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ORIGINAL ARTICLE

Anastomosis groups and sensitivity to fungicides of *Rhizoctonia solani* strains isolated from potato in Russia

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Abstract

A survey of *Rhizoctonia solani* isolates from potato tubers and stems grown in European and Far Eastern regions of Russia in 2012–2020 was conducted. Ten isolates from German and two from Australian seed potato tubers were analyzed, too. Species-specific region ITS1/2 of rDNA was sequenced and analyzed for all 53 isolates. Out of those, 51 isolates were classified as multinucleate and two as binucleate *Rhizoctonia*. Among the tested multinucleate isolates, 49 belong to AG 3PT, the most frequent group on potato, and two isolates to AG 5 group. Binucleate *Rhizoctonia* were determined as AG K. All AG 5 and AG K isolates were isolated from Russian potato tubers (AG 5) and stems (AG K).

A study of fungicide resistance showed that all tested isolates were susceptible to thiabendazole ($EC_{50} < 7.2$ mg/l) and fludioxonil ($EC_{50} < 42$ mg/l). Resistance to benzoic acid varied between isolates, and EC_{50} ranged from 5.38 to 362 mg/l. After long-term (1 month and more) cultivation on Petri dishes with fludioxonil, several strains developed resistant sectors. Almost all strains were sensitive to pencycuron ($EC_{50} < 5$ mg/l), while three isolates with very high resistance ($EC_{50} > 1000$ mg/l) were detected. Two resistant isolates belonged to the anastomotic group AG 5 and one to AG 3. To our knowledge, this is the first report of pencycuron-resistant *R. solani* strains in Russia. Three resistant to pencycuron multinucleate and binucleate isolates grow at temperature + 34 °C. After a week of incubation, no growth of sensitive multinucleate isolates at + 34 °C was registered.

Keywords *Rhizoctonia solani* · Anastomosis group · rDNA polymorphism · Rhizoctonia potato disease · *Solanum tuberosum* · *Thanatephorus cucumeris*

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Introduction

The potato (*Solanum tuberosum* L.) is one of the most cultivated agricultural crop in Russia. The annual crop losses are usually caused by the basidiomycetous fungus *Rhizoctonia solani* var. *solani* J.G. Küch 1858, which infects tubers, stems, stolons and roots of potato. It is the causal agent of the widespread potato disease called rhizoctoniosis or black scurf. This disease is widespread in Russia as well as in other potato producing regions of the world. The *Rhizoctonia* disease on potato is extremely difficult to control due to the fact that the pathogen is able to outlast saprotrophic in soil and subsequently infect different hosts, e.g., tobacco, tomato, soybean (Muzhinji et al. 2015; Ajayi-Oyetunde and Bradley 2018). Potato cultivars with high levels of resistance to the disease are not yet available (Tsror 2010).

Rhizoctonia solani is a species complex containing several genetically distinct subspecific groups. Multinuclear *R. solani* (teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk 1956) nowadays comprise 13 anastomosis groups (AG) (Carling et al. 2002). Isolates of *R. solani* that infect potatoes belong predominantly to AG 3, but AG 2–1, AG 4 and AG 5 also occur on potato. Based on molecular analysis, the most frequent AG 3 was divided into three subgroups depending on the main host plant, isolates from potato form AG 3PT. This was confirmed in studies in the UK (Woodhall et al. 2008), Finland (Lehtonen et al. 2008), France (Campion et al. 2003; Fiers et al. 2011), Poland (Woodhall et al. 2013), Cyprus (Kanetis et al. 2016) and China (Yang et al. 2017); see also the review by Tsror (2010). Over the last years, isolates of new groups (AG 1, AG 2–2, AG 7, AG 9, AG 11) were isolated from damaged potato plants (stems, roots or tubers) (Carling et al. 2002; Truter and Wehner 2004; Lehtonen et al. 2008; Abd-Elsalam et al. 2009; Das et al. 2014). Groups of binucleate *Rhizoctonia* (AG A, E, G and K) (teleomorph *Ceratobasidium* sp.) were isolated from potato in Turkey (AG A and AG K, Ozer and Bayraktar, 2015), South Africa (AG A and AG R, Muzhinji et al., 2018), and Brazil (AG A and AG R, Inokuti et al., 2019). However, the intraspecific diversity of the pathogen in Russia has not been investigated properly (Pilshchikova and Gannibal 2016). In previous studies of Russian *Rhizoctonia*, only AG 3 group was detected from the potato (Shaldyaeva, Pilipova 2005).

The main method of rhizoctoniosis control is the fungicide treatment of tubers with chemical or biological fungicides. Due to high variability connected with the coexistence of different nuclei in each cell, *Rhizoctonia* can develop resistance against fungicides. Several chemicals were registered to control *R. solani*. Pencycuron, thiabendazole, benzoic acid, and fludioxonil are of most common in Russia for tuber treatment. Different anastomosis groups differ in their fungicide sensitivity (Kataria et al., 1991; Campion et al. 2003; Lehtonen et al. 2008; Özer and Bayraktar 2015).

The details of AG compositions of *R. solani* and the effectiveness of fungicides remain unclear in Russia. Our study aims to analyze AG composition of Russian *R. solani* isolates from potato and evaluate their sensitivity to several widespread in Russia fungicides. Several isolates obtained from German and Australian tubers were analyzed to evaluate the possible differences between potentially imported *R. solani* isolates. This is the first study from Russia identifying the AG of *Rhizoctonia* isolates on potato using ITS sequencing.

Materials and methods

Fungal isolates

The tubers with typical black sclerotia were collected in different potato growing regions in Russia, Germany, and Australia from 2012 to 2020 (Fig. 1, Table 1).

Axenic cultures were obtained by transfer of hyphae tips, sclerotia or diseased stem parts onto fresh PDA plates with penicillin and incubated at 25 °C. Only isolates showed typical for *R. solani* macro- and micromorphological characteristics (typical hyphal branching, sclerotia formation) (Parmeter, Whitney, 1970) were used in investigation.

In vitro assessment of fungicide sensitivity

Four chemical fungicides (fludioxonil, pencycuron, benzoic acid, and thiabendazole) were evaluated in vitro for their efficacy against R. *solani* (Table 2). The fungicides were selected based on their current usage in Russia and were obtained from local agro-chemical suppliers. The sensitivity of *Rhizoctonia* isolates to fungicides was evaluated in mycelial growth assays. The fungicides were added at different concentrations to autoclaved PDA medium to produce a concentration series of 0, 0.01, 0.1, 1, 10, 100 and 1000 mg/l for each fungicide (active ingredient). Mycelial plug (5 mm in diameter) of each *Rhizoctonia* isolate was punched from the margin of an actively growing colony of a 5-day old culture with a sterile cork-borer and placed at the center of a 90 mm PDA plate amended with fungicide as well as on non-amended PDA plates. Per treatment three replicates were produced, and the plates were incubated at 24 ± 1 °C for 4 days, the radius of the fungal colony of each plate was measured at perpendicular angles, and the average of the two measurements was used for data analysis. The fungicide concentration that inhibits

Fig. 1 Collection sites of infected potato tubers (see Tables 1, 2)

Table 1 Collecting sites of *R. solani* isolates

Region	Location of collection site in Fig. 1	Diseased part of the plant	Year	Cultivar	Number of isolates
Smolensk region	1	PT	2012	Udacha	10
Moscow region	2	PT	2012	Jubiley Zhukova	2
Moscow region	2	PT	2013	Sante	11
Germany, Field 1	3	PT	2013	Delfine	2
Germany, Field 1	3	PT	2013	Estrella	2
Germany, Field 2	4	PT	2013	Safia	1
Kostroma region, field 1	6	PT	2014	Manifest	6
Vladimir region	5	PT	2014	Red Scarlett	4
Kostroma region, field 2	7	PT	2017	Unknown	5
Magadan region	8	PT	2017	Unknown	2
Kaluga region	9	PT	2018	Romano	1
Moscow region	2	PT	2018	Safia	1
Moscow region	2	PT	2019	Gala	2
Astrakhan region	10	PS	2020	Unknown	2
Australia, Victoria	11	PT	2020	Unknown	2

*PT potato tuber, *PS* potato stem

Table 2 Description of fungicides

Active ingredient (a.i.)	Chemical name	Trade name (manufacture)
Pencycuron*	N-[(4-chlorophenyl)-methyl]-N-cyclopentyl-N′-phenylurea	Prestige® Emulsifiable concentrate 150 g/l (Bayer Crop Science)
Fludioxonil	4-(2,2-difluoro-1,3-benzdioxol-4-yl)-1 h-pyrrole-3-carbonitrile	Maxim® Suspension concentrate 25 g/l (Syngenta)
Benzoic acid	Triethanolamine benzoate	Kagatnic® Soluble concentrate 300 g/l (Shchelkovo Agrohim)
Thiabendazole	4-(1H-benzimidazol-2-yl)-1,3-thiazol	Tecto® Soluble concentrate 500 g/l (Syngenta)

*Prestige preparation (pencycuron 150 g/l + imidacloprid 140 g/l) was used for tests. According our previous experiments, imidacloprid has no influence on the growth of *R. solani* mycelium

linear growth of colony of 50% over control (EC_{50}) was determined for each isolate (Horsfall, 1956).

Growth rate measurements

The ability of several strains to grow at high temperature (+ 34 °C) was evaluated to estimate the possible level of the disease development in summer, when the temperature of the upper level of soil increases significantly. The growth of each isolate was observed on maltagar in Petri dishes. Small agar inoculum blocks (5 mm in diameter) were placed in the center of Petri dishes and incubated at + 34 ± 1 °C (experiment) and 24 ± 1 °C (control) during 5 days in the dark. After the incubation period, the colony radiuses were measured (in two directions per colony).

DNA extraction, PCR amplification, DNA sequencing and phylogenetic analyses

For DNA extraction, mycelium of fungal isolates was grown on pea-broth liquid nutrient media. DNA isolation and PCR amplification were performed as described by Kutuzova et al. 2017. ITS1 and ITS4 primers were used for the amplification of the ITS region (White et al. 1990). DNA concentration was determined using a spectrophotometer NanoDrop 2000 (Thermo Scientific, USA) by measuring absorbance at 260 nm. PCR amplicons were sequenced using the BigDye®Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and the Applied Biosystems 3730 xl automated sequencer (Applied Biosystems, USA). Each fragment was sequenced in both directions using the same primers described above. Contigs sequences were used to identify the fungal isolates AG based on the similarity using the BLASTn program (version 2.0, NCBI United States National Institutes of Health, Bethesda, MD, USA).

The nucleotide sequences were aligned using MEGA 7 (ClustalW algorithm), followed by manual checking and editing where necessary. The sequence data were submitted into NCBI GenBank database (Fig. 2). Phylogenetic analysis was performed with the maximum likelihood method in MEGA 7. Bootstrap method was performed with 1000 replications. Reference *R. solani* sequences were gained from Fiers et al. (2011), Muzhinji et al. (2018), Yang et al. (2017), and GenBank database.

To determine the anastomosis groups of tested isolates, ITS sequences of isolates with known AG classification were used. This method gives the same results as earlier developed method of division *R. solani* isolates into AG based on anastomosis reactions (Sharon et al., 2008).

Results

The total 55 isolates of *Rhizoctonia* spp. were obtained from diseased tubers and stems of potato plants from Moscow, Kaluga, Smolensk, Kostroma, Vladimir, Astrakhan, Magadan regions of Russia, Germany and Australia (Fig. 1, Table 1). Out of those, 51 isolates were classified as multinucleate, two—as binucleate Rhizoctonia by ITS analysis (Fig. 2). Two anastomosis groups were determined among the tested multinucleate isolates. Forty-nine belonged to AG 3PT, the most frequent group. Two isolates from Moscow and Smolensk regions – to AG 5 group. Two isolates from potato stems collected in Astrakhan region were identified as binucleate *Rhizoctonia* AG K group. In Russia, isolates of the group AG 5 were detected on potato tubers for the first time. Isolates of other AG groups were not detected in our study.

Fungicide sensitivity and growth under different temperature

A study of fungicide sensitivity has shown that all 46 tested isolates were susceptible to thiabendazole ($EC_{50} < 7.2$ mg/l, while the recommended chemical concentration for tuber treatment is 4800–5600 mg/l)) (Table 3). Sensitivity to benzoic acid varied between isolates, and EC_{50} ranged from 5.38 to 362 mg/l (recommended concentration 15,000 mg/l). Most of strains were sensitive to fludioxonil ($EC_{50} < 1.5$ mg/l, recommended concentration 1000 mg/l) (Fig. 3), but three strains (R12S2PT32, R13M2PT1, R12M1PT3) showed reduced sensitivity toward fludioxonil, their EC_{50} were 7.6; 20.0; 41.7 correspondingly. After long-term (1 month and more) cultivation on Petri dishes with fludioxonil, several strains developed resistant sectors.

Almost all strains were sensitive to pencycuron ($EC_{50} < 5$ mg/l, recommended concentration 10,500 mg/l), while three isolates (R12S2PT7, R13M2PT1, R14VMrs6) with very high resistance ($EC_{50} > 1000$ mg/l) were detected. EC_{50} values for these isolates could not be accurately estimated because their growth was not inhibited by more than 50% even on the highest concentration (1000 mg/l) tested. Two (R12S2PT7, R13M2PT1) resistant isolates belonged to the anastomotic group AG 5 and one (R14VMrs6) to AG 3.

Table 3 shows growth of 46 isolates under temperatures 24 and 34 °C, where colony diameters at optimal temperature (24 °C) after 48 h of incubation ranged from 24 to 59 mm. Among multinucleate isolates, only three resistant to pencycuron were able to grow at high temperature (34 °C). Both binucleate *R. solani* isolates grew at 34 °C as well as at 24 °C. After a week of incubation, no growth of sensitive multinucleate isolates at 34 °C was registered.

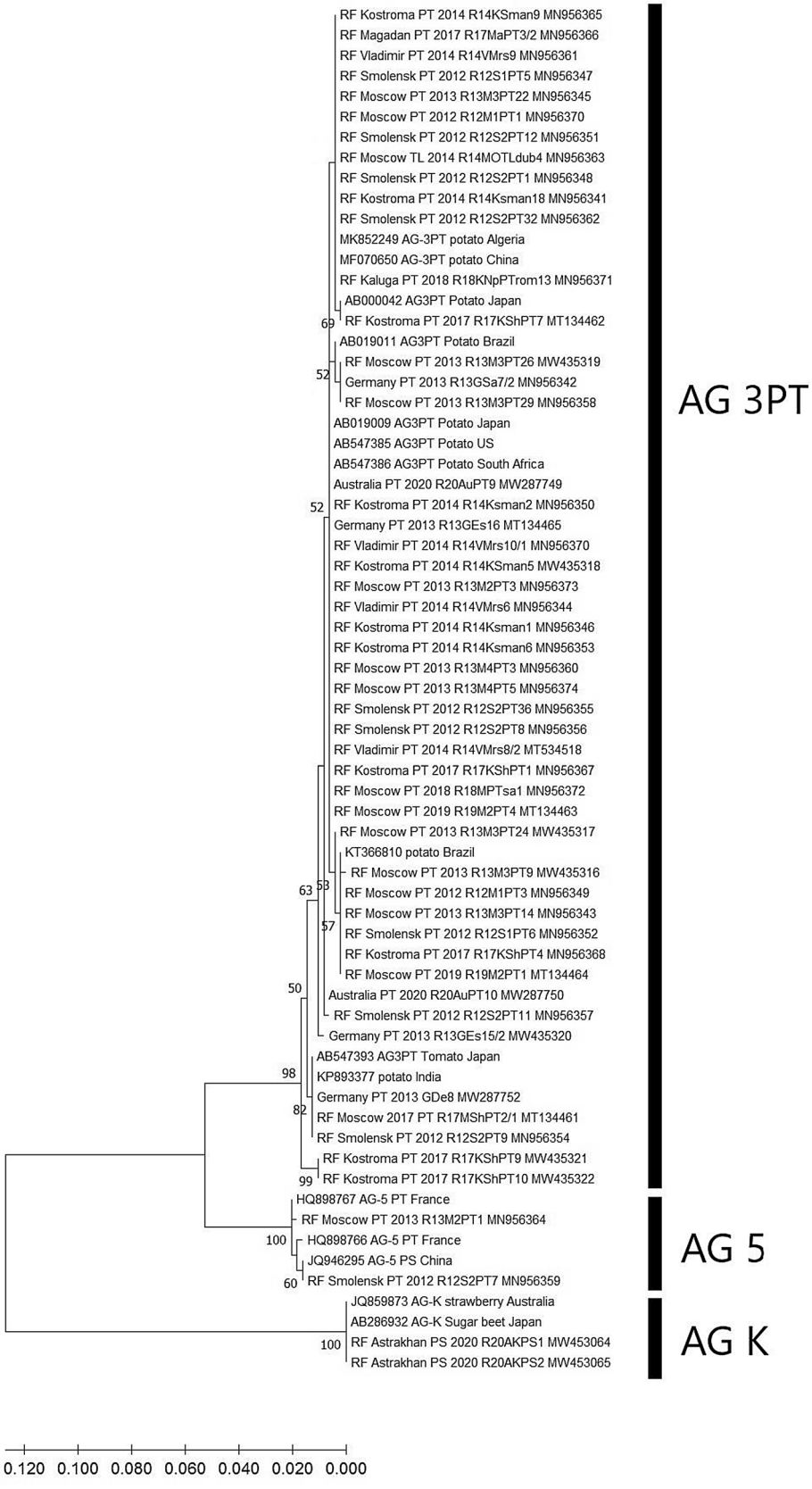

Fig. 2 Maximum likelihood tree of ribosomal internal transcribed spacer (ITS) sequences

Table 3 Sensitivity to fungicides and growth at high temperatures for tested R. solani strains

Isolate	Location of collection site in Fig. 1	AG	Fludioxonil EC_{50}, mg/l	Pencycuron EC_{50}, mg/l	Benzoic acid EC_{50}, mg/l	Thiabendazole EC_{50}, mg/l	Colony radius[a], mm	
							24 °C	34 °C
R12S2PT1	1	3	0.01	0.01	61.21	4.23	44.20	0.00
R12S2PT7	1	5	0.02	> 1 000.00	60.63	0.52	53.00	12.00
R12S2PT8	1	3	0.01	0.01	67.86	4.88	46.30	0.00
R12S2PT9	1	3	0.02	0.02	41.30	7.14	16.00	0.00
R12S2PT11	1	3	0.01	0.02	7.80	4.84	32.50	0.00
R12S2PT12	1	3	0.01	0.02	7.22	4.68	25.70	0.00
R12S2PT32	1	3	7.6	0.02	60.29	5.38	25.00	0.00
R12S2PT36	1	3	0.01	0.02	5.64	0.76	33.30	0.00
R12S1PT5	1	3	0.01	0.01	53.66	5.05	26.00	0.00
R12S1PT6	1	3	0.01	0.01	362.50	0.56	33.50	0.00
R12M1PT1	2	3	0.01	0.01	6.47	0.58	26.00	0.00
R12M1PT3	2	3	41.7	0.02	219.12	4.69	43.00	0.00
R13M3PT14	2	3	0.01	0.01	66.74	3.20	30.70	0.00
R13M3PT22	2	3	0.05	0.02	50.00	3.25	17.00	0.00
R13M3PT29	2	3	0.45	1.09	6.25	0.68	12.00	0.00
R13M2PT1	2	5	0.03	> 1 000.00	10.00	6.21	59.00	26.00
R13M2PT3	2	3	20.0	0.02	55.00	0.12	12.00	0.00
R13M4PT3	2	3	0.01	0.02	6.38	0.63	18.00	0.00
R13M4PT5	2	3	0.04	0.01	36.54	0.53	15.00	1.00
R13GDe8	3	3	0.07	0.25	61.84	0.56	17.50	0.00
R13GDe21	3	3	1.50	0.02	87.14	4.55	33.30	0.00
R13GEs15	3	3	0.07	0.07	5.56	0.65	35.00	0.00
R13GEs16	3	3	0.06	0.03	93.57	2.50	32.50	0.00
R13GSa7/2	4	3	1.04	0.05	49.60	0.58	44.00	0.00
R14KSman1	6	3	0.05	0.06	5.56	5.50	31.70	0.00
R14KSman2	6	3	0.95	0.06	37.00	3.21	45.50	0.00
R14KSman6	6	3	1.15	0.07	250.00	0.57	15.00	0.00
R14KSman9	6	3	0.02	1.07	51.54	0.51	14.00	0.00
R14KSman18	6	3	1.33	0.06	55.00	0.48	17.30	0.00
R14VMrs6	5	3	0.35	> 1 000.00	55.67	0.61	24.00	18.00
R14VMrs8/2	5	3	0.05	0.02	6.15	0.57	19.00	0.00
R14VMrs9	5	3	0.55	1.08	5.38	0.60	25.00	0.00
R14VMrs10/1	5	3	0.05	0.06	34.55	3.21	52.30	0.00
R17KShPT1	7	3	0.06	0.05	333.04	5.00	23.00	0.00
R17KShPT4	7	3	0.06	0.11	18.08	3.48	39.00	0.00
R17KShPT7	7	3	1.04	0.05	27.56	3.94	28.30	0.00
R17MShPT2/1	2	3	0.06	0.08	6.51	0.59	26.00	0.00
R17MaPT3/2	8	3	0.05	0.08	5.86	0.62	19.30	0.00
R18KNpPTrom13	9	3	0.08	0.14	7.13	0.67	31.00	0.00
R18MPTsa1	2	3	0.05	5.13	6.61	0.58	25.50	0.00
R19M2PT1	2	3	1.08	0.01	5.98	0.50	29.00	0.00
R19M2PT4	2	3	1.03	0.00	6.31	0.55	24.00	0.00
R20AuPT9	11	3	0.05	0.07	7.08	0.50	30.80	0.00
R20AuPT10	11	3	0.05	0.07	5.55	0.55	21.30	0.00
R20AKPS1	10	K	0.66	4.50	71.29	0.58	22.00	22.00
R20AKPS2	10	K	0.66	2.50	65.89	0.52	22.00	23.00

[a]after 48 h of incubation

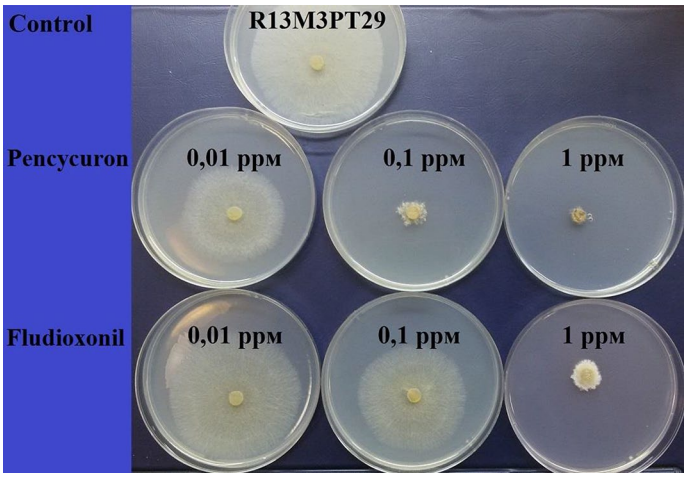

Fig. 3 Sensitive isolate growth on different concentrations of fungicides (1 ppm = 1 mg/l)

Discussion

The predominance of *R. solani* potato-infecting subgroup AG 3PT on potato has been reported worldwide by various authors (Tsror 2010; Das et al. 2014; Muzhinji et al. 2015; Yang et al. 2017, Fiers et al. 2011; Ozer, Bayraktar 2015). In Russia, most of tested isolates belonged to this group too, some genetic differences between them were found. Almost all isolates, including those collected in Russian Far East, Australia and Germany, had identical (or nearly identical) sequences with the submitted to NCBI AG 3PT strains from Japan, China, Algeria, Brazil, and South Africa. No geographic grouping of strains was identified, which is consistent with other authors' (González et al. 2006; Fiers et al. 2011) observations.

Sequences of two AG 5 isolates differed by 2 nucleotides (similarity 99.05%), and they shared 99.2–100% genetic similarity with the AG 5 reference isolates from France and China (Yang, Wu, 2012; Fiers et al. 2011).

Sequences of two binucleate (AG K) isolates were identical; they were similar to the sequences of AG K isolates from Australia (isolated from strawberry) and Japan (from sugar beet).

The only strain from tomato (R14MOTL Dub4, MN956363) belonged to AG 3PT group and was completely similar to many potato isolates obtained in Russia. It was also similar to AG 3 PT strains, isolated from tomato in Pakistan (according to submitted in GenBank sequences, the similarity was 97,1–98,89%) and in Japan (98.89–99.63%) (Gondal et al. 2019; Misawa et al. 2020). In Pakistan, it was the most frequent group in tomato (Gondal et al. 2019).

Our results show that ITS1 sequences are more variable than ITS2 ones. Most of discovered ambiguities belonged the standard variable sites detected by a French group (Fiers et al. 2011); in several isolates, atypical nucleotide replacements were found. No variation was found in 5,8 S rRNA.

The coexistence of multiple sequence types within individual isolates was confirmed before, by cloning and sequencing of French isolates (Fiers et al. 2011). It was found also for AG 2–1 (Pannecoucque and Hofte 2009) and AG 3 (Justesen et al. 2003) within *R. solani* species complex. Among tested AG 3 isolates, variable sites were detected as double peaks in sequence file. Such peaks occurred in ITS1 and ITS2 regions of 30 of 48 AG 3 isolates tested. Within analyzed ITS region, 12 polymorphic sites were detected: nine in ITS1 region and three in ITS2 region. The number of variable sites ranged from 1 (for example, for isolate R12S2PT12) to 7 (R12S2PT8) for different isolates within AG 3PT.

It has been previously reported that some isolates from AG 5 have a higher level of resistance to pencycuron in comparison with strains of other AG groups. Strains of the group AG 5 with elevated level of resistance to pencycuron (EC_{50} up to 5 mg/l) were noted among French isolates (Campion et al. 2003). Pencycuron, developed for special control of *R. solani*, is successfully used in combination with other chemical agents as a part of various preparations (Prestige®, Monceren®). A high level of efficiency was registered to this chemical agent (Thind et al. 2002; Djébali and Belhassen 2010). AG 5 and AG 3 strains highly resistance to pencycuron ($EC_{50} > 1000$ mg/l) were found for the first time.

Fludioxonil provides effective control against all *R. solani* isolates; similar results were obtained in Canada (Bains et al. 2002), Tunisia (Djébali et al. 2014), and South Africa (Muzhinji et al. 2018). Fludioxonil has proved to be more effective against rhizoctoniosis in field conditions compared to other fungicides (Bains et al. 2002; Truter 2005). Since sensitive isolates are predominant on potato, application of fludioxonil and pencycuron seems to be appropriate.

Special attention should be paid to the ability of strains to grow at high temperature. Considering development of potato and other Solanaceae plants cultivation in South regions (Devaux et al. 2020) temperature- and

pencycuron-resistant aggressive strains of *R. solani* may cause great loss of potato crop. Within Tunisian isolates, three AG 3 strains were found which were able to grow at high temperature (30 °C), but they were sensitive to pencycuron (Djébali et al. 2014).

Potato varieties differ in resistance to rhizoctoniosis (Bains et al. 2002). In the European Cultivated Potato database, there are eight varieties with high to very high resistance to *Rhizoctonia*, some of them are widely used in agriculture (Ackersegen), but no completely resistant variety exists. In our previous work (Pronicheva and Elansky, 2014), we estimate the resistance of cutted stems of 10 potato cultivars to three *R. solani* strains: one resistant to pencycuron and high temperature AG 5 isolate (R13M2PT1) and two sensitive AG 3 isolates (R12S1PT3 and R13M3PT22). The potato cultivars differed in levels of their resistance, but in all experiments resistant strain R13M2PT1 was the most aggressive to all cultivars tested.

The research has shown the presence of extremely harmful *R. solani* isolates in Russian field populations: resistant to fungicides and high temperature, and aggressive to potato. The continuous control of harmful strains in regional populations is required to prevent the disease, reduce yield losses and improve the quality of potato seeds and tubers.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Consent for publication All authors agree with this publication.

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