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Genetic Diversity Assessment of the *Diaporthe cinerascens* Causal Agent of Fig Canker Disease Using ISSR Markers

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Abstract: *Diaporthe cinerascens* is a significant plant pathogen that causes canker disease in fig trees. This pathogen is notably dominant in major fig-production areas in Iran, as well as in other fig-producing countries such as Bulgaria, USA (California), Canada, and Italy. In the present study, six Inter-Simple Sequence Repeats (ISSR) markers were used to investigate genetic diversity among representative *D. cinerascens* isolates, selected based on the variety of their host cultivars, aggressiveness, and morphological features. The discriminating power of each ISSR marker was assessed using 10 parameters including effective multiplex ratio, expected heterozygosis, observed heterozygosity, polymorphic information content, marker index, resolving power, Nei's diversity, Shannon index, polymorphic percentage, and allele number. Among them, M1 primer was the best to detect the variability of the *D. cinerascens* isolates. The isolates exhibited a high degree of genetic similarity, as evidenced by Jaccard's similarity coefficients, which ranged from 79% to 100%. Despite the high values of pairwise Jaccard's similarity coefficient among the isolates (at least 79% similarity), they were grouped into four distinct genetic clusters, based on Principal Coordinate Analysis (PCoA) analysis. The observed grouping pattern was found to be partially influenced by their geographical origins and the specific fig cultivars from which the isolates were recovered. Based on ISSR fingerprinting, we provided genetic evidence regarding the clonal structure of *D. cinerascens*. These results could be critical to understanding Diaporthe canker epidemiology and adopting sustainable management practices for the disease.

Keywords: Diaporthe canker; *Ficus carica*; Inter-simple sequence repeat (ISSR); Population genetics.

INTRODUCTION

The genus *Diaporthe* Nitschke (family *Diaporthaceae*) has often been reported as saprobic, endophytic, and important pathogenic species with tropical and temperate distributions (Gao et al. 2017). Many *Diaporthe* species colonize diverse plant hosts as opportunists, while some, such as *D. cinerascens* Sacc., appear to be strictly host-specific (Gomes et al. 2013; Bolboli et al. 2022). The fig canker disease, first documented in Italy in 1878 by Saccardo, was found to be caused by the *Phomopsis cinerascens* (Sacc.) Traverso, as identified by Grove in 1935. This disease led to a significant epidemic across all commercial cultivars, particularly the Kadota in California, exacerbated by extensive pruning (Ferguson et al. 1990). The edible fig (*Ficus carica* L.) is the only reported host for this canker-causing pathogen. Fig trees infected by *D. cinerascens* show symptoms of canker type A, including trunk lesion with zonation originating from pruning wounds, death of bark and woody tissues, limb dieback, leaf yellowing, defoliation, and consequently, death of the trees (Ogawa & English, 1990). This pathogen is also a significant and dominant fig canker-causing pathogen in the largest fig production hub in Iran, Fars Province (Banihashemi & Javadi, 2009; Bolboli et al. 2022).
Understanding the genetic structure of the *D. cinerascens* population is needed for assessing disease epidemiology, characterizing the pathogen's reproductive system, evaluating non-susceptible cultivars in breeding programs, and consequently, adopting sustainable management practices of the disease (McDonald & Linde, 2002; Koenick et al.

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2019). The Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeat (ISSR), and Simple Sequence Repeat (SSR) markers have been successfully used in genetic variations studies among some *Diaporthe* species such as *D. helianthi* Munt.-Cvetk., Mihaljč. & M. Petrov, (Says-Lesage et al. 2002), *D. amygdali* (Delacr.) Udayanga, Crous & K.D. Hyd (Carlier et al. 2009), *D. asparagi* Fuckel (Yang et al. 2020), and *D. citri* (H.S. Fawc.) F.A. Wolf. (Xiong et al. 2021). While the significance of fig Diaporthe canker and the observed phenotypic variation in the aggressiveness of the pathogen are well-documented (Bolboli et al. 2022a), the genetic structure of the causative agent remains unknown. Among PCR-based genetic markers, ISSRs can rapidly, efficiently, and sensitively detect the polymorphism of genomic DNA. This dominant marker has also good repeatability, is cost-effective, and is suitable for the detection of large samples (Yang et al. 2020).

Our previous studies showed distinct differences in the aggressiveness of the *D. cinerascens* population recovered from different fig cultivars in the Fars and Khuzestan Provinces (Bolboli et al. 2022c) We employed ISSR markers to investigate the genetic structure of the *D. cinerascens* population in southern Iran. The present study aimed to reveal the genetic structure of the pathogen in southern Iran, and discover the possible genetic evidence to confirm the phenotypic variation among pathogen aggressiveness.

MATERIALS AND METHODS

Fungal isolates

From 218 *D. cinerascens* isolates identified in Bolboli et al. (2022) that cause canker in fig cultivars, 35 representative isolates were selected. Selected isolates represent various clusters, each exhibiting varying degrees of aggressiveness, as per the findings of Bolboli et al. (2022). These isolates were recovered from different fig cultivars showing Diaporthe canker symptoms including 'Sabz' and 'Payves' (commercial dried figs), 'Pouzdonbali' (a caprifig), and 'Dehdez' (a wild fig) cultivars in various counties within the Fars and Khuzestan Provinces (Table 1, Table 1S, Fig 1S).

DNA extraction and PCR amplification

All selected isolates were cultured on malt extract agar (MEA, 20 g/L of malt extract, 15 g/L agar, and distilled water) (Crous et al. 2019), and incubated at 25 °C for 10–15 days. Total fungal DNA was extracted from harvested and freeze-dried mycelia using the DNG-PLUS extraction kit (CinnaGen, Tehran, Iran) by following the instructions provided by Mirsoleimani & Mostowfizadeh-Ghalamfarsa (2013). The quality and quantity of the extracted DNA were evaluated using an MD-1000 Nanodrop spectrophotometer (NanoDrop Technologies, Delaware, USA). Six ISSR primers were used to investigate the isolates. Polymerase chain reaction (PCR) mixtures were carried out in a total volume of 25 µl, containing one µl genomic DNA (~ 100 ng), one µl forward and reverse primers (10 pM), 12.5 µl Taq DNA Polymerase 2× Master Mix RED (Ampliqon, Denmark), and 9.5 µl PCR-quality distilled water. The PCR amplifications were performed on a Peltier Thermal Cycler (Bio-Techne, Minneapolis, MN, USA), which was programmed for initial DNA denaturation at 95 °C for two min, followed by 35 cycles of one min denaturation at 94 °C, one min annealing at the specific temperature to the primer and one min extension at 72 °C, with a final extension at 72 °C for 10 min (Haghi et al. 2020). The ISSRs primers were selected based on their appropriate discriming power in the previous studies (Haghi et al. 2020). Features of these primers, including their sequences and annealing temperatures used in the PCR reactions are listed in Table 2. Amplified products were resolved by electrophoresis on 1.5% agarose gel in tris-borate EDTA (TBE) buffer stained with 0.5 µg/ml ethidium bromide, and photographs were taken by using the gel documentation system (Syngen, USA).

Genetic diversity analysis using inter simple sequence repeat (ISSR)-PCR

The size of the amplified-fragments was estimated through comparison against the molecular weight ladder using GelAnalyzer 19.1 software (www.gelanalyzer.com, by Istvan Lazar Jr. and Istvan Lazar Sr.). The ISSR data scoring for computer-based analysis was made based on the presence (1) or absence (0) of amplified products for each primer. The genetic associations were evaluated by calculating the Jaccard's similarity coefficient for pairwise comparisons based on the proportion of shared bands produced by the primers. The similarity matrix was subjected to the cluster analysis of the unweighted pair group method with arithmetic averages (UPGMA), and corresponding dendrograms were constructed by NTSYS-pc version 2.1 software (Rohlf, 2000). The discriminating power of each ISSR marker was assessed using 10 parameters including percentage of polymorphic loci (*PPL*), Shannon index (*I*), Nei's gene diversity index (*H*), polymorphic information content (*PIC*), expected heterozygosis (*He*), observed heterozygosis (*Ho*), effective allele number (K_e), allele number (*A*), effective multiplex ratio (*EMR*), marker index (*MI*). The polymorphic information content (*PIC*) of each ISSR marker was calculated according to the formula $PICi = 2fi\ (1–fi)$, where *PICi* is the polymorphic information content of marker i, fi the frequency of the marker bands that was present, and 1–fi is the frequency of marker bands that was absent (Smith et al. 1997). The *MI* is a statistical tool to estimate the total utility of the marker system which can be calculated to characterize the capacity of each primer to detect polymorphic loci among the isolates (Varshney et al. 2007) based on the formula: $MI=EMR\times PIC$. Where *EMR* (effective multiplex ratio) $= n \times \beta$, where n is the average number of fragments amplified by accession and β is estimated

fragments amplified by accession and β is estimated from the number of polymorphic loci (PB) and the number of non-polymorphic loci (MB); Thus, β = PB/(PB + MB) or *EMR* = np (np/n) (Powell et al. 1996). Furthermore, expected heterozygosis (*He*), observed heterozygosis (*Ho*), Nei's diversity (*H*), Effective allele number (K_e), and the Shannon information index (*I*), were calculated by Popgen v. 1.32 software (Francis & Yang, 1999). PCoA was performed using DARwin 5.0 to show the distribution of the accessions in a scatter plot (Altıntaş et al. 2008).

RESULTS

Analysis of genetic similarity

A total of 109 DNA fragments were amplified using six ISSR primers. The features of each ISSR primer are displayed in Table 2. Among these primers, 31 distinct and scorable bands were observed, ranging in size from 180 bp to 3,490 bp (Table 2). The P10 primer produced a total of 20 common fragments, representing the maximum number of bands detected in our experiment. The M1 primer exhibited the highest *PPL* (46.66%) (Fig. 1), while both the M1 and PCMS primers had the highest Shannon index values (*I*) (0.54–0.55). Upon evaluating the discriminatory capacity of ISSR markers, it was observed that the *MI* exhibited the least value for M2 (3.08), while it was highest for M1 (8.68). *PIC* ranged from 0.174 (for M2) to 0.350 (for M1), and the *EMR* varied from 24.33 (for M2) to 28.50 (for P10). Nei's Diversity Index (*H*) ranged from 0.047 (for M2) to 0.36 (for M1) and 0.377 (for PCMS). *He* ranged from 0.161 (for M2) to 0.369 (for M1), while *Ho* ranged from 0.15 (for M2) to 0.35 (for M1). K_e were 1.58 and 1.24 for M1 and M2, respectively, and the *A* ranged from 1.50 (for M2) to 1.92 (for M1) (Table 3).

The UPGMA analysis of ISSR data classified the isolates of *D. cinerascens* into two primary groups. Group I comprised 11 isolates associated with 'Sabz', a cultivar of commercial significance. These isolates were obtained from Estahban County, Fars Province. On the other hand, Group II included 24 isolates that were recovered from infected fig cultivars 'Sabz,' 'Peyves', 'Puzdonbali', and 'Dehdez'. These isolates were found in the Jahrom, and Kazerun counties in Fars Province, as well as Dezpart County in Khuzestan Province (Fig. 2). Within these two primary groups, four distinct clusters were identified based on a 95% similarity in the UPGMA tree. Cluster A comprised four isolates retrieved from the 'Sabz' and 'Pouzdonbali' cultivars in Kazerun County, Fars Province. Cluster B encompassed 20 isolates, which were obtained from the 'Sabz', 'Payves', and 'Dehdez' cultivars in Kazerun and Jahrom, located in Fars and Khuzestan Provinces, respectively. Cluster C consisted of two isolates, ES013-10 and ES013-6, from Estahban County. Lastly, Cluster D included nine isolates recovered from Estahban County (Fig. 2). A similarity matrix based on Jaccard's coefficient revealed that the pairwise value between *D. cinerascens* isolates indicated at least 79% similarity. Also, the pairwise value between KDB43W and ES232 (the most distinct isolates in the UPGMA dendrogram) revealed an 89% similarity (Table 2S). Thirty-five *D. cinerascens* isolates were categorized into four clusters, namely A, B, C, and D, based on the PCoA of ISSR data. Cluster A consisted of 23 isolates recovered from the 'Sabz' and 'Payves' cultivars in Kazerun County, Fars Province as well as from 'Dehdez' (wild fig cultivar) in Khuzestan Province. Cluster B included nine isolates recovered from Estahban county, cluster C included 2 isolates, ES013-10 and ES013-6, from Estahban county, and Cluster D included only the KDB43W isolate (the most aggressive isolate) from a caprifig cultivar in Fars Province (Fig. 3).

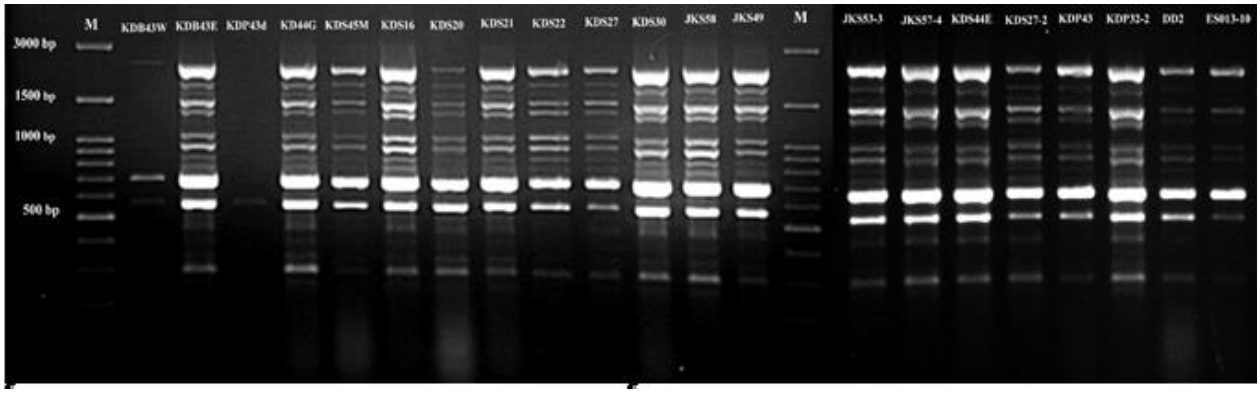

Fig. 1. Representative electrophoresis gel results showing ISSR-PCR amplification of genomic DNA from different isolates of *Diaporthe cinerascens* using M1 ISSR primer. Lane M: 100-bp ladder marker DNA.

Table1. List of *Diaporthe cinerascens* isolates used in this study, recovered from infected fig trees in Fars and Khuzestan Provinces of Iran.

Isolate	Location	Host cultivar	Date	Latitude	Longitude
ES013-10	Fars-Estahban	*Ficus. carica* cv. Sabz	2018.11.06	29°09'0.581"N	054°05'0.561"E
ES013-6	Fars-Estahban	*F. carica* cv. Sabz	2018.11.06	29°09'0.581"N	054°05'0.561"E
ES227-1	Fars-Estahban	*F. carica* cv. Sabz	2023.02.07	29°09'0.482"N	054°05'0.622"E
ES227-2-1	Fars-Estahban	*F. carica* cv. Sabz	2023.02.07	29°09'0.482"N	054°05'0.622"E
ES227-3	Fars-Estahban	*F. carica* cv. Sabz	2023.02.07	29°09'0.482"N	054°05'0.622"E
ES228	Fars-Estahban	*F. carica* cv. Sabz	2023.02.07	29°09'0.465"N	054°05'0.612"E
ES229	Fars-Estahban	*F. carica* cv. Sabz	2023.02.07	29°09'0.471"N	054°05'0.609"E
ES229-2	Fars-Estahban	*F. carica* cv. Sabz	2023.02.07	29°09'0.471"N	054°05'0.609"E
ES231	Fars-Estahban	*F. carica* cv. Sabz	2023.02.07	29°09'0.478"N	054°05'0.592"E
ES232	Fars-Estahban	*F. carica* cv. Sabz	2023.02.07	29°09'0.495"N	054°05'0.591"E
ES232-2	Fars-Estahban	*F. carica* cv. Sabz	2023.02.07	29°09'0.495"N	054°05'0.591"E
KDS106	Fars-Kazerun, Dosiran	*F. carica* cv. Sabz	2018.12.11	29°49'0.414"N	051°47'0.754"E
KDS018	Fars-Kazerun, Dosiran	*F. carica* cv. Sabz	2018.12.11	29°44'0.414"N	052°35'0.359"E
KDS20-9	Fars-Kazerun, Dosiran	*F. carica* cv. Sabz	2018.12.11	29°49'0.479"N	051°47'0.681"E
KDP21	Fars-Kazerun, Dosiran	*F. carica* cv. Payves	2018.12.11	29°49'0.479"N	052°47'0.681"E
KDS22-2	Fars-Kazerun, Dosiran	*F. carica* cv. Sabz	2018.12.11	29°49'0.482"N	052°47'0.688"E
KDS27-2	Fars-Kazerun, Dosiran	*F. carica* cv. Sabz	2018.12.11	29°49'0.479"N	052°47'0.625"E
KDS28-2	Fars-Kazerun, Dosiran	*F. carica* cv. Sabz	2018.12.11	29°49'0.493"N	051°47'0.630"E
KDS30-D	Fars-Kazerun, Dosiran	*F. carica* cv. Sabz	2018.12.11	29°48'0.597"N	051°46'0.598"E
KDP31-2	Fars-Kazerun, Dosiran	*F. carica* cv. Payves	2018.12.11	29°49'0.886"N	051°47'0.602"E
KDP32-2	Fars-Kazerun, Dosiran	*F. carica* cv. Payves	2018.12.11	29°48'0.030"N	051°45'0.594"E
KDS34-1	Fars-Kazerun, Dosiran	*F. carica* cv. Sabz	2018.12.11	29°49'0.886"N	051°47'0.610"E
KDB43E	Fars-Kazerun, Dosiran	*F. carica* cv. Pouzdonbali	2018.12.11	29°50'0.150"N	051°47'0.721"E
KDB43W	Fars-Kazerun, Dosiran	*F. carica* cv. Pouzdonbali	2018.12.11	29°50'0.150"N	051°47'0.721"E
KDS28-2D	Fars-Kazerun, Dosiran	*F. carica* cv. Sabz	2018.12.11	29°49'0.493"N	051°47'0.630"E
KDP43D	Fars-Kazerun, Dosiran	*F. carica* cv. Payves	2018.12.11	29°50'0.150"N	051°47'0.721"E
KDS44G	Fars-Kazerun, Dosiran	*F. carica* cv. Sabz	2018.12.11	29°50'0.150"N	051°47'0.727"E
KDS45M	Fars-Kazerun, Dosiran	*F. carica* cv. Sabz	2018.12.11	29°50'0.148"N	051°47'0.729"E
KDS44B	Fars-Kazerun, Dosiran	*F. carica* cv. Sabz	2018.12.11	29°50'0.150"N	051°47'0.727"E
JKS49-8	Fars-Jahrum, Kereft	*F. carica* cv. Sabz	2018.01.08	28°59'0.452"N	052°50'0.519"E
JKS53-3	Fars-Jahrum, Kereft	*F. carica* cv. Sabz	2018.01.08	28°59'0.465"N	052°50'0.492"E
JKS54-4	Fars-Jahrum, Kereft	*F. carica* cv. Sabz	2018.01.08	28°59'0.479"N	052°50'0.507"E
JKS57-5	Fars-Jahrum, Kereft	*F. carica* cv. Sabz	2018.01.08	28°59'0.551"N	052°50'0.349"E
DD2	Khuzestan-Dezpart	*F. carica* cv. Dehdez	2019.05.15	31°45'0.423"N	050°13'0.119"E
DD5	Khuzestan-Dezpart	*F. carica* cv. Dehdez	2019.05.15	31°45'0.423"N	050°13'0.119"E

Table 2. Inter-simple sequence repeat (ISSR) primers used for the analysis of *Diaporthe cinerascens* genetic variation.

Primer	*PPL*	*I*	*H*	*PIC*	*He*	*Ho*	K_e	*A*	*EMR*	*MI*
M1	65.35	0. 541	0.360	0.350	0.369	0.348	1.58	1.92	26.14	8.68
M2	60.83	0.09	0.047	0.174	0.161	0.151	1.24	1.50	24.33	3.08
P5	68.75	0.411	0.252	0.306	0.278	0.261	1.42	1.91	27.50	7.81
P10	71.25	0.239	0.164	0.277	0.201	0.189	1.34	1.50	28.50	7.28
P17	67.50	0.263	0.140	0.342	0.265	0.249	1.42	1.87	27	8.56
PCMS	64.28	0.555	0.377	0.336	0.319	0.301	1.52	1.85	25.71	7.57
Mean	66.32	0.350	0.224	0.279	0.265	0.250	1.42	1.76	26.53	7.19

A: Allele number, *EMR*: Effective multiplex ratio, *He*: Expected heterozygosis, *Ho*: Observed heterozygosis, *H:* Nei's gene diversity index, *I*: Shannon index, K_e: Effective allele number, *MI*: Marker index, *PIC*: Polymorphic information content, *PPL*: Percentage of polymorphic loci

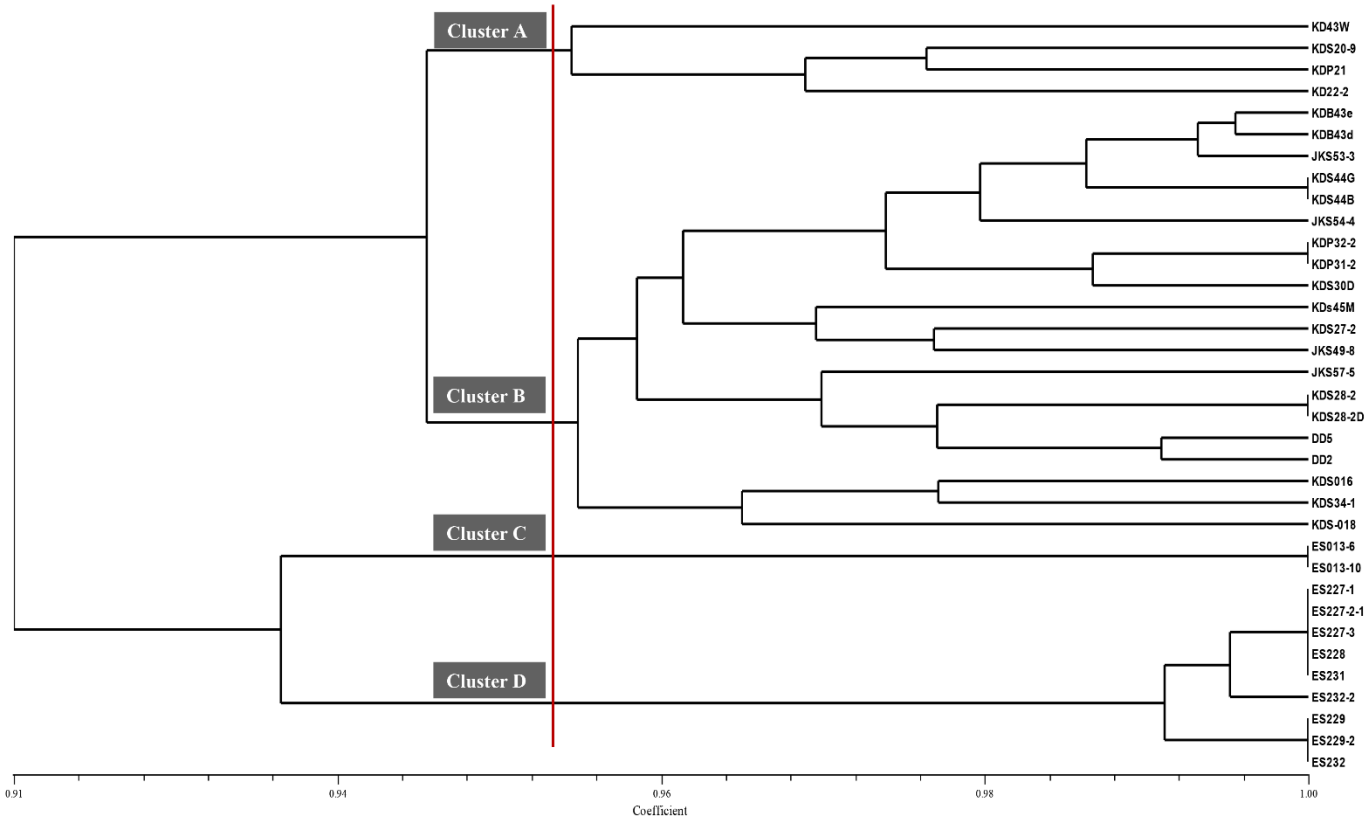

Fig. 2. Dendrogram generated by UPGMA-based cluster analysis of ISSR markers of the *Diaporthe cinerascens* isolates from fig trees in southern Iran. **Cluster A:** KD43W, KDS20-9, KDP21, KD22-2; **Cluster B:** KDB43e, KDB43d, KDS44G, KDS44B, KDs45M, KDP32-2, KDS016, KDS-018, KDS27-2, KDS30D, KDP31-2, KDS34-1, JKS53-3, JKS54-4, JKS57-5, JKS49-8, KDS28-2, KDS28-2D, DD5, DD2; **Cluster C:** ES013-6, ES013-10; **Cluster D:** ES227-1, ES227-2-1, ES227-3, ES228, ES229, ES229-2, ES231, ES232, ES232-2.

Table 3. Different indexes for the assessment of the discriminating power of ISSR markers used in this study.

Primer name	Sequence	Annealing temperature (°C)	Total no. of bands	Rang of bands (bp)	No. of polymorphic bands	Polymorphism percentage
M1	$(ACTG)_4$	55	15	304-2266	7	46.66
M2	$(GACAC)_4$	45	15	257-2800	4	26.66
P5	$(ACTG)_3ACG$	46	17	300-2820	7	41.17
P10	$(GACC)_4$	63	20	180-3450	4	20
P17	$(GGAGA)_3$	45	19	197-2150	5	26.31
PCMS	(GTC)7	52	13	187-3490	4	30.76

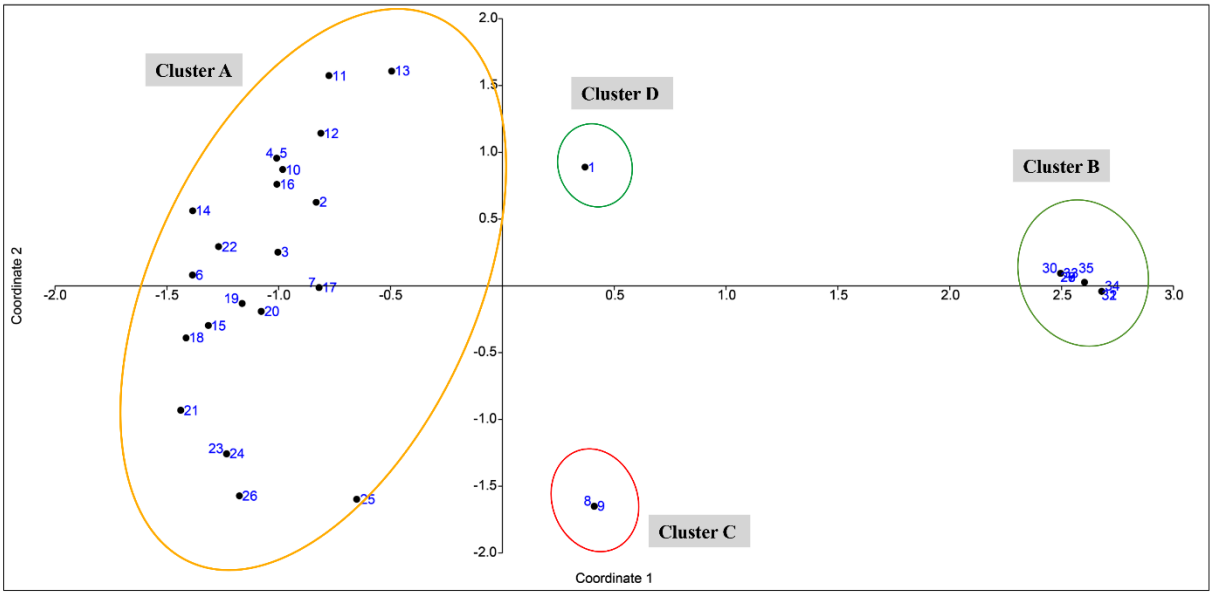

Fig. 3. The results of the Principal Coordinate Analysis (PCoA) in the grouping of 35 *Diaporthe cinerascens* isolates from fig trees in southern Iran, based on ISSR markers. **Cluster A:** 2. KDB43e, 3.KDB43d, 4. KDS44G, 5. KDS44B, 6. KDs45M, 7. KDP32-2, 10. KDS016, 11. KDS20-9, 12. KDS-018, 13. KDP21, 14. KD22-2, 15. KDS27-2, 16. KDS30D, 17. KDP31-2, 18. KDS34-1, 19. JKS53-3, 20. JKS54-4, 21. JKS57-5, 22. JKS49-8, 23. KDS28-2, 24. KDS28-2D, 25. DD5, 26. DD2, **Cluster B:** 1. 27. ES227-1, 28. ES227-2-1, 29. ES227-3, 30. ES228, 31. ES229, 32. ES229-2, 33. ES231, 34. ES232, 35. ES232-2. **Cluster C:** 8. ES013-6, 9. ES013-10, **Cluster D:** 1. KD43W.

DISCUSSION

This study represents the inaugural exploration into the genetic structure of *D. cinerascens*, the primary agent responsible for fig tree canker disease in Iran. This pathogen was initially reported in Italy (Ogawa & English, 1990). Despite its prevalence in Iran, particularly in the Fars Province (Bolboli et al. 2023), it has also made a significant impact on other major fig-producing regions including California (Ferguson et al. 1990), Canada (Hampson, 1981), and Bulgaria (Gomes et al. 2013). The screened isolates were intentionally selected to encompass the diversity of their host cultivars and aggressiveness. However, our investigation revealed low genotypic diversity among *D. cinerascens* isolates from southern Iran. This high degree of similarity was further corroborated by the results of a phylogenetic study, which utilized nuclear genes (ITS, *his*, and *tub2*) sequences from representative isolates recovered across different cultivars and regions, alongside their morphological characterization (Bolboli et al. 2022). Despite extensive investigations, no sexual stage of *D. cinerascens* has been observed under either natural or laboratory conditions (Banihashemi & Javadi, 2009; Bolboli et al. 2022). The significant genetic similarity within the *D. cinerascens* population is likely due to the absence of teleomorphs, which have not been observed either naturally or artificially in the pathogen. This absence may lead to a consequent uniformity in the composition of mating types. Consequently, clonal reproduction assumes paramount importance, leading to the gradual erosion of genetic diversity in the *D. cinerascens* population.

Diaporthe cinerascens initiate epidemics each season just via the asexual stage (Banihashemi & Javadi, 2009). The production of conidia within mucilaginous exudate from pycnidium contributes to dispersal via rain, irrigation splash, and pruning tools, but primarily over short distances or at the plant level (Koenick et al. 2019). In contrast, wind-borne ascospores play a crucial role in longer-distance aerial dispersal in other *Diaporthe* species (Burt et al. 1998; Rieux et al. 2014). This phenomenon may explain the limited distribution of fig Diaporthe canker disease in Iran and other fig plantation regions worldwide. However, further molecular investigations, such as employing a MAT-specific PCR assay in conjunction with microsatellites, could provide valuable insights into the sexual reproductive strategies of the pathogen. Similar approaches have been successfully applied to study other pathogenic species of this genus, like *D. citri* (H.S. Fawc.) F.A. Wolfi, in Japan, *D. longicolla* (Hobbs) J.M. Santos, Vrandečić & A.J.L. Phillips, *D. unshiuensi* F. Huang, K.D. Hyde & Hong Y. Li, and *D. ueckerae* Udayanga & Castl. in Arkansas (Al Shuwaili et al. 2020; Xiong et al. 2021)

Upon comparing the discriminatory power of six tested ISSR markers, using ten parameters, it was found that marker M1 displayed the most significant discriminatory ability. In this study, M1 is highlighted as the optimal selection for examining the genetic variation within the scrutinized group of isolates, relative to other ISSR markers in use. Jaccard's similarity coefficients, which measure the genetic similarity between isolates, ranged from 79% to 100%. Interestingly, our findings align with a previous study by Carlier et al. (2009) on *D. amygdali*, the causal agent of almond Diaporthe canker disease. They reported a high similarity of 88% among *D. amygdali* isolates, emphasizing the conservation of genetic traits within this pathogen. PCoA results corroborated these findings, mirroring the outcomes of a separate study by Bolboli et al. (2022a) that employed Principal Component Analysis (PCA) based on aggressiveness data. Notably, the most aggressive isolate, KDB43W, formed a distinct single-member group in both approaches.

In the present study, utilizing ISSR fingerprinting, we have provided novel genetic evidence regarding the clonal structure of the fig Diaporthe canker-causing pathogen, *D. cinerascens*. These findings are crucial for understanding disease epidemiology across various phases of the disease cycle. The observed lack of a sexual stage and the limited distribution of the pathogen suggest that Diaporthe canker management can be achieved through straightforward practices such as disinfecting pruning tools and preventing the transfer of contaminated plant materials between fig orchards. Given the low genetic diversity of *D. cinerascens* and the lack of evidence supporting sexual reproduction, this pathogen has been classified as a low-risk pathogen (McDonald & Lind, 2002). This classification is indicative of its relatively limited potential for rapid evolution and more durability of recently introduced non-susceptible cultivars, such as 'Matti' (Bolboli et al. 2022a), in managing Diaporthe canker disease. However, several important questions remain unanswered, including the genetic structure of *D. cinerascens* populations in other regions worldwide, the distribution of mating-type loci, and the genetic basis underlying variable aggressiveness among *D. cinerascens* isolates from fig tree. Future investigations could focus on advanced molecular techniques, such as identifying single nucleotide polymorphisms (SNPs) via genotyping by sequencing (GBS) and comparative genomics approaches.

CRediT authorship contribution statement

Conceptualization, Zeinab Bolboli (Z.B.) and Reza Mostowfizadeh-Ghalamfarsa (R.M.-G); methodology, Z.B. and R.M.-G; software, Z.B; validation, Z.B. and R.M.-G, and Hossein Masigol (H.M.); formal analysis, Z.B.; investigation, Z.B.; resources, R.M.-G; data curation, Z.B.; writing—original draft preparation, Z.B. and R.M.-G; writing—review and editing, Z.B., R.M.-G. and H.M.; visualization, Z.B.; supervision, R.M.-G.; project administration, R.M.-G.; funding acquisition, Z.B. and R.M.-G. All authors have read and agreed to the published version of the manuscript.

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ارزیابی تنوع ژنتیکی *Diaporthe cinerascens* عامل بیماری شانکر درخت انجیر با استفاده از نشانگرهای ISSR

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چکیده: بیمارگر *Diaporthe cinerasce* عامل مهم و غالب بیماری شانکر در مناطق مهم کشت درختان انجیر در ایران و همچنین برخی از کشورهای تولیدکننده انجیر مانند ایالات متحده آمریکا (کالیفرنیا)، کانادا، ایتالیا و بلغارستان است. در مطالعه‌ی حاضر از شش نشانگر نواحی بین توالی‌های تکراری ساده (آی‌اِس‌اِس‌آر)، برای بررسی تنوع ژنتیکی ۳۵ جدایه‌ی *D. cinerascens* منتخب بر اساس تنوع در ارقام انجیر میزبان، میزان تازندگی و ویژگی‌های ریخت‌شناختی، استفاده شد. قدرت تمایز هر نشانگر با استفاده از ۱۰ شاخص نشانگری شامل، نسبت چندگانه‌ی موثر، چندشکلی مورد انتظار، چندشکلی مشاهده شده، محتوای اطلاعات چندشکلی، شاخص نشانگری، قدرت تفکیک، شاخص تنوع نی، شاخص شانون، درصد چندشکلی و تعداد آلل‌های چندشکلی مورد ارزیابی قرار گرفت. بر اساس نتایج این واکاوی، نشانگر M1، به عنوان بهترین نشانگر، برای ردیابی تنوع در میان جدایه‌های *D. cinerascens* شناسایی شد. میزان بالای ضرایب تشابه جاکارد با دامنه‌ی تغییر ۷۹ تا ۱۰۰ درصد، نشان‌دهنده‌ی میزان بالایی از شباهت ژنتیکی میان جدایه‌های مورد بررسی بود. همچنین بر اساس نتایج واکاوی تجزیه به مختصات اصلی (PCoA)، جدایه‌های مورد بررسی با وجود میزان بالای ضرایب جاکارد، به چهار خوشه‌ی ژنتیکی مجزا گروه‌بندی شدند. این الگوی گروه‌بندی به میزان جزئی با خاستگاه جغرافیایی جدایه‌ها و نوع رقم انجیر میزبان مرتبط بود. بنابراین در این مطالعه بر اساس انگشت‌نگاری نشانگرهای آی‌اِس‌اِس‌آر، شواهدی مبنی بر وجود ساختار ژنتیکی همسانه‌ای در میان جدایه‌های مورد بررسی به دست آمد. نتایج حاصل از این تحقیق در مطالعات همه‌گیری‌شناختی و همچنین اتخاذ راه‌بردهای مدیریتی پایدار در مهار این بیماری کاربرد خواهد داشت.

کلمات کلیدی: ژنتیک جمعیت، شانکر دیاپورته، نواحی بین توالی‌های تکراری ساده (ISSR)، *Ficus carica*

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