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ORIGINAL ARTICLE

Genetic variability and virulence of some Iranian *Rhizoctonia solani* isolates associated with stem canker and black scurf of potato (*Solanum tuberosum* L.)

Mehdi Nasr Esfahani*

Plant Protection Research Department, Isfahan Agricultural and Natural Resources Research and Education Center (AREEO), Isfahan, Iran

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*Corresponding address:
mne2011@gmail.com

Abstract

Stem canker and black scurf of potato (*Solanum tuberosum* L.) caused by *Rhizoctonia solani* Kühn are important and epidemic diseases in potato-growing regions worldwide, including Iran. In this study, 120 isolates were retrieved from infected stem canker from six potato-growing regions in Iran (Isfahan, Ardebil, Fars, Hamedan, Kurdestan and Kerman). Out of these, 30 isolates were selected as representatives for genetic and virulence analysis. The isolates were analyzed by one sequence analyzes of the ITS-rDNA region, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR), as well as virulence studies. Based on sequence analysis of the ITS-rDNA region, all 30 isolates were assigned to the anastomosis group (AG) and all were assigned to AG-3 PT. Cluster analysis using the unweighted pair group method with the arithmetic averages (UPGMA) method for both RAPD and ISSR markers revealed that they were divided into three main groups, with no correlation to geographical regions of the isolates. Pathogenicity tests showed that all isolates were pathogenic on potato cv. Agria; however, virulence variability was observed among the isolates. The grouping based on RAPD analysis and virulence variability was not correlated.

Keywords: genetic diversity, isolates, ISSR, RAPD, virulence variability

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important crops that plays a vital role in ensuring food security in many countries globally, and has a high degree of genetic diversity. Many varieties are grown commercially around the world. Potato is the fourth most staple crop after wheat, rice and corn around the world (Dubey *et al.* 2012).

Potato plants can be infected with different pathogens such as stem canker. Stem canker and black scurf caused by *Rhizoctonia solani* Kühn are some of the most important diseases on potatoes, and causes considerable damage (Cubeta and Vilgalys 2000; Misawa and Kuninaga 2010). This species can cause disease on around 500 plant species (Gush *et al*. 2019). In general, the isolates, AG-1, AG-2-1, AG-3, AG-4, AG-5 and AG-9 with many subgroups, have been reported as the main isolates causing stem canker and black scurf on potato worldwide (Tsror 2010; Fiers 2011; Muzhinji *et al*. 2015). However, AG-3 is considered the predominant anastomosis group associated with Rhizoctonia diseases on potatoes (Carling and Leiner 1986; Bandy *et al*. 1988; Woodhall *et al*. 2008; Das *et al*. 2014; Ito *et al*. 2017), including Iran (Balali *et al.* 2007).

At least 13 related, but genetically distinct, anastomosis groups (AGs) are known to form the species complex of *R. solani* (Carling *et al*. 2002a, 2002b; Yang *et al.* 2017). They vary in hyphal anastomosis ability, host symptoms, host range, biochemical patterns,

geographical distribution, fatty acid composition, and DNA sequences (Ogoshi 1987; Carling 1996; Cubeta and Vilgalys 1997). Recently, sequence analyzes of ribosomal DNA (rDNA) regions have been employed for the accurate identification of *Rhizoctonia* species and their anastomosis groups (Kuninaga *et al.* 1997; 2002; Sharon *et al.* 2006, 2008; Yang *et al.* 2017).

Genetic analysis of plant pathogen populations is fundamental to understanding epidemiology, resistance management and host-pathogen coevolution (McDonald and Linde 2002). Understanding genetic variation within pathogen populations is necessary and should be considered as the first step for developing disease management programs (Godoy-Lutz *et al.* 2008). In Iran, genetic diversity and virulence variability among *R. solani* isolates causing stem canker and black scurf of potato has not been studied. Therefore, the objective of this study was to determine the genetic and virulence variability among *R. solani* isolates associated with potato plants collected from various potato-growing regions of Iran.

Materials and Methods

Isolation

Rhizoctonia infected samples were collected from six potato growing regions in Iran: Isfahan, Ardebil, Fars, Hamedan, Kerman and Kordestan states (Fig. 1). Sixty fields from all major potato-cultivating areas of Iran were surveyed, and potato plants exhibiting pronounced stem canker symptoms were collected. Two infected plants were arbitrarily selected per field. To isolate the causal agent of the disease, 5-mm^2 sections were taken from the edge of lesions and surface sterilized in 1% NaOCl for 2 min. The infected sections were rinsed in sterile distilled water twice, and then dried using sterilized filter paper for 10 min and eventually plated on potato dextrose agar (PDA) (Nasr Esfahani 2018a). The media were incubated in an incubator in the dark at 25°C for 10 days (Caesar *et al.* 1993). A total of 120 isolates were obtained, out of which 30 were selected randomly based on variations

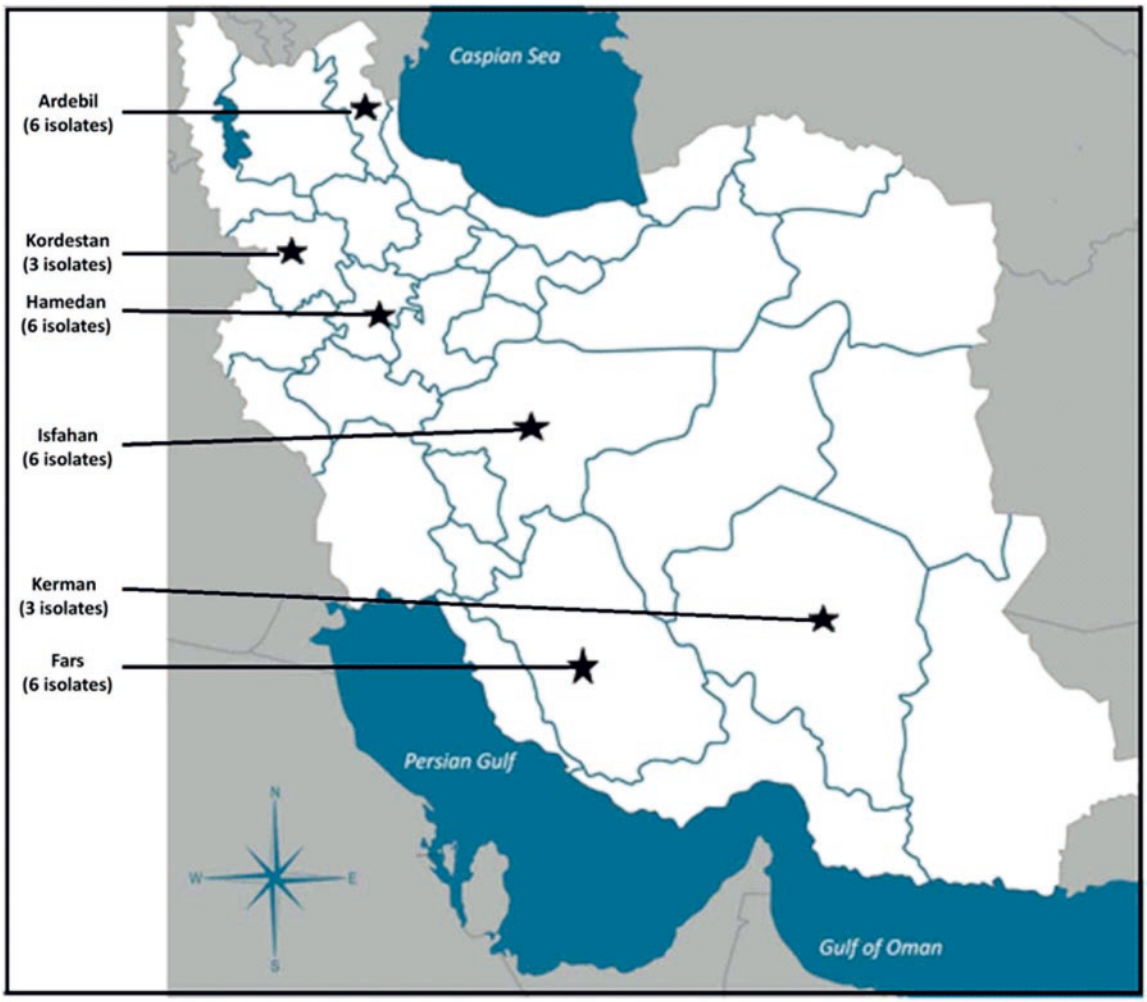

Fig. 1. Map of Iran shows the locations of potato plantations where 30 *Rhizoctonia solani* isolates were collected, including, the state name and the numbers of the isolates

in morphology and phenology as the representatives to cover almost all the sample isolates which were to be subjected to genetic and virulence analysis. The hyphal tip technique was used to purify the isolates for further studies (Boysen *et al.* 1996; Linde *et al.* 2005; Nasr Esfahani 2019).

DNA extraction, PCR amplification and sequencing

A semi-synthetic liquid medium was used to prepare mycelial mass production for DNA extraction (Romain *et al.* 1999; Nasr Esfahani 2018b). Nutrient medium (40 ml) was poured into 100-ml Erlenmeyer flasks, which were closed with sterile cotton. After sterilization, two discs of the fungus (diameters of 5 mm) were placed in the medium. The flasks were put on a rotary shaker at a speed of 120 rpm for 4 days. Finally, a MM vacuum pump was used to get dried mycelia. The mycelia of all isolates were stored at –80°C. Total genomic DNA was extracted from all isolates using the CTAB method (Romain *et al.* 1999). A NanoDrop ND-1,000 spectrophotometer (LMS Co., Ltd., Tokyo, Japan) was used to check the quality and concentration of genomic DNA. The internal transcribed spacer-ribosomal DNA (ITS-rDNA) region was used to characterize the isolates using the universal primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAA GG-3') (Alghuthaymi 2018). PCR amplifications were performed as described by Yang *et al.* (2017). PCR products were then purified using Gene JETTM commercial PCR Purification Kit (Fermentas, Axon Scientific, Malaysia) and sequenced using commercial sequencing service provider, according to the producer's recommendations (Iraizol Old Extraction DNA Kits, Rona Bio-Fanavaran Co., Iran).

Sequence alignment and phylogenetic analysis

DNA sequences of each isolate were refined using BioEdit sequence Alignment Editor (Hall 1999), in which the sequences obtained from reverse primers were transformed to the reverse complement orientation and aligned with the sequences obtained from forward primers to obtain consensus sequences. To analyze the relationship of all 30 isolates to known *R. solani* AGs, the sequences from this study and sequences of 13 reference *R. solani* AGs (AG-2-1, AG-3, AG-4 and AG-5) (Alghuthaymi 2018; Yang *et al.* 2017) were initially aligned using Clustal W Multiple alignment (Thompson *et al.* 1994), checked visually, and improved manually where necessary. The sequence of *Athelia rolfsii* isolate FSR-052(AY684917) was used as an out-group.

Phylogenetic analysis of the rDNA-ITS region was performed with a Kimura 2-parameter model in MEGA 7.0 (Kumar *et al.* 2016). Branch support of the trees obtained from the neighbor-joining analysis was assessed by boot-strapping with 1,000 replications to estimate the reliability of inferred monophyletic groups. All gaps were treated as missing data.

Identification of anastomosis groups using a specific primer

A specific primer, which was designed by Woodhall *et al.* (2007) for the identification of the anastomosis group AG-3 PT (potato type), was used to confirm the identification of all 30 isolates.

RAPD and ISSR analyses

Ten random amplified polymorphic DNA (RAPD) primers, including OPA-03, OPB-17, OPC-08, OPC-9, OPP-16, OPP-17, OPP-18, OPP-19, OPX-14 and OPF-10 were used for genetic analysis of polymorphisms among the 30 *R. solani* isolates. For the inter-simple sequence repeat (ISSR) analysis, nine primers (EZ1, EZ2, EZ7, EZ9, EZ11, EZ13, EZ18, EZ25, and EZ27) were used for genetic analysis polymorphisms among the isolates. PCR amplification of RAPD loci were carried out in a 25 ml volume mixture, containing 0.5 µM primer, 2.5 µl of a 10× buffer (200 mM Tris–HCl, 500 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 U Taq DNA polymerase and 2 µl of DNA template (10 ng). RAPD and ISSR analyses were carried out as described by Nasehi *et al.* (2014).

RAPD analysis was performed in three replications to confirm the consistency of amplification and only repeatable bands were scored. Monomorphic and polymorphic bands were considered as binary characters and were scored as 1 for the presence and 0 for the absence of DNA bands. The scores were then entered into a matrix for analysis by the numerical taxonomy and multivariate analysis system, NTSYS-pc 1.8 program (Applied Biostatistics Inc., Setauket, NY, USA) (Rohlf 1993). The similarity matrix was calculated using Jaccard's similarity coefficient. Clustering was performed using the unweighted pair group method with arithmetic averages (UPGMA) to generate the dendrogram.

Pathogenicity tests

Pathogenicity tests were performed for all isolates in a greenhouse with an average temperature of

17 ± 2°C, relative humidity of about 60% and a photoperiod of approximately 12–14 h daylight (Nasr Esfahani *et al.* 2012). The experiment was arranged in a completely randomized design in 10 replications of infected and control plants. Seed tubers (mean size 50 ± 5 g) from commonly grown and certified cv. Agria sprouted potatoes from the Seed and Seedling Department, Isfahan, Iran were planted individually in plastic pots filled with a mixture of pasteurized sand, soil and peat at a 1 : 2 : 1 ratio. Each replication consisted of one potato plant. For the inoculation, potato tubers initially were surfaced sterilized in 1% sodium hypochlorite for 10 min, and then inoculated with five discs (5 mm diameter) of each isolate, which were grown on PDA media for 10 days, and were placed 10 mm above the tuber and covered with a 10-mm layer of sand. Control pots were inoculated with PDA media only. The plants were irrigated thrice a week to ensure normal moisture, and all pots were fertilized on two occasions with a complete fertilizer (Blomstra NPK: 100 : 18 : 86 and micronutrients, Orkla Care, Solna, Sweden). In total, each plant received 220 mg nitrogen and 240 mg nitrogen, respectively.

Plants from each replicate were sampled 30 days after inoculation for stem canker disease index assessment. To confirm Koch's postulates, *R. solani* isolates were re-isolated from infected potato plants on PDA media and re-inoculated as above, and the infections were observed visually (no scoring). Disease rating was scored based on a scale of 0–6 points, where: 0 – no disease symptoms, 1 – <10% of infection on the underground stems, 2 – ≤10.1 to 25% of infection on the underground stems, 4 – ≤25.1 to 50% of infection on the underground stems, 5 – ≤50.1 to 75% of infection on the underground stems, and 6 – ≤75.1 to 100% of infection on the underground stems (NIAB 1985; Padasht-Dehkaei *et al.* 2013; Nasr Esfahani *et al.* 2014). The experiments were repeated twice. Re-isolation of the inoculated fungi was performed to fulfill Koch's postulate. Percent disease severity (PDS) in each replication was calculated using the following formula:

$$PDS = \frac{\sum RT \times 100}{S \times N},$$

where: T – the total number of underground stems in each category; R – the disease severity scale; N – the total number of underground stems tested; S – the highest number in the scale.

Data were transformed to arcsine square-root and then subjected to analysis of variance (ANOVA, $p < 0.05$), and the means were compared by Duncan's multiple range test using SAS software version 9.2 (Moghaddam *et al.* 2019).

Results

Isolation

A total of 120 isolates were retrieved from potatoes infected with stem canker from six potato-growing regions in Iran. Out of these 30 isolates were selected as representatives for further studies on genetic and virulence analysis.

Anastomosis group determination by PCR

PCR amplification of the ITS-rDNA regions of all 30 isolates produced a 711 bp fragment. Sequences of the ITS-rDNA regions of all 30 isolates were identical on the basis of the initial alignment using the Clustal W Multiple in Mega 7.0 (Kumar *et al.* 2016); therefore, the sequences of representative isolates Rs-5, Rs9, and Rs-12 were deposited in DDBJ/EMBL/GenBank databases as MH113808, H113809 and MH113810, respectively. These three sequences were only used in the phylogenetic analysis. Phylogenetic analysis of the ITS-rDNA indicated that the isolates were grouped in a cluster that included reference isolates of *R. solani* AG-3 with a bootstrap value of 94% (Fig. 2). The results also revealed that the isolates were clustered in a sub-distinct cluster with reference isolates of *R. solani* AG-3 PT (potato type) with a high bootstrap value of 97%. Additionally, the identification of the isolates was confirmed by positive specific PCR amplification with the 474 bp fragment using the specific primer set Rs1F2/AG-3PR2.

RAPD

A total of 116 consistently amplified DNA bands were generated from ten RAPD primers, of which 87.93% were polymorphic. The mean number of bands per primer was 11.6, which ranged in size from approximately 100 to 3,000 bp. The dendrogram produced from UPGMA analysis based on Jaccard's coefficient revealed that the variability was relatively high among the 30 *R. solani* isolates, and the similarity index ranged from 100 to 52%. This result also indicated that the isolates obtained from the main potato growing regions of Iran, (Ardebil, Hamadan, Fars, Isfahan, Kerman and Kurdestan provinces) were clustered into three main groups at a similarity index of 64%, with no correlation with the geographical regions of the isolates (Fig. 3).

ISSR

Application of nine ISSR primers generated a total of 44 consistently amplified fragments (100–3,000 bp),

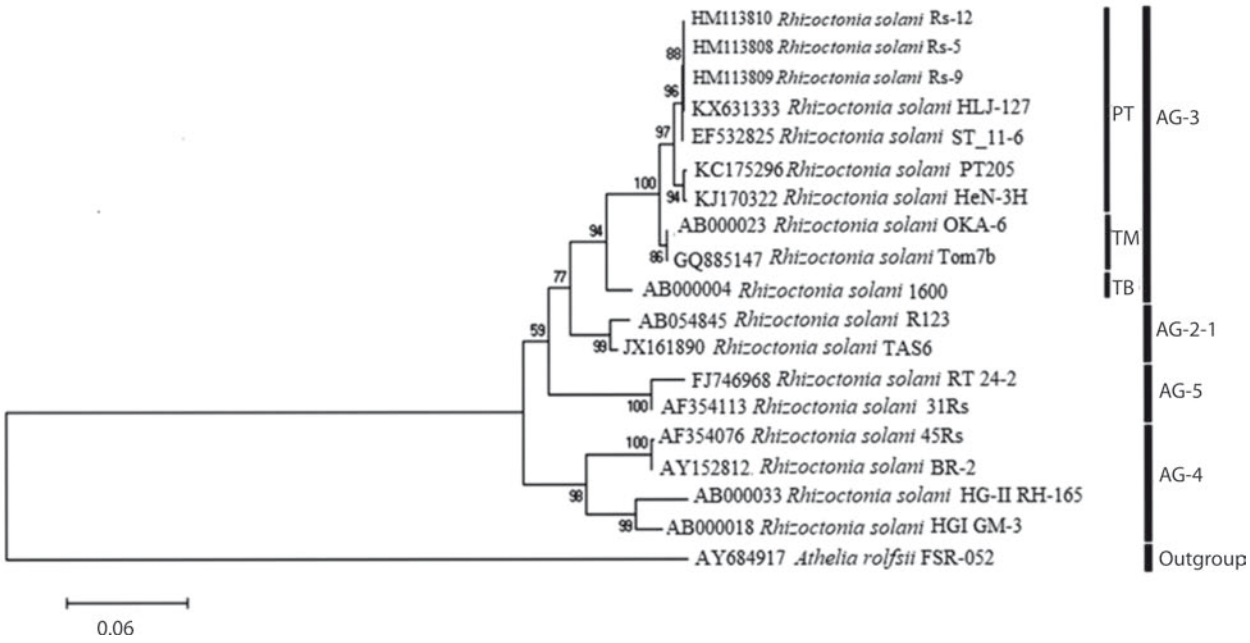

Fig. 2. Phylogenetic tree constructed with the ITS-rDNA region of the three representative isolates from this study, and *Rhizoctonia solani* reference sequences retrieved from GenBank (MH113808 (Rs-5); MH113809 (Rs-9) and MH113810 (Rs-428 12): BankIt and seq1–3 were not accession number of GenBank. The *R. solani* isolates AG-3 from potato, tomato and tobacco were indicated with PT (potato type), TM (tomato type), and TB (tobacco type), respectively. *Athelia rolfsii* (FSR-052) was used as the out-group taxon. The bar indicates nucleotide substitutions per site. Numbers of bootstrap support values ≥50% based on 1,000 replicates

of which 42 (95.45%) fragments were polymorphic. The mean number of bands per primer was 4.89. The dendrogram showed that the variability was relatively high among the isolates, and the similarity index ranged from 100 to 60% (Fig. 4). The results indicated that 30 *R. solani* isolates were also clustered into three main groups at a similarity index of 78%, with no correlation with the geographical regions of the isolates.

Pathogenicity tests

Typical symptoms of the stem canker disease started 30 days after inoculation. The results of pathogenicity indicated that the 30 isolates were pathogenic on potato (cv. Agria). No symptoms were observed on potato plants grown in the control treatment. The pathogenicity of the isolates was fulfilled according to Koch's postulate visually, but not from potato plants grown in the control treatment. The results also revealed that PDS was significantly different, and the isolates were clustered in different groups with high virulence variability (Table 1). This result showed that the isolate Ji5 with 86.1 PDS (from Jiroft, Kerman) had the highest pathogenicity on potato, while the isolate Se4 with 15.0 PDS (from Semirom, Isfahan) had the lowest pathogenicity. Other isolates were grouped between these two groups into different groups.

Discussion

Molecular markers have been widely adopted to determine the genetic characteristics of fungi, plants and animals (Nasehi *et al.* 2014; Moghaddam *et al.* 2019). Sequencing of different DNA regions has been employed as an alternative to morphological identification of *R. solani* (Kuninaga *et al.* 1997; Gondal *et al.* 2019). The ITS-rDNA regions have proven to be powerful tools for revealing the heterogeneity of AG-3 from potato, tomato and tobacco (Kuninaga *et al.* 2002), AG-1 IA isolates – from soybean (Fenille *et al.* 2003), AG-1 IA – from rice (Wang *et al.* 2015), AG-1 IB – from lettuce (Grosch *et al.* 2007), and AG-2-1 – from cauliflower (Pannecoucque and Höfte 2009). In the present study, phylogenetic analysis of the ITS-rDNA region confirmed the identification of the isolates as *R. solani* AG-3 PT. There were no differences among all isolates based on the ITS-rDNA regions sequenced. The identification was also confirmed using the specific primer for AG-3 PT.

RAPD and ISSR markers are also potential tools to separate individuals having intraspecific and interspecific variability (Nasehi *et al.* 2014). Previous studies have shown that RAPD and ISSR markers reveal the existing genetic variability among populations of *R. solani* (Dubey *et al.* 2012; Zheng *et al.* 2013; Shu *et al.* 2014; Chikara *et al.* 2015), as well as other plant

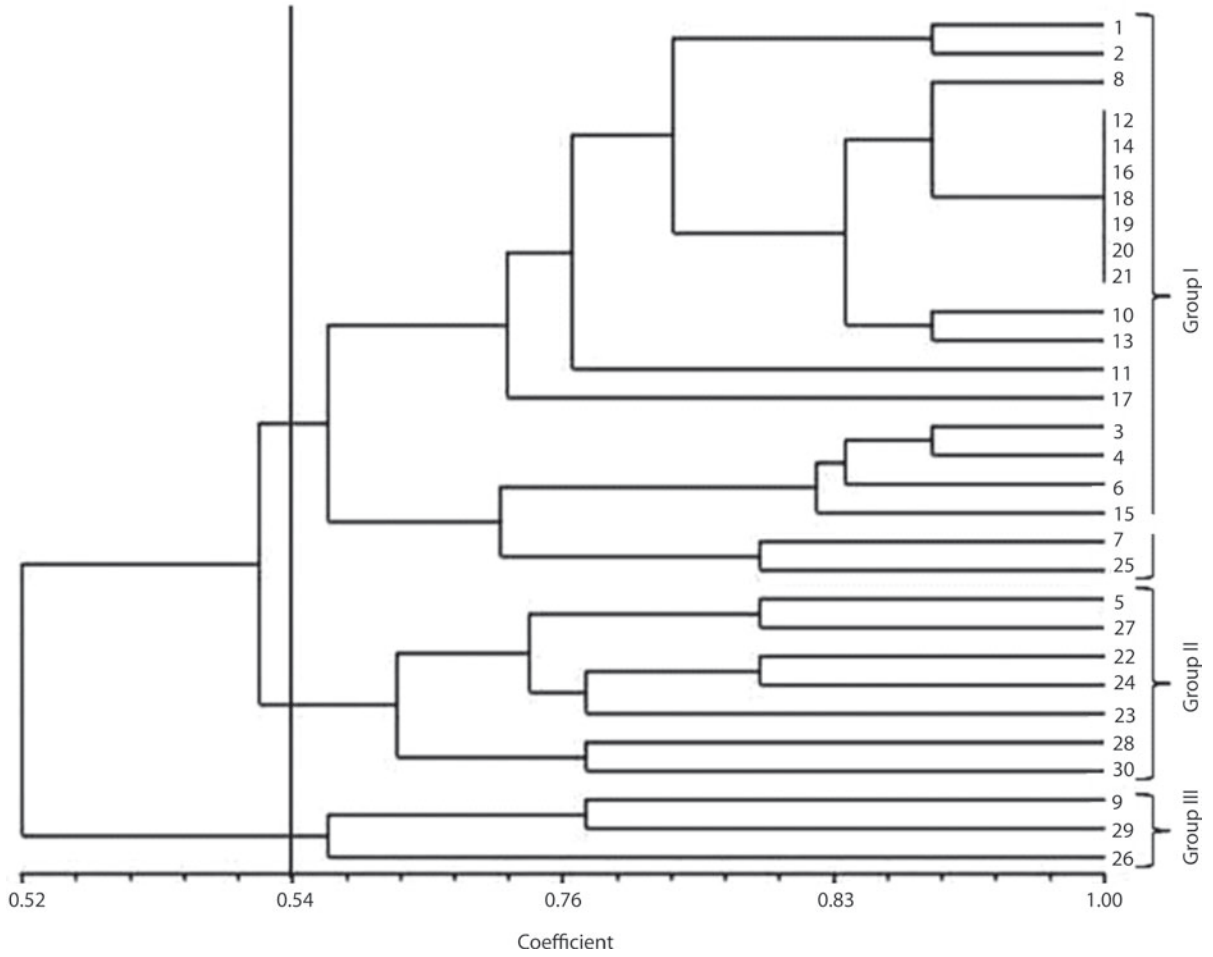

Fig. 3. Dendrogram generated by UPGMA showing the genetic variability of 30 *Rhizoctonia solani* isolates derived from a combination of the 10 RAPD primers, including OPA-03, OPB-17, OPC-08, OPC-9, OPP-16, OPP-17, OPP-18, OPP-19, OPX-14 and OPF-10. The numbering of the isolates is based on Table 1

pathogenic fungi (Mahmodi *et al*. 2014; Nasehi *et al*. 2014). In this study, 30 isolates of *R. solani* AG-3 collected from main potato-growing provinces of Iran, including Isfahan, Ardebil, Fars, Hamedan, Kurdestan and Kerman were studied on the basis of genetic diversity using RAPD and ISSR markers. Both RAPD and ISSR analyzes showed that the 30 *R. solani* AG-3 isolates had relatively high diversity, and the isolates were grouped into three main groups, with no correlation to geographical origins of the isolates.

Pathogenicity tests revealed that all 30 *R. solani* AG-3 isolates were pathogenic on potato (cv. Agria), which confirmed that these isolates were the causal agent of stem canker on potato, however virulence variability was observed between them. The virulence variability between the isolates was relatively high and the isolates were clustered into different groups, however these variations were not correlated to geographical origins of the isolates. The mean PDS % of three to eight isolates obtained from each state was as follows: Isfahan – 60.3%; Ardebil – 61.6%; Hamedan – 53.5%; Fars – 59.7%; Kerman – 68.5% and Kordestan – 67.6%. There was about 17% difference between the highest (Kerman state) and the lowest (Hamedan state). The mean PDS% of Kordestan state (67.6%) and Kerman state (68.5%) were also high. The groupings based on RAPD and ISSR analyzes and virulence variability were also not correlated.

In this study, it was shown that RAPD and ISSR markers could successfully discriminate between the 30 various Iranian *R. solani* isolates from potato. In general, consensus data provided reliable information related to the isolates, indicating new information with RAPD and ISSR markers having similar polymorphism with 88 and 95% similarities, and similarity index ranges of 52–100 and 60–100%, respectively. In addition, there was a very close clustering of the *R. solani* isolates within the three main groups at similarity indices of 64 and 78% for RAPD and ISSR. Our data are not in agreement with the statement that ISSR is the best choice to study the genetic diversity in comparison to RAPD by Mahdizadeh *et al.* (2012) on *Macrophomina phaseolina* on sesame and Sornakili *et al.* (2017) on *Colletotrichum gloeosorioides* isolates on mango. It has already been concluded that RAPD is a more powerful marker than ISSR (Nasehi *et al.* 2014).

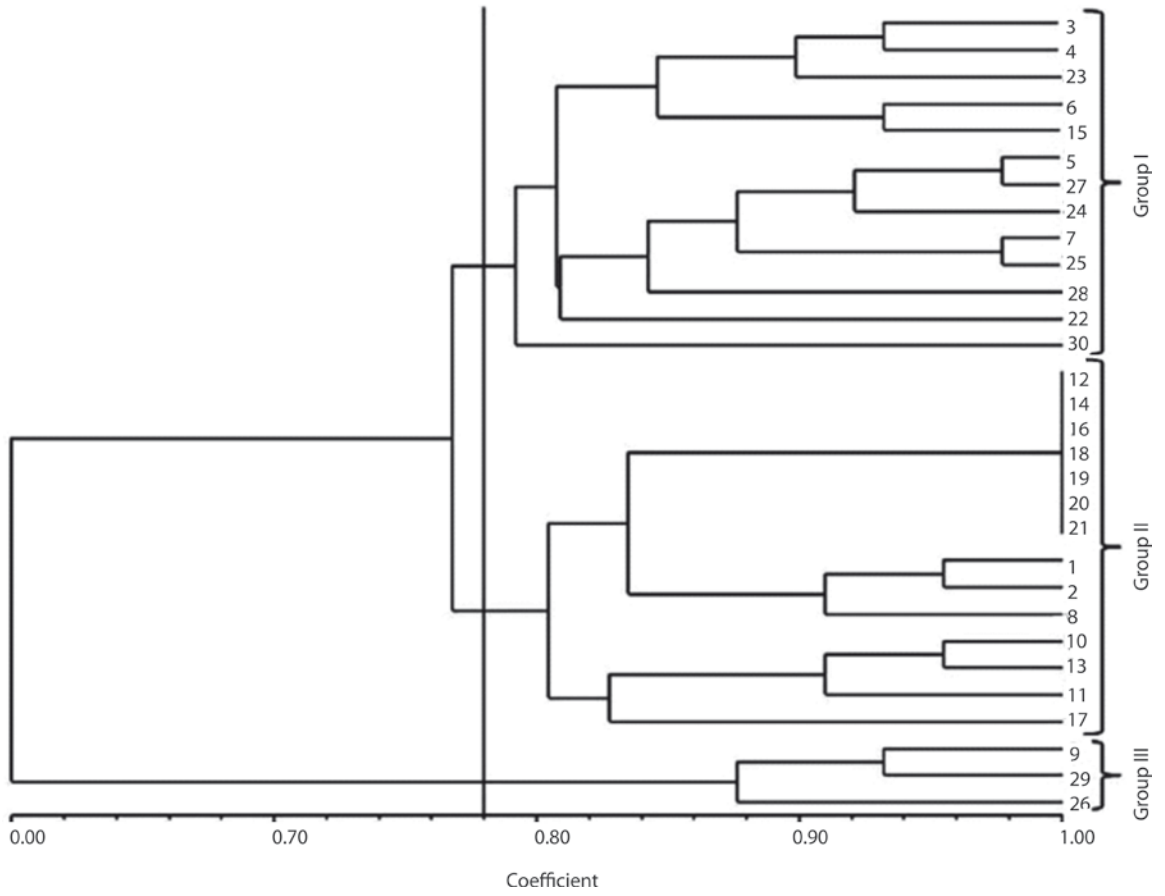

Fig. 4. Dendrogram generated by UPGMA (the unweighted pair group method with arithmetic averges) showing the genetic variability of 30 *Rhizoctonia solani* isolates derived from a combination of the nine ISSR primers, including EZ1, EZ2, EZ7, EZ9, EZ11, EZ13, EZ18, EZ25, and EZ27

Table 1. Origins and the percent disease severity of *Rhizoctonia solani* isolates used in this study

S/No.*	Isolate	Species	Location	States	RAPD groups	ISSR groups	Percent disease severity [%]
1	Es1	*Rhizoctonia solani*	Isfahan	Isfahan	Group 1	Group 2	49.98 e–i
2	Ch1	*R. solani*	Chadeghan	Isfahan	Group 1	Group 2	74.99 abc
3	Se3	*R. solani*	Semirom	Isfahan	Group 1	Group 1	69.44 a–e
4	Se4	*R. solani*	Semirom	Isfahan	Group 1	Group 1	15.00 j
5	Gh2	*R. solani*	Gholpayeghan	Isfahan	Group 2	Group 1	72.22 a–d
6	Es5	*R. solani*	Isfahan	Isfahan	Group 1	Group 1	80.55 ab
7	Na3	*R. solani*	Namin	Ardebil	Group 1	Group 1	66.66 a–f
8	Na5	*R. solani*	Namin	Ardebil	Group 1	Group 2	63.88 b–g
9	Ni3	*R. solani*	Nir	Ardebil	Group 3	Group 3	72.22 a–d
10	Ni2	*R. solani*	Niaz village	Ardebil	Group 1	Group 2	44.44 ghi
11	Na8	*R. solani*	Namin	Ardebil	Group 1	Group 2	66.66 a–f
12	Ag3	*R. solani*	Aghabagher village	Ardebil	Group 1	Group 2	55.55 c–h
13	As1	*R. solani*	Asadabad	Hamedan	Group 1	Group 2	55.55 c–h
14	Ra1	*R. solani*	Razan	Hamedan	Group 1	Group 2	58.33 c–h
15	De1	*R. solani*	Dehpiaz	Hamedan	Group 1	Group 1	41.66 hi
16	Ra2	*R. solani*	Razan	Hamedan	Group 1	Group 2	33.33 i

*Numbers of obtained *Rhizoctonia solani* isolates causing stem canker and black surf of potato in the said states

Table 1. Origins and the percent disease severity of *Rhizoctonia solani* isolates used in this study – *continuation*

S/No.*	Isolate	Species	Location	States	RAPD groups	ISSR groups	Percent disease severity [%]
17	Ka1	*R. solani*	Kabodarahang	Hamedan	Group 1	Group 2	63.88 b–g
18	De2	*R. solani*	Dehpiaz	Hamedan	Group 1	Group 2	63.88 b–g
19	Ka3	*R. solani*	Kazeron	Fars	Group 1	Group 2	69.44 a–e
20	Ma2	*R. solani*	Marvdasht	Fars	Group 1	Group 2	58.33 c–h
21	Eg5	*R. solani*	Eghlid	Fars	Group 1	Group 2	47.22 f–i
22	Ab1	*R. solani*	Abadeh	Fars	Group 2	Group 1	52.77 d–i
23	Eg4	*R. solani*	Eghlid	Fars	Group 2	Group 1	55.55 c–h
24	Eg1	*R. solani*	Eghlid	Fars	Group 2	Group 1	75 abc
25	Ji2	*R. solani*	Jiroft	Kerman	Group 1	Group 1	44.44 ghi
26	Ji5	*R. solani*	Jiroft	Kerman	Group 3	Group 3	86.10 a
27	Ji3	*R. solani*	Jiroft	Kerman	Group 2	Group 1	74.99 abc
28	Sa2	*R. solani*	Sanandaj	Kordestan	Group 2	Group 1	66.66 a–f
29	Sa1	*R. solani*	Sanandaj	Kordestan	Group 3	Group 3	66.66 a–f
30	Sa5	*R. solani*	Sanandaj	Kordestan	Group 2	Group 1	69.44 ae

*Numbers of obtained *Rhizoctonia solani* isolates causing stem canker and black surf of potato in the said states
Values followed by the same letter in the column did not differ significantly (0.05 level) in Duncan's multiple range test
The difference between the mean disease severity of the states was about 17%, with the highest in Kerman by 69%, the lowest one was 52% in Hamedan state, and others were with about 61% *R. solani* disease severity

In conclusion, in this study it was shown that both the RAPD and ISSR markers could effectively distinguish between the Iranian *R. solani* isolates, with new and more reliable information on the viability of the isolates. In addition, the disease severity showed considerable variation within and between the states. This indicates that due to the existence of relatively high diversity in genetics and virulence of *R. solani*, a single isolate could not be used for screening resistance of potato varieties. These results might help in selecting appropriate breeding strategies against this potato pathogen. The results of the present study could also be expanded to examine a wider genetic diversity of *R. solani* on different host plants from different geographical regions. Thus, it is suggested that both markers, RAPD and ISSR, may be used as complementary tools for reconfirmation of the results for genetic analysis of *R. solani*. This study also suggests that well-chosen primers could result in an accurate and quick analysis of genetic diversity of *R. solani* isolates and their geographical distribution studies. The Iranian *R. solani* population is genetically highly diverse, which is most likely due in part to the exposure of the pathogen to diverse environments and possibly to a wider host range. To our knowledge it is the first report on analyzing *R. solani* isolates covering the main potato growing areas in Iran, by RAPD, ISSR, pathogenicity and phylogenetic analysis.

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References

Alghuthaymi MA. 2018. Cultural, anastomosis, and universally primed PCR typing of *Rhizoctonia solani* from potato. Biosciences Biotechnology Research Asia 15: 119–130. DOI: http://dx.doi.org/10.13005/bbra/2614

Balali G.R., Neate S.M., Kasalkheh A.M., Stodart B.J., Melanson D.L., Scott E.S. 2007. Intraspecific variation of *Rhizoctonia solani* AG 3 isolates recovered from potato fields in Central Iran and South Australia. Mycopathologia 163: 105–115. DOI: https://doi.org10.1007/s11046-006-0089-7

Bandy B.P., Leach S.S., Tavantzixs S.M. 1988. Anastomosis group 3 is the major cause of *Rhizoctonia* disease of potato in maine. Plant Disease 72: 596–598. DOI: https://link.springer.com/chapter/10.1007/978-94-017-2901-7_29

Boysen M., Borja M., del Moral C., Salazar O., Rubio V. 1996. Identification at strain level of *Rhizoctonia* solani AG4 isolates by direct sequence of asymmetric PCR products of the ITS regions. Current Genetic 29: 174–181. DOI: https://link.springer.com/article/10.1023/A:1008736404263.

Caesar J., Rees N.E., Spencer N.R., Quimby P.C. 1993. Characterization of *Rhizoctonia* spp. causing disease of leafy spurge in the Northern Plains. Plant Disease 77 (7): 681–684. DOI: https://doi.org 10.1094/PD-77-0681

Carling D.E., Leiner R.H. 1986. Isolation and characterization of *Rhizoctonia solani* and binuclate *Rhizoctonia solani*-like fungi from aerial stems and subterranean orangs of potato plants. Phytopathology 76: 725–729. DOI: https://www.apsnet.org/publications/phytopathology/backissues/Documents/1992Articles/Phyto82n12_1387.PDF

Carling D. 1996. Grouping in *Rhizoctonia solani* by hyphal anastomosis interactions. p. 37–47. In: "Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology, and Disease Control" (B. Senh, S. Jabaji-Hare, S. Neate, G. Dijst, eds.). Dordrecht: Kluwer Academic Publishers. DOI: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-99652012000200007

Carling D.E., Kuninaga S., Brainard K.A. 2002a. Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among subsets of *Rhizoctonia solani* anastomosis group-2 (AG-2) and AG-BI. Phytopathology 92: 43–50. DOI: https://apsjournals.apsnet.org/doi/pdf/10.1094/PHYTO.2002.92.1.43

Carling D.E., Baird R.E., Gitaitis R.D., Brainard K.A., Kuninaga S. 2002b. Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani*. Phytopathology 92: 893–899. DOI: https://www.jstor.org/stable/26458599?seq=1.

Chikara R.K., Malik V., Kumar U. 2015. Identification of RAPD based genetic variability in *Rhizoctonia solani* isolates from Northern India. International Journal of Biotechnology and Recent Advances 3: 25–30. DOI: https://link.springer .com/book/10.1007%2F978-981-10-1917-3

Cubeta M.A., Vilgalys R. 2000. Rhizoctonia. p. 109–116. In: "Encyclopedia of Microbiology" (J. Lederberg, ed.). Academic Press, San Diego, CA, USA, 3848 pp.

Cubeta M., Vilgalys R. 1997. Population biology of the *Rhizoctonia solani* complex. Phytopathology 87: 480–484. DOI: https://doi.org/10.1094/PHYTO.1997.87.4.480

Das S., Falloon R.P., Stewart A., Pitman A.R. 2014. Molecular characterisation of an endornavirus from *Rhizoctonia solani* AG-3PT infecting potato. Fungal Biology 118 (11): 924–934. DOI: https:// doi.org/10.1016/j.funbio.2014.08.003

Dubey S.C., Tripathi A., Upadhyay B.K. 2012. Molecular diversity analysis of *Rhizoctonia solani* isolates infecting various pulse crops in different agro-ecological regions of India. Folia Microbiol (Praha) 57: 513–524. DOI: https://doi.org/10.1007/s12223-012-0165-y

Fenille R.C., Ciampi M.B., Kuramae E.E., Souza N.L. 2003. Identification of *Rhizoctonia solani* associated with soybean in Brazil by rDNA-ITS sequences. Fitopatologia Brasileira 28: 413–419. DOI: https://doi.org/10.1590/S0100-41582003000400011

Fiers M., Edel-Hermann V., Héraud C., Gautheron N., Chatot C., Le Hingrat Y., Bouchek-Mechiche K., Steinberg Ch. 2011. Genetic diversity of *Rhizoctonia solani* associated with potato tubers in France. Mycologia 103: 1230–1244. DOI: https://doi.org/10.3852/10-231

Godoy-Lutz G., Kuninaga S., Steadman J.R., Powers K. 2008. Phylogenetic analysis of *Rhizoctonia solani* subgroups associated with web blight symptoms on common bean based on ITS-5.8 S rDNA. Journal of General Plant Pathology 74: 32–40. DOI: http://dx.doi.org/10.1094/PDIS-02-14-0146-RE

Gondal G., Rauf A., Naz F. 2019. Anastomosis groups of *Rhizoctonia solani* associated with tomato foot rot in Pothohar Region of Pakistan. Scientific Reports 9: 3910. DOI: https://doi.org/10.1038/s41598-019-40043-5

Grosch R., Schneider J.H.M., Peth A., Waschke A., Franken P., Kofoet A., Jabaji-Hare S.H. 2007. Development of a specific PCR assay for the detection of *Rhizoctonia solani* AG 1-IB using SCAR primers. Journal of Applied Microbiology 102: 806–819. DOI: 10.1111/j.1365-2672.2006.03125.x

Gush S., Muzhinj N., Truter M., van der Waals J.E. 2019. First report of *Rhizoctonia solani* AG 2-2IIIB causing elephant hide on potato tubers in South Africa. Plant Disease 103 (7): 1776. DOI: https://doi.org/10.1094/pdis-10-18-1850-pdn

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98NT. Nucleic Acids Symposium Series 41: 95–98. DOI: http://brownlab.mbio.ncsu.edu/JWB/papers/1999hall1.pdf

Ito M., Meguro-Maoka A., Maoka T., Akino S., Masuta C. 2017. Increased susceptibility of potato to *Rhizoctonia* diseases in potato leaf roll virus-infected plants. Journal of General Plant Pathology 83: 169–172. DOI: https://doi.org/10.1094/PDIS-07-17-1124-RE

Kumar S., Stecher G., Tamura K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Evolutionary Genetics Analysis 33: 1870–1874. DOI: 10.1093/molbev/msw054

Kuninaga S., Natsuaki T., Takeuchi T., Yokosawa R. 1997. Sequence variation of the rDNA its regions within and between anastomosis groups in *Rhizoctonia solani*. Current Genetics 32 (3): 237–243. DOI: 10.1007/s002940050272

Kuninaga S., Carling D.E., Takeuchi T., Yokosawa R. 2002. Comparison of rDNA-ITS sequences between potato and tobacco strains in *Rhizoctonia solani* AG-3. Journal of General Plant Pathology 66 (1): 2–1. DOI: https://doi.org/10.1007/pl00012917

Linde C.C., Zala M., Paulraj R.S.D., McDonald B.A., Gnanamanickam S.S. 2005. Population structure of the rice sheath blight pathogen *Rhizoctonia solani* AG-1 IA from India. European Journal of Plant Pathology 112: 113–121. DOI: https://doi.org 10.1007/s10658-005-2231-7

Mahdizadeh V., Safaie N., Goltapeh E.M. 2012. Genetic diversity of sesame isolates of *Macrophomina phaseolina* using RAPD and ISSR markers. Trakia Journal of Sciences 10 (2): 65–74.

Mahmodi F., Kadir J.B., Puteh A., Pourdad S.S., Nasehi A., Soleimani N. 2014. Genetic diversity and differentiation of *Colletotrichum* spp. isolates associated with Leguminosae using multigene loci, RAPD and ISSR. Plant Pathology Journal 30: 10–24. DOI: https://doi:10.5423/PPJ.OA.05.2013.0054.

McDonald B.A., Linde C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology 40: 349–379. DOI: https://doi.org/10.1146/annurev.phyto.40.120501.101443

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. Journal of General Plant Pathology 76: 310–319. DOI: http://dx.doi.org/10.5197/j.2044-0588.2017.035.016

Misawa T., Kuninaga S. 2010. The first report of tomato foot rot caused by *Rhizoctonia solani* AG-3 PT and AG-2-Nt and its host range and molecular characterization. Journal of General Plant Pathology 76: 310–319. DOI: https://doi.org/10.1007/s10327-010-0261-2

Moghaddam G.A., Rezayatmand Z., Nasr Esfahani M., Khozaei M. 2019. Genetic defense analysis of tomatoes in response to early blight disease, *Alternaria alternata*. Plant Physiology and Biochemistry 142: 500–509. DOI: https://doi.org/10.1016/j.plaphy. 2019.08.011

Muzhinji N., Truter M., van der Waals J.E. 2015. First report of *Rhizoctonia solani* AG 4HG-III causing potato stem canker in South Africa. Plant Disease 100: 555–564. DOI: https://doi.org/10.1094/pdis-11-13-1131-pdn

Nasehi A., Kadir J.B., Nasr Esfahani M., Abed Ashtiani F., Wong M.Y., Rambe S.K., Golkhandan E. 2014. Analysis of genetic and virulence variability of *Stemphylium lycopersici* associated with leaf spot of vegetable crops. European Journal of Plant Pathology 140: 261–273. DOI: https://doi.org/10.1007/s10658-014-0460-3

Nasr Esfahani M. 2018a. Genetic and virulence variation in *Fusarium oxysporum* f. sp. *cepae* causing root and basal rot of common onion in Iran. Journal of Phytopathology 166: 572–580. DOI: https://doi.org/10.1111/jph.12720

Nasr Esfahani M. 2018b. Analysis of genetic and virulence variability of *Alternaria alternate* associated with leaf spot of potato in Iran. Acta Mycologia 53: 1–9. DOI: https://doi.org/10.5586/am.1105

Nasr Esfahani M. 2019. Morphological, virulence and genetic variability of *Ulocladium atrum* causing potato leaf blight disease in Iran. Journal of Plant Protection Research 59: 41–49. DOI: https://doi.org/10.24425/jppr.2019.126034

Nasr Esfahani M., Chatraee M., Shafizadeh S., Jalaji S. 2012. Evaluation of resistance of cucurbit and cucumber cultivars to *Phytophthora drechsleri* in Greenhouse. Iranian Seed and Plant Improvement Journal 28: 407–417. DOI: https://www.cabdirect.org/cabdirect/abstract/20193162748

Nasr Esfahani M., Nasehi A., Rahmanshirazi P., Ghadirian H., Ashtiani F.A. 2014. Susceptibility assessment of bell pepper genotypes to crown and root rot disease. Archives of Phytopathology and Plant Protection 47: 944–953. DOI: https://doi.org/10.1080/ 03235 408.2013.826541

National Institution of Agriculture Botany (NIAB). 1985. Diseases assessment manual for crop variety trials. Cambridge, UK. Available on: https://www.niab.com/sitemap

Ogoshi A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. Annual Review of Phytopathology 25: 125–143. DOI: https://doi.org/10.1146/annurev.py.25.090187.001013

Padasht-Dehkaei F., Ceresini P.C., Zala M., Okhovvat S.M., Nikkhah M.J. 2013. Population genetic evidence that basidiospores play an important role in the disease cycle of rice-infecting populations of *Rhizoctonia solani* AG-1 IA in Iran. Plant Pathology 62: 49–58. DOI: https://doi.org/10.1111/j.1365-3059.2012.02613.x

Pannecoucque J., Hofte M. 2009. Detection of rDNA ITS polymorphism in *Rhizoctonia solani* AG 2-1 isolates. Mycologia 101: 26–33. DOI: https://doi.org/10.3852/08-084

Rohlf E.J. 1993. NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 1.80. Applied Biostatistics Inc., Setauket, New York. Applied Biostatistics Inc., Setauket, New York. DOI: https://ci.nii.ac.jp/naid/10011286550

Romain R.F., Mabbayad M.O., Poulitz T.C., Watson A.K. 1999. Host range of *Alternaria alternata* f.sp. *sphenocleae* causing leaf blight *Sphenoclea zeylanica*. Canadian Journal of Botany 77: 103–112. DOI: https://doi.org/10.1139/cjb-77-1-103

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

Sharon M., Sneh B., Kuninaga S., Hyakumachi M., Naito S. 2008. Classification of *Rhizoctonia* spp. using rDNA-its sequence analysis supports the genetic basis of the classical anastomosis grouping. Mycoscience 49: 93–114. DOI: https://doi.org/10.1007/s10267-007-0394-0

Shu C.W., Zou C.J., Chen J.L., Tang F., Yi R.H., Zhou E.X. 2014. Genetic diversity and population structure of *Rhizoctonia solani* AG-1 IA, the causal agent of rice sheath blight, in South China. Canadian Journal of Plant Pathology 36: 179–186. DOI: https://doi.org/10.1080 /07060661.2014.913685

Sornakili A., Rathinam P.K., Thiruvengadum R., Kuppusamy P. 2017. Comparative assessment of RAPD and ISSR markers to study genetic polymorphism in *Colletotrichum gloeosorioides* isolates of mango. Asian Journal of Plant Pathology 11: 130–138. DOI: https://doi.org/10.3923/ajppaj.2017.130.138

Thompson J.D., Higgins D.G., Gibson T.G. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680. DOI: https://doi.org/10.1093/nar/22.22.4673

Tsror L. 2010. Biology, epidemiology and management of *Rhizoctonia solani* on potato. Journal of Phytopathology 158 (10): 649–658. DOI: https://doi.org/10.1111/j.1439-0434.2010. 01671.x

Wang L., Liu L.M., Hou Y.X., Li L., Huang S.W. 2015. Pathotypic and genetic diversity in the population of *Rhizoctonia solani* AG–1IA causing rice sheath blight in China. Plant Pathology 64: 718–728. DOI: https://doi.org/10.1111/ppa.12299

Woodhall J., Lees A.K., Edwards S.G., Jenkinson P. 2007. Characterization of *Rhizoctonia solani* from potato in Great Britain. Plant Pathology 56: 286–295. DOI: https://doi.org/10.1111/j.1365-3059.2006.01545.x

Woodhall J.W., Lees A., Edwards S.G., Jenkinson P. 2008. Infection of potato by *Rhizoctonia solani*: Effect of anastomosis group. Plant Pathology 57: 897–905. DOI: https://doi.org/10.1111/j.1365-3059.2008.01889.x

Yang S., Min F., Wang W., Wei Q., Guo M., Gao Y., Dong X., Lu D. 2017. Anastomosis group and pathogenicity of *Rhizoctonia solani* associated with stem canker and black scurf of potato in Heilongjiang province of China. American Journal of Potato Research 94: 95–104. DOI: https://doi.org/10.1007/s12230-016-9535-3

Zheng L., Shi F., Hsiang T. 2013. Genetic structure of a population of *Rhizoctonia solani* AG 2-2 IIIB from *Agrostis stolonifera* revealed by inter-simple sequence repeat (ISSR) markers. Canadian Journal of Plant Pathology 35: 476–481. DOI: https://doi.org/10.1080/ 07060661.2013.830148