Saudi Journal of Biological Sciences 27 (2020) 1863–1870

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

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

Ewa Moliszewska *, Małgorzata Nabrdalik, Zbigniew Ziembik

Faculty of Natural and Technology, Opole University, Poland

ARTICLE INFO

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

ABSTRACT

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. One of them formed light-colored sclerotia. 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.

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, 2009; Buddemeyer et al. 2004).

Rhizoctonia solani Kühn (the teleomorph *Tanatephorus cucumeris* (Frank) Donk) is a complex group of soil fungi that can infect many plant species. In Europe, isolates of *R. solani* with pathogenic properties are investigated in sugar beets, potatoes, forestry and orchards, and vegetable cultivation (Grosh et al. 2004; Hietala et al. 1994; Moliszewska 2009; Ogoshi 1987, 1996).

R. solani as a fungal complex is composed of genetically isolated groups, and their identification and classification are based on anastomosis behavior (Carling 1996; Ogoshi 1987, 1996). Currently, 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 sclerotia found on rice and soybean fields and also on rice stem lesions in Texas. They are pathogenic to the foliage of young rice and soybean plants. A mean hyphal diameter of the isolates was 5.1 μm, the average number of nuclei was 8.3 per cell, and the mean growth of their hypha was 0.53 mm/h at 28 °C on potato dextrose agar (PDA). Until now, AG11 had not been isolated from or identified on 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 Burgieł 2002; Moliszewska and Schneider 2002; Moliszewska 2009, Skonieczek et al. 2016).

---

* Corresponding author.
E-mail address: ewamoli@uni.opole.pl (E. Moliszewska).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.05.026

This study aims to describe *R. solani* AG11 as a pathogen of sugar beet seedlings as well as, due to its rare occurrence in the world, to review the worldwide appearance of this anastomosis group on other crops.

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 Nabben (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 1985; 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):

$$\boldsymbol{Ip}[\%] = [\sum(n_k \times k)/N \times k_{max}] \times 100\%$$

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 not infected, and the whole test plot was divided randomly into three replications. Seedlings were counted once a week with diseased seedlings being noted. The diseased seedlings were tested in the laboratory to determine the causal organism of the disease.

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,G.E., Shaw,M.W., Lambourne,C., Jennings,P., Clayburn,R., Boonham,N. and 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., Vilgalys,R. and Cubeta,M.A.	Australia	2002
6	LC215404	Roth1 (AG11)	soil	Misawa,T., Kayamori,M., Kurose,D., Sasaki,J. and Toda,T.	USA	2017
7	LC215406	Roth26 (AG11)	*Oryza sativa*			
8	LC215405	Roth11 (AG11)				
9	LC215410	89–740 (AG11)	*Triticum aestivum*		Australia	
10	LC215409	ZN163 (AG11)	*Lupinus angustifolius*			
11	LC215408	ZN56 (AG11)				
12	LC215407	R942 (AG11)	*Trifolium repens*			
13	LC215402	31 (AG11)	*Lilium* spp. Oriental lily Sheila		Hokkaido, Japan	
14	LC215403	L5 (AG11)	*Lilium* spp. Oriental lily Sheila			
15	AF354114	Roth16 (AG11)	Soybean	Gonzalez,D., Carling,D.E., Kuninaga,S., Vilgalys,R. and Cubeta,M.A.	USA	2006
16	AF354115	Roth24 (AG11)				
17	AB019027	Roth25 (ATCC 90858) (AG11)	Rice	Kuninaga,S., Carling,D.E., Takeuchi,T. and Yokosawa,R.	USA	2000
18	KX118394	X12SDSa (AG11)	Soybean seedlings	Ajayi,O.O. and Bradley,C.A.	USA	2019
19	KX118382	SP_19b (AG11)				
20	KX118381	SP_19a (AG11)				
21	KX118336	BVT_18 (AG11)				
22	KX118355	HPIN22A (AG11)				
23	KX118356	KARS02_1_11 (AG11)				
24	KX118358	KARS02_1_20 (AG11)				
25	KX118367	K_4_18b (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	KX810069	ID11 (AG11)				2016
29	FJ392702	CSL1846 (ATCC 90857) (AG11) beta-tubulin-like gene, partial sequence	*Lupinus* sp.	Budge,G.E., Shaw,M.W., Colyer,A., Pietravalle, S. and Boonham,N.	FERA culture collection	2009
30	AY154313	(AG11)	Not determined	Kuramae,E.E., Buzeto,A.L., Ciampi,M.B. and Souza,N.L.	Not determined	2009
31	KJ380831 (large subunit)	ZG-3 R1013 (AG11)	*Lupinus angustifolius*	Gonzalez,D.	Australia	2015
32	KJ380830 (large subunit)	Roth16 (AG11)	*Glycine* max	Gonzalez,D.	USA	2015
33	KJ380757 (small subunit)	Roth16 (AG11)	*Glycine* max	Gonzalez,D.	USA	2015
34	JX989021 (28S ribosomal RNA)	ZG-3_R1013 (AG11)	*Lupinus angustifolius*	Gonzalez,D.	Australia	2015
35	JX989020 (28S ribosomal RNA)	Roth16 (AG11)	*Glycine* max	Gonzalez,D.	USA	2015

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 coats – 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/dm^3 or 35 mg/dm^3 of hymexazole and 7.5 mg/dm^3 or 37.5 mg/dm^3 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 confidence level was considered in testing and the critical p-value was set at 0.05.

The trends of changes in time were estimated with the ANCOVA model (Hay-Jahans 2011; Kutner et al. 2004). The *p*-values of structural parameters β were calculated using the t test. For statistical computations, the R language (R Development Core Team (2015)) was utilized.

3. Results

The anastomosis group for both tested isolates was identified after pairing with tester isolates of *R. solani* AG1 through AG13. They anastomosed with the *R. solani* testers of AG11 – 11–01 and 11–03 giving distinct C2 reactions. The zymogram patterns confirmed that ID3 and ID11 isolates belonged to *R. solani* AG11. The comparison of their sequences, at the region ITS1-5,8S-ITS2 of their DNA, to the sequences from the GenBank database confirmed *R. solani* AG11 affiliation. Isolate ID3 showed an identity of 99.15% with Japanese *R. solani* isolates L5 (LC215403) and 31 (LC215402), isolated from *Lilium* sp., and with an isolate PT321 from China, obtained from potato (KC146421). The *R. solani* isolate ID11 showed 95.48% identity with the sequence of the *R. solani* isolate of 31 (LC215402) and 96.05% with PT321 from China, (KC146421). The phylogenetic analysis closely clustered the *R. solani* isolate ID11 together with both of the above mentioned isolates. There are 30 *R. solani* AG11 sequences submitted to GenBank (Table 1). For the phylogenetic analyses we used 28 (one was too short, one was a partial sequence of a beta-tubulin-like gene). The relationships of all AG11 isolates mentioned above were confirmed by constructing a phylogenetic tree on which two groups are recognizable as two subclades. One of them included mostly sequences from the USA obtained from soybean isolates, and the second one included other isolates with our ID11 among them. The accessions of the ITS1-5,8S-ITS2 of the rDNA nucleotide sequence for our isolates to GenBank were registered under the Nos. KU837255 (ID3 isolate) and KX810069 (ID11 isolate).

The daily rate of hyphal growth of tested isolates at 21 °C was 22.8 mm and 22.6 mm, respectively for isolate ID3 and ID11 (0.95 mm/h and 0.94 mm/h). The number of nuclei in young hyphae ranged from 13 to 15. The mean diameter of running hyphae was 6.6 µm and 7.0 µm for ID3 and ID11 respectively, and 7.25 µm for a tester isolate 11–01. Also, for both isolates, a description of mycelium on PDA was prepared. It was found that on the PDA medium, the mycelia of ID3 and ID11 were not identical; ID11 formed light-colored sclerotia after two weeks of growth. The mycelium was white-beige (ID11) to creamy (ID3), and each isolate showed both flat and puffy mycelia, with wide and indistinct concentric zonation. The colony reverse was creamy-beige for ID11 and yellowish-beige for ID3.

The metabolites of both isolates contained toxic compounds that influenced the seedlings' condition. After two days of exposure to post-culture filtrates of tested isolates, most healthy seedlings showed symptoms of necrosis (Fig. 2). The index of the disease [Ip] was 66% for ID3 and almost 50% for ID11, and the results of the experiment were significantly different compared to the control, which was not treated with post-culture filtrates (Fig. 2). Statistical analysis showed no significant difference between the action of ID3 and ID11 nor for the time of observation.

In the field test, it was distinctly seen that plants treated with both isolates showed worse emergence than control ones. The emergence of treated plants was slower, and three weeks after the sowing date, the damping-off was evident, especially for ID3 (Fig. 3). Finally, on the 39th day after sowing, the average stands were 7.8% for ID3 and 16.7% for ID11, compared to 51.1% for the unprotected and uninfected control. The diseased seedlings from plots infected by tested fungi showed infection by *R. solani*, as 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).

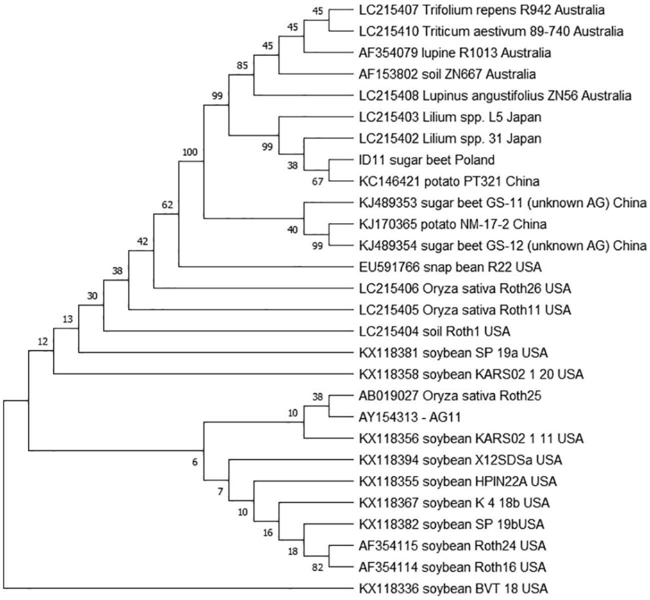

Fig. 1. Evolutionary relationships of different isolates of *R. solani* AG 11.

Using the ANCOVA method, trends in germination inhibition were estimated. The dependence of changes in sprout number on time of infection emergence was analyzed for ID3, ID11 and control treatments.

Equation (1) below shows the proposed linear description:

$$n = \beta_0 + \beta_{1.k} \times t \tag{1}$$

where n is the number of sprouts at time t.

In the model, the intercept β_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 β_0 confirms the presumed 0 value of this parameter. Significantly lower values of β_1 for ID3 and ID11 than for the control demonstrate inhibitory activity of isolates on the sugar beet germination process.

Seedlings 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

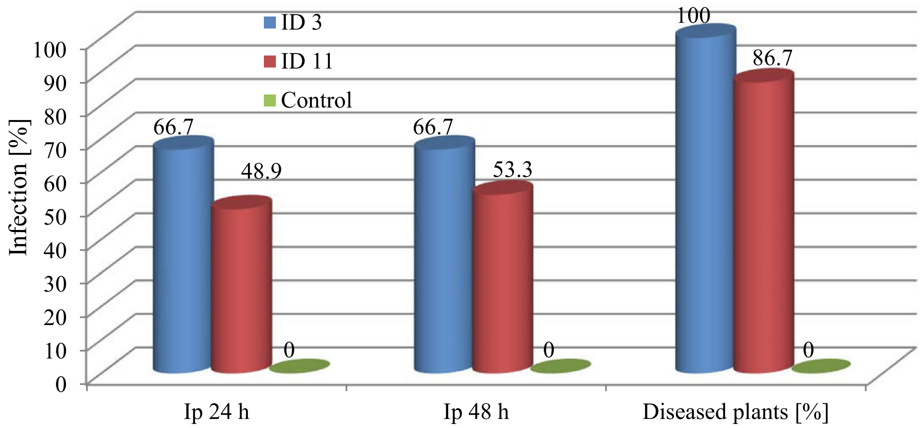

Fig. 2. The influence of fungal metabolites on the sugar beet seedlings, observations after 24 and after 48 h.

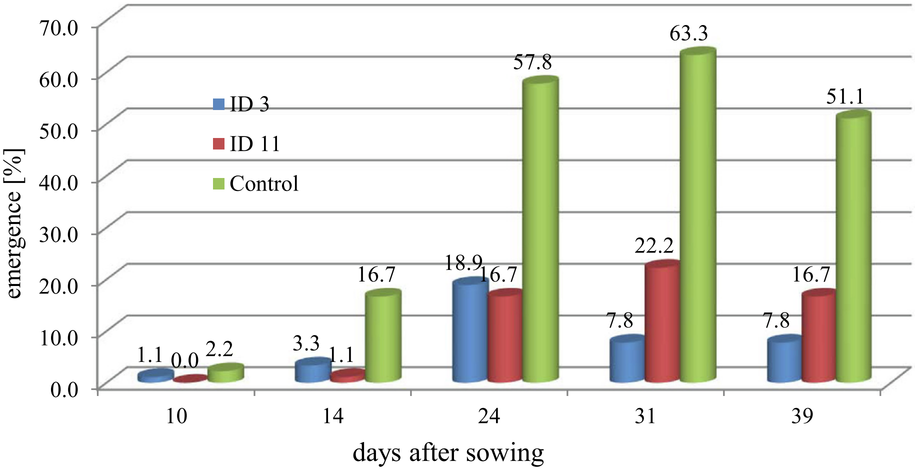

Fig. 3. The emergence of sugar beet seedlings in the field test under the influence the isolates ID3 and ID11 (data are the results of the emergence and damping-off).

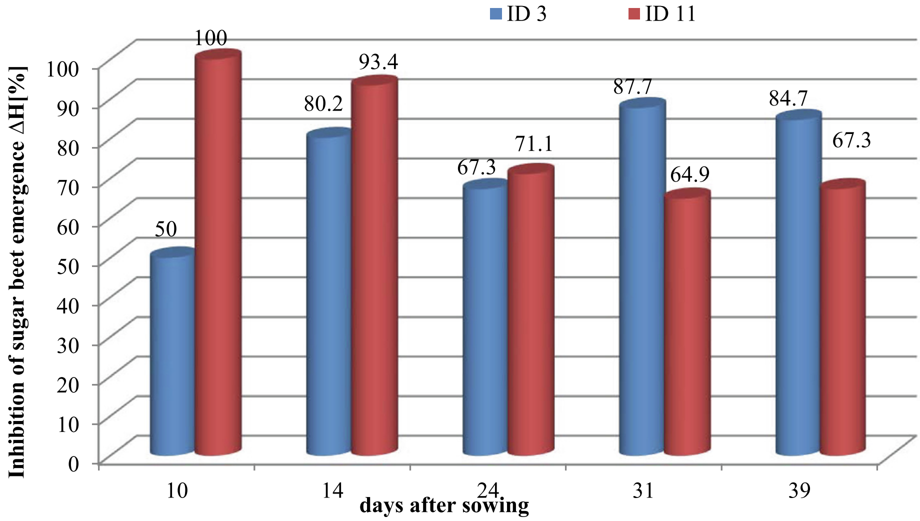

Fig. 4. The inhibition of sugar beet emergence, according the infection by isolates ID3 and ID11.

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

Table 2
Values of the structural parameters β, their standard errors and p-values for H_0: $\beta = 0$.

parameter	estimate	Standard error	p-value
β_0	−1.1	1.4	0.43
$\beta_{1,ID3}$	0.13	0.061	0.035
$\beta_{1,ID11}$	0.20	0.061	0.003
$\beta_{1,contr.}$	0.54	0.061	0.000

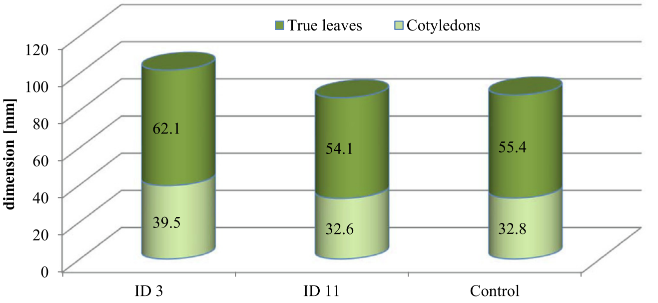

Fig. 5. The influence of isolates ID3 and ID11 on the dimensions of cotyledons and true leaves of survived sugar beet seedlings.

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 average hyphae diameter for ID3 ranged from 6.11 to 7.15 μm on media with fungicides and PDA, for ID11 – from 6.44 to 6.96 μm, and for tester 11–01 the average hyphae diameter on PDA was 7.25 μm. But, the results of hyphae diameter under the influence of fungicides were not statistically different for all combinations.

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.

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-

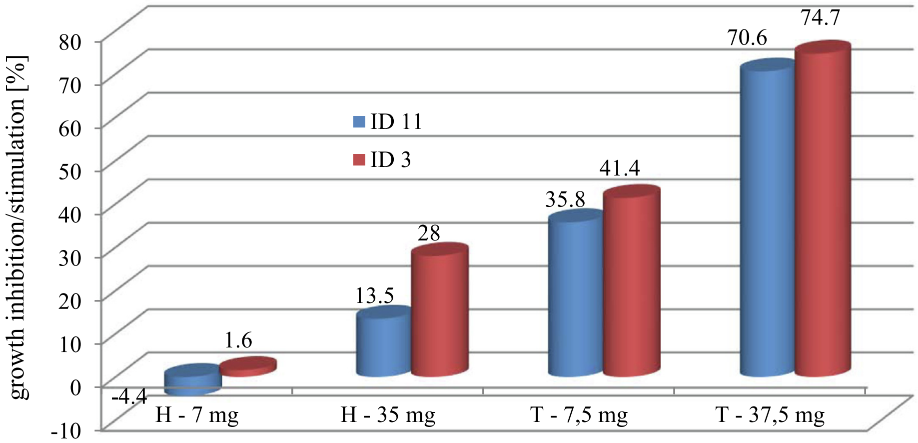

Fig. 6. The influence of two commercially used fungicides on the growth of isolates of AG11 (H - hymexazole, T – thiram; "-" means the stimulation of the growth).

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 (Moliszewska 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 (Chołuj and Moliszewska 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 gen 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 as legumes, 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.

References

Ajayi-Oyetunde, O.O., Bradley, C.A., 2017. Identification and Characterization of *Rhizoctonia* Species Associated with Soybean Seedling Disease. Plant Disease 101, 520–533. https://doi.org/10.1094/PDIS-06-16-0810-RE.

Broders, K.D., Parker, M.L., Melzer, M.S., Boland, G.J., 2014. Phylogenetic diversity of *Rhizoctonia solani* associated with canola and wheat in Alberta, Manitoba, and Saskatchewan. Plant Dis 98, 1695–1701.

Buddemeyer, J., Pfähler, B., Petersen, J., Märländer, B., 2004. Genetic variation in susceptibility of maize to *Rhizoctonia solani* (AG 2–2IIIB) - symptoms and damage under field conditions in Germany. Z. PflKrankh. PflSchutz 111, 521–533.

Carling, D.E., 1996. Grouping in *Rhizoctonia solani* by hyphal anastomosis reaction. In: Sneh, B., Jabaji-Hare, S., Neate, S., Dijst, G. (Eds.), *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control. Kluwier Academic Publishers, Dordrecht/Boston/London, pp. 35–38.

Carling, D.E., Rothrock, C.S., MacNish, G., Sweetingham, W., Brainart, A., Winters, S. W., 1994. Characterization of anastomosis group 11 (AG11) of *Rhizoctonia solani*. Phytopathol 84 (12), 1387–1393.

Chołuj, D., Moliszewska, E.B., 2012. The influence of *Aphanomyces cochlioides* on selected physiological processes in sugar beet leaves and yield parameters. Eur J Plant Pathol 132 (1), 59–70. https://doi.org/10.1007/s10658-011-9848-5.

Cruickshank, R.H., 1990. Pectic zymograms as criteria in taxonomy of *Rhizoctonia*. Mycological Research 94, 938–948.

Dalgaard, P., 2008. Introductory Statistics with. Springer.

Eken, C., Demirci, E., 2004. Anastomosis groups and pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia* isolates from bean in Erzurum. Turkey. J Plant Pathol 86 (1), 49–52.

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39, 783–791.

Garret, S.D., 1970. Pathogenic root infecting fungi. Cambridge University Press.

Gonzalez, D., Carling, D.E., Kuninaga, S., Vilgays, R., Cubeta, M.A., 2001. Ribosomal DNA systematics of *Ceratobasidium* and *Tanatephorus* with *Rhizoctonia* anamorphs. Mycologia 93, 1138–1150.

Grosh, R., Schneider, J.H.M., Kofoet, A., 2004. Characterization of *Rhizoctonia solani* anastomosis groups causing bottom rot in field-grown lettuce in Germany. Eur J Plant Pathol 110, 53–62.

Hay-Jahans, Ch., 2011. An R Companion to Linear Statistical Models. Chapman and Hall/CRC, Boca Raton, FL.

Herr, L.J., 1996. Sugarbeet diseases incited by *Rhizoctonia* spp. In: Sneh, B., Jabaji-Hare, S., Neate, S., Dijst, G. (Eds.), *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control. Kluwier Academic Publishers, Dordrecht/Boston/London, pp. 35–38.

Hietala, A.M., Sen, R., Lilja, A., 1994. Anamorphic and teleomorphic characteristic of a uninucleate *Rhizoctonia* sp. isolated from the roots of nursery grown conifer seedlings. Mycol Res 98, 1044–1050.

Jaaffar, A.K.M., Paulitz, T.C., Schroeder, K.L., Thomashow, L.S., Weller, D.M., 2016. Molecular Characterization, Morphological Characteristics, Virulence, and Geographic Distribution of *Rhizoctonia* spp. Washington State. Phytopathology 106 (521), 459–473.

Jones, R.K., Carling, D.M., 1999. Identification of *Rhizoctonia solani* AG-UNK from Rice and Rice Fields in Texas as AG-11. Plant Dis 83 (9), 880. https://doi.org/10.1094/PDIS.1999.83.9.880D.

Kronland, W.C., Stanghellini, M.E., 1988. Clean slide technique for the observation of anastomosis and nuclear condition of *Rhizoctonia solani*. Phytopathol 78, 820–822.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol. 35 (6), 1547–1549. https://doi.org/10.1093/molbev/msy096.

Kumar, S., Sivasithamparam, K., Sweetingham, M.W., 2002. Prolific production of sclerotia in soil by *Rhizoctonia solani* anastomosis group (AG) 11 pathogenic on lupin. Annals of Applied Biology 141 (1), 11–18. https://doi.org/10.1111/j.1744-7348.2002.tb00190.x.

Kutner, M.H., Nachtsheim, Ch., Neter, J., Li, W., 2004. Applied Linear Statistical Models. McGraw-Hill/Irwin.

Lamprecht, S.C., Tewoldemedhin, Y.T., Hardy, M., Calitz, F.J., Mazzola, M., 2011. Effect of cropping system on composition of the *Rhizoctonia* populations recovered from canola and lupin in a winter rainfall region of South Africa. Eur J Plant Pathol 131, 305–316. https://doi.org/10.1007/s10658-011-9809-z.

MacNish, G.C., Carling, D.E., Brainard, K.A., 1993. Characterization of *Rhizoctonia solani* AG-8 from bare patches by pectic isozyme (zymogram) and anastomosis techniques. Phytopathol 83, 22–927.

Misawa, T., Kayamori, M., Kurose, D., Sasaki, J., Toda, T., 2017. First report of *Rhizoctonia* disease of lily caused by *Rhizoctonia solani* AG-11 in Japan. J Gen Plant Pathol 83, 406–409. https://doi.org/10.1007/s10327-017-0735-6.

Moliszewska, E.B., Burgieł, Z.J., 2002. Badania wybranych szczepów grzyba *Rhizoctonia solani* Kűhn wyizolowanych z zamierających siewek buraka cukrowego (in Polish). Z. Nauk. AR w Krakowie 82, 101–104.

Moliszewska, E.B., Schneider, J.H.M. (2002). Some pathogenic properties of Rhizoctonia solani to sugar beet seedlings. Proc. 6th Conf. EFPP 2002, Prague. Plant Protect Sci, 38 (Special Issue 2), 322-324.

Moliszewska, E.B., 1999. Wpływ wybranych herbicydów na zdrowotność siewek buraka cukrowego, praca doktorska (The influence of herbicides on the sugar beet seedlings, doctoral dissertation, in Polish). Uniwersytet Opolski – Akademia Rolnicza. w Krakowie, Opole-Kraków.

Moliszewska, E.B., 2000. The influence of some herbicides on species variation of fungi associated with rotted tissue of sugar beet seedlings. The Polish Phytopathol Society Phytopathologia Polonica 20, 85–95.

Moliszewska, E.B., 2002. Preliminary anastomosis grouping by modified technique for *Rhizoctonia solani* isolated from sugar beet seedlings. The Polish Phytopathol Society Phytopathologia Polonica 26, 85–90.

Moliszewska, E.B., 2009. Etiologia wybranych chorób korzeni buraka cukrowego (The aethiology of diseases sugar beet roots, in Polish). Studia i Monografie nr 412, Wydawnictwo. Uniwersytetu Opolskiego, Opole.

Ogoshi, A., 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. Ann Rev Phytopathol 25, 125–143.

Ogoshi, A., 1996. Introduction – the genus *Rhizoctonia*. In: Sneh, B., Jabaji-Hare, S., Neate, S., Dijst, G. (Eds.), *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control. Kluwier Academic Publishers, Dordrecht/Boston/London, pp. 35–38.

Ohkura, M. (2008). Characterization of Rhizoctonia solani and Rhizoctonia-like fungi infecting vegetables in New York and their pathogenicity to corn. A Thesis presented to the Faculty of the Graduate School of Cornell University, in partial fulfillment of the requirements for the degree of Master of Science. http://hdl.handle.net/1813/10362 accesed 5 Jan 2020.

Ohkura, M., Abawi, G.S., Smart, C.D., Hodge, K.T., 2009. Diversity and aggressiveness of *Rhizoctonia solani* and *Rhizoctonia*-like fungi on vegetables in New York. Plant Dis. 93, 615–624.

Pisarek, I., Moliszewska, E.B., 1999. Badanie wpływu właściwości fizyko-chemicznych gleb na populację *Rhizoctonia solani* Kühn w obecności wybranych herbicydów (Studies of the influence of soil physical and chemical properties on the *R. solani,* in the presence in the soil of herbicides, in Polish). Zeszyty Problemowe Postępów Nauk Rolniczych 467, 579–585.

Development Core Team, R., 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Saitou, N., Nei, M., 1987. The Neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4, 406–425.

Schneider, J.H.M., Salazar, O., Rubio, V., Keijer, J., 1997. Identification of *Rhizoctonia solani* associated with field – grown tulips using ITS rDNA polymorphism and pectic zymograms. Eur J Plant Pathol 103 (7), 607–622.

Shao, J., 1999. Mathematical statistics. Springer-Verlag, New York.

Sharon, M., Kuninaga, S., Hyakumachi, M., Sneh, B., 2006. The advancing identification and classification of *Rhizoctonia* spp. using molecular and biotechnological methods compared with the classical anastomosis grouping. Mycoscience 47, 299–316. https://doi.org/10.1007/s10267-006-0320-x.

Sharon, M., Kuninaga, S., Hyakumachi, M., Naito, S., Sneh, B., 2008. Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. Mycoscience 49, 93–114.

Skonieczek, P., Nowakowski, M., Piszczek, J., Żurek, M., Matyka, Ł., 2016. Influence of selected *Rhizoctonia solani* isolates on sugar beet seedlings. Journal Of Plant Protection Research 56 (2), 116–121.

Tamura, K., Nei, M., Kumar, S., 2004. Prospects for inferring very large phylogenies by using the Neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101, 11030–11035.

Tewoldemedhin Y. T. (2005), Characterisation of Rhizoctonia in cropping systems in the Western Cape Province, on-line manuscript (accesed on November 2018)

Tewoldemedhin, Y.T., Lamprecht, S.C., McLeod, A., Mazzola, M., 2006. Characterization of Rhizoctonia spp. recovered from crop plants used in rotational cropping systems in the Western Cape province of South Africa. Plant Dis. 90, 1399–1406.

Windels, C.E., Nabben, D.J., 1989. Characterization and pathogenicity of anastomosis groups of *Rhizoctonia solani* isolated from *Beta vulgaris*. Phytopathol 79, 83–88.

Windels, C.E., Kuznia, R.A., Call, J., 1997. Characterization and pathogenicity of *Tanatephorus cucumeris* from sugar beet in Minnesota. Plant Dis 81, 245–249.

Yang, Y., Zhao, C., Guo, Z., Wu, X., 2015. Anastomosis group and pathogenicity of *Rhizoctonia solani* associated with stem canker and black scurf of potato in China. Eur J Plant Pathol 143 (1), 99–111. https://doi.org/10.1007/s10658-015-0668-x.

Yu J.F., Li X.N. (2013), Thanatephorus cucumeris isolate PT321 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence. https://www.ncbi.nlm.nih.gov/nuccore/KC146421 (Accessed on November 2016, 2018)

Zhang, Z., Schwartz, S., Wagner, L., Miller, W., 2000. A greedy algorithm for aligning DNA sequences. J Comput Biol 7 (1–2), 203–214.