Diversity of *Macrophomina phaseolina* Based on Morphological and Genotypic Characteristics in Iran

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Fifty two *Macrophomina phaseolina* isolates were recovered from 24 host plant species through the 14 Iranian provinces. All isolates were confirmed to species using species-specific primers. The colony characteristics of each isolate were recorded, including chlorate phenotype, relative growth rate at 30°C and 37°C, average size of microsclerotia, and time to microsclerotia formation. The feathery colony phenotype was the most common (63.7%) on the chlorate selective medium and represented the chlorate sensitive phenotype of the Iranian *Macrophomina phaseolina* population. Mean-time, inter simple sequence repeats (ISSR) Markers were used to assess the genetic diversity of the fungus. Unweighted pair-group method using arithmetic means (UPGMA) clustering of data showed that isolates did not clearly differentiate to the specific group according to the host or geographical origins, however, usually the isolates from the same host or the same geographic origin tend to group nearly. Our results did not show a correlation between the genetic diversity based on the ISSR and phenotypic characteristics. Similar to the *M. phaseolina* populations in the other countries, the Iranian isolates were highly diverse based on the phenotypic and the genotypic characteristics investigated and needs more studies using neutral molecular tools to get a deeper insight into this complex species.

**Keywords**: charcoal rot, chlorate phenotype, ISSR

The causal agent of charcoal rot, *Macrophomina phaseolina* (Tassi) Goidanich, is a soil- and seed-borne pathogen of over 500 host plant species (Dhingra and Sinclair, 1978) and it causes significant damage in Iran to soybean (Raeyatpanah and Forootan, 1993; Raeyatpanah et al., 2002) and sunflower (Razavi and Pahlavani, 2004). The high levels of morphological variability of *M. phaseolina* across different hosts and geographical regions suggest that this species may be divided into subgroups (Aboshosha et al., 2007; Beas-Fernandez et al., 2006; Hawatema and Hameed, 2006; Karunanithi et al., 1999; Mayek-Perez et al., 1997; Mihail and Taylor, 1995; Omar et al., 2007). However, grouping of isolates by *formeae specialis*, subspecies, or physiological race was challenged due to variation in the morphology of isolates from a single host (Dhingra and Sinclair, 1972).

Molecular markers are powerful tools for assessing genetic variation and elucidating genetic relationships within and among species (Chakravarthi and Naravaneni, 2006). Different molecular methods have been used for differentiating *M. phaseolina* populations including Restriction Fragment Length Polymorphism (RFLP) of rDNA-ITS regions (Aghakhani and Dubey, 2009; Almeida et al., 2003; Purkayastha et al., 2006; Su et al., 2001), Random Amplified Polymorphic DNA (RAPD) (Aboshosha et al., 2007; Aghakhani and Dubey, 2009; Almeida et al., 2003; Almeida et al., 2008; Babu et al., 2010; Das et al., 2006; Jana et al., 2003; Omar et al., 2007; Purkayastha et al., Rajkumar and Kuruvinashetti, 2007; Su et al., 2001; 2006; Zade et al., 2009), Amplified Fragment Length Polymorphism (AFLP) (Brooker et al., 2008; Mayek-Perez et al., 2001; Reyes-Franco et al., 2006; Saleh et al., 2010; Vandemark et al., 2000), Universal Rice Primer PCR (URP-PCR) (Jana et al., 2005b), Inter simple sequence repeats (ISSR) (Jana et al., 2005a; Purkayastha et al., 2008), Repetitive Sequence-Based Polymerase Chain Reaction (Rep-PCR) (Purkayastha et al., 2008) and SSR (Baird et al., 2010). Inter simple sequence repeat (ISSR) markers are powerful tools which can be utilized to access the variation in the flanking regions of microsatellite loci that are dispersed throughout all genomes (Zietkiewicz et al., 1994). In this study we used some conventional techniques and ISSR markers to assess the morphologic and genetic variability among 52 isolates of *M. phaseolina* from different hosts and origins within Iran and identifying insights to host specialization.

**Material and Methods**

**Fungal isolates.** Isolates of *M. phaseolina* were recovered from 24 different host species showing typical symptoms.
Morphological and Genotypic Diversity of *Macrophomina phaseolina* and signs of charcoal rot in 14 Iranian provinces (Fig. 1 and Table 2). To isolate *M. phaseolina* from infected tissue, five small (0.5 cm) epidermal sections were excised from symptomatic roots or stems. Tissue sections were surface disinfested with 75% ethanol for 30 s and 2.5% sodium hypochlorite for 30 then washed in sterile water for 1 min. and placed on potato dextrose agar (PDA, Merck, Darmstadt, Germany) containing chloramphenicol (0.1 mg/ml). Plates were incubated at 30 °C in the dark for 5 days. Pure cultures were obtained by the hyphal tip technique and maintained on sterile toothpicks at room temperature (Edmunds, 1964). Isolates were identified to species using species-specific primers (MpKFI/MpKRI) according to Babu et al. (2007). Controls included a no DNA negative control, DNA of isolates of *Trichoderma harzianum*, and *Cytospora schusleri*, *Sclerotinia sclerotiorum*. A known marker isolate of *M. phaseolina* was used as a positive control (Fig. 2).
Morphological analysis. The morphological characteristics of the recovered isolates were investigated, including colony phenotype on medium containing 120 mM potassium chloride (Fig. 3) (Pearson et al., 1986), relative growth rate at 30 °C and 37 °C (Beas-Fernandez et al., 2006; Mayek-Perez et al., 1997), height of aerial mycelium, average length and width of 50 microsclerotia, and time to microsclerotia formation.

Genomic DNA extraction. Isolates were grown in the dark at 28°C for 5 days in 250 ml glass bottles containing 50 ml potato dextrose broth (PDB). Mycelia were filtered through Whatman No. 1 filter paper and lyophilized by vacuum pump. Genomic DNA was extracted according to Lee and Taylor (1990) with some modifications (excluding phenol-chloroform step) as described by Safaie et al. (2005). DNA concentrations were determined by BioPhotometer at 260 nm and were stored at −20°C for further use.

PCR amplification and gel electrophoresis. ISSR analysis was carried out amplifying the genomic DNA using ISSR primers (Table 1). The PCR amplification conditions were: initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 94°C for 2 min, primer annealing according to Table 1 for 1 min, primer extension at 72°C for 2 min, and a final primer extension at 72°C for 7 min. The amplification product was separated in a 1.5% agarose gel. The gel was stained with ethidium bromide (0.50 µg/ml) and visualized under UV to confirm DNA amplification. Amplifications and gel separation were repeated at least twice with each primer. A total of nine primers were screened initially. Six primers were selected for final analysis based on informative banding patterns, clarity, and repeatability.

Statistical analysis. Statistical analysis of morphological characteristics was performed in SAS.9.1.3.Service Pack 2. Means comparisons were performed by Duncan’s Multiple Range Test. Relatedness among the 52 isolates was estimated by comparing the amplified DNA bands across the six

| Primer | Primer sequence ('5–3') | Annealing temperature (°C) | No. of amplified DNA fragments | No. of polymorphic fragments | Percent polymorphic fragments | Reference |
|--------|--------------------------|-----------------------------|-------------------------------|-------------------------------|------------------------------|-----------|
| ISSR2  | 5'-ACTGACTGACTGACTGACT-3' | 48                          | 26                            | 22                            | 84.6                         | Jana et al., 2005 |
| ISSR5  | 5'-GACACGACACGACACAC-3'  | 50                          | 25                            | 21                            | 84.0                         | Jana et al., 2005 |
| ISSR10 | 5'-CCACCCACCCACCC-3'     | 52                          | 20                            | 18                            | 90.0                         | Jana et al., 2005 |
| PeMs   | 5'-GTCGTCGTCGTCGTCGTCGTC-3' | 57                          | 20                            | 18                            | 90.0                         | Islam et al., 2003 |
| ISSR09 | 5'-CCACCCACCCACCC-3'     | 47                          | 17                            | 14                            | 82.4                         | Vasserur et al., 2005 |
| P4     | 5'-ATGATGATGATGATGATGAT-3' | 47                          | 16                            | 14                            | 87.5                         | Stepansky et al., 1999 |
| P5     | 5'-ACACACACACACACACACAC-3' | -                           | -                             | -                             | -                            | Stepansky et al., 1999 |
| P6     | 5'-GAGAGAGAGAGAGAGAGAG-3' | -                           | -                             | -                             | -                            | Stepansky et al., 1999 |
| LB-B   | 5'-GACAGACAGACAGACATT-3   | -                           | -                             | -                             | -                            | Arbaoui et al., 2008 |
| Total  |                          |                             | 124                           | 108                           | 87.1                         | -         |
Morphological and Genotypic Diversity of *Macrophomina phaseolina*

### Table 2. Host-, geographic-origins, and morphological characteristics of the studied *M. phaseolina* isolates of Iran

| Isolate No. | Host       | Location            | Colony morphology | Chlorate sensitivity | Growth rate at 30°C (mm/hr) | A     | B     |
|-------------|------------|---------------------|-------------------|----------------------|-----------------------------|-------|-------|
|             |            |                     |                   |                      |                             |       |       |
| 1           | Sunflower  | Khozestan           | Feathery          | Sensitive            | 1.385                       | M     |       |
| 2           | Sunflower  | Fars                | Restricted        | Sensitive            | 1.156                       | L     |       |
| 3           | Sunflower  | Markazi             | Restricted        | Sensitive            | 0.906                       | L     | +     |
| 4           | Soybean    | Golestan            | Feathery          | Sensitive            | 1.021                       | M     | +     |
| 5           | Soybean    | Golestan            | Restricted        | Sensitive            | 0.833                       | M     | +     |
| 6           | Soybean    | Golestan            | Restricted        | Sensitive            | 1.115                       | M     |       |
| 7           | Olive      | Khozestan-bagh malek| Feathery          | Sensitive            | 1.323                       | H     | +     |
| 8           | Olive      | Khozestan-haftapeh  | Feathery          | Sensitive            | 1.448                       | M     | +     |
| 9           | Olive      | Khozestan-ahlavaz    | Feathery          | Sensitive            | 1.135                       | M     | +     |
| 10          | Cantaloupe | Khorasan            | Restricted        | Sensitive            | 1.198                       | M     |       |
| 11          | Cantaloupe | Fars                | Dense             | Resistant            | 1.083                       | L     | +     |
| 12          | Cantaloupe | Isfahan-kashan      | Dense             | Resistant            | 1.240                       | M     |       |
| 13          | Common Bean| Khorasan            | Feathery          | Sensitive            | 1.302                       | M     | +     |
| 14          | Common Bean| Isfahan             | Feathery          | Sensitive            | 1.155                       | L     | +     |
| 15          | Common Bean| Ghazvin             | Restricted        | Sensitive            | 1.010                       | H     | +     |
| 16          | Tomato     | Khorasan            | Restricted        | Sensitive            | 1.240                       | L     |       |
| 17          | Tomato     | Qom                 | Feathery          | Sensitive            | 1.219                       | H     |       |
| 18          | Tomato     | Ghazvin             | Feathery          | Sensitive            | 1.125                       | M     | +     |
| 19          | Cucumber   | Tehran-karaj        | Dense             | Resistant            | 1.542                       | L     |       |
| 20          | Cucumber   | Hamedan             | Restricted        | Sensitive            | 1.010                       | L     |       |
| 21          | Cucumber   | Qom                 | Feathery          | Sensitive            | 1.292                       | H     |       |
| 22          | Melon      | Khorasan            | Restricted        | Sensitive            | 1.458                       | H     |       |
| 23          | Melon      | Semnan              | Dense             | Resistant            | 1.104                       | L     |       |
| 24          | Melon      | Qom                 | Feathery          | Sensitive            | 1.260                       | M     | +     |
| 25          | Sesame     | Isfahan-kashan      | Feathery          | Sensitive            | 1.406                       | H     |       |
| 26          | Sesame     | Khozestan           | Feathery          | Sensitive            | 1.500                       | M     | +     |
| 27          | Sesame     | Kerman              | Feathery          | Sensitive            | 0.844                       | L     | +     |
| 28          | Kiwi       | Golestan            | Feathery          | Sensitive            | 1.010                       | M     | +     |
| 29          | Kiwi       | Golestan            | Feathery          | Sensitive            | 0.990                       | M     | +     |
| 30          | Kiwi       | Golestan            | Feathery          | Sensitive            | 0.927                       | M     | +     |
| 31          | Hemp       | Isfahan-kashan      | Feathery          | Sensitive            | 1.385                       | M     | +     |
| 32          | Hemp       | Isfahan             | Restricted        | Sensitive            | 1.125                       | M     |       |
| 33          | Hemp       | Isfahan             | Feathery          | Sensitive            | 1.188                       | L     |       |
| 34          | Pumpkin    | Markazi             | Feathery          | Sensitive            | 1.344                       | M     |       |
| 35          | Pumpkin    | Ghazvin             | Feathery          | Sensitive            | 1.208                       | M     |       |
| 36          | Pumpkin    | Ghazvin             | Feathery          | Sensitive            | 1.177                       | M     | +     |
| 37          | Mungbean   | Kerman              | Feathery          | Sensitive            | 1.146                       | M     |       |
| 38          | Mungbean   | Kerman              | Feathery          | Sensitive            | 1.250                       | L     |       |
| 39          | Mungbean   | Kerman              | Feathery          | Sensitive            | 1.208                       | M     |       |
| 40          | Okra       | Khozestan           | Feathery          | Sensitive            | 1.406                       | M     |       |
| 41          | Okra       | Yazd                | Feathery          | Sensitive            | 1.073                       | L     |       |
| 42          | Sugar beet | Khorasan-fariman    | Dense             | Resistant            | 1.240                       | L     |       |
| 43          | Canola     | Qom                 | Feathery          | Sensitive            | 0.948                       | H     |       |
| 44          | Datura     | Markazi             | Feathery          | Sensitive            | 1.021                       | L     |       |
| 45          | Eggplant   | Hormozgan           | Feathery          | Sensitive            | 1.260                       | L     |       |
| 46          | Sonchus    | Tehran-karaj        | Restricted        | Sensitive            | 1.219                       | H     | +     |
| 47          | Maize      | Isfahan-kashan      | Feathery          | Sensitive            | 1.115                       | H     |       |
| 48          | Maize      | Golestan            | Feathery          | Sensitive            | 1.333                       | M     | +     |
| 49          | Watermelon | Isfahan-kashan      | Feathery          | Sensitive            | 1.021                       | H     | +     |
| 50          | Marigold   | Golestan            | Restricted        | Sensitive            | 1.198                       | H     | +     |
| 51          | Sorghum    | Golestan            | Feathery          | Sensitive            | 1.271                       | M     |       |
| 52          | Turnip     | Isfahan-kashan      | Feathery          | Sensitive            | 1.115                       | L     | +     |

A: Height of aerial mycelium: L=low (0 mm), M=moderate (1–2 mm), H=high (> 2 mm). B: Isolates with microsclerotia formed before 48 h of incubation at 37°C. C: Isolates with microsclerotia formed after 60 hr of incubation at 37°C.
ISSR primers. Each band was considered either present (coded as 1) or absent (coded as 0). The binary data were analyzed using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) package (Rholf, 2000) to determine the similarities among individuals. Similarities were estimated using Jaccard’s (J) coefficient (Jaccard, 1908). Clustering analysis was performed using NTSYS-PC software programs by the unweighted pair-group method using arithmetic averages (UPGMA).

Results and Discussion

**Fungal isolates.** Fifty two *Macrophomina phaseolina* isolates were recovered from 24 plant species collected from the 14 Iranian provinces (Fig. 1 and Table 2). This was the first report of *M. phaseolina* from hemp, turnip, okra, mungbean, marigold and *Sonchus* in Iran. The species-specific primers designed by Babu et al. (2007) amplified one 350 bp band in all isolates of *Macrophomina*, confirming that the isolates obtained belonged to the species *M. phaseolina*. No bands were present in the negative controls, confirming that the primers were specific to *M. phaseolina*. In most cases, we selected three isolates from each host, but from different regions, to try to determine whether isolates of one host from three regions have host-specific fingerprint profiles, this is an insight into host specialization.

**Morphological analysis.** Most *M. phaseolina* isolates (67.3%) were characterized by feathery colony phenotype when grown on PDA medium containing 120 mM potassium chlorate while 9.6% of the isolates showed dense growth and 23.1% of the isolates showed restricted growth (Table 2). Isolates characterized with a dense growth phenotype were considered resistant to chlorate, while isolates characterized with feathery or restricted growth phenotype were considered resistant to chlorate. All resistant isolates, except one, were collected from cucurbiteaceus host plants, the exception was collected from sugar beet. All isolates from olive, kiwi, pumpkin, mungbean, okra and maize had a feathery phenotype, while two isolates from sunflower and soybean had a restricted phenotype (Table 2). Isolates were characterized according to the height of aerial mycelium when grown in PDA culture. Low aerial mycelium measured 0 mm, medium aerial mycelium measured 1–2 mm, and high aerial mycelium measured greater than 2 mm. Among the isolates collected in Iran, 30.8% had low aerial mycelium, 48.1% were moderate, and 21.2% had high aerial mycelium (Table 2). Isolates No.10 and No.12 had the largest microsclerotia, and isolates No.41 and No.27 had the smallest microsclerotia. Isolates from common bean (No.13, No.14 and No.15), maize (No.47 and No.48), mungbean (No.38 and No.39), kiwi (No.29 and No.30), cucumber (No.19 and No.20), olive (No.8 and No.9), sunflower (No.4 and No.5), soybean (No.26 and No.27), sunflower (No.1 and No.2), and mungbean (No.36 and No.37) had the smallest microsclerotia.

![Fig. 4. Dendrogram constructed with UPGMA clustering method for 52 *Macrophomina phaseolina* recovered from different provinces in Iran. Dissimilarities were computed from chlorate phenotype, relative growth rate at 30°C and 37°C, height of aerial mycelium, average length and width of 50 microsclerotia, and time of microsclerotia formation.](image-url)
flower (No.1 and No.3), soybean (No.4 and No.6) and hemp (No.31 and No.33) have the same size of sclerote and grouped according to host. Finally, isolates were clustered using all morphological characteristics (Fig. 4).

All the dense chlorate-resistant isolates, except one, were recovered from cucurbitaceous hosts, the exception was collected from sugar beet in Khorasan province. Chlorate is an analog of nitrate, and the reduction of chlorate via nitrate reductase can result in chlorate toxicity in plants, algae, bacteria and fungi. Chlorate-sensitive strains can reduce nitrate to nitrite, and the chlorate resistant strains cannot (Pearson et al., 1986). All isolates from olive, kiwi, pumpkin, mungbean, tomato, okra and maize were sensitive to chlorate, as recognized by the feathery phenotype on potassium chlorate medium. These results support reports that isolates of the same host species usually have the same colony phenotype (Cloud and Rupe, 1994; Pearson et al., 1987; Purkayastha et al., 2006; Su et al., 2001). As observed among M. phaseolina isolates from olive, kiwi, pumpkin, mungbean, tomato, okra and maize, the association of chlorate sensitivity and host may be due to the selection pressure posed by the host over time. Host-specific isolates may have had time to establish a genetic change based on the physiology of nitrogen utilization in the host. However, colony phenotype of M. phaseolina isolates of cucumber and melon grown on chlorate medium was not consistent within each host. The host from which an isolate was collected may not be the only host to which the isolate was exposed (Pearson et al., 1986). Inorganic nitrate serves as an excellent nitrogen source for many fungi, including M. phaseolina, but it is generally not utilized unless the cells lack a favored nitrogen source, such as ammonia, glutamine or glutamate (Dhingra and Sinclair, 1978; Marzluf, 1997). Maximum invasion in Macrophomina took place during the grain development stages because of competition for carbohydrates with developing grains that physiologically weaken the plant (Dodd, 1980). Under such conditions it is highly probable that fungi might utilize nitrate as a source of nitrogen (Das et al., 2006).

**ISSR analysis.** DNA fragments that resulted from amplification with six ISSR primers ranged in size from 250 to 3,300 bp (Fig. 5). The number of bands ranged from 16 to 26, of which 84–90% were polymorphic (Table 1). Dendrogram of pooled six primers data is presented in Fig. 6. Based on the resulting dendrogram, isolates did not clearly differentiate to the specific group according to the host or geographical origins, however, usually the isolates from the same host or the same geographic origin tend to group nearly. For example, isolates from hosts olive (No.7 and No.8), sunflower (No.2 and No.3), Kiwi (No.28, No.29 and No.30), mungbean (No.38 and No.39), hemp (No.32...
and No.33) and isolates from provinces Qom (No.17 and No.24), Golestan (No.4, No.28, No.29, No.30 and No.5), Ghazvin (No.18 and No.26), Kerman (No.38 and No.39), Isfahan (No.32 and No.33) appeared to be closely related (Fig. 6).

The ISSR technique is an appropriate molecular analysis approach to identify genetic differences in *M. phaseolina* populations (Jana et al., 2005a). The results of the present study clearly demonstrated that *M. phaseolina* from different parts of the country were highly variable and ISSR markers are suitable to reflect the genetic diversity among the populations. A number of molecular studies elsewhere have shown a high level of polymorphism in this fungus when isolates from different host or geographical origins were compared using different molecular tools (Almeida et al., 2003; Jones et al., 1998; Mayek-Perez et al., 2001; Su et al., 2001; Vandemark et al., 2000). Other recent studies have demonstrated the genetic diversity among *M. phaseolina* isolates (Aboshosha et al., 2007; Aghakhani and Dubey, 2009; Babu et al., 2010; Baird et al., 2010; Beas-Fernandez et al., 2006; Das et al., 2006; Jana et al., 2005a; Jana et al., 2005b; Omar et al., 2007; Purkayastha et al., 2008; Rajkumar and Kuruvinashetti, 2007; Reyes-Franco et al., 2006; Saleh et al., 2010). The high genetic diversity of this pleomorphic fungal species is reflected not only in isolates from distinct hosts and geographical origins but also within isolates collected from a single host or geographical origin. The activity of transposons and a high mutation rate of microsatellites could be crucial for genetic variability generated by parasexual reproduction of the fungi (Purkayastha et al., 2008). Based on the resulting dendrogram for ISSR markers, the isolates were not completely differentiated to specific groups according to host or geographical origins. However, usually isolates from the same host or origin tend to group together. Several studies have found no association between DNA genotype and host origin in populations (Almeida et al., 2003; Reyes-Franco et al., 2006; Vandemark et al., 2000). Recently Saleh et al. (2010) used AFLP markers and ITS region sequences to assess the genetic diversity and relationships of *M. phaseolina* isolates from maize, sorghum, tallgrass prairie and soybean. Using Bayesian cluster analysis based on the estimation of co-ancestry coefficients they demonstrated the incomplete specialization by host in charcoal rot fungus, in contrast to results reported by earlier studies (Jana et al., 2003; Jana et al., 2005a; Jana et al., 2005b; Mayek-Perez et al., 2001; Su et al., 2001). Recently, Baird et al. (2010) used twelve simple sequence repeat (SSRs) loci to evaluate genetic diversity of *M. phaseolina* collected from different geographical regions and host species throughout the United States. Their results showed some clustering by host and geography, but the dendrogram generally grouped isolates independent of host or geography. Three Primers in the present study, ISSR2, ISSR5, and ISSR10, were used by Jana et al. (2005a) to separate
isolates according to their hosts and geographical origins. Unlike the present study, these primers did not completely separate isolates according to their hosts. The controversial results could be due to the high number of isolates and hosts in our study compared to theirs. Isolates from hosts olive (No.7 and No.8), sunflower (No.2 and No. 3), Kiwi (No.28, No.29 and No. 30), mungbean (No.38 and No.39), hemp (No.32 and No.33) and isolates from provinces Qom (No.17 and No.24), Golestan (No.4, No.28, No.29, No.30 and No.5), Ghazvin (No.18 and No.26), Kerman (No.38 and No.39), Isfahan (No.32 and No.33) were closely related (Fig. 6). In part similar to our results, several other studies have found an association between DNA genotype and host or geographic origin in populations (Jana et al., 2003; Jana et al., 2005a; Jana et al., 2005b; Mayek-Perez et al., 2001; Purkayastha et al., 2006; Su et al., 2001). The present findings are partially in accordance with the earlier results that the *M. phaseolina* isolates of same host were genetically similar and differed distinctly from the isolates of other hosts (Purkayastha et al., 2006; Su et al., 2001). The existence of genetic diversity among the isolates from the same provinces might be due to the movement of *M. phaseolina* through infested seeds and soil (Aghakhanli and Dubey, 2009). Some of the isolates within a province showed similarity suggesting that these isolates may be considered as a part of the same ancestral population (Almeida et al., 2003).

**Conclusion**

A considerable degree of diversity was revealed in the Iranian population of *M. phaseolina* in the present study. It is suggest that this enhanced its ability to adapt to diverse conditions and overcome host resistance deployed by growers (Trigiano et al., 2008). Basic morphological characteristics are the primary tools to detect diversity in all organisms; however, such morphological phenotypes are subjected to modification by environmental factors or by interaction with products of unknown genes in the genetic background. Molecular markers are ideal tools for assessing variation rapidly within and among species (Chakrarvathi and Naravane, 2006). In the present study, 52 *M. phaseolina* isolates were recovered from 24 host plant species throughout 14 Iranian provinces surveyed. All isolates were confirmed to species based on species-specific primers according to Babu et al. (2007).

Our results revealed that like *M. phaseolina* populations in other countries; the Iranian populations are highly diverse based on phenotypic and genotypic data. High levels of variability are most likely due to the exposure of the pathogen to diverse climates, hosts, cropping patterns and movement of the pathogen within the country. In recent years the country has experienced severe drought conditions possibly due to global warming. *M. phaseolina* capable of becoming a severe problem in many regions and on several host plants, it is tempting to call it a “global warming plant pathogen” as this may influence its current importance in countries such as Iran.

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