J. Crop Prot. 2022, 12 (4): 495-507

Research Article

Population genetic structure of *Ustilago maydis* in Iran

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Abstract: The fungus *Ustilago maydis* causes common smut disease in corn. Under favorable conditions, it can cause severe damage to corn. In this study, the genetic structure of *U. maydis* populations in Iran from the most corn-growing regions of seven provinces, including Ardebil, Fars, Isfahan, Kerman, Kermanshah, Khuzestan, and Qazvin, was evaluated using rep-PCR with primers; BOX, ERIC, and REP. Rep-PCR reactions with 109 isolates of *U. maydis* produced seven distinct clusters consistent with their geographical origin with few exceptions. The results of AMOVA revealed significant genetic differences within and between pathogen populations. The Euclidean similarity coefficient and the UPGMA algorithm indicate five distant clusters based on the disease severity index. The mean comparison of the disease severity index grouped target isolates into 18 clades using the Tukey test. Our findings showed that the pathogenicity assay-based grouping was not consistent with those of the geographical origin of the isolates nor their genetic similarity.

Keywords: corn, common smut, pathogenicity, genetic diversity

Introduction

The fungal pathogen *Ustilago maydis* (DC) Corda, causes common smut disease in corn. The disease was first reported in 1982 in cornfields in Iran (Mehrian 1982). *Ustilago maydis* is an opportunistic fungus that can survive as saprophytic and dormant teliospores in the soil and debris (Barnes *et al*. 2004). Any stress or injury, such as hailstorms, drought, improper fertilizers, and herbicides, can increase the occurrence of the pathogen on exposed corn plants (Christensen 1963). During the pathogenic process, *U. maydis* secretes effectors that suppress the plant immune system and alter the plant metabolism in favor of the pathogen. Since effectors play a vital role in fungal pathogenesis, their identification and characterization are essential to understanding the various aspects of disease development in plants (Darino *et al*., 2021). During the colonization process, this fungus causes the expression of several genes, including six in the chitin deacetylase (CDA) family and a pseudogene (Rizzi *et al*., 2021). Weiland and Altegoer (2021) examined six transmembranes (TM) proteins whose expression increased during disease progression. Two of these six proteins, Vmp1 and Vmp2, are essential for complete plant infection. Ludwig *et al*. (2021) found that five effectors that bind to two proteins form a

Handling Editor: Rasoul Zare

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Received: 09 November 2022, Accepted: 09 January 2023
Published online: 13 January 2023

[DOR: 20.1001.1.22519041.2022.11.4.6.3]

stable complex in *U. maydis* and that entering the pathogen into the host triggers the expression of associated genes.

Although ancient hosts of *U. maydis* are from Mexico, to support the demographic history, Munkacsi *et al*. (2008) showed that domestication and the onset of agriculture affected the pathogen's genetic diversity and geographical dispersal. The pathogen has followed corn as the natives moved the new crop plant to South America and, more recently, to the United States (Munkacsi *et al*. 2008). Schweizer *et al*. (2021) sequenced the genome of 22 isolates of *U. maydis* from Mexico to review the evolutionary history of the species. They found two populations whose date of divergence coincided with corn domestication. They also showed that effector genes had more virulence-associated mutations, suggesting their importance in disease development (Schweizer *et al*., 2021). We still have little information about the link between virulence potential and genetic diversity. In addition, scientists need to elucidate the genetics and evolution of virulence at the population level in *U. maydis* (Mueller *et al*. 2013; Zhang *et al*. 2015). By studying the genetic structure of a population, we can understand its evolutionary history and developmental potential (McDonald 1997). Experts can use genetic structure to understand how genetic diversity distributes within and between populations. Genetic markers can provide insight into genetic variation and diversity distribution (Valverde *et al*. 2000). The genetic interaction of *U. maydis* with corn and the site history in the field may influence the genetic structure of the *U. maydis* population (Zambino *et al*. 1997). Valverde *et al*. (2000) identified genetic diversity among the Mexican strains of *U. maydis*. Their results showed no correlation between the genetic diversity and geographic distance of *U. maydis* isolates. Zhang *et al*. (2015) used SSR markers to study the population structure among 40 isolates of *U. maydis*. Their results revealed the unique genetic diversity among *U. maydis* populations in corn fields in China. Using two molecular markers, ISSR and AFLP, Menzies *et al*. (2003) achieved genetic diversity among several *Ustilago* strains. According to their conclusion, the differences among isolates within species were low. Zambino *et al*. (1997) used a mixture of plate mating techniques and a polymerase chain reaction (PCR)-based assay to analyze the population diversity at the *U. maydis b* mating type locus in samples from four Minnesota locations. They observed high variability in locus b.

In an investigation, Choukan *et al*. (2007) crossed two resistant and susceptible inbred lines to study the genetic control of resistance to common smut in corn. Their findings demonstrated that additive, dominance, and epistasis effects are involved in the genetic control of resistance to common corn smut. There is no documented study on the population structure and genetic diversity of the pathogen in Iran, and most of the studies conducted in Iran have been focused only on the evaluation of corn germplasm resistance to common smut. Therefore, it is necessary to make some investigations in the field of fungal genetic structure.

The objective of this study was to evaluate population genetic structure among Iranian isolates of *U. maydis* using rep-PCR as a molecular marker. We also investigated the relationship between fungal genetics and pathogenicity diversity.

Materials and Methods

Isolates of *Ustilago maydis*

A collection of 109 isolates was obtained from naturally infected corn plants in seven provinces (35 locations) of Iran, including Ardebil, Fars, Isfahan, Kerman, Kermanshah, Khuzestan, and Qazvin (Fig. 1). Teliospores of *U. maydis* were cultured on potato dextrose agar (PDA) containing 10 g/l dextrose in 9 cm diameter Petri dishes (Thakur *et al*., 1989) and incubated for five days at 23 °C until enough sporidia were obtained. The resulting colonies of haploid sporidia were removed and streaked on PDA two more times to obtain pure colonies. The surface of the final medium was scraped and lyophilized in a freeze-dryer before DNA extraction.

[DOR: 20.1001.1.22519041.2022.11.4.6.3]

Figure 1 Seven provinces and 35 locations (black squares) in Iran where the isolates of *Ustilago maydis* were collected.

Pathogenicity tests

Pope and McCarter (1992) method was used to perform the pathogenicity test. Sporidial concentration of 10^6/ml in ddH_2O at a volume of 3 ml per ear was tip injected with 12-ml syringes equipped with a needle seven days after the initial tassel emergence in the susceptible Mo17 corn line. Unwounded ears, which were inoculated using only ddH_2O, were considered controls. The experiment was performed based on a completely randomized design with three replicates on 109 isolates of *U. maydis*. Twenty-one days after inoculation, the disease severity was recorded using a 0-7 scale scoring-based system (Pope and McCarter 1992). SAS version 9.1 was used to analyze the data.

DNA extraction

Genomic DNA was extracted from 40 mg of lyophilized sporidia by a microextraction protocol according to Mueller *et al*. (1992) and one extraction with chloroform isoamyl alcohol (24:1 v/v). DNA concentration and quality were determined spectrophotometrically at A260 and adjusted to a final concentration of 10 ng μL^{-1} in TE buffer.

Primers and PCR amplification

Three primers were used, including BOX (5´-CTA CGG CAA GGC GAC GCT GAC G-3´), ERIC (ERIC1: 5´-ATG TAA GCT CCT GGG GAT TCA C-3´ ERIC2: 5´-AAG TAA GTG ACT GGG GTG AGC G-3´) and REP (REP1: 5´-IIII CGI CGI CAT CIG GC-3´ REP2: 5´-ICG ICI TAT CIG GCC TAC-3´) (Versalovic *et al*. 1994) for rep-PCR reactions. Polymerase chain reactions were performed in 96-well plates in a thermocycler (Biorad, USA) at a total volume of 25 µl, containing 30 ng of genomic DNA, 1X PCR buffer (Fermentas, Germany), 0.1 mM of each dNTPs, 1.5 mM MgCl2, 5 pmol of each pair of forward and reverse primers, and 1 U Taq

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DNA polymerase (Fermentas, Germany). The reaction conditions included an initial denaturation step at 95 ºC for 2 min, followed by 35 cycles at 94 ºC for 1 min, 36 ºC for 1 min, and 72 ºC for 2 min, and a final extension step at 72 ºC for 7 min. The fragment analysis was performed on a 2% agarose gel in 1XTBE buffer.

Genetic diversity

Genetic diversity was evaluated with POPGENE version 1.31 (Yeh *et al*. 1999), and the following parameters were calculated: H (genetic diversity), Nm (number of migrants), GD (genetic distance), and Shannon's information index (I) as a measure of gene diversity.

Analysis of Molecular Variance (AMOVA) was used to examine genetic variation within and between the seven geographic populations of Iran using GenAlEx6 (Peakall and Smouse 2006). AMOVA indicates what percentage of the observed genetic variance occurred within the sites regardless of population grouping.

Cluster analysis

Fingerprint patterns were analyzed using NTSYSpc-2.02e software. The banding patterns for each isolate were converted to a binary matrix, indicating the presence or absence. Dice similarity coefficient (1945) was selected to calculate the similarity matrix and UPGMA (unweighted pair group method with arithmetic averages) for cluster analysis. Each isolate was considered an operational taxonomic unit (OTU).

Results

Pathogenicity tests

Analysis of variance showed a significant difference among the isolates of *U. maydis* with a confidence level of 99% (Table 1). Using Tukey (P = 0.01), we divided the means of disease severity into 18 groups (A to R) (Supplementary Table S1). Isolate Um 64, with the highest mean of disease severity (6.80), was classified in group A, and isolate Um 12, with the lowest virulence (Mean of DS = 0.07), was classified in group R. However, isolates with mean of disease severity below one, are commonly considered as very weak pathogens.

The results of the Euclidean similarity coefficient and UPGMA algorithm showed five distance groups (Fig. 2), including VW (very weak: VW), W (Weak: W), MV (Moderately Virulent: MV), V (Virulent: V), and VV (very virulent: VV) based on disease severity index. Members of the VW cluster with 15 isolates had the least DS among all isolates, from 0.07 for Um12 to 0.9 for Um52, Um73, and Um99. Group W, with 33 isolates, ranged from 1.1 (Um14) to 1.7 (Um18). The largest cluster was MV, with 42 isolates, and the range of DS was from 2.03 (Um19) to 2.9 (Um27 and Um74). Group V included 11 members. The most significant DS in this cluster belonged to Um17 (DS = 3.9), and the least (DS = 3.1) belonged to the two isolates, Um37 and Um65. The fifth group (VV) contained isolates with a disease severity index of 4.2 to 6.8.

Table 1 Analysis of variance obtained from pathogenicity tests of 109 isolates of *Ustilago maydis* on the corn line Mo17.

Source of variation	DF	Sum of Squares	Mean Squares	F
Isolate	108	479.57	4.44	**10.80
Error	218	89.66	0.41	
Total	326	569.23		

**: Significant at 1%.

Genetic diversity and pathogen populations

Rep-PCR reaction with three primers produced 79 polymorphic bands among 109 isolates belonging to the seven populations. Polymorphic loci within a population ranged from 69.62% for Fars isolates to 100% for Ardebil, Kerman, and Khuzestan isolates (Table 2).

Genetic diversity was higher in Ardebil, Kerman, Khuzestan, and Isfahan populations than in the Fars, Qazvin, and Kermanshah populations (Table 2). We estimated the total genetic diversity (Ht) for the entire isolates to be 0.44. Nei's analysis (1973) of gene diversity estimated the gene flow (*Nm*) to be 1.0024 and *Gst* to be 0.3328.

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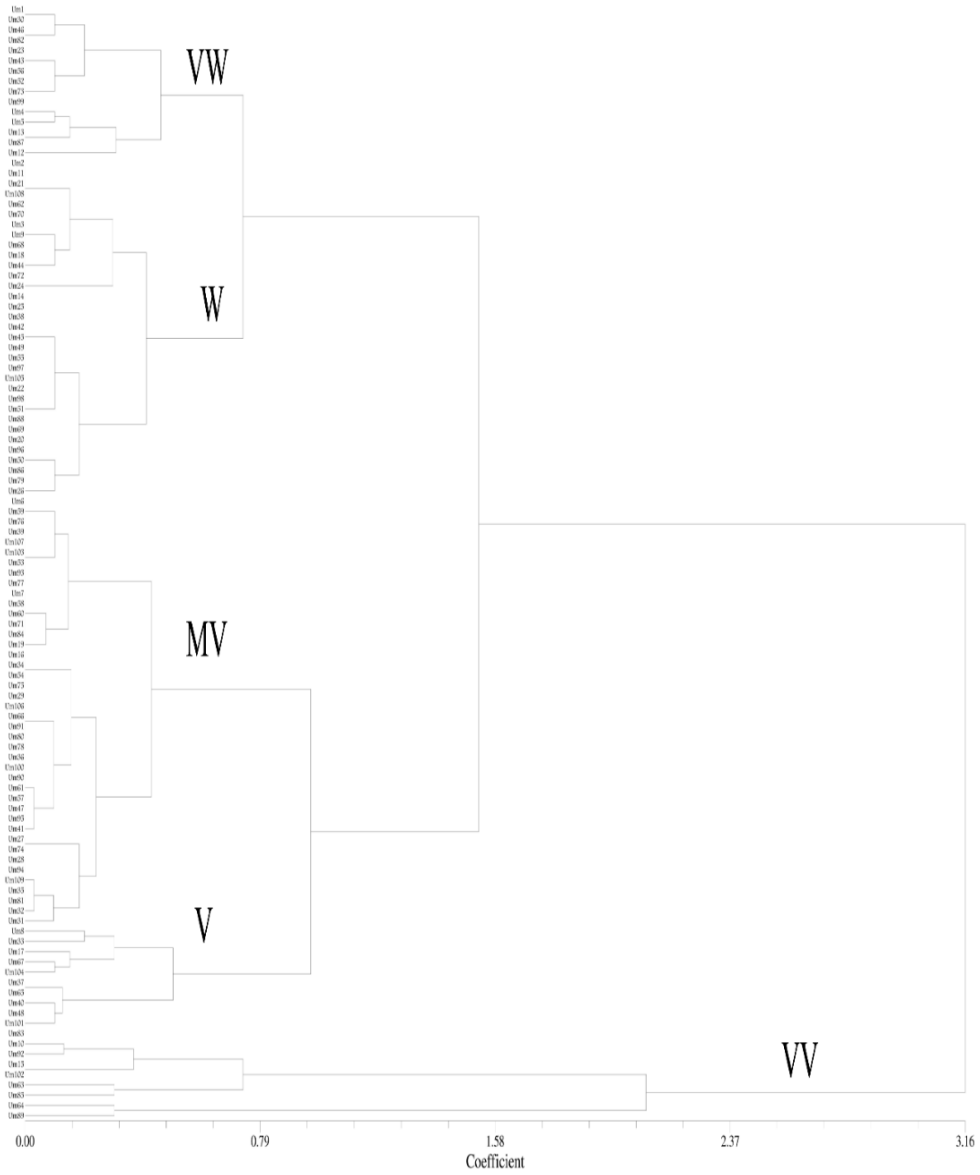

Figure 2 Cluster analysis of 109 isolates of *Ustilago maydis* based on disease severity using Euclidean similarity coefficient and UPGMA algorithm. VW (very weak), W (weak), MV (moderately virulent), V (virulent), and VV (very virulent).

Table 2 Polymorphic loci and genetic diversity in seven populations of *Ustilago maydis* in Iran.

Ustilago maydis population	No. of isolates	No. of polymorphic loci	% polymorphic loci	H_s*
Fars	16	55	69.62	0.2259(0.1867)
Qazvin	15	58	73.42	0.2397(0.1833)
Kermanshah	16	67	84.81	0.2959(0.1772)
Isfahan	16	78	98.73	0.3213(0.1219)
Ardebil	16	79	100.00	0.2976(0.0496)
Kerman	15	79	100.00	0.3175(0.0507)
Khuzestan	15	79	100.00	0.3364(0.0505)

*: Hs: average genetic diversity within population; the number in parenthesis refers to the standard deviation.

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Genetic distance

An unrooted tree was generated based on Nei's genetic distance that revealed the clustering of the seven populations into different groups (Fig. 3). The smallest genetic distance (Ds = 0.0722) was observed between Qazvin and Kermanshah populations, while we detected the most significant genetic distance between Isfahan and Ardebil populations (Ds = 0.3547) (Table 3).

Analysis of Molecular variance (AMOVA)

Results from AMOVA (Table 4) revealed significant genetic differences ($p <= 0.001$) within and between the pathogen populations of the seven provinces of Iran. AMOVA calculations showed that 32% of the variation occurred between populations and 68% between isolates within populations. According to the results, PhiPT was estimated to be 0.324 ($p <= 0.001$).

Similarity cluster analysis

The genetic similarity dendrogram was constructed for 109 isolates of *U. maydis* (Fig. 4) and resulted in the distinction of seven major clusters, which were arbitrarily named C1 to C7. Cluster 1 consisted mainly of isolates from Fars province, with three isolates from Kermanshah. Cluster 2 included isolates from Kermanshah province, with two isolates from Qazvin. Only two isolates from cluster 3 belonged to Fars, and the other belonged to Qazvin. Most of the isolates in Cluster 4 were from Isfahan, and four were from Kermanshah. All isolates in Clusters 5, 6, and 7 belonged to Khuzestan, Ardebil, and Kerman provinces. Nine isolates, including Um57, Um58, Um62, Um63 (from Isfahan), Um37, Um40, Um42 (from Kermanshah), Um29 (from Qazvin), and Um103 (from Khuzestan) did not belong to any group and were considered as single isolates outside of the seven main clusters.

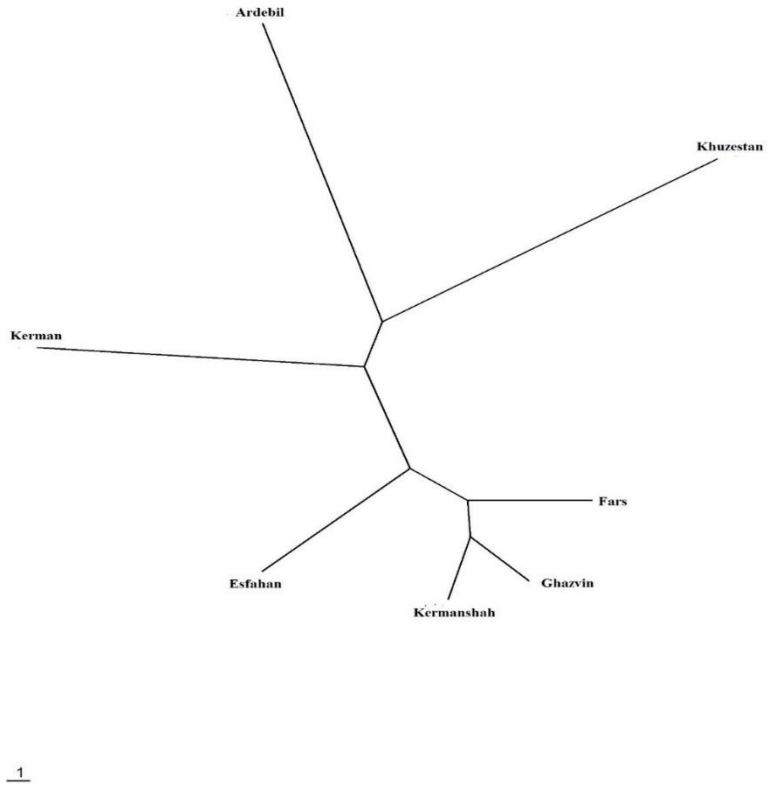

Figure 3 Unrooted UPGMA tree based on Nei's genetic distance between populations of *Ustilago maydis* from seven provinces of Iran. The scale bar represents 1 substitution per site.

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Table 3 Nei's genetic identity (above diagonal) and genetic distance (below diagonal) between seven populations of *Ustilago maydis* in this study.

POP ID	Fars	Qazvin	Kermanshah	Isfahan	Ardebil	Kerman	Khuzestan
Fars	****	0.8879	0.8987	0.7786	0.7101	0.7277	0.6532
Qazvin	0.1189	****	0.9303	0.8444	0.7101	0.7558	0.6774
Kermanshah	0.1069	0.0722	****	0.8988	0.7036	0.7584	0.7201
Isfahan	0.2502	0.1691	0.1067	****	0.7014	0.7360	0.7785
Ardebil	0.3423	0.3424	0.3515	0.3547	****	0.7071	0.7011
Kerman	0.3179	0.2800	0.2765	0.3066	0.3465	****	0.7050
Khuzestan	0.4259	0.3895	0.3284	0.2504	0.3551	0.3496	****

Table 4 Analysis of molecular variance among and within populations of *Ustilago maydis* originated from seven provinces of Iran.

Source of variation	df	Sum of Squares	Mean Squares	Estimated Variance	Variation (%)
Among populations	6	622.007	103.668	5.871	32%
Within populations	102	1250.342	12.258	12.258	68%
Total	108	1872.349		18.130	100%

*: P value = 0.001 and number of permutations is 999.

Discussion

Rep-PCR was used to assess genetic diversity within a collection of *U. maydis* isolates in Iran. We determined genetic diversity in samples from seven provinces and found high levels of diversity in populations. This diversity may be important because pathogen populations with high genetic variability can rapidly evolve to changing environmental conditions (McDonald *et al*. 1994).

Our results are consistent with other findings from other researchers worldwide who have studied the genetic diversity of *U. maydis* isolates (Zambino *et al*. 1997; Valverde *et al*. 2000; Barnes *et al*. 2004; Zhang *et al*. 2015). However, direct comparisons are impossible with different molecular markers, geographic locations, and sample sizes used in various studies. According to Barnes *et al*. (2004), the breeding structure of the Common Smut fungus maintains high population genetic variability across the diverse host cultural practices used throughout the Americas. Since corn is the sole host of *U. maydis*, so Barnes *et al*. (2004) believe that genetic divergence between the North American (NA) and South American (SA) populations occurred over a short period.

Zambino *et al*. (1997) also found high variability at the *b* mating type locus in populations of *U. maydis* collected from three fields in Minnesota, USA. They found 18 different mating types with equal frequency in all localities. Using PCR-RFLP, they demonstrated that each *b* locus had a unique fingerprint. One explanation for the very high genetic diversity among *b* mating type populations (0.86 to 0.96) in their study is that the heterozygosity and diversity at *b* are required for teliospore formation and, afterward, completion of its life cycle. In contrast to Tilletia with high inbreeding, this phenomenon is not common in *U. maydis*. Zambino *et al*. (1997) did not repeatedly recover the same *b* mating type, indicating that populations of *U. maydis* are outbreeding, increasing genetic diversity among populations. We also observed significant genetic diversity, and this outbreeding could significantly impact the diversification of the Iranian population of *U. maydis*.

Similar to our findings, Zhang *et al*. (2015) obtained significant genetic variation at both the population and the isolate levels, indicating a relatively high degree of gene exchange within and between different populations in the sample. They developed a set of SSR markers in *U. maydis* to analyze genotype and genetic diversity, assess genetic relationships among isolates, and assess the correspondences to isolates mating type and geographic origin. Comparing with their study using only 40 isolates from China, we used 109 isolates collected from seven provinces of Iran, which may help to increase the accuracy of the results.

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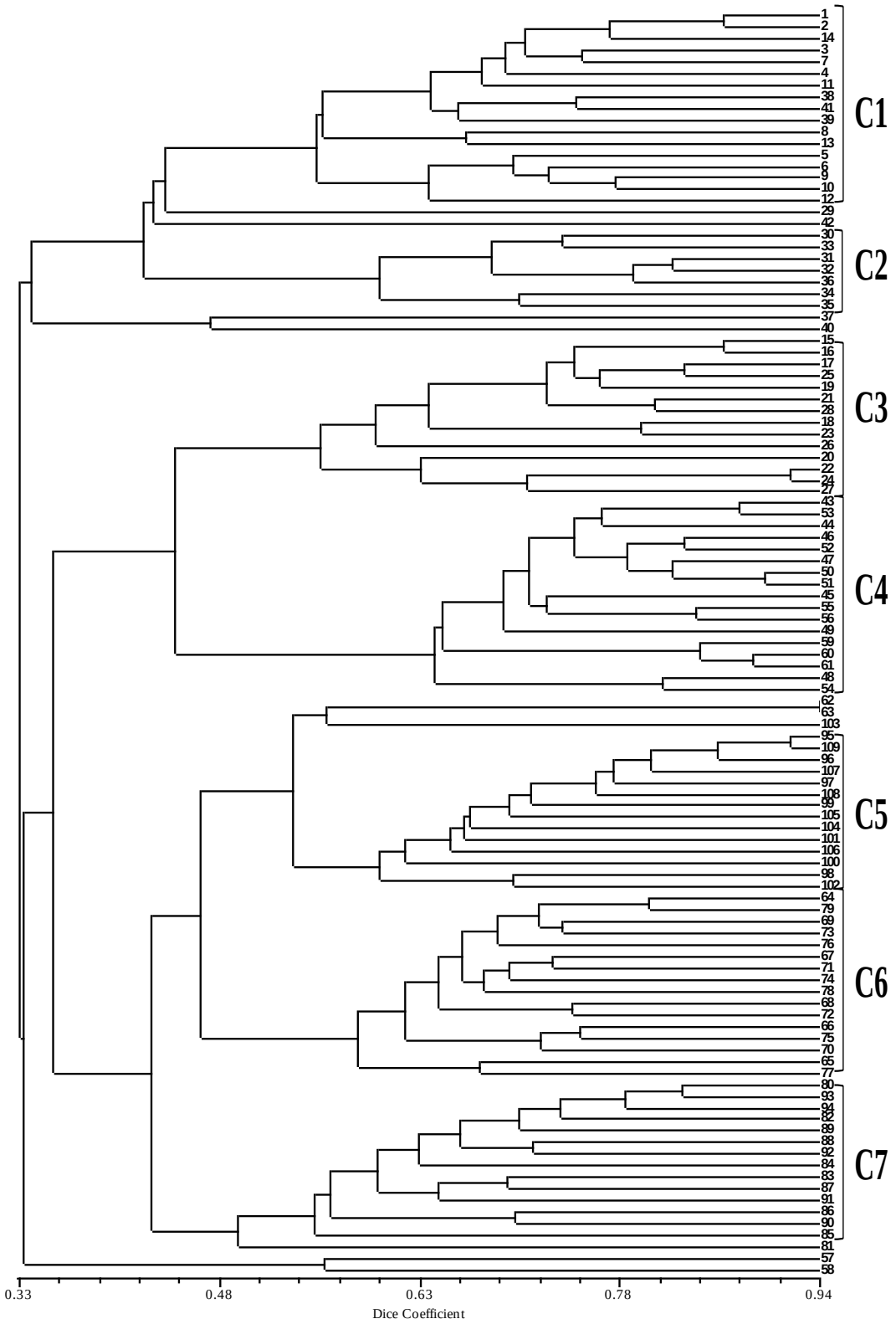

Figure 4 Dendrogram obtained by UPGMA analysis showing the genetic similarity among isolates of *Ustilago maydis*.

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The reason for the high genetic diversity found among isolates of *U. maydis* in our study could be natural mutations, sexual recombination, selection, or strong bottlenecks leading to drift (Barnes *et al*. 2004). Unlike crop plants, the genetic diversity of plant pathogen populations will rebound from bottlenecks and support higher levels of genetic diversity than wild plant populations (Munkacsi *et al*. 2008). According to Barnes *et al*. (2004) infection of a corn plant resulting from a diverse inoculum, including secondary sporidia surviving on debris or multiple teliospores might cause greater diversity in future natural populations of *U. maydis*.

The isolates of *U. maydis* in this study were grouped into seven distinct groups, corresponding to their geographical origins, based on Nei's analysis of genetic distance (Fig. 4). This finding is consistent with the results of Zhang *et al*. (2015) in which isolates collected from the same city were genetically similar and often clustered together. However, they found a few exceptions, including strains isolated from Jiangsu scattered across most clusters. We also found some exceptions in our results, including three isolates from Kermanshah (Um38, Um41, and Um39) in Cluster1 (C1) (Fig. 4) among Fars isolates, two isolates of Qazvin (Um30 and Um31) among all the isolates from Kermanshah in C2, two isolates from Fars (Um15 and Um16) in Cluster 3 contained all the isolates from Qazvin and finally are the five isolates from Kermanshah (Um43, Um44, Um45, Um46, and Um47) among Isfahan isolates in cluster 4. Qazvin and Kermanshah populations clustered together in the genetic distance, while we found the most significant distance between the Ardebil and Isfahan populations (Fig. 3). Valverde *et al*. (2000) also reported this agreement between the genetic and geographical origins of the *U. maydis* isolates, where the standard strains provided from the USA, were placed distant from other Mexican isolates in the dendrogram obtained in their study.

The fungal isolates used in this study were mainly collected from SC 704, which is the most common hybrid of corn cultivated in Iran; therefore, in this research, the type of variety did not have a significant impact on the obtained genetic diversity. After the establishment of the pathogen in different regions, it seems that geographical and climatic differences could be considered as the main factor in creating genetic diversity among corn common smut isolates of Iran.

Sanchez-Alonso *et al*. (1996) found highly polymorphic patterns among strains of *U. maydis*, and our findings agree with their results. They used probes from the telomeric region of the chromosomes during Southern hybridization. Their results showed significant differences among three groups of wild isolates belonging to different geographical areas.

According to our results, UPGMA cluster analysis and AMOVA are consistent with the results of Nei's analysis of genetic distance. AMOVA calculations showed that 32% of the variations occurred between populations and 68% between isolates within populations. The current findings agree with the previous study by Valverde *et al*. (2000), who used restriction fragment length polymorphism (RFLP) analysis to examine the genetic diversity among different isolates of *U. maydis* belonging to five locations in Mexico. They used 23 probes with highly variable banding patterns to study populations of *U. maydis*. Based on their results, most variation (77.46%) was distributed within populations and a small proportion (10.37%) was distributed among populations. Our findings also indicate that most of the genetic diversity in Iranian isolates of *U. maydis* is distributed in different provinces rather than between populations. Valverde *et al*. (2000) found 32 haplotypes among 32 strains indicating that each strain produces a unique banding pattern and the fungus exhibited high genetic diversity. According to their results, the polymorphic RFLP loci and the numbers of alleles per locus were high in isolates of *U. maydis* that originated from all regions of Mexico. All populations exhibited more genetic distance than genetic identity in their study.

Contrary to our findings, Saleh *et al*. (2006) did not observe a correlation between genetic

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and geographic distance for *U. maydis* populations. They used RAPD to study genetic diversity among strains of *U. maydis* and found high genetic diversity among ten samples of *U. maydis* that they collected from four geographic regions. Their samples belonged to different *b* mating types.

Munkacsi *et al*. (2006) have developed tandem repeat markers applicable in population studies of *U. maydis*. They used 36 isolates from the United States, Mexico, and South America. Their findings showed that the number of alleles for each locus was not predictable only with the length of the repeat motif. High polymorphic markers can reveal diversity in a small geographical area.

Karaoglu *et al*. (2005) analyzed and compared the characteristics of simple sequence repeats of *U. maydis* and eight other fungal species. Their findings provided molecular markers for population genetic studies. They demonstrated that the relative abundance of SSRs was inconsistent with the genome size.

Munkacsi *et al*. (2008) believe that the domestication of maize in Mexico and its subsequent expansion across the Americas, influenced the evolution of *U. maydis* and the history of the pathogen population. It had a high impact on the genetic structure of *U. maydis* populations because the Mexican populations showed no greater genetic diversity than more recent populations of the fungus in USA and South America. They suggested that the establishment of the US population of *U. maydis* and the introduction of maize to the USA happened at the same period of time.

Based on pathogenicity tests, we clustered isolates of *U. maydis* into five distanced groups, including VW (very weak: VW), W (Weak: W), MV (Moderately Virulent: MV), V (Virulent: V), and VV (very virulent: VV) based on disease severity index. The clustering of the pathogenicity assays was inconsistent with the geographical origin of the isolates and their genetic similarity produced by the REP, REIC, and BOX PCR reactions. This conclusion is natural because these molecular markers do not replicate only pathogenesis-related genes. They used primers corresponding to the repetitive extragenic sequences (repetitive extragenic palindromic) (REP), enterobacterial repetitive intergenic consensus (ERIC), and BOX element to generate the fingerprint of DNA.

Munkacsi *et al*. (2008) stated that agricultural practices for the host and *U. maydis* create far-apart host and geographical populations. Still, enough gene flow has maintained virulence polymorphism and prevented speciation. Although a broad spectrum of resistance in recently developed corns leads to slow virulence evolution (McDonald and Linde 2002).

Kellner *et al*. (2014) examined the genetic diversity of two virulence gene clusters, 2A, and 19A, of *U. maydis*. Their results demonstrated an overall low intraspecific genetic variation of these virulence-related genes across large geographic distances and subpopulation barriers that reflect the demographics of *U. maydis* populations.

According to our results, rep-PCR with REP, ERIC, and BOX primers are effective tools to study the population genetic structure among isolates of *U. maydis*. It can provide us with valuable information about the characterization and distribution of genetic diversity within and between different populations with diverse geographical origins. This work indicated that populations from seven provinces in Iran are composed of genetically and pathogenically diverse isolates.

Conflict of Interests

The authors declare that they have no conflict of interest.

Authors' contribution

This research paper was accomplished with the collaboration of all authors. Sample collection, study design, pathogenicity tests, and analysis were performed by Hassan Momeni. PCR reactions and analysis were performed by Fahimeh Nazari. The first draft of the manuscript was written by Hassan Momeni, and Fahimeh Nazari read and approved the final version of the manuscript.

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Acknowledgments

We acknowledge Iranian Research Institute of Plant Protection (IRIPP) for the financial support.

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[DOR: 20.1001.1.22519041.2022.11.4.6.3]

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[DOR: 20.1001.1.22519041.2022.11.4.6.3]

ساختار ژنتیکی قارچ *Ustilago maydis* در ایران

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دریافت: ۱۸ آبان ۱۴۰۱؛ پذیرش: ۱۹ دی ۱۴۰۱

چکیده: قارچ *Ustilago maydis* باعث ایجاد بیماری سیاهک معمولی در ذرت می‌شود. در شرایط مساعد قارچ می‌تواند آسیب شدیدی به ذرت وارد کند. در این مطالعه، ساختار ژنتیکی جمعیت‌های *Ustilago maydis* در ایران در عمده مناطق کشت ذرت شامل استان‌های اردبیل، فارس، کرمانشاه، کرمان، اصفهان، قزوین و خوزستان با استفاده از نشانگر مولکولی rep-PCR با آغازگرهای BOX، ERIC و REP بررسی شد. با چند مورد استثنا، ۱۰۹ جدایه‌ی قارچ در هفت خوشه‌ی مجزا مطابق با منشأ جغرافیایی آن‌ها قرار گرفتند. نتایج آنالیز واریانس مولکولی تفاوت‌های ژنتیکی قابل‌توجهی را در داخل و بین جمعیت‌های بیمارگر نشان داد. با عنایت به ضریب تشابه اقلیدسی و الگوریتم UPGMA پنج خوشه مجزا براساس شاخص شدت بیماری به‌دست آمد. براساس میانگین شدت بیماری، جدایه‌ها با استفاده از الگوریتم توکی در ۱۸ گروه قرار گرفتند. گروه‌بندی حاصل از بررسی‌های بیماری‌زایی با منشاء جغرافیایی جدایه‌ها و نیز مشابهت ژنتیکی آن‌ها منطبق نبود.

واژگان کلیدی: *Ustilago maydis*، ذرت، سیاهک معمولی ذرت، ساختار ژنتیکی جمعیت

[DOR: 20.1001.1.22519041.2022.11.4.6.3]