Original article

*Rhizoctonia solani* AG 11 isolated for the first time from sugar beet in Poland

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**A R T I C L E   I N F O**

**Article history:**
Received 6 January 2020  
Revised 13 May 2020  
Accepted 14 May 2020  
Available online 22 May 2020

**Keywords:**  
*Rhizoctonia solani*  
Sugar beet  
AG11  
Fungicide  
Plant disease  
Poland

**A B S T R A C T**

Two isolates of *Rhizoctonia solani* AG11 were isolated from sugar beet seedlings from South-west Poland. Both isolates gave C2 reactions in anastomose pairings with the tester isolates of AG11. The membership of both isolates to AG11 was confirmed by analysis of pectic isozyme profiles, and by verification that the internal transcribed spacer sequences of both isolates matched the references in the GenBank database. Both AG11 isolates formed white-beige to creamy-colored mycelium with wide concentric zonation. The average daily rate of hyphal growth at 21 °C was 22.8 mm and 22.6 mm on PDA. They were mildly pathogenic to sugar beet seedlings due to the mycelial and secondary metabolites’ activity. The sensitivity to fungicides typically used in sugar beet protection was different for each isolate; one of them (isolate ID11) was less sensitive to thiram than the other (isolate ID3). This article discusses the worldwide occurrence of *R. solani* AG11, expands the currently known host range, shows its broad world distribution in regions of moderate climate, and confirms the isolates’ low frequency.

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1. Introduction

*Rhizoctonia solani* is one of the fungi that causes both sugar beet root rot and seedlings’ damping-off. In Europe, crop losses caused by *R. solani* are significant; it is one of the primary causes of damage to sugar beet crops in Poland and Germany (Moliszewska 1999, 2000, 2002; Moliszewska and Burgiel 2002; Moliszewska and Schneider 1999; Pisarek and Moliszewska 1999; Skonieczek et al. 2016).

13 groups called anastomosis groups (AG) have been recognized. The isolates from different groups do not anastomose with each other (Carling 1996; Ogoshi 1987, 1996). Many of these groups have been divided into subgroups based on host range, cultural morphology, and biochemical and molecular characteristics (Ogoshi 1987). Groups AG1 to AG5, as well as some anastomosis groups of binucleate *Rhizoctonia* spp., are pathogenic to sugar beets. Some of them are reported as significant reductants of stands on sugar beet fields, while other isolates are capable of causing root rot in mature plants (Windels and Nabben 1989; Windels et al. 1997). The first AG to be identified on the rotten tap root of sugar beets was AG2, and the next was AG4 (Herr 1996).

*R. solani* AG11 was described by Carling et al. (1994) for the first time, and shown to be mildly pathogenic to different plants, but not to sugar beets. Jones and Carling (1999) identified their *R. solani* AG-UNK isolates as AG11, which were collected as atypical *Rhizoctonia* isolates from sugar beets, and since then, only a few investigations have been done on *R. solani* and sugar beets in Poland (Moliszewska 1999, 2000, 2002; Pisarek and Moliszewska 1999; Moliszewska and Burgiel 2002; Moliszewska and Schneider 2002; Moliszewska 2009; Skonieczek et al. 2016).

https://doi.org/10.1016/j.sjbs.2020.05.026
2. Materials and methods

2.1. The procedure of isolation, identification, and characterization of isolates

Seedlings of sugar beets with damping-off symptoms were taken from field experiments in order to isolate fungal pathogens. The fields were localized near Opole in the Southwest region of Poland. The isolation of fungi associated with diseased seedlings was conducted according to typical phytopathological procedures, using PDA medium supplemented with 50 mg/dm$^3$ of ampicillin. Newly obtained fungal colonies were transferred onto the fresh PDA medium. Clean isolates of $R$. solani, as well as other isolated fungi, were stored on PDA slants for further use. All fungal strains were identified to the species name. The identification of $R$. solani among all the isolated fungi was made according to the characteristic hyphal structure of $R$. solani. The AGs of all $R$. solani isolates were then identified using typical sugar beet tester strains (AG1 to AG5). Next, the growth rate in 21°C, hyphal diameter and the number of nuclei in the cells of young mycelium were observed, with the purpose of distinguishing typical $R$. solani from binucleate Rhizoctonia sp. (BNR) (Moliszewska 2009). The number of nuclei in vegetative cells was determined using a DAPI staining. Isolates of $R$. solani were sorted into their AGs according to morphological features and hyphal anastomoses, obtained with tester isolates using a method described by Windels and Naben (1989) and Grosh et al. (2004), avoiding staining of hyphae (Kronland and Stanghellini 1988, Carling 1996; MacNish et al. 1993). Among strains of $R$. solani assigned as AG 1, 2, 4 and 5, two isolates were assigned as unidentified (UN isolates), because they did not anastomose with typical sugar beet AGs of $R$. solani. The UN isolates were coded as ID3 and ID11, and they were later identified after pairing with tester isolates from groups AG6 through AG13. Tester isolates of $R$. solani were obtained in 2002 from the collection of the Institute of Sugar Beet Research – IRS (dr JHM Schneider) – in the Netherlands. The AG11 testers used were isolates coded 11–01 and 11–03 – obtained from Glycine max in Western Australia (personal information from JHM Schneider).

To confirm the anastomosis group, molecular identification was also used ( Gonzalez et al. 2001; Sharon et al. 2006, 2008). DNA was extracted from the freeze-dried samples (50 mg) using a DNeasy Mini Kit (QiAGEN) in accordance with the producer instructions. The region of rDNA was amplified in a PCR reaction using ITS1 and ITS4 primers with the following procedure: PCR reaction was carried out with 25 μl total volume, containing 12.5 μl Taq mastermix (1x PCR buffer, 0.2 mM each dNTP mix, mM MgCl$_2$, Taq DNA polymerase 2.5 U), 1 μl MgCl$_2$ 25 mM, 0.5 μl each of ITS1 and ITS4 10 μM, 9.5 μl double distilled water, and 1 μl template DNA 5 ng/μl.

The PCR was performed for 30 cycles of denaturation at 93°C for 1 min, annealing at 57°C for 1 min, extension at 72°C for 2 min, initial denaturation at 93°C for 2:30 min and final polishing at 72°C for 10 min. PCR products were stored at 4°C or frozen at −20°C. A portion of product (10 μl) for each isolate was electrophoresed in 1.5% agarose gel with 0.5x TBE buffer and visualised under UV light following ethidium bromide staining. The rDNA-ITS regions were sequenced in Macrogen (www.macrogen.com) for UN isolate ID3 and in Genomed (www.genomed.pl/Poland) for UN isolate ID11. Alignments and phylogenetic analyses were done using AliView and Mega X software. The sequences were compared to GenBank nucleotide (NCBI) databases using the BLAST algorithm to determine sequence identity and to find the closest match based on the maximal percentage identity (Zhang et al. 2000). The sequences of the rDNA-ITS regions of both UN isolates were submitted to GenBank (accessions KU837255 and KX810069).

To determine the phylogenetic relation among isolates marked as $R$. solani AG11, a phylogenetic tree was constructed based on data of the $R$. solani AG11 rDNA-ITS sequences, including two isolates of unknown AGs obtained from sugar beets in China (which sequences matched to ours) (Table 1). The phylogenetic tree was generated by the Neighbor-Joining method with 2000 bootstrap replicates using MEGA X (Kumar et al. 2018). The evolutionary distances were computed using the Maximum Composite Likelihood method for 28 nucleotide sequences with total 663 positions in the final dataset and were given in units of the number of base substitutions per site. All ambiguous positions were removed for each sequence pair (pairwise deletion option) (Saitou and Nei 1987; Felsenstein 1983; Tamura et al., 2004). The ID3 sequence was not used in phylogenetic analysis because of its short length.

2.2. Pectic zymograms

Pectic zymograms were prepared for rapid assignment of $R$. solani isolates to AG groups and subgroups (Cruickshank 1990; MacNish et al. 1993; Schneider et al. 1997). The enzyme activity was checked on the polyacrylamide gel electrophoresis according to Schneider et al. (1997) and Grosh et al. (2004).

2.3. Pathogenicity of culture filtrates

Tested isolates were grown on liquid Czapek-Dox medium during fourteen days at 22–23°C. 10 ml of each fungal culture filtrate was transferred into sterile Petri dishes (9 cm diameter) filled with blotting-paper discs. In each Petri dish, 10 pre-germinated seeds of sugar beets with healthy sprouts were inserted. After 24 and 48 h, the health condition of sprouts was estimated using the following scale: 0 – no disease symptoms, 1 – small spots, 2 – distinct and large necrotic spots, 3 – necrosis on a whole sprout.

The test was carried out with three replications at room temperature.

Results were transformed into an infection coefficient [Ip] using the following formula (Moliszewska 2009):

$$Ip\% = \frac{\left(\sum (n_k \times k)\right) \times N}{k_{max}} \times 100\%$$

$k$ – a scale degree (for estimation of health condition; $k = 0–3$), $N$ – a total number of observed sprouts (10), $n_k$ – number of diseased sprouts observed in each degree of the scale.

2.4. Pathogenicity of tested isolates against sugar beets in a field test

For both UN isolates, ID3 and ID11, a field test was arranged to measure their pathogenic activity on sugar beet seedlings and young plants. Pathogens' inocula were prepared on a corn-sand medium (Garret 1970, Moliszewska 2009). They were incubated for three weeks at room temperature. Non-coated sugar beet seeds (cv. Janka, kindly provided by Kutnowska Hodowla Buraka Cukrowego, Poland) were sown in natural conditions in the test field at the beginning of the third week of April. In each row (1.5 m) thirty seeds were planted every 5 cm. The inocula of fungi were put into the soil in rows in the amount of 150 cm$^3$. The control rows were obtained from JHM Schneider).

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2.5. Influence of fungicides on the growth of tested isolates

This test was prepared to check the influence of conventionally used fungicides for protection against damping-off in sugar beet—Tachigaren 70 WP (70% hymexazole) and Zaprawa Nasienna T 75 DS/WS (75% thiram) on the hyphal growth. Fungicides were added to the PDA medium to achieve 7 mg/dm³ or 35 mg/dm³ of hymexazole and 7.5 mg/dm³ or 37.5 mg/dm³ of thiram. Modified media were poured into Petri dishes, then inoculated with the appropriate 1-week old isolate and incubated in the dark at room temperature. The inhibition or stimulation (%) of fungal growth was estimated via comparison data obtained by growing both fungi on fungicide-amended and unamended control PDA media.

2.6. Statistics

Since the distribution of the measurement results was unknown, for comparison between results the Wilcoxon signed-rank test was utilized (Shao 1999; Dalgaard 2008). This non-parametric statistical hypothesis test was used for assessing whether one of the two samples of independent observations tended to have larger values than the other. The two-tailed critical

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Table 1

Rhizoctonia solani AG11 sequences deposited in GenBank.

| No. | Accession number | Isolate | Host | Author/s | Country/Source | Year of publication |
|-----|------------------|---------|------|----------|----------------|---------------------|
| 1   | KC146421         | PT321 (AG11) | potato | Yu,J.F. and Li,X.N. | China | 2013 |
| 2   | KJ170365         | NM-17–2 (AG11) | potato | Yang,Y. and Wu,X. |  | 2015 |
| 3   | FJ435120         | CSL1945:11_1945 ATCC 90857, 11R01 (AG11) | Not determined | Budge,C.E., Shaw,M.W., Lambourne,C., Jennings,P., Clayburn,R., McPherson,M. | Australia / Plant Research International, the Netherlands | 2009 |
| 4   | AF153802         | ZN667 (AG11) | isolated from soil core | Pope,E.J. and Carter,D.A. | Western Australia | 2001 |
| 5   | AF354079         | (ZG-3)R1013 (AG11) | lupine | Gonzalez,D., Carling,D.E., Kuninaga,S., Vilgaly,R. and Cubeta,M.A. | Australia | 2002 |
| 6   | LC215404         | Roth1 (AG11) | soil | Misawa,T., Kayamori,M., Kurose,D., Sasaki,J. and Toda,T. | USA | 2013 |
| 7   | LC215406         | Roth26 (AG11) | Oryza sativa |  |  |  |
| 8   | LC215405         | Roth11 (AG11) |  |  |  |  |
| 9   | LC215410         | 89–740 (AG11) | Triticum aestivum |  |  |  |
| 10  | LC215409         | ZN163 (AG11) | Lupinus angustifolius |  |  |  |
| 11  | LC215408         | ZN56 (AG11) |  |  |  |  |
| 12  | LC215407         | R942 (AG11) | Trifolium repens |  |  |  |
| 13  | LC215402         | 31 (AG11) | Lilium spp. |  |  |  |
| 14  | LC215403         | L5 (AG11) | Oriental lily |  |  |  |
| 15  | AF354114         | Roth16 (AG11) | Soybean | Gonzalez,D., Carling,D.E., Kuninaga,S., Vilgaly,R. and Cubeta,M.A. | USA | 2006 |
| 16  | AF354115         | Roth24 (AG11) | Soybean seedlings | Ajayi,O.O. and Bradley,C.A. | USA | 2019 |
| 17  | AB019027         | Roth25 (ATCC 90858) (AG11) | Rice | Kuninaga,S., Carling,D.E., Takeuchi,T. and Yokosawa,R. | USA | 2000 |
| 18  | KK118394         | X12SDs (AG11) | Soybean |  |  |  |
| 19  | KK118382         | SP 19b (AG11) |  |  |  |  |
| 20  | KK118381         | SP 19a (AG11) |  |  |  |  |
| 21  | KK118336         | BVT_18 (AG11) |  |  |  |  |
| 22  | KK118355         | HPIN22A (AG11) |  |  |  |  |
| 23  | KK118356         | KARS02_1_11 (AG11) |  |  |  |  |
| 24  | KK118358         | KARS02_1_20 (AG11) |  |  |  |  |
| 25  | KK118367         | K_4_11b (AG11) |  |  |  |  |
| 26  | EU591766         | R22 (AG11) | Snap bean | Ohkura,M., Abawi,G.S., Smart,C.D. and Hodge, K.T. | USA | 2009 |
| 27  | KU837255         | ID3 (AG11) | Sugar beet | Moliszewska E., Maculewicz D. | Poland | 2016 |
| 28  | KX810089         | ID11 (AG11) |  |  |  |  |
| 29  | FJ392702         | CSL1846 (ATCC 90857) (AG11) | Lupinus sp. | Budge,C.E., Shaw,M.W., Colyer,A., Pietravalle, S. and Boonham,N. | FERA culture collection | 2009 |
| 30  | AY154313         | Not determined | beta-tubulin-like gene, partial (AG11) | Not determined |  |  |
| 31  | KJ380831         | ZG-3_R1013 (AG11) (large subunit) | Lupinus angustifolius | Gonzalez,D. | Australia | 2015 |
| 32  | KC380830         | Roth16 (AG11) (large subunit) | Glycine max | Gonzalez,D. | USA | 2015 |
| 33  | KJ380757         | Roth16 (AG11) (small subunit) | Glycine max | Gonzalez,D. | USA | 2015 |
| 34  | JX989021         | ZG-3_R1013 (AG11) (28S ribosomal RNA) | Lupinus angustifolius | Gonzalez,D. | Australia | 2015 |
| 35  | JX989020         | Roth16 (AG11) (28S ribosomal RNA) | Glycine max | Gonzalez,D. | USA | 2015 |
lates. There are 30 isolates ID11 together with both of the above mentioned isolates – ID11 showed 95.48% identity with the sequence of the DNA, to the sequences from the GenBank database confirmed by constructing a phylogenetic tree on which two groups were formed. The relationships of all AG11 isolates mentioned above were confirmed by microscopic observation. In control plants the infection was produced by other fungal species, mainly belonging to the genera of Fusarium and Pythium. The percentage of inhibition of the emergence of sugar beet seedlings by our AG11 isolates was very high and differed in time, ranging from 50% to 87.7% for ID3, to even 100% for ID11 (Fig. 4). Using the ANCOVA method, trends in germination inhibition were estimated. The dependence of changes in sprout number on time of inhibition emergence was analyzed for ID3, ID11 and control treatments.

Equation (1) below shows the proposed linear description:

\[ n = n_0 + \beta_{1,k} \times t \]

where \( n \) is the number of sprouts at time \( t \).

In the model, the intercept \( \beta_{0} \) value which represents the starting number of sprouts and no inhibition at \( t = 0 \), is expected to be the same for all treatments, and its value should be 0. The inhibition effect would be represented by the slope \( \beta_{1,k} \), when \( k= \) ID3, ID11, control.

In Table 2 values of the structural parameters \( b \), standard errors and \( p \)-values are presented.

The \( p \)-value for \( \beta_{0} \) confirms the presumed 0 value of this parameter. Significantly lower values of \( \beta_{1} \), for ID3 and ID11 than for the control demonstrate inhibitory activity of isolates on the sugar beet germination process.

Seeds that survived in the field experiment were not influenced by the tested fungi and showed almost the same dimensions as the control seedlings. The seedlings obtained from plots infected with ID3 showed slightly larger true leaves than the control ones (Fig. 5); however, an analysis by the Wilcoxon test showed no statistically significant differences between these seedlings when compared to the control.

Additionally, both isolates were tested for their ability to grow under the influence of commercially used fungicides in coatings of sugar beet seeds. Hymexazole in a lower dose did not have an influence on the growth of either tested isolate. On the contrary, even a slight stimulation was observed for ID11. This fungicide used in a higher dose slightly inhibited the growth of both fungal strains – ID11 at 13.5% and ID3 at 28% (Fig. 6). Thiram inhibited the growth of both isolates, but the activity was different and
depended on the concentration of fungicide and fungus isolate. It ranged from 35.8% of growth inhibition of the ID11 isolate, when a lower dose of thiram was applied, to 74.7% for a higher dose for ID3 isolate. The isolate ID11 was less sensitive to both tested fungicides than the isolate ID3. The tested fungicides did not exhibit strong fungitoxic activity against either fungi (Fig. 6), but rather acted only fungistatically. This conclusion is supported by the Wilcoxon
test, which did not reveal statistically significant differences in fungal growth under fungicide treatment. For both tested isolates, the diameter of running hyphae was measured under the influence of fungicides. Most of the tested combinations of fungicides did not influence on the hyphae diameter, but in some cases, minor changes were observed. The ID11 reacted with both preparations achieving 9% thinner hyphae under the influence of a lower dose of hymexazole, and 5% thinner ones under the influence of a higher dose of thiram. The ID3 isolate showed 7% thinner hyphae under the influence of hymexazole in a lower dose and almost 8% greater diameter of hyphae for 35 mg of hymexazole as well as for 7.5 mg of thiram comparing to the control. The ID3 isolate produced light brown mycelia darkening with age, two of them released yellow pigment to PDA medium. The isolates abundantly produced light tan to brown sclerotia densely distributed on the colony surface. Those isolates had an average 8 number of nuclei per cell with distribution from 5 to 10, it is less than our isolates which had 13–15 nuclei per cell. Our isolates also were light brown, one of them caused yellowish of the reverse side of the colony, but sclerotia were not produced so abundantly.

Isolates of AG11 do not typically cause bare path symptoms, but they are capable of causing damage to legumes, cotton, radishes, as well as damage of hypocotyl and coleoptile of wheat. The distinct AG11 populations of Western Australia and Arkansas, were indicated as a comparatively homogenous group (Carling et al. 1994). The first known isolates of AG11 were collected from seedlings of Glycine max, Oryza sativa in Arkansas and from Lupinus angustifolius in Western Australia (Carling et al. 1994). Kumar et al. (2002) named the AG11 as legume-specific, in accordance with isolates from Western Australia. The AG11 isolates from lupine were also obtained from 2000 to 2003, and also investigated in 2005–2007 in Western Cape (South Africa). In Tewoldemedhin (2005) research, only two isolates AG11 were obtained from lupine in 2000 (accession numbers PPRI 7440 and PPRI 7441 in the National Collection of Fungi at the ARC – Plant Protection Research Institute in Pretoria, South Africa), and they were moderately or weakly virulent on canola, clover, lucerne, and lupine. In the next experiments realized in Western Cape, AG11 were 1.6% and 4% of total Rhizoctonia spp. population obtained in different tillage and crop rotation systems. This group of fungi was suggested as an important pathogen of the lupine crop in Western Cape province (Tewoldemedhin 2005; Tewoldemedhin et al. 2006; Lamprecht et al. 2011). Lamprecht et al. (2011) noticed that the incidence of multinucleate Rhizoctonia, with AG11 among others, were not influenced by different tillage systems and crop rotation, and that they were most frequently isolated at the beginning and the end of the season. Kumar et al. (2002) pointed out a possibility of survival of sclerotia in hot and dry soil in extended periods, as well as the possibility of producing abundant sclerotia in the field soil. They pointed out that regular cropping of lupine will contribute to building up a high sclerotial inoculum in the soil. Kumar et al. (2002) found that isolates were able to the abundant production of sclerotia when mycelium was covered by sand. After drying, they were still alive and about 50% loss of vigor was observed after four drying cycles. The radial growth of the mycelium from sclerotia declined with each drying and germination cycle. Mycelium aggressiveness increased with the size of sclerotia resulting in more severe rot of lupine hypocotyls. The number of sclerotia pro-

### Table 2

| Parameter Estimate | Standard Error | p-value |
|-------------------|----------------|---------|
| $b_0 / C_0$       | 1.1            | 1.4     | 0.43    |
| $b_1, ID3$        | 0.13           | 0.061   | 0.035   |
| $b_1, ID11$       | 0.20           | 0.061   | 0.003   |
| $b_1, contr$      | 0.54           | 0.061   | 0.000   |

4. Discussion

Ajayi-Oyetunde and Bradley (2017) described distinctly the morphological features of their isolates AG11 obtained from soybeans, from Illinois (6 isolates) and Arkansas (2 isolates). Their isolates produced light brown mycelia darkening with age, two of them released yellow pigment to PDA medium. The isolates abundantly produced light tan to brown sclerotia densely distributed on the colony surface. Those isolates had an average 8 number of nuclei per cell with distribution from 5 to 10, it is less than our isolates which had 13–15 nuclei per cell. Our isolates also were light brown, one of them caused yellowish of the reverse side of the colony, but sclerotia were not produced so abundantly.

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Fig. 5. The influence of isolates ID3 and ID11 on the dimensions of cotyledons and true leaves of survived sugar beet seedlings.

Fig. 6. The influence of two commercially used fungicides on the growth of isolates of AG11 (H – hymexazole, T – thiram; “-“ means the stimulation of the growth).
duced in soil increased with increasing density of lupine seedlings. These findings can explain the differences between pathogenicity of AG11 isolates however, they do not match with our observations. In our research, sclerotia production on PDA was uniform for the isolate ID11, what produced distinct light-colored sclerotia. The isolate ID3 did not produce any sclerotia on PDA.

A single isolate of AG11 was obtained from the snap bean in the region of New York by Ohkura (2008, 2009), as well as by Eken and Demirci (2004) from the bean in the Erzum region from Turkey. Broders et al. (2014) found only one isolate on canola which was the most similar to an isolate of AG11 used in the study by Gonzalez et al. (2001). Yu and Li (2013) registered a single isolate (PT321) obtained from potato in China, and next Yang and Wu (2015) also found R. solani AG11 (only one isolate) on potato in China causing stem canker (Yang et al. 2015). In 2017 Ajayi-Oyetunde and Bradley (2017) reported the next eight AG11 isolates from seedlings of soybean in the USA. The isolates were weakly pathogenic to soybeans and mildly to corn. Their R. solani AG11 collection seems to be the biggest one. Further, in 2017 two other isolates were obtained from Lilium sp. in Japan (Misawa et al. 2017). Jaaffar et al. (2016) found only one AG11 isolate between 498 R. solani isolates collected from cereal cropping systems in 2000–2011 in the Inland Pacific Northwest of the USA, which only confirms a rare occurrence of AG11. All of those findings show the wide pathogenic potential of R. solani AG11 isolates against different plant species, however, they have not been registered from sugar beet seedlings until now. It is notable that all researchers, including Carling and coworkers (1994), observed infections caused by AG11 isolates on the early developmental stages of infested plants (seedlings). Our isolates were initially isolated from sugar beet seedlings, which is adequately similar to other observations for isolates of this group. Most AG11 isolates have not shown the distinct pathogenic activity which was partly confirmed by our experiment. Pathogenic activity for our AG11 isolates was due to the mycelium activity what was observed in the field experiment, and due to the phytotoxic metabolites produced by mycelium. This observation seems to be characteristic for pathogenic isolates of R. solani. We were expecting some visible changes in surviving plants, as we had observed in plants under the influence of AG5 (Moliszewksa and Schneider 2002). However, the surviving plants presented normal or slightly enlarged dimensions, as compared to the control plants. Some disturbances in plant development were suspected, because observation for other sugar beet pathogens showed us the influence of the pathogen even if the disease symptoms were not created (Choluj and Moliszewksa 2012). The AG11 is a rather unusual group of R. solani as a pathogen of sugar beets. Our two isolates (ID3 and ID11) are currently the only ones isolated in both Poland and Europe. This findings presents a new and distinct group of R. solani to consider in sugar beet seedlings diseases, especially for European producers. This report also expands the known geographic range of AG11 in the world (Carling et al. 1994; Jones and Carling 1999), and the range of the host plants (Carling et al. 1994). Presently, there are 30 isolates registered in the NCBI database, including our two isolates and one isolate for which β-tubulin like gene partial sequence is banked (Table 1). This group should be expanded to isolates from South African collection, although the number of surviving ones (e.g. in any collections) is not known.

In our investigation, we included two sequences of R. solani isolated from sugar beets in China as potentially new AG11 (see Fig. 1). This shows that it is possible to find other strains of AG11 between the GanBank accessions, although the lack of anastomosis data will not improve the information, because some sequences are also comparable to AG2, and real determination should be confirmed by anastomosis reaction. Misawa et al. (2017) described for the first time AG11 on lilies’ bulbs, stems and leaves. They finally obtained only two isolates of R. solani AG11. Both isolates showed an optimum temperature of growth at 20–25 °C, what was in accordance with Tewoldemedhin (2005) observations, but their growth rates seemed to be about a half slower than that of our AG11 isolates. The determination of AG for both Japanese isolates was done only according to a comparison of internal transcribed spacer regions of ribosomal DNA.

Our search also showed us the insufficiency of information about this group. Finding the isolates of AG11 in North America, Australia, South Africa, Turkey, and Europe suggests that AG11 occurs worldwide, and that its preference is for rather warm or partly warm / partly moderate climate conditions. However, the range of host plants seems to be broad, from Poaceae as rice and wheat, lily, corn (monocots), to different dicotyledonous aslegumes, cotton, radish, canola, and sugar beet. Our research also showed the significant influence of AG11 isolates on sugar beet seedlings, although their effect on mature roots is still unknown. The findings are also important for future European agriculture initiatives e.g soybeans cultivation, which recently has been started to cultivate in Poland and the neighboring countries. We also showed that Polish isolates of AG11 are significantly sensitive for thiram, although this fungicide could not completely inhibit their growth. This is the first report of AG11 reaction to fungicides. We also suggest that further searching for other AG11 isolates of R. solani will enable to uncover their real and full significance, and occurrence across global agricultural regions.

Acknowledgments

Authors would like to thank J.H.M. Schneider PhD (IRS) for his kindness in providing tester isolates, and consultation on the results. The authors also acknowledge Dagna Maculewicz for her technical support, and Ms. Julia Dickenson, a Fulbright grantee at the University of Opole, for her language corrections.

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