after staining with aniline blue in lactophenol or safranin O (Herr, 1979).

Testing for AG grouping was done using the modified clean slide technique (Kronland & Stranghellini, 1988; Martin, 2000) by pairing unknown *Rhizoctonia* isolates with tester isolates of nine different anastomosis groups and subgroups of multinucleate *Rhizoctonia,* AG-1-1C (R62), AG-2-1 (00269), AG-2-2 (01336), AG-3 (R14 1/97 T1), AG-4 HGII (2319), AG-5 (B8), AG-6 (06-01), AG-8 (R28) and AG-9 (CBS970.96) (kindly provided by Dr James Woodhall, University of Idaho, USA, and Dr Véronique Edel-Herman, INRA France). The anastomosis reactions were classified as frequent (more than five fusions observed), weak to intermediate (up to 5 fusions observed) or incompatible (no fusion observed) (Manici & Bonora, 2007).

Pathogenicity testing

Pathogenicity of two selected Serbian sugar beet *Rhizoctonia* isolates (270-15 and 275-15) was tested using inoculations of non-wounded sugar beet seedlings. Superficially sterilized commercial seeds of sugar beet were placed on PDA slants in 20 cm glass tubes and incubated at 23-25°C in a cycle of 12 h light/12 h darkness. After seed germination and cotyledon development, mycelial plugs (5 mm in diameter) from 7-day old cultures were placed on the roots (mycelial surface face down), while seedlings inoculated with sterile PDA served as negative control. The pathogenicity of the isolates was estimated 7 days post inoculation (dpi). Each isolate was inoculated onto five seedlings, and the experiment was repeated twice. Re-isolations from symptomatic seedlings were performed using the same methods as for fungal isolation.

DNA amplification and sequencing

For molecular identification of two selected *Rhizoctonia* isolates (270-15 and 275-15), total genomic DNA was extracted from 100 mg of dry mycelium from 5-7-day old cultures grown in potato dextrose broth (PDB) by the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The selected genome region ITS rDNA was amplified using the primers ITS1-F (Gardes & Bruns, 1993) and ITS4 (White et al., 1990) (Table 1). Amplification reactions were performed in a total reaction volume of 25 µl, consisting of 12.5 µl 2 × PCR Master mix (Fermentas, Lithuania), 9 µl RNase-free water, 2.5 µl of both forward and reverse primers (100 pmol/µl, Metabion International, Deutschland) and 1 µl template DNA. Amplification conditions were as follows: initial denaturation step of 2 min at 95°C, followed by 35 cycles of 35s at 94°C, 1 min at 52°C and 2 min at 72°C, with final extension period of 10 min at 72°C. PCR products were stained in ethidium bromide, analyzed by 1% agarose gel electrophoresis and visualized using a UV transilluminator. The PCR products were

Table 1. Primers used in the study

Primer name	Primer sequence (5'-3')	Reference	Amplicon size
ITS 1F	CTTGGTCATTTAGAGGAAGTAA	Gardes & Bruns, 1993	700 bp
ITS 4	TCCTCCGCTTATTGATATGC	White et al., 1990	
AG-2-2_F	CAC CTT TTG CTC TTT TTT TAA TCC A	Budge et al., 2009	150 bp
AG-2-2_R	ATA AAT TGG GTT TAT ATT AGA GTT GAG TAG ACA	Budge et al., 2009	

sequenced in both directions in an automated sequencer (ABI 3730XL Automatic Sequencer Macrogen Inc., Korea), using the same primers as for the amplification. Consensus sequences were computed using the ClustalW (Thompson et al., 1994), integrated in MEGA6 software (Tamura et al., 2013), and deposited in the GenBank (http://www.ncbi.nlm.nih.gov). Generated sequences were compared with each other by calculating nucleotide (nt) identities, as well as with previously deposited *Rhizoctonia* spp. isolates available in the GenBank, using the similarity search tool BLAST.

Conventional and real-time PCR detection

Protocols for specific detection of *Rhizoctonia* AG-2-2 based on conventional and real-time PCR were developed and compared. Both protocols are based on a previously published specific primer pair (Table 1) designed for detection of AG-2-2 using TaqMan chemistry (Budge et al., 2009).

Conventional PCR was performed in a total reaction volume of 5 µl, consisting of 2.5 µl 2 × PCR Master mix (Fermentas, Lithuania), 1.3 µl Nuclease-free water, 0.5 µl of both forward and reverse primers (100 pmol/µl, Metabion International, Deutschland) and 0.2 µl template DNA. Amplification conditions were as follows: initial denaturation step of 4 min at 94°C, followed by 35 cycles of 1 min at 95°C, 1 min at 49°C and 2 min at 72°C, with final extension period of 10 min at 72°C. PCR products were stained in ethidium bromide, analyzed by 1% agarose gel electrophoresis and visualized using a UV transilluminator.

Real-time PCR was conducted using a magnetic induction MIC qPCR cycler (Bio Molecular Systems, Australia) in the total reaction volume of 5 µl, containing 0.2 µl of target DNA, 2.5 µl FastGene IC Green 2x qPCR Universal mix (Nippon Genetics Europe Gmbh), 0.5 µl of both forward and reverse primers (100 pmol/µl, Metabion International, Deutschland), and 1.3 µl Nuclease-free water. The amplification program consisted of 95°C for 2 min, followed by up to 40 cycles consisting of 95°C 15 s, 60°C 30 s, and 72°C 2 s. Fluorescence was monitored after each annealing step. Amplicon melting profiles were generated by increasing the temperature of the reaction from 72 to 95°C (0.3^0 C/s).

Specific detection using both protocols was tested against 10 different anastomosis groups and subgroups of binucleate (AG-A, AG-G, AG-F and AG-U), as well as multinucleate (AG-2-1, AG-3, AG-4-HGI, AG-4-2, AG-6 and *Waitea*) *Rhizoctonia* isolates from the Fungal Collection of the Department of Phytopathology, Institute of Phytomedicine, University of Belgrade - Faculty of Agriculture. The sensitivity of both conventional and real-time PCR were compared using 10^{-1} to 10^{-5}dilutions of target DNA. All samples in all experiments were amplified in duplicates or triplicates and all PCRs included NTCs (no template control).

RESULTS

Disease symptoms, pathogenicity and conventional identification

During 2015, samples were collected from two sugar beet crops with root rot disease incidence estimated at 30%. Only *Rhizoctonia*-like isolates were recovered from plants exhibiting typical symptoms of Rhizoctonia root rot (Fig 1A, B). A total of 17 hyphal-tip isolates from both localities (8 and 9 isolates from localities of Vašica

Figure 1. *Rhizoctonia solani* AG-2-2: Root rot (A) and pith discoloration (B) of sugar beet

and Adaševci, respectively) had uniform morphological features, and one isolate from each locality was selected for further studies, i.e. isolates designated as 270-15 (locality of Vašica) and 271-15 (locality of Adaševci) (Table 2).

Pathogenicity testing revealed that both Serbian *Rhizoctonia* isolates were highly pathogenic, causing prominent root necrosis 7 dpi on all inoculated sugar beet seedlings. Control seedlings showed no symptoms. Isolates were successfully recovered from all symptomatic seedlings thus fulfilling Koch's postulates.

Both characterized *Rhizoctonia* isolates from sugar beet had uniform macroscopic and microscopic features (Table 2, Figure 2) with multinucleate nuclear cell condition and typical hyphal branching pattern. The colonies were fast growing, buff to dark brown, with abundant aerial mycelia and visible sclerotia distributed zonally on the periphery (Figure 2A, B). Typical monilioid cells were present on colony surface 3 dpi (Figure 2B), and brown sclerotia 7 dpi (Figure 2C). The isolates differed by their average growth rate ($p<0.01$), which was 17.08 and 17.38 mm for isolates 270-15 and 271-15, respectively. The presence of hyphal anastomosis was observed in all pairings of both 270-15 and 271-15 isolates against the AG-2-2 tester isolate (isolate 01336). No anastomosis was observed

Table 2. Isolates and characteristics of *Rhizoctonia* from sugar beet in Serbia

Isolate	Year of isolation	Locality	Colony color	Growth rate	Nuclear condition	Moniliod cells presence
270-15	2015	Vašica	Brown	17.08±0.084 a	>2	+
271-15	2015	Adaševci	Brown	17.38±0.130 b	>2	+

a, b. Average growth rate (MS±SD) in mm/day - the letter indicates significant difference

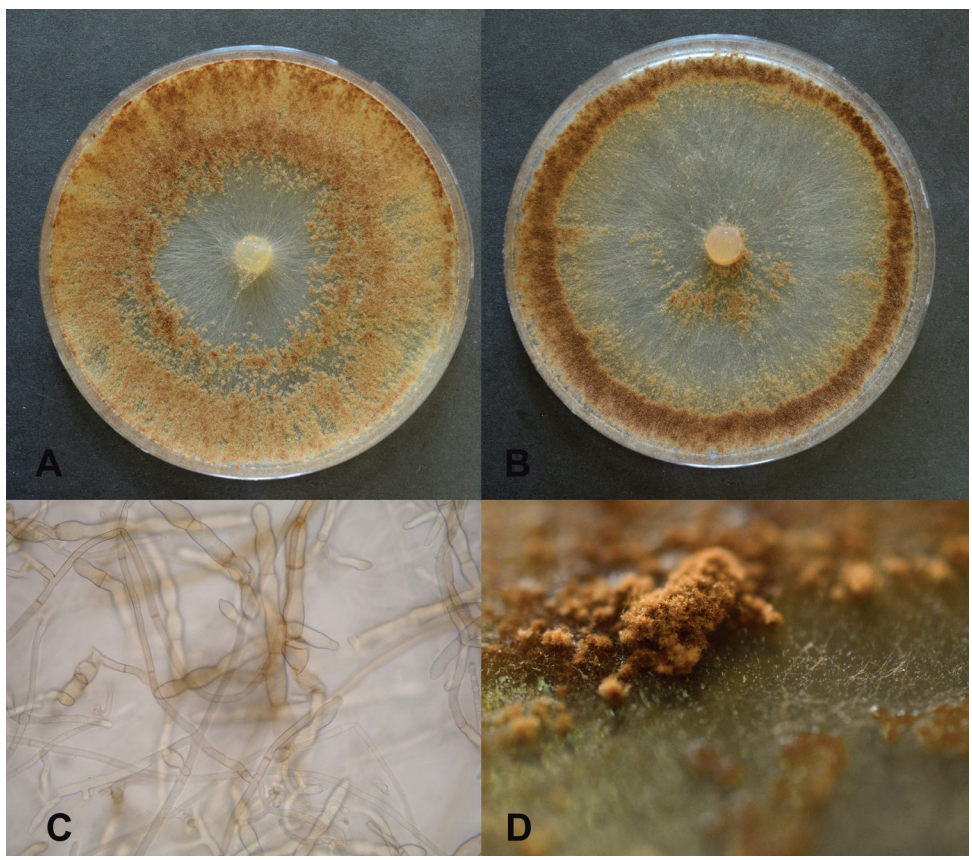

Figure 2. *Rhizoctonia solani* AG-2-2: Buff to dark brown colonies with abundant mycelia (A, B), monilioid cells (C), brown sclerotia formation (D).

after pairings against the AG-1-1C, AG-2-1, AG-3, AG-4 HGII, AG-5, AG-6, AG-8 and AG-9 tester isolates.

Molecular identification

Successful amplification of the ITS region of rDNA of both Serbian *Rhizoctonia* isolates resulted in obtaining amplicons of the predicted size of 700 bp, and after sequencing, manually edited consensus sequences of the isolates 270-15 and 271-15 were deposited in the GenBank database (Acc. Nos. MK123309 and MK123311, respectively). Sequence analyses revealed that the isolates share 99.6% similarity (with 3 bp differences), while BLAST analysis revealed the highest nt sequence homology of 99% (100% query coverage) of Serbian sugar beet isolates with over 50 sequences of *R. solani* mainly AG-2-2 but also with some of related AGs from different parts of the world and different host plants. Preliminary phylogenetic analyses (data not shown) confirmed that both Serbian sugar beet isolates belonged to *R. solani* AG-2-2.

Conventional and real time PCR specific detection

Using extracted DNA from pure cultures as the template, both conventional and real-time PCR protocols specifically amplified only the isolates of *R. solani* AG-2-2, exhibiting high specificity of both protocols. Using real-time PCR, the isolates 270-15 and 271-15 *Rhizoctonia* AG-2-2 were detected as early as in the 8-10th cycles, which is at least 15 cycles earlier than any other detectable amplification (Figure 3). Melting temperatures of Tm 81.3°C and 81.27°C, respectively, provided clear differentiation from any other test *Rhizoctonia* AG at amplification, thus additionally confirming specific detection. Sensitivity of the conventional vs. real-time PCR differed significantly (Figure 4). Real-time PCR detection was successful in all tested dilutions including the highest of 10^{-5}, while no products were visible in conventional PCR after amplification of 10^{-3} dilution and higher.

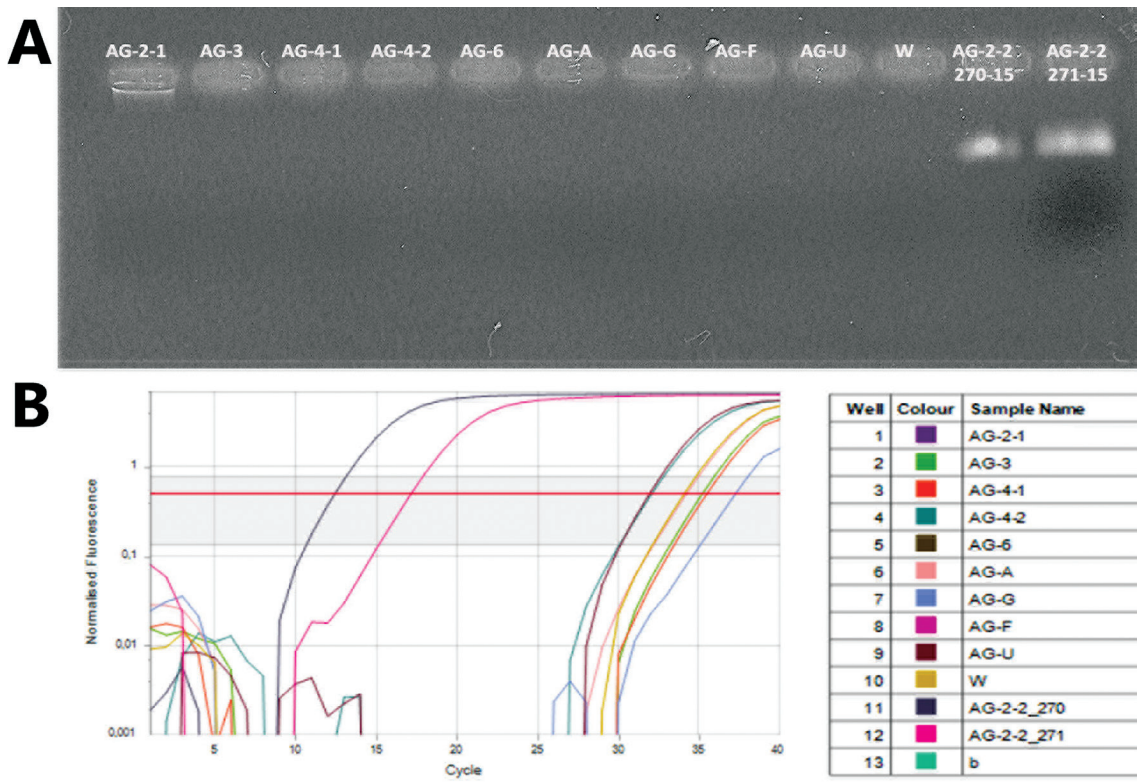

Figure 3. Primer specificity test for *Rhizoctonia solani* AG-2-2: isolates of *Rhizoctonia* AG-2-1, AG-3, AG-4-1, AG-4-2, AG-6, AG-A, AG-G, AG-F, AG-U, *Waitea*, Serbian AG-2-2 isolates 270-15 and 271-15 and non-target control, amplified using conventional PCR (A) and real-time PCR (B).

DISCUSSION

The genus *Rhizoctonia* comprises a group of soil-borne and highly heterogeneous, species and isolates, some still with ubiquitous taxonomic status. The AG concept has been adopted for grouping isolates that share some common features, including host range, pathogenicity (Lakshman & Amaradasa, 2014) and fungicide sensitivity (Amaradasa et al., 2014). *Rhizoctonia* AG-2 is one of the most diverse AG (Ogoshi, 1976, 1987; Naito & Kanematsu, 1994; Carling et al., 2002) with several defined subgroups, including AG-2-2, which is a well-established and widespread pathogen in Europe, reported in the Netherlands (Schneider et al., 1997), Germany (Buddemeyer et al., 2004), Spain (Gonzalez et al., 2011), Serbia (Stojšin et al., 2011), Poland (Skonieczek et al., 2016), and Italy (Aiello et al., 2017). The host range of *R. solani* AG-2-2 is wide and besides sugar beet (Kiewnick et al., 2001; Jacobsen, 2006; Bolton et al., 2010, Stojšin et al., 2011, Abbas et al., 2014), it also includes tobacco (Gonzalez et al., 2011), bean (Mora-Umana et al., 2013), potato (Muzhinji et al., 2015), soybean (Ajayi-Oyetunde & Bradley, 2018), onion (Misawa et al, 2017), ornamentals (Aiello et al., 2017), as well as maize (Fähler & Petersen, 2004). Further increase in the significance of *R. solani* AG-2-2 in forthcoming years could therefore be expected, coupled with substantial yield reductions in different crops.

Rhizoctonia spp. are considered to be some of the most destructive sugar beet pathogens in Serbia and worldwide and so far the presence of *R. solani* AG-4 and AG-2-2 has been confirmed in Serbia (Stojšin et al., 2011). During 2015, we collected samples from two sugar beet crops with disease incidence estimated at 30% and plants exhibiting damping-off of sugar beet seedlings and roots, crown rot, and foliar blight. Two selected isolates representing a group of hyphal tip isolates from each of the localities were identified and characterized and only the presence of *R. solani* AG-2-2 was detected. In the majority of sugar beet production areas in Europe, *R. solani* AG-2-2 is also the most frequently isolated AG (Buddemeyer et al., 2004; Skonieczek et al., 2016) and it is considered to be favored by narrow crop rotation (Ithurrart et al., 2004).

Serbian isolates obtained in this study exhibited uniform morphological features consistent with published *R. solani* AG-2-2 isolates (Misawa et al., 2015). In pathogenicity testing, our isolates exhibited uniform pathogenicity with no differences in symptom appearance or intensity, and caused seedling necrosis similar to USA and Japan isolates (Carling et al., 2002;

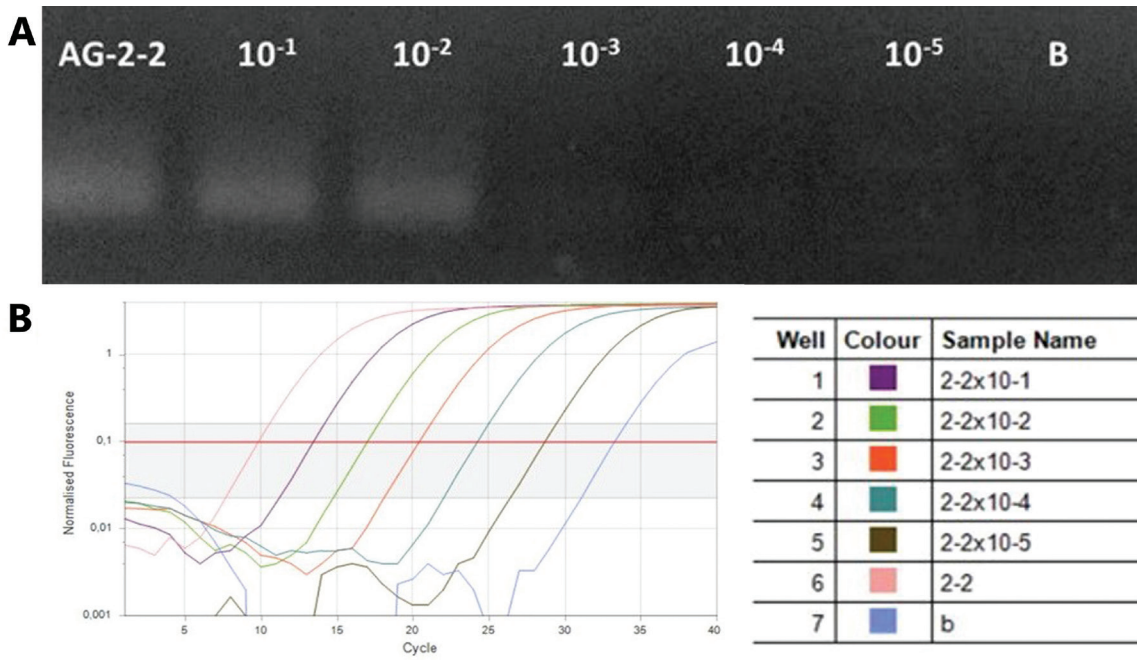

Figure 4. Primer sensitivity test for *Rhizoctonia solani* AG-2-2: template DNA dilutions of 0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} of *R. solani* AG-2-2 Serbian isolate 270-15 and non-target control, amplified using conventional PCR (A) and real-time PCR (B).

Bolton et al., 2010). Conventional identification by successful AG pairing with a known tester of AG-2-2 isolate, designated our isolates to be *R. solani* AG-2-2, which was further confirmed by ITS sequencing and sequence analyses. Two isolates originating from different localities and sugar beet crops exhibited a low level of 0.4% nt difference (3 bp), which correlates with a previously detected level of variability within the AG-2-2 population (Carling et al., 2002).

Fast, specific and sensitive *Rhizoctonia* detection and AG identification is important and needed in practical, every day disease diagnosis, as well as in decision-making support for implementing control measures. In this paper we report a highly specific and sensitive real-time PCR protocol for the detection of *R. solani* AG-2-2 in which results can be obtained in a couple of hours. The use of already published primers for specific detection of *R. solani* AG-2-2 (Budge et al., 2009), and modification of the protocol by using SYBR Green chemistry instead of TaqMan significantly reduced the cost of analyses per sample. All real-time PCR reactions were conducted in a MIC thermal cycler in small reaction volumes of 5 µl recommended by the producer which additionally contributed to cost-effectiveness of the analyses. Abbas et al., (2014) also designed primers for detection of *R. solani* AG-2-2 in conventional, as well as in real-time PCR, with some limitations in amplicon size or cross-reactivity with closely and distantly related fungi including some *Fusarium* species. Budge et al. (2009) reported that primers designed in their research, which we also used in our protocols, exhibited a low level of cross-reactivity with *R. solani* AG-2-1. Nevertheless, in all our experiments, primers showed a high level of specificity after testing against 10 different anastomosis groups and subgroups, including the AG-2-1 isolate from our collection. Moreover, conventional PCR assay with the same primers showed in our study the same specificity but proved to be at least 100x less sensitive, probably due to the previously described limitation of post PCR visualization of amplicons (Lees et al., 2002).

Real-time PCR is a tool which can be successfully used for the fast and specific detection of *Rhizoctonia* at the species and AG levels, as well as for quantification of a wide range of pathogen DNA concentrations (Lees et al., 2002; Lievens et al., 2006). In this paper we are proposing a sensitive and specific real-time PCR protocol for direct identification and detection of *R. solani* AG-2-2. Future research will include further testing and adaptation of this protocol for direct detection and quantification of *R. solani* AG-2-2 in different substrates, including plant tissue and soil samples.

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Konvencionalni i real-time PCR protokoli za detekciju i identifikaciju *Rhizoctonia solani* AG-2-2 prouzrokovača truleži korena šećerne repe u Srbiji

REZIME

Smatra se da su gljive iz roda *Rhizoctonia* koje se održavaju u zemljištu, najdestruktivniji patogeni šećerne repe. Mada je širom sveta višejedarna *R. solani* AG-2-2 najčešće označena kao najvažniji prouzrokovač truleži korena šećerne repe, nekoliko dvojedarnih (AG-A, AG-E i AG-K) kao i višejedarnih *Rhizoctonia* (*R. solani* AG-4, AG-5 i AG-8) takođe mogu da učestvuju u kompleksu bolesti. Kako se održavaju u zemljištu i imaju širok krug domaćina, suzbijanje vrsta *Rhizoctonia* koje izazivaju trulež korena šećerne repe veoma je zahtevno. Identifikacija anastomoznih grupa prouzrokovača oboljenja predstavlja neophodan prvi korak u uspostavljanju uspešnog suzbijanja. U ovom radu razvijen je visokospecifičan i osetljiv real-time PCR protokol za detekciju i identifikaciju *R. solani* AG-2-2 koji se pokazao kao visokospecifičan nakon testiranja koja su obuhvatila 10 različitih AG grupa i podgrupa, uključujući i AG-2-1 kao najsrodniju. Sličan konvencionalni PCR protokol ispoljio je istu specifičnost, ali i najmanje 100 × manju osetljivost. Buduća istraživanja uključiće dalje testiranje i adaptaciju ovog protokola za direktnu detekciju i kvantifikaciju *R. solani* AG-2-2 u različitim uzorcima, uključujući biljno tkivo i zemljište.

Ključne reči: *Rhizoctonia solani* AG-2-2; šećerna repa; morfologija; molekularna identifikacija; specifična detekcija; real-time PCR