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Full Length Research Paper

Genetic diversity among crown and root rot isolates of *Rhizoctonia solani* isolated from cucurbits using PCR-based techniques

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Twenty three (n=23) isolates of *Rhizoctonia solani* were obtained from root, crown and rhizosphere of cucumber, pumpkin, watermelon and melon plants. Investigation of the anastomosis groups (AGs) showed that all of the isolates belonged to AG4. Twenty three of *R. solani* AG4 were measured for disease severity according to their geographical origins and host plants. The pathogenicity test revealed that the disease severity varied from 8.3 to 91.6%, when all isolates were inoculated on their corresponding hosts. The disease severity indicated that *R. solani* isolates were classified into five groups. Genetic diversity was performed among isolates of *R. solani* AG4 by combination of random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) techniques. Amplicon size of six RAPD primers out of fifteen and four ISSR primers out of ten, ranged from 0.5 to 3 Kb in isolates. All isolates were divided into seven groups at >30% similarity level. The results exhibited a high genetic variability in the *R. solani* AG4 population, without any correlation between genetic diversity, the host plants and geographical regions.

Key words: Cucurbits, genetic diversity, random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), pathogenicity, *Rhizoctonia solani.*

INTRODUCTION

Rhizoctonia solani Kuhn, (Teleomorph: *Thanatephorus cucumeris* (A.B. FRANK) DONK), is a widely distributed, soil borne plant parasitic-saprophytic fungus. Its characterization as a collective species is based on encompassing many related but genetically distinct groups (Sneh et al., 1991). Isolates of *R. solani* of anastomosis group 4 (AG4) cause seed rot, post-emergence damping off, limb rot and root rots of important crop plants. Fruit rot of cucumber as well is caused by isolates characterized as AG4 (Lewis and Papavizas, 1992). In the absence of stable morphological and physiological characteristics and anastomosis groups, the identification of *R. solani* isolates has proved to be very difficult and unspecific.

Therefore, problems associated with studying different levels of genetic diversity in *R. solani* can be best solved by application of molecular techniques (Toda et al., 1999). To date, no reportable data on the genetic diversity among crown and root rot isolates of *R. solani* in Iran. Thus, the objective of the present study is to determine the genetic variability of Iranian isolates of *R. solani* collected from cucurbits with crown and root rot symptoms in four provinces of Iran. Random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) analysis as well as the relationship of genetic diversity and pathogenic variability of the isolates were applied as tools for differentiation.

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Table 1. Pathogenic variability of *Rhizoctonia solani* AG4 isolates on cucurbits.

Isolate number	Host	Origin	Location	Disease severity (%)
1VCu	Cucumber	root	Pishva/Tehran	16.6^{def*}
3VPu	Pumpkin	root	Pishva/Tehran	16.6^{def}
5VPu	Pumpkin	Crown and root	Varamin/Tehran	16.6^{def}
8VPu	Pumpkin	root	Pishva/Tehran	41.6^{abcde}
9VPu	Pumpkin	root	Pishva/Tehran	25^{def}
12EPu	Pumpkin	root	Dastgerd/Ghaemiyeh/Esfehan	16.6^{def}
14EPu	Pumpkin	root	Dastgerd/Ghaemiyeh/Esfehan	41.6^{abcd}
16EPu	Pumpkin	soil	Dastgerd/Ghaemiyeh/Esfehan	16.6^{def}
25ECu	Cucumber	root	Esfehanak/Ichi/Esfehan	25^{cdef}
27ECu	Cucumber	Crown and root	Esfehanak/Ichi/Esfehan	16.6^{def}
30ECu	Pumpkin	Crown and root	Esfehanak/Ichi/Esfehan	75^{ab}
35KhPu	Pumpkin	root	Shooshtar/Khouzestan	66.6^{ab}
36KhPu	Pumpkin	root	Sarbisheh/Dezful/Khouzestan	8.3^{ef}
40KhPu	Pumpkin	root	Sarbisheh/Dezful/Khouzestan	33.3^{bcdef}
43KhCu	cucumber	soil	Shooshtar/ Khouzestan	16.6^{def}
47KhCu	Cucumber	soil	Shooshtar/ Khouzestan	16.6^{def}
49KhCu	Cucumber	soil	Sarbisheh/Dezful/Khouzestan	75^{ab}
52MMe	Melon	soil	Goharbaran/Anarein/Mazandaran	83.3^{a}
53MMe	Melon	soil	Goharbaran/Anarein/Mazandaran	75^{ab}
54MMe	Melon	soil	Goharbaran/Anarein/Mazandaran	25^{cdef}
55MWa	Watermelon	soil	Goharbaran/Anarein/Mazandaran	16.6^{def}
57MWa	Watermelon	soil	Goharbaran/Anarein/Mazandaran	91.6^{a}
58MWa	Watermelon	soil	Goharbaran/Anarein/Mazandaran	83.3^{a}

*Data followed by different letters differ significantly at P≤0.05 (Duncaǹ's multiple range test).

MATERIALS AND METHODS

Fungal isolates and culture maintenance

In this study, twenty-three (n=23) isolates of *R. solani* were isolated from different cucurbits including cucumber, melon, pumpkin, watermelon and rhizosphere soils. These isolates were collected from diverse geographic regions in Tehran, Mazandaran, Khuzestan and Isfahan Provinces as tabulated in Table 1. Isolates were further individualized by hyphal tip culture on potato dextrose agar (PDA) and designated as RS1-RS23.

Characterization of isolates

Mycelium was stained with Safranin O and 3% (w/v) KOH and examined microscopically at 400 fold magnification (Bandony, 1979). Determination of anastomosis group was done according to a clean glass slide technique (Kroland and Stanghellini, 1988) and anastomosis reactions were observed.

Pathogenicity test

Cucumber, melon, pumpkin and watermelon plants were used to test the pathogenic behavior of all twenty-three isolates (*R. solani* AG4). To study pathogenicity of the isolates, the inoculum of each isolate prepared on wheat grains: the mycelial culture of *R. solani* AG4 isolates were grown in water soaked wheat grains in separate flasks and sterilized in three consecutive days (Sneh et al., 1991). Each flask was inoculated with two mycelial plugs, taken from three-day-old culture of each of the isolates grown on PDA medium. After incubation, the colonized wheat grains were air dried on sterilized paper and stored at 4°C until use. Pathogenicity of the isolates to roots of seedlings was tested as follows: cucurbits seeds were sown separately in pots and kept in greenhouse at 25±1°C temperature.

Plants were inoculated with the mycelial mats of *R. solani* AG4 beside crown four weeks after planting. All isolates were inoculated on their corresponding hosts and were regularly (every two days) observed for the appearance of root and crown rot symptoms. Seedlings in each pot were uprooted and washed under running tap water and rated. Pathogenicity was rated 14 days after inoculation using the following 0-5 scale: 0= healthy, 1= 1-20% diseased, 2= 21-40% diseased, 3= 41-60% diseased, 4= 61-80% diseased and 5= 81-100% diseased (Mathew and Gupta, 1996). Pathogenicity was calculated as:

$$\sum \left(\frac{\text{Disease index} \times \text{No. of inoculated root samples in each ind}}{\text{Maximum index} \times \text{Total no. of inoculated root}} \right.$$

Set up of all inoculation trials consisted of a Randomized Complete Block Design (RCBD) with four replicate randomized for each treatment. Pathogenicity test was repeated three times and virulence was measured using disease severity.

Cross-interaction between host-pathogen

A total of 23 isolates were examined for cross pathogenicity test, four isolates with high disease severity were selected for this test. Each

isolate inoculated on all hosts including cucumber, melon, pumpkin and watermelon plants. This experiment was examined the similar way as described earlier. Set up of all inoculation trials consisted of a Randomized Complete Block Design (RCBD) with four replicate randomized for each treatment. Also, cross-interaction test was repeated three times.

RAPD analysis

Total genomic DNA was extracted from the mycelia grown on potato dextrose broth (PDB) (Safaie et al., 2005). In preliminary experiments, a total of 15 primers were screened for RAPD analysis of which, six primers amplified fragments of 0.5 to 3.0 kb and were further used for all *R. solani* isolates. These primers were: OPA-07: 5´-GAAACGGGTG-3´, OPA-11: 5´-TTCCCGTCAT-3´, OPA-16: 5´-AGCCAGCGAA-3´, R-28: 5´- ATGGATCCGC-3´, OPC-18: 5´-TGAGTGGGTG-3´ and OPC-19: 5´-GTTGCCAGCC-3´ (Williams et al., 1990). Amplification was performed in an Eppendorf Master-Cycler ep-gradient (Eppendorf AG, Hamburg, Germany), with temperature profiles of 92°C for 2 min, 40 additional cycles at 93°C for 1 min, 36°C for 1 min and 70°C for 2 min. Finally, a final incubation at 70°C for 5 min was performed (Sharma et al., 2005).

ISSR analysis

Four out of ten ISSR primers which were reproducible and polymorphic were used for the amplification of genomic DNA isolates as described by Sharma et al. (2005). These primers were: ISSR-02: 5´- ACTGACTGACTGACTG-3´, PCMS: 5´-(GTC)7-3´, ISSR-P1: 5´-(TC)8C-3´ and ISSR P4: 5´- (ATG)6-3´ (Sharma et al., 2005). Amplification was performed with temperature profiles of 95°C for 5 min, 45°C for 45 s and 72°C for 1 min. Thirty eight additional cycles were carried out at 95°C for 45 s, 50°C (for ISSR-02 and ISSR P4), 67°C (for PCMS) and 47°C (for ISSR-P4) for 45 s and 72°C for 1 min. Finally, a final incubation at 72°C for 10 min was performed (Sharma et al., 2005). Polymerase chain reaction (PCR) was performed in 20 µl reaction volumes containing 1 to 1.5 unit Taq DNA polymerase, dNTP mix (0.2 mM each of dCTP, dGTP, dATP and dTTP), 1X PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$; Bangalore Genei, Pvt Ltd, Bangalore, India), 0.5 µM primer and 25 to 50 ng of genomic DNA. The reaction mixture was vortexed and centrifuged briefly.

Data analysis

The data were analyzed by using MVSP (Multi Variate Statistical Package- version 2.0). To evaluate the effectiveness of DNA markers in differentiating genetic variability in *R. solani* population, it was assumed that each DNA band position corresponds to a locus with two alleles, which appears as presence or absence of the band, respectively. A combined data sets of 151 amplified bands obtained for 23 isolates and all primers (RAPD and ISSR), and also similarity coefficients were clustered to generate trees by using the unweighted pair-group method with arithmetic average (UPGMA) and Jaccard´s coefficient. Expected heterozygosity (H_n) was computed from the square sum of allele frequencies.

The arithmetic mean heterozygosity and marker index (MI) were calculated according to Powell et al. (1996). The effective multiplex ratio (EMR) was calculated as $n_p\beta$, where np is the number of polymorphic loci in different isolates that were analyzed in each experiment and $\beta = n_p/(n_p + n_{np})$ is the fraction of polymorphic loci. MI which is the product of expected heterozygosity (H_n) and the EMR, that is MI= EMR×H_n. Also, MI for each class of marker was also calculated from the average expected heterozygosity, $H_{av(p)}$ and EMR (Sharma et al., 2005).

RESULTS

Characterization of isolates

Twenty three isolates with hyphae characteristic of *R. solani* were recovered from different cucurbits including cucumber, melon, pumpkin and watermelon showing root and crown rot. All isolates were multinucleate, containing 3 to 7 nuclei with the average of 4±1.08 nuclei per hyphal cell. Also, these isolates anastomosed with the control isolate belonging to AG4, but did not anastomose with other AG test isolates. Accordingly, the isolates were identified as *R. solani* AG4.

Pathogenicity test

All isolates were inoculated on cucurbits (cucumber, melon, pumpkin and watermelon) and observations on percentage of disease severity were recorded (Table 1). The disease severity varied from 8.3 to 91.6%. One *R. solani* isolate (no. 36) collected from pumpkin was the least disease severity (8.3%) on pumpkin. While, an isolate (no. 57) isolated from watermelon developed high disease severity (91.6%) on watermelon. On the other hand, the disease severity was <20% in ten isolates, between 20 and 80% in ten isolates and three isolates produced a disease severity of more than 80% (Table 1). Hence, these may be pathogenic on widely grown cucurbits in the pathogenicity tests.

Clustering the results of disease severity indicated that 23 isolates were divided into five groups: Seven *R. solani* isolates (58MWa, 57MWa, 52MMe, 53MMe, 49KhCu, 35KhPu and 30EPu) were categorized as high virulence (HV) group. Three *R. solani* isolates (14EPu, 9VPu and 9VPu) were grouped as virulence (V). The MV groups with moderate virulence comprise three *R. solani* isolates (40KhPu, 54MMe and 25ECu). The group with weak virulence (W) comprise nine *R. solani* isolates (55MWa, 47KhCu, 43KhCu, 27ECu, 16EPu, 12EPu, 5VPu, 3VPu and 1VCu), and a single isolate (36KhPu) were grouped into very weak virulence (VW) (Figure 1).

Cross-interaction between host-pathogen

Disease severity of the selected isolates of *R. solani* AG4 (52MMe, 49KhCu, 57MWa and 30EPu) varied considerably among the four hosts (Table 2). The range of disease severity was from 40.0 to 98.8% in melon, cucurbit**,** pumpkin and watermelon. The highest disease severity of roots by the *R. solani* AG4 isolate 30EPu, ranging from 86.5 to 98.8%, observed in hosts; while those by the *R. solani* AG4 isolate 49KhCu was from 40.0 to 72.0%. Significantly, the maximum disease severity of root (98.8%) due to the isolate 30EPu was recorded in cucumber, which was statistically similar to watermelon. Also, the minimum disease severity of root (40.0%) due to the isolate 49KhCu recorded in melon (Table 2).

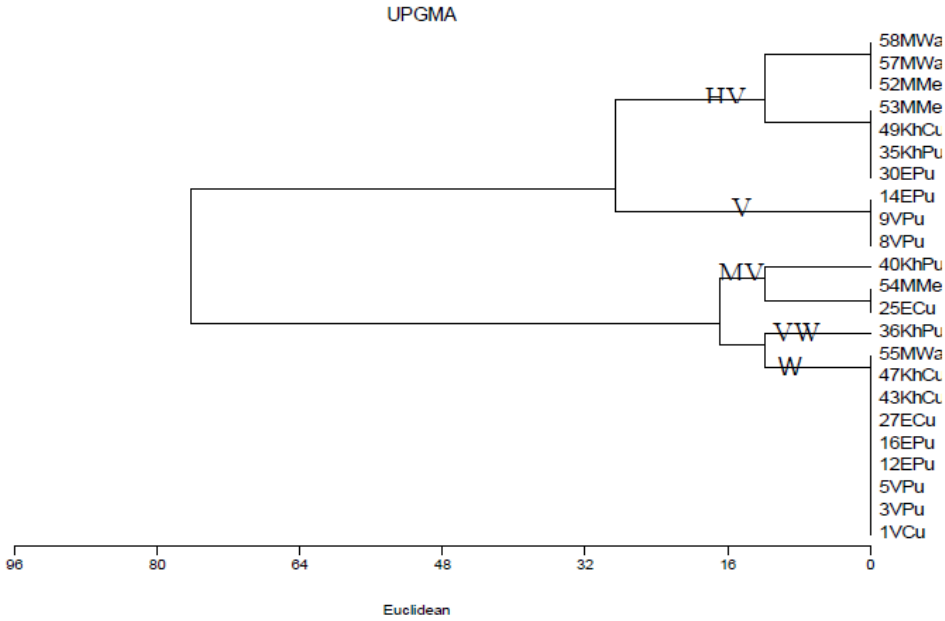

Figure 1. The clustering of *Rhizoctonia solani* AG4 isolates based on pathogenicity test results. HV: High virulence, V: virulence, MV: moderate virulence, W: weak virulence, VW: very weak virulence. Isolates number are given on the end of the branches.

Table 2. Pathogenic variability of four isolates of *Rhizoctonia solani* AG4 on four hosts.

Isolate number	Host			
	Melon	Cucumber	Pumpkin	Watermelon
	Disease severity (%)			
52MMe	75^{g}*	80^{f}	75^{g}	75.5^{g}
49KhCu	40^{k}	72^{h}	50^{j}	55.5^{i}
57MWa	85.6^{d}	81.5^{g}	75^{g}	80^{f}
30EPu	95.8^{b}	98.8^{a}	86.5^{c}	98.8^{a}

52MMe: obtained from melon; 49KhCu; obtained from cucurbit; 57MWa: obtained from watermelon; 30EPu: obtained from pumpkin. *Data followed by different letters differ significantly at P≤0.05 (Duncan's multiple range test).

DNA profile analysis

Genetic variation was detected among 23 *R. solani* isolates using RAPD and ISSR profiles. All PCRs with 15 RAPD primers and 10 ISSR primers were repeated three times. The results showed monomorphic and polymorphic DNA banding patterns in the size range of 0.5 to 3.0 kb for different isolates. Most bands obtained with RAPD primer OPC19 (Figure 2a) and least from OPA16 (Figure 2b). Also, primers ISSRP1 and ISSR02 gave multiple products (Figure 3a, b). Combined data set of 151 DNA fragments obtained from all the isolates with RAPD and ISSR markers were analyzed with UPGMA and a dendrogram was constructed as indicated in Figure 4. Genetic similarity coefficient was relatively low among all the isolates, which ranged from 0.04 to 0.84.

Based on cluster analysis, all *R. solani* isolates collected from different geographical areas of northern, central and southern of Iran, were classified into seven groups at >30% similarity level. Group I, consisting of seven isolates, which constituted two subgroups. Subgroup Ia contained three isolates (numbers 1VCu, 3VPu and 40KhPu) with approximately >30% genetic similarity. Subgroup 1b including four isolates (numbers 8VPu, 9VPu, 54MMe and 57MWa) obtained from Tehran and Mazandaran. Group II; consisting of one isolate (number 43KhCu) obtained from Khouzestan. Also Group III, consisting of one isolate (number 12EPu) recovered from Esfehan. Isolates of 52MMe and 55MWa obtained from Mazandaran and placed in Group IV with approximately >50% genetic similarity. Group V consisting of six isolates (numbers 5VPu, 16EPu, 30ECu, 25ECu, 47KhCu and 35KhPu), obtained from Esfehan and Khouzestan. Group VI consisting of five isolates (numbers 14EPu, 27ECu,

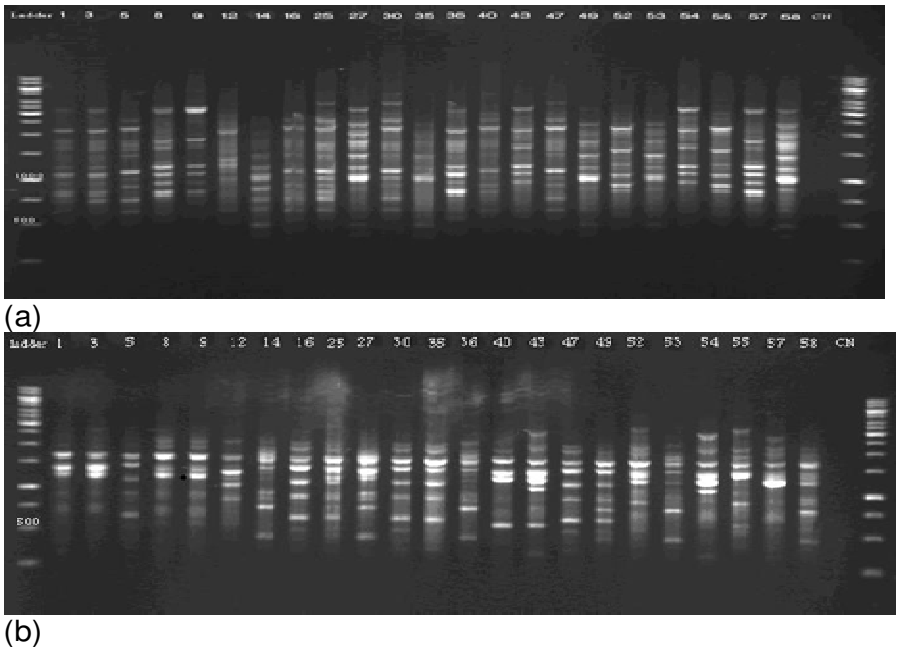

Figure 2. DNA profiles of 23 isolates of *Rhizoctonia solani* obtained with random amplified polymorphic DNA (RAPD) analysis. RAPD profiles obtained with primer OPC19 (a) and OPA16 (b). Lanes 1–23 are *R. solani* isolates; on the right and left: DNA Ladder 1 Kb. lanes: 1: 1VCu, 3: 3VPu, 5: 5VPu, 8: 8VPu, 9: 9VPu, 12: 12EPu, 14: 14EPu, 16: 16EPu, 25: 25ECu, 27: 27ECu, 30: 30ECu, 35: 35KhPu, 36: 36KhPu, 40: 40KhPu, 43: 43KhCu, 47: 47KhCu, 49: 49KhCu, 52: 52MMe, 53: 53MMe, 54: 54MMe, 55: 55MWa, 57: 57MWa, 58: 58MWa.

36KhPu, 49KhCu and 53MMe), collected from Esfehan, Khouzestan and Mazandaran. Totally, isolate of 58MWa obtained from Mazandaran and placed in Group VII with approximately 30% genetic similarity (Figure 4).

Comparison of different analysis

The aspects of RAPD and ISSR DNA polymorphisms were compared. These were the efficiency of detecting polymorphisms in different isolates of *R. solani* AG4, the overall utility of bands for detecting genetic variation and the ability of different assays established between isolates. Expected heterozygosity (H_n) and the arithmetic mean (H_{av}) were calculated for each band (Table 3). Loci monomorphic in our set of 23 isolates were excluded from this analysis and included in calculation of EMR and MI as described in Materials and Methods. ISSR profiles showed the highest levels of polymorphism (0.37) in *R. solani* AG4, while the lowest levels of polymorphism (0.32), was detected with RAPD profiles.

A convenient estimate of marker utility may therefore be devised from the product of information as measured by mean expected heterozygosity and EMR (EMR = $n_p\beta$). Based on experimental data, MI was calculated for each band (Table 3). Due to a higher EMR component to the calculation (12.74), this calculation showed that RAPD profile having 4.07 MI which is slightly higher than ISSR profile (3.98); while the mean expected heterozygosity is actually lower than ISSR marker system (Table 3).

DISCUSSION

In this study, the hyphal width of 23 isolates ranged from 4.46 to 6.86 µm, which was in agreement with other authors (Singh et al., 2002; Sneh et al., 1991). Also, changes in mycelium color and sclerotia formation grown on PDA observed after 20 days incubation, as described by Stevens-Jonk and Jones (2001). These isolates were anastomosed with AG4 isolate. So, the isolates were identified as *R. solani* AG4. The isolates of AG4 are commonly isolated from soil and various infected hosts in warmer countries (Sneh et al., 1991). Many scientists observed that AG-4 isolates caused seed, root, crown, and hypocotyls rots while AG-1 caused pre and post emergence damping-off, hypocotyls rots (Sneh et al., 1991; Muyolo et al., 1993). Results showed that, AG4 isolates produced vary considerably according to the severity of symptoms in the same geographical regions. Accordingly, there does not seem to be any relationship between disease severity and environment. The disease cross-interaction of the selected isolates varied considerably among the hosts. The roots of all hosts were found to be

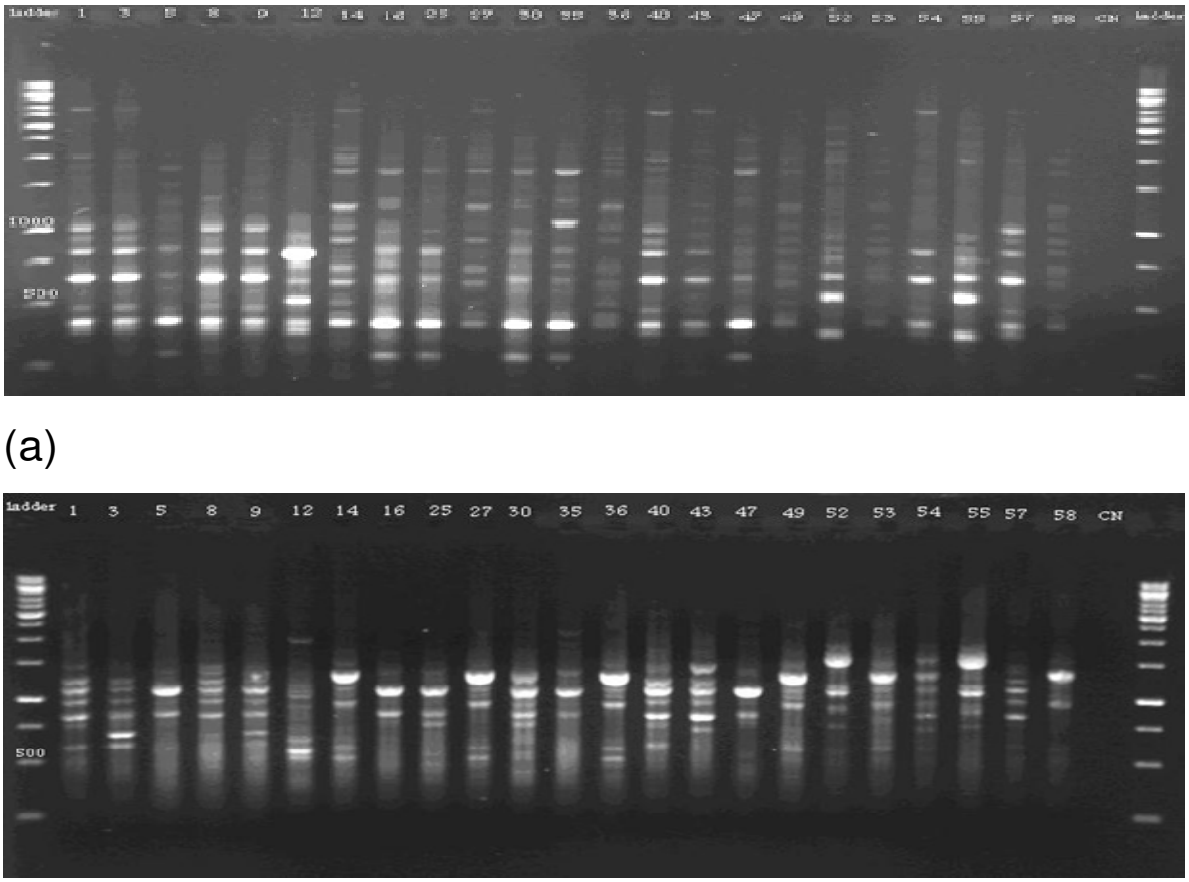

Figure 3. DNA profiles of 23 isolates of *Rhizoctonia solani* AG4 obtained with inter simple sequence repeat (ISSR) analysis. ISSR profiles obtained with primer PMCS (a) and ISSRP1 (b). Lanes 1–23 are *R. solani* isolates; DNA Ladder 1 Kb. lanes: 1: 1VCu, 3: 3VPu, 5: 5VPu, 8: 8VPu, 9: 9VPu, 12: 12EPu, 14: 14EPu, 16: 16EPu, 25: 25ECu, 27: 27ECu, 30: 30ECu, 35: 35KhPu, 36: 36KhPu, 40: 40KhPu, 43: 43KhCu, 47: 47KhCu, 49: 49KhCu, 52: 52MMe, 53: 53MMe, 54: 54MMe, 55: 55MWa, 57: 57MWa, 58: 58MWa.

susceptible and produced symptoms when mycelial mats were inoculated on roots. Earlier reports indicated that, isolate of *R. solani* isolated from a host might be virulence, less virulence or avirulence for other hosts (Yang et al., 1996). The aforementioned findings are in close agreement with the present study; the pathogenicity of the test isolates varied on different hosts and same host.

Morphological characters are influenced by environmental and cultural conditions. Where morphological characteristics are either absent or not able to differentiate isolates properly, molecular approaches are used as important tools for the characterization of genetic diversity in the pathogens (Sharma et al., 2005). A total of 104 RAPD profiles were obtained of which 89 were polymorphic and 15 were monomorphic. These results support the findings of Toda et al. (1999) which also showed that, dendrograms constructed from RAPD analysis led to one cluster of isolates of web blight of European pear belonging to anastomosis group AG1-BI. Moumeni et al. (2005) separated AG2 and AG4 by using RAPD-PCR with seven random primers. Also, they reported that correlation between geographical region and pathogenicity could be detected by RAPD-PCR. Our own RAPD analyses of *R. solani* AG4 on cucurbits; however, did not reveal any correlation between pathogenicity and polymorphism. In contrast, Sharma (2003) revealed a correlation between disease severity and polymorphism in RAPD analyses.

Moreover, the ISSR-analysis did not produce host-specific fingerprint profiles in cucumber, melon, pumpkin and watermelon isolates from diverse geographic regions. The evaluation of genetic diversity of *R. solani* AG4 by RAPD and ISSR profiles only showed high genetic diversity between different isolates and different regions. However, Singh et al. (2002) reported that pathogenic variation is related to the distribution of the isolates in

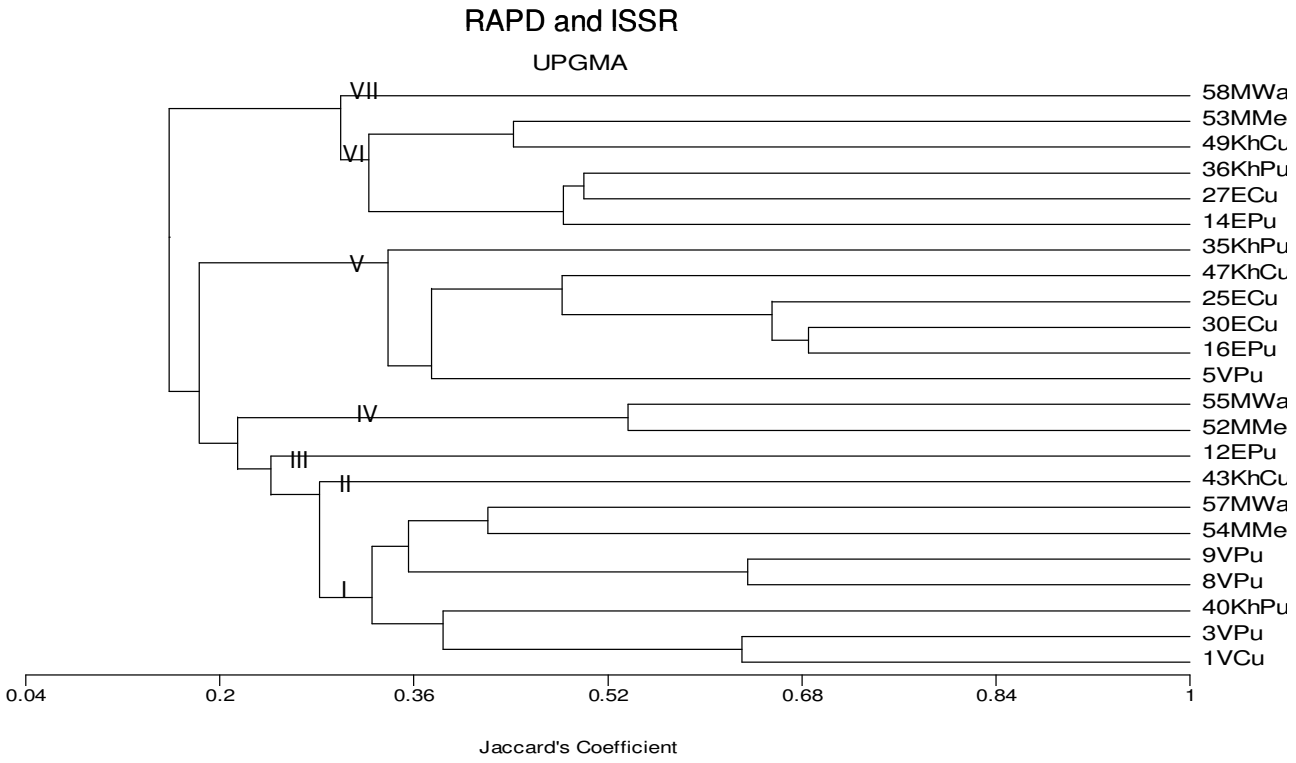

Figure 4. Dendrogram obtained from the combined data sets of 23 *Rhizoctonia solani* AG4 isolates with RAPD and ISSR profiles. Isolate number, host and place of collection are given on the end of the branches.

Table 3. Total numbers of bands, polymorphic bands and monomorphic bands expected heterozygosity (H_n), fraction of polymorphic loci (β), marker index (MI) and effective multiplex ratio (EMR) obtained from RAPD and ISSR analysis.

Marker system	Total number of bands	Polymorphic bands	Monomorphic bands	Expected heterozygosity (H_n)	β	MI	EMR
ISSR							
ISSR02	14	13	1	0.36	0.93	4.40	12.07
ISSRP1	9	8	1	0.36	0.89	2.59	7.11
ISSRP4	12	12	0	0.38	1.00	4.52	12.00
PMCS	12	12	0	0.37	1.00	4.41	12.00
Total	47	45	2	$H_{av(p)}$:0.37	0.86	3.98	10.80
RAPD							
OPA07	18	15	3	0.29	0.83	3.62	12.50
OPA11	13	11	2	0.35	0.85	3.26	9.31
OPA16	11	10	1	0.41	0.91	3.74	9.09
OPC18	18	17	1	0.29	0.94	4.70	16.06
OPC19	26	22	4	0.29	0.85	5.38	18.62
R28	18	14	4	0.28	0.78	3.07	10.89
Total	104	89	15	$H_{av(p)}$:0.32	0.96	4.07	12.74
Total Markers	151	134	17				

$H_{av(p)}$: Average expected heterozygosity.

different climatic regions as environment might be influencing pathogenic variability. While, Mahmoodi et al. (2005) grouped sugar beet isolates collected from different geographical regions of Iran into two anastomosis groups,

that is, AG-1 and AG-4. Combined data set of 151 DNA fragments (RAPD and ISSR profiles) indicated the high genetic diversity in the pathogen population prevalent in different epidemiological regions of Iran. These results are in accordance with Mahmoodi et al. (2005) who reported that no relationship between genetic diversity, disease severity and geographic regions within *R. solani* AG4 based on RAPD analysis. Similar as Yang et al. (1996) who reported the considerable variation within *R. solani* AG9.

Comparison of the DNA profile performance aspects indicated that ISSR technique was more informative in detecting genetic diversity in *R. solani* AG4 than RAPD technique. The utility of a given marker system is a balance between the level of polymorphism detected and the extent of an assay which can identify multiple polymorphisms (Sharma et al., 2005). While testing the utility of two techniques used in this study in terms of their efficiency in detecting polymorphisms among the isolates, we found that the ISSR analysis were more efficient than the RAPD technique. ISSR profile is more specific rather than the RAPD, because of short primers and high annealing temperature (Kang et al., 2002). These techniques can be used for further analysis of more numbers of *R. solani* isolates collected from different epidemiological regions.

Conclusion

High genetic diversity was observed within *R. solani* AG4 isolates; however, there was no relationship between genetic diversity, disease severity and geographical origins of isolates based on their DNA profiles.

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