Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

Morphological, virulence and genetic variability of *Ulocladium atrum* causing potato leaf blight disease in Iran

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Vol. 59, No. 1: 41–49, 2019

DOI: 10.24425/jppr.2019.126034

Received: July 30, 2018
Accepted: January 28, 2019

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Abstract

Potato leaf blight disease caused by *Ulocladium atrum* (Syn. *Stemphylium atrum*) is an important and epidemic disease in potato-growing regions of Iran. In this study, 30 isolates of the disease were collected from the main potato-growing regions of Iran and were analyzed on the basis of morphological characterization and pathogenicity. Based on morphological characteristics, all isolates were identified as *U. atrum*. Pathogenicity studies indicated that all 30 isolates were pathogenic on potato "Agria" to varying degrees. Five *U. atrum* isolates causing potato leaf blight disease, obtained from the Plant Pathology Laboratory, Isfahan Research Center for Agriculture and Natural Resources, Isfahan, Iran, were also examined in this study. A total of 35 isolates were genetically analyzed using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers. Cluster analysis using the un-weighted pair group method with the arithmetic average (UPGMA) method for RAPD marker revealed no clear grouping of the isolates obtained from different geographical regions. The groupings, based on morphological characteristics, virulence variability and RAPD analysis, were not correlated. Cluster analysis using Jaccard's coefficient for ISSR divided the *U. atrum* isolates into four main groups, in which there was no significant correlation between the isolate groupings regarding their geographic location and pathogenicity. Using molecular techniques genetic variability was detected among the accessions, with cophenetic correlation coefficients (CCC) of 0.80 for RAPDs and 0.89 for ISSRs. The RAPD and ISSR marker results corresponded well, with a correlation of 0.55.

Keywords: genetic diversity, ISSR, pathogenicity, potato, RAPD, *Ulocladium atrum*

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important strategic products that play a vital role in feeding the world and has a high degree of genetic diversity. Many varieties are grown commercially around the world. Various pests and diseases can cause disease on potato and other plants worldwide. Leaf blight caused by *Ulocladium atrum* is one of the most important diseases on potato plants and causes considerable damage to the products of this plant in Iran. Symptoms of the disease were mostly at the edges of the leaves, first as small, dark brown to black lesions, and then extending into irregular patches that, in some cases, completely covered infected leaves or entire plants (Nasr Esfahani 2018a). It has also been reported from other places (De Hoog *et al*. 2010; HADB 2018). *Ulocladium atrum* was reported as the causal agent of leaf blight disease on potato plants in Iran (Nasr Esfahani 2018b).

Knowledge from genetic analysis of plant pathogen populations is fundamental to understanding epidemiology, resistance management and host-pathogen coevolution (Hubballi *et al.* 2010). Understanding genetic variations within pathogen populations is imperative and should be considered as one of the first steps for disease management strategy (McDonald

and Linde 2002). In Iran, morphological characterization, genetic diversity and virulence variability among *U. atrum* isolates causing leaf blight of potato is not well known.

The most commonly adopted control measure is the use of fungicides and resistant cultivars. In Iran, inter-simple sequence repeats (ISSR) was used only on 11 closely related, *A. alternata* isolates from potato in Hamedan provinces (El-Mougy 2009; Bagherabadi *et al.* 2015). No comprehensive study has analyzed the genetic diversity among the Iranian *U. atrum* isolates from various potato-growing regions for control planning programs. Thus, because of the importance of leaf blight disease of potato, *U. atrum* in Iran (Nasr Esfahani 2018a, b), it is necessary to have more information on the present status of the disease.

Therefore, the objective of the study was to estimate the morphological, genetic and virulence variability among *U. atrum* isolates associated with leaf blight disease of potato collected from various potato-growing regions of Iran.

Materials and Methods

Isolation

Samples of the disease were taken from infected potato regions of Iran, including Esfahan, Fars and Hamadan provinces. To isolate the causal agent of the disease, 5 mm^2 sections were removed from the edge of lesions and surface sterilized in 1% NaOCl for 2 min. The leaf sections were rinsed twice in sterile distilled water, and then dried on sterilized filter paper for 10 min and eventually plated on potato dextrose agar (PDA). The media were incubated under natural light at 25°C for 10 days. A single spore technique was used to purify the isolates for further studies.

Morphological characterization

For this purpose, the diameter and number of spores per unit area of each isolate were examined on PDA medium after 10 days of incubation at 25°C. They were assessed visually or with a stereo microscope, and the shape and size of conidia were determined with a compound microscope. The color of the spore masses was observed with a stereo microscope.

Pathogenicity tests

Pathogenicity tests were conducted for all 30 isolates in a greenhouse at an average temperature of 27 ± 2°C, relative humidity of about 75–85%. The experiment was arranged in a completely randomized design in 10 replications of infected and control plants. Each replication consisted of one potato plant (cv. Agria). One-month old potato plants were inoculated with spore suspension of 10^3 per ml. Disease rating was scored 2 weeks after inoculation based on a scale of 0–6 points, where: 0 = no disease symptoms, 1 = lesions as pinpoints and non-measurable, 2 = <10% of the leaves with brown necrotic lesions, 4 = ≤10 to 25%, 8 = ≤25 to 50%, 16 = ≤50 to 75% of the leaves with brown necrotic lesions, and 32 = ≤75 to ≥100% of the leaves with brown necrotic lesions or completely brown (Nasr Esfahani 2018a). 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:

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

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

Data were arcsine transformed prior to statistical analysis. Percent disease severity was subjected to analysis of variance using SAS (ver. 9.2, SAS, Inc., Cary, NC, USA).

DNA extraction

Semi-synthetic liquid medium was used to prepare mycelial mass production for DNA extraction. Forty ml of nutrient medium was poured into a 100-ml Erlenmeyer flask, and was closed with sterile cotton. After sterilization, two discs of the fungus with diameters of 5 mm were placed in the medium. The flasks were put on a rotary shaker at a speed of 120 revolutions per min for 4 days. Eventually, 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 (Noelting *et al.* 2016). A NanoDrop ND-1,000 spectrophotometer (LMS Co., Ltd., Tokyo, Japan) was used to check the quality and concentration of genomic DNA.

Random amplified polymorphic DNA (RAPD) analysis

A universal primer UcharF1-1 (5'-AGCGGGCT-GGAATCCaTT) was used for genetic analysis of polymorphisms among the isolates. In this analysis, 35 isolates of *U. atrum* were used (Fig. 1). Five more isolates of *U. atrum* were obtained from the Plant Pathology Laboratory, Isfahan Research Center for Agriculture and Natural Resources, Isfahan, Iran (Table 1). Polymerase chain reaction (PCR) amplification of RAPD loci was carried out in 25 ml containing 0.5 µM

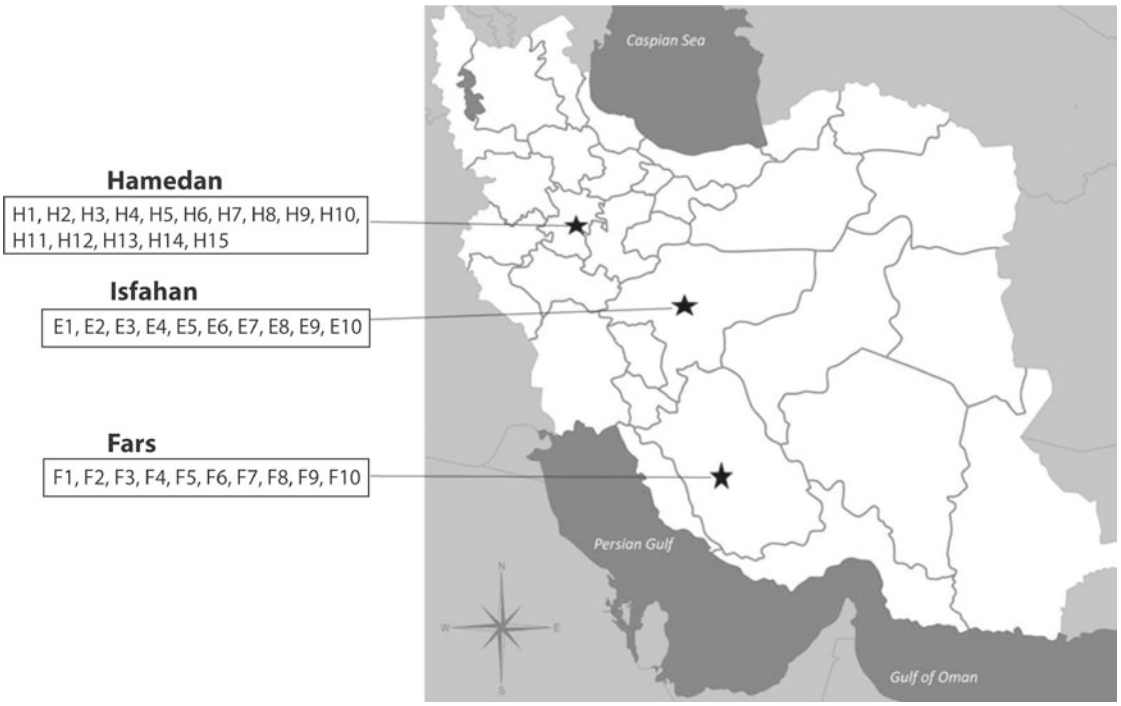

Fig. 1. Map of Iran showing the locations of potato plantations where 35 *Ulocladium atrum* isolates were collected. The name of the isolates is based on Table 1. Five isolates of *U. atrum* (H11, H12, H13, H14 and H15) were obtained from the Plant Pathology Laboratory, Isfahan Research Center for Agriculture and Natural Resources, Isfahan, Iran

primer, 2.5 µl of a 10X 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 analysis was carried out as described by Nasehi *et al.* (2014). PCR amplification was conducted in a thermocycler programed with the following parameters: 45 cycles at 94°C for 1 min (denaturation), 35°C for 1.5 min (annealing) and 72°C for 2 min (extension) with initial denaturing at 94°C for 4 min and final extension at 72°C for 10 min. All PCR reactions were performed in three replications to confirm the consistency of amplification.

Inter-simple sequence repeats (ISSRs) analysis

For ISSR, 14 UBC primers were used for PCR (Table 2). At the time, small amounts of master mix needed for the PCR were prepared, except for the DNA sample and primer (Table 2). First, 2 µl of DNA from each sample was poured into PCR specific micro-tubes, followed by the addition of 2 µl primers at the rate of 10 pmol · μl^{-1} and were kept in the refrigerator. Immediately, 21 µl of the master mix was added to each micro-tube containing DNA and primer. A thermocycler device (Techne model TC-512) was used. The amplified fragments were separated in 1.5% agarose gel, stained with gel red, and visualized under UV light. The amplification conditions were optimized for each primer to determine the best temperature for amplification.

RAPD and ISSR analyses

All PCR reactions were performed in three replications to confirm the consistency of amplification. The bands of RAPD and ISSR analysis were considered as binary characters and were scored as 1 for the presence of 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 2000). The similarity matrix was calculated using Jaccard's similarity coefficient. Clustering was performed using the un-weighted pair group method with arithmetic averages (UPGMA) to generate the dendrogram.

Results

Morphological characterization

Thirty isolates of the disease were collected from the main potato-growing regions of Iran, including Hamadan, Fars and Isfahan provinces. Based on the conidial morphology, including variations in conidial

Table 1. Origins and morphological characterization of *Ulocladium atrum* isolates used in this study, including isolates, species, location, province, years 2013–2014 number and size of spores and pathogenicity[c] tests, analyzed by Duncan's multiple range tests. The diameter and number of spores per unit area of each isolate was examined after 10 days of incubation at 25°C. They were assessed visually or with a stereo microscope, and conidia size was determined with a compound microscope. The spore masses were observed with a stereo microscope

No.	Isolates[a]	Species	Location	State	Number of spores[b]	Size of spores[b]
1	H1	*U. atrum*	Chahardoli Asadabadi	Hamadan	20.00 a	9.73 b
2	H2	*U. atrum*	Shireensoo	Hamadan	15.00 abcd	9.16 bcdef
3	H3	*U. atrum*	Kaboodarahang	Hamadan	18.33 ab	9.36 bcdef
4	H4	*U. atrum*	Baharosalehabad	Hamadan	15.00 abcd	9.73 b
5	H5	*U. atrum*	Hamadan	Hamadan	15.00 abcd	9.40 bcde
6	H6	*U. atrum*	Ghobaghtapeh	Hamadan	18.33 ab	10.56 a
7	H7	*U. atrum*	Famnin	Hamadan	16.66 abc	9.36 bcdef
8	H8	*U. atrum*	Lalehjeen	Hamadan	16.66 abc	9.36 bcdef
9	H9	*U. atrum*	Joorghan	Hamadan	18.33 ab	9.73 b
10	H10	*U. atrum*	Bahar	Hamadan	20.00 a	9.53 bcd
11	F1	*U. atrum*	Kooshk Moola village, Dari area	Fars	10.00 de	9.10 bcdefg
12	F2	*U. atrum*	Borouj village, Dari area	Fars	10.00 de	9.36 bcdef
13	F3	*U. atrum*	Daryon village, Dari area	Fars	10.00 de	9.26 bcdef
14	F4	*U. atrum*	Dehbid area	Fars	11.66 cde	8.96 cdefg
15	F5	*U. atrum*	Shireenabad village, Dehbid area	Fars	10.00 de	8.83 defgh
16	F6	*U. atrum*	Hassanabad village, Eqlid area	Fars	13.33 bcde	8.66 fgh
17	F7	*U. atrum*	Bavanat village, Abadeh	Fars	11.66 cde	8.90 defgh
18	F8	*U. atrum*	Sisakht	Fars	13.33 bcde	8.73 efgh
19	F9	*U. atrum*	Marvdasht	Fars	15.00 abcd	8.83 defgh
20	F10	*U. atrum*	Kaftarak	Fars	11.66 cde	8.73 efgh
21	E1	*U. atrum*	Chadegan	Esfahan	20.00 a	7.13 kl
22	E2	*U. atrum*	Daran	Esfahan	8.33 e	6.65 l
23	E3	*U. atrum*	Rezveh	Esfahan	20.00 a	7.10 kl
24	E4	*U. atrum*	Semiroom	Esfahan	15.00 abcd	7.43 jk
25	E5	*U. atrum*	Golpayegan	Esfahan	16.66 abc	8.46 ghi
26	E6	*U. atrum*	Mahdiabad	Esfahan	16.66 abc	7.55 jk
27	E7	*U. atrum*	Nissian	Esfahan	15.00 abcd	7.91 ij
28	E8	*U. atrum*	Freidan	Esfahan	13.33 bcde	8.00 ij
29	E9	*U. atrum*	Fereydonshahr	Esfahan	15.00 abcd	8.26 hi
30	E10	*U. atrum*	Esfahan	Esfahan	13.33 bcde	9.63 bc
31	H11	*U. atrum*	Kaboodarahang	Hamadan	–	–
32	H12	*U. atrum*	Baharosalehabad	Hamadan	–	–
33	H13	*U. atrum*	Hamedan	Hamadan	–	–
34	H14	*U. atrum*	Ghobaghtapeh	Hamadan	–	–
35	H15	*U. atrum*	Famnin	Hamadan	–	–

[a] Isolates 1–30 were used for morphological characterization. Isolates 1–35 were used for genetic diversity.

[b] Values followed by the same letter in the column did not differ significantly (0.01 level) by Duncan's multiple range tests (DMRT)

[c] The results of pathogenicity tests indicated that the 30 isolates tested were statistically different and were divided into various groups. The two isolates F7 and F10 with 35 PDS (group c) had the lowest pathogenicity on potato. The three isolates F4 (70 PDS), H1 (65 PDS), and E1 (60 PDS) were median virulent isolates and clustered into group b. The other 25 isolates were the most virulent isolates (group a) and had 100 PDS. The additional five isolates of *U. atrum* were obtained from the Plant Pathology Laboratory, Isfahan Research Center for Agriculture and Natural Resources, Isfahan, Iran

shape, size, length/width ratio, color, septation, and ornamentation, all 30 isolates were identified as *U. atrum* (Badenoch *et al*. 2006; Nasr Esfahani 2018a). These results also revealed that the isolates were clustered into different groups on the basis of the spore diameters (Table 1). The isolates H6 (from Hamadan)

Table 2. Inter-simple sequence repeats (ISSRs) primers analysis used to assess interaspecific genetic diversity among 35 *U. atrum* isolates, including primers, sequence, amplified fragment, polymorphic fragment. For ISSR, 14 UBC primers were used for polymerase chain reaction (PCR), as are listed in the table. At the time, small amounts of master mix needed for the PCR was prepared, except for the DNA sample and primer as in Table 2

No.	Primer	Sequence	Amplified fragment	Polymorphic fragment
1	UBC 807	$(AG)_8T$	10	10
2	UBC 808	$(AG)_8C'$	5	5
3	UBC 809	$(AG)_8G'$	6	6
4	UBC 818	$(CA)_8G$	10	9
5	UBC 822	$(TC)_8A$	0	0
6	UBC 834	$(AG)_8CT$	9	9
7	UBC 835	$(AG)_8CC$	11	11
8	UBC 840	$(GA)_8TT'$	9	9
9	UBC 841	$(GA)_8CC$	11	11
10	UBC 842	$(GA)_8TG$	4	4
11	UBC 846	$(CA)_8$ AT	1	1
12	UBC 849	$(GT)_8$ CA	11	11
13	UBC 850	$(GT)_8CA$	8	8
14	UBC 856	$(AC)_8CA$	0	0

All PCR reactions were performed in three replications to confirm the consistency of amplification. The bands of RAPD (universal primer UcharF1-1) and ISSR analysis 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 un-weighted pair group method using arithmetic averages (UPGMA) to generate the dendrogram. For random amplified polymorphic DNA (RAPD) analysis a universal primer UcharF1-1 (5'-AGCGGGCTGGAATCCaTT) was used for genetic analysis polymorphisms among the isolates. In this analysis, 35 isolates of *U. atrum* were used. The additional five isolates of *U. atrum* were obtained from the Plant Pathology Laboratory, Isfahan Research Center for Agriculture and Natural Resources, Isfahan, Iran

and E2 (from Esfahan) had the maximum and minimum diameters among the isolates with averages of 10.56 and 6.56 µm, respectively. The other isolates were clustered between these two isolates.

The number of spores per cm^2 for each isolate was counted in PDA medium, and the results indicated that the isolates H1, F1, E1, and E3 with the number of spores being 20.00 and E2 with the number of spores being 8.33 had the highest and the lowest number of spores among the isolates, respectively. The other isolates were clustered between these isolates.

Pathogenicity tests

Symptoms of the leaf blight disease began 2–3 days after inoculation. Our results revealed that all isolates were pathogenic on potato and were the causal agents of the leaf blight disease. After 2 weeks, the results of pathogenicity tests indicated that the 30 isolates tested were statistically different and were divided into various groups. The two isolates F7 and F10 with 35 PDS (group c) had the lowest pathogenicity on potato. The three isolates F4 (70 PDS), H1 (65 PDS), and E1 (60 PDS) were median virulent isolates and clustered into group b. The other 25 isolates were the most virulent isolates (group a) and had 100 PDS.

RAPD analysis

A total of 13 consistently amplified DNA bands were generated from universal primer UcharF1-1, in which 100% were polymorphic. The bands ranged in size from approximately 200 to 2,000 bp. The dendrogram produced from UPGMA analysis based on Jaccard's coefficient revealed that the polymorphism among 35 *U. atrum* isolates ranged from 0 to 25%. This result also indicated that the isolates obtained from main potato-growing regions of Iran, including Hamadan, Fars and Isfahan provinces were clustered into different groups with no correlation to geographical regions of the isolates (Fig. 2).

ISSR analysis

The twelve ISSR primers generated a total of 95 consistently amplified DNA bands, in which 100% were polymorphic. The average number of bands per primer was 7.92 which ranged in size from approximately 200 to

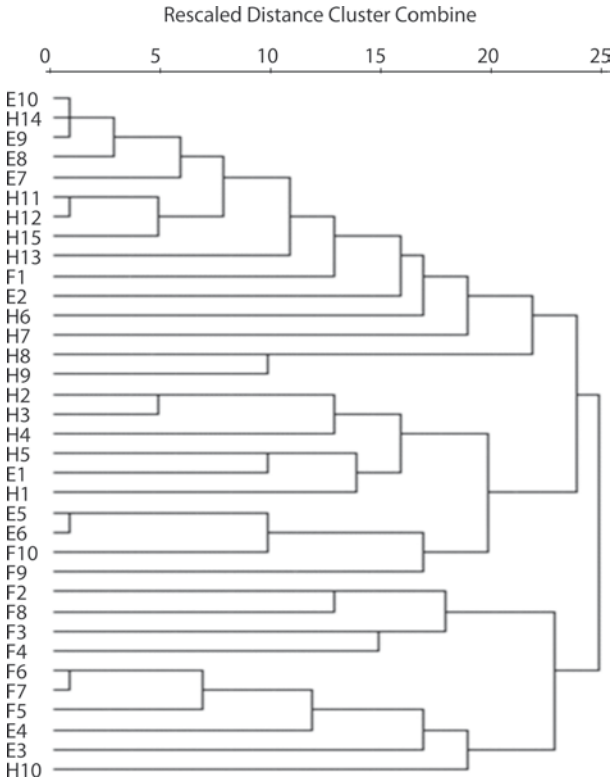

Fig. 2. Dendrogram generated by UPGMA showing the genetic variability of 35 *Ulocladium atrum* isolates derived from a combination of RAPD universal primer UcharF1-1. The name of the isolates is based on Table 1. All PCR reactions were performed in three replications to confirm the consistency of amplification. The bands of RAPD analysis 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 2000). The similarity matrix was calculated using Jaccard's similarity coefficient. Clustering was performed using the un-weighted pair group method using arithmetic averages (UPGMA) to generate the dendrogram

1,600 bp. The dendrogram also indicated that the variability among the 30 *U. atrum* isolates was relatively high and grouped the isolates into different groups with no correlation to geographical regions of the isolates. The similarity index ranged from 18 to 80% with a mean value of the Jaccard's similarity coefficient of 0.42 (Fig. 3).

Discussion

The main objective of this study was to investigate and demonstrate the variation among the Iranian *U. atrum* isolates, causing potato leaf blight in Iran. In this study, 30 isolates of *U. atrum* causing potato leaf blight obtained from the main potato-growing regions of Iran, including Hamadan, Fars and Isfahan provinces were studied using morphology and pathogenicity tests. Based on the conidial morphology, including variations in conidial shape, size, length/width ratio, color, septation, and ornamentation, all 30 isolates were identified as *U. atrum* (Badenoch *et al.* 2006; Nasr Esfahani 2018a, b). The results of morphological characterization also revealed that the 30 isolates had high variability on the basis of the size and number of spores, and were clustered in different groups. The variations observed among the 30 isolates were not correlated to the geographical origins of the isolates.

Pathogenicity tests indicated that all 30 isolates were pathogenic on the susceptible cultivar potato "Agria", however virulence variability was observed among the isolates. According to the pathogenicity tests, the isolates were clustered into three pathogenicity groups: low (isolates F7 and F10 with 35 PDS), moderate (isolates F4, H1 and E1 with 70, 65 and 60 PDS, respectively), and highly pathogenic (25 other isolates with 100 PDS). Virulence assays indicated that all the isolates

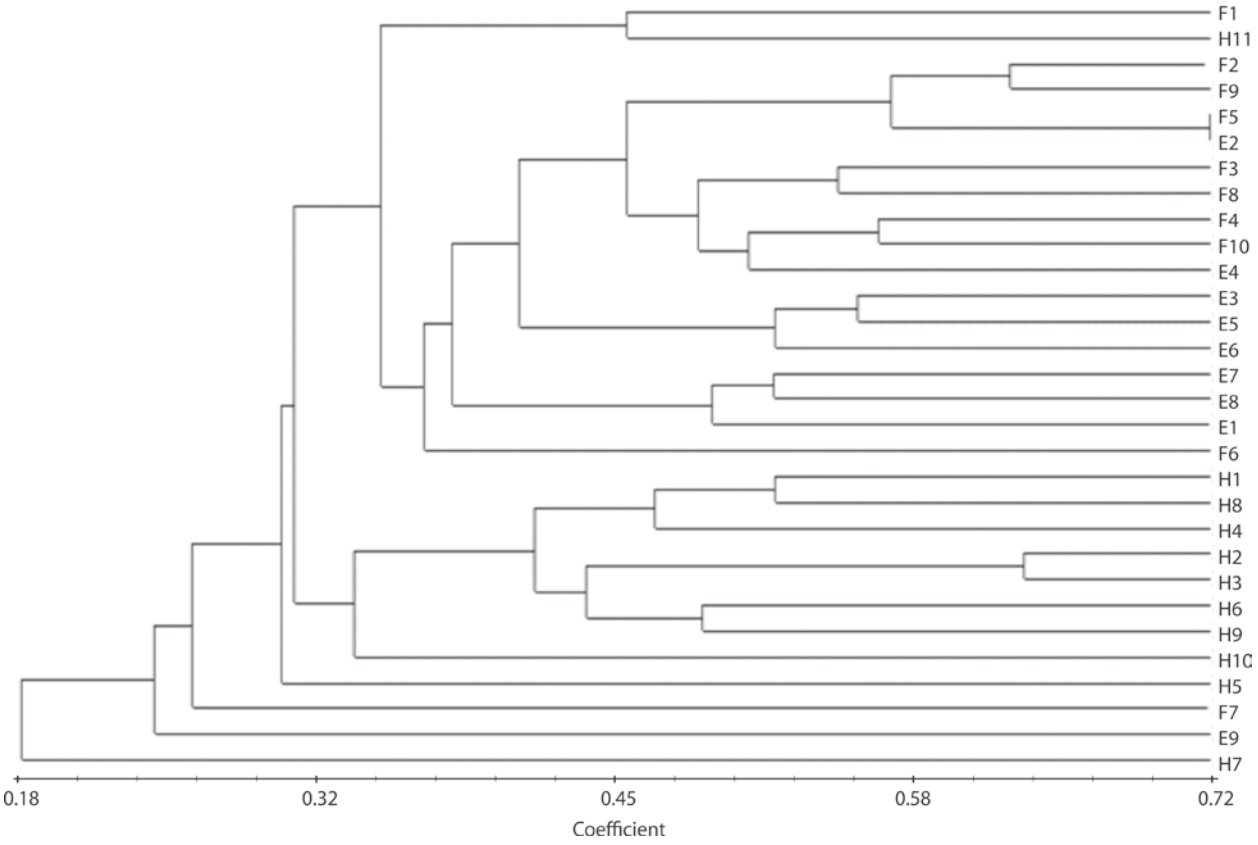

Fig. 3. Dendrogram generated by UPGMA showing the genetic variability of 35 *Ulocladium atrum* isolates derived from a combination of ISSR primers. The name of the isolates is based on Table 1. All PCR reactions were performed in three replications to confirm the consistency of amplification. The bands of RAPD and ISSR analysis were considered as binary characters and were scored as "1" for the presence of 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 2002). The similarity matrix was calculated using Jaccard's similarity coefficient. Clustering was performed using the un-weighted pair group method using arithmetic averages (UPGMA) to generate the dendrogram

were pathogenic on "Agria" potato leaves. Symptoms on leaves were observed 7 days after inoculation of the *U. atrum* isolates on all inoculated potato plants. Leaf blight symptoms were observed mainly on the edges of the leaves, initially as small, dark brown to black lesions, which extended into irregular patches with time and in some cases, completely covered all of the infected leaves or entire plants (Nadia *et al*. 2008; Nasr Esfahani 2018a). Very similar results have also been reported elsewhere (De Hoog *et al*. 2010). *Ulocladium atrum* was also reported as the causal agent of leaf blight disease on potato plants in Iran and other potato growing areas (Morante 2016; Nasr Esfahani 2018b; Pourarian *et al*. 2018). Re-isolation of *U. atrum* from all inoculation treatments confirmed Koch's postulates. Therefore, these *U. atrum* isolates were confirmed to be the causal agent of the disease on potato. The results also revealed that there was virulence variability among the isolates, and the grouping based on virulence variability did not correlate with the results of RAPD and ISSR analyses, as well as morphological characterization and geographical regions. These results agreed with previous studies on *Alternaria alternate*, a species close to *U. atrum,* obtained from citrus hybrids of Iran (Kakvan *et al.* 2012), potato and tomato (Soleimani and Kirk 2012; Bagherabadi *et al.* 2015).

Molecular markers have been widely adopted to determine the genetic characteristics of fungi, plants and animals. The RAPD marker is an extremely powerful tool to separate individuals having intraspecific and interspecific variability. This marker has been used to reveal the genetic variability existing among populations of *U. atrum* (Abd-El-Karreem *et al.* 2004; Badenoch *et al.* 2006). The result of RAPD analysis indicated that the 35 *U. atrum* isolates used in this study had a relatively high diversity with no correlation to geographical origins of the isolates. The grouping based on RAPD analysis did not correlate with the results of virulence variability as well as morphological characteristics.

The results on the implications of the ISSR marker showed that 12 primers out of 14 primers used in this study, generated consistently high amplified DNA

bands, indicating 100% polymorphism and a similarity index of 18 to 80%. The average number of bands per primer was approximately eight. There was a relatively high variability with no correlation to geographical regions of the isolates.

In this study, it was shown that RAPD and ISSR markers could successfully identify 35 various Iranian *U. atrum* isolates from potato. In general, consensus data provided more reliable information related to the isolates, indicating new information from RAPD and ISSR markers having similar polymorphism with 100% similarities. 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). These two markers have been employed to provide comprehensive information regarding the genetic variation in related species populations including *A. alternata* on citrus (Kakvan *et al.* 2012), linseed (Kale *et al.* 2012) potato (Bagherabadi *et al.* 2015; Cwalina-Ambroziak *et al.* 2015; Pourarian *et al.* 2018), tangerine (Demartelaere *et al.* 2018), tomato (Soleimani and Kirk 2012) and other fungal species, *Stemphylium lycopersici* associated with leaf spot of vegetable crops (Nasehi *et al.* 2014). Although, the level of polymorphism revealed by RAPD and ISSR may be lower than amplified fragment length polymorphism (AFLP) and other molecular markers, in comparison with the more labor and expense involved, the use of radioactivity, and the time-consuming nature of AFLP, these too can be appropriate options, since they are easier to implement and are less costly.

In conclusion, it was found that both RAPD and ISSR markers could successfully differentiate *U. atrum* isolates genetically. It was also shown that, because of the relatively extant diversity in morphology, pathogenicity and genetics of *U. atrum* isolates, not every isolate could be used for evaluating resistance of potato varieties. Due to the breakdown and decline of the resistance, the new and aggressive isolates should be screened for evaluating resistance of potato varieties. These results might help in selecting appropriate breeding strategies against this pathogen of potato plants. The results of the present study could also be expanded for a wider genetic diversity of *U. atrum* on different host plants from different geographical regions. RAPD and ISSR markers could also be extended to a wider genetic diversity of *U. atrum* from other regions of the world, as well as other host plants. Since temperature, wind speed, potato cultivars and other factors may affect the population structure of fungi, future studies should address the population structure of *U. atrum* under different growing conditions. In addition, studies are required to address pathogen specialization on different cultivars. To our knowledge this is the first analysis of *U. atrum* isolates covering the main potato growing areas in Iran, by RAPD, ISSR, pathogenicity and morphological analysis.

Acknowledgements

We wish to thank the plant protection division, Isfahan Center for Agricultural and Natural Resources Research, Isfahan, Iran, for providing the necessary funds and facilities for the project.

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