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# Morphological and Molecular Characterisation of *Campylocarpon fasciculare* and *Fusarium* spp., the Cause of Black Disease of Grapevine in Iran

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## ABSTRACT

In 2014, disease symptoms of yellowing, foot rot and drying of leaves were observed in vineyards in Hormozgan province, Iran. The goal of the present study was to characterise fungal isolates associated with black foot of grapevines (*Vitis* spp.) using multi-gene DNA analysis (partial translation elongation factor-1 [*tef1*], internal transcribed spacers [ITS rDNA] and β-tubulin) and pathogenic characteristics of the isolates from the grapevines. Twenty-five isolates were obtained from diseased plants and identified as *Campylocarpon fasciculare* (14), *Fusarium solani* (7) and *F. decemcellulare* (4) through morphological characteristics. The three DNA regions analysed supported the morphological concept. All fungal isolates were evaluated for their pathogenicity on one-year-old rooted grapevine cultivar Askari in the planthouse. Typical root rot symptoms were observed within 90 days after inoculation. *Campylocarpon fasciculare* and an unnamed phylogenetic species of FSSC 20 were reported for the first time for Iranian mycoflora, indicating that grapevine vineyards have become the new host plants for *F. decemcellulare*.

*Keywords*: Grapevine, black disease, fungal species, multi-locus analysis, morphology

## INTRODUCTION

Black disease, a disease affecting grapevines, is one of the most serious diseases to take note if in grape-producing plantations throughout the world. The disease is caused by different species of *Cylindrocarpon*, *Cylindrocladiella*, *Ilyonectria* and *Campylocarpon* (Alaniz et al., 2007; Auger et al., 2007). Black disease is characterised by root hairs, necrotic lesions on the outside of the foot and root reduction in root biomass. Mature grapevines presenting this disease are

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usually weak with small leaves, shortened internode, and uneven wood maturity (Halleen et al., 2006).

Black disease of grapevine was first described in 1961 (Grasso & Magnano, 1975). Grapevine black disease pathogens have been intensively investigated based on comprehensive morphological comparisons and molecular phylogeny analyses of multilocus DNA sequence (Halleen et al., 2004, 2006; Alaniz et al., 2007; Schroers et al., 2008; Lombard et al., 2012). According to previous studies, four distinct genera of *Cylindrocarpon*, *Cylindrocladiella*, *Ilyonectria* and *Campylocarpon* were reported as the causal agents of black disease of grapevine in most grapevine production areas of the world. Moreover, it is well known that these pathogens are distributed in soils and plant debris and usually act as decomposers. Therefore, control of these pathogens is difficult (Auger et al., 2007; Alaniz et al., 2007; Schroers et al., 2008; Lombard et al., 2012). Until now, no grapevine cultivar is sufficiently resistant to black disease (Casieri et al., 2009). Also, chemical control alone is not efficient to eradicate black disease pathogens in vineyards (Agustí-Brisach & Armengol, 2013). In addition, these pathogens have also been associated with different diseases of other economically important hosts, such as common olive (Úrbez-Torres et al., 2012), avocado (Vitale et al., 2012), and Scots pine (Menkis & Burokiene, 2012). Therefore, the correct identification of the causal agents of black disease of grapevine is necessary in order to develop proper management strategies to control pathogenic species (Agustí-Brisach & Armengol, 2013).

Mohammadi et al. (2009) introduced *Cylindrocarpon liriodendri* as the black disease of grapevine in Iran. But, until today, little attempt has been made to classify fungal isolates associated with black disease of grapevines in Iran. Therefore, the objectives of this study were (1) to identify fungal isolates associated with black disease of grapevines (Rishbaba, Askari and Black cultivars) in southern Iran by using morphological characteristics and sequencing of ITS regions, *tef1*and β-tubulin to determine genetic relationship among them, and (2) to determine their pathogenicity in grapevine cultivar, Askari.

## MATERIALS AND METHOD

### Fungal Cultures

A total of 25 fungal isolates were obtained from 21 diseased grapevines (Rishbaba, Askari and Black cultivars) in southern Iran that were showing severe symptoms of early decline (Table 1).

Table 1
*Place of sample collection, Genbank Accession Numbers of fungal species isolated from black foot disease of grapevine vineyards collected from southern Iran*

| No. | Isolate Number | Species Identified | Location in Southern Iran (gps) | | | [a]*tef1* | [a]ITS | [a]*tub2* |
|---|---|---|---|---|---|---|---|---|
| 1 | CSC 1 | *C. fasciculare* | Bandar Abbas | 27º 10' 59' N | 56º 16' 59' E | KT935554 | KT935546 | KT935542 |
| 2 | CSC 2 | *C. fasciculare* | Bandar Abbas | 27º 10' 59' N | 56º 16' 59' E | KT935555 | KT935547 | KT935543 |
| 3 | CSC 3 | *C. fasciculare* | Bandar Abbas | 27º 10' 59' N | 56º 16' 59' E | - | - | - |
| 4 | CSC 4 | *C. fasciculare* | Bandar Abbas | 27º 10' 59' N | 56º 16' 59' E | - | - | - |
| 5 | CSC 5 | *C. fasciculare* | Bandar Abbas | 27º 10' 59' N | 56º 16' 59' E | - | - | - |
| 6 | CSC 6 | *C. fasciculare* | Bandar Abbas | 27º 10' 59' N | 56º 16' 59' E | - | - | - |
| 7 | CSC 7 | *C. fasciculare* | Bashagard | 26º 45 30' N | 55º 46' 48' E | - | - | - |
| 8 | CSC 8 | *C. fasciculare* | Bashagard | 26º 45 30' N | 55º 46' 48' E | - | - | - |
| 9 | CSC 9 | *C. fasciculare* | Bashagard | 26º 45 30' N | 55º 46' 48' E | - | - | - |
| 10 | CSC 10 | *C. fasciculare* | Bashagard | 26º 45 30' N | 55º 46' 48' E | - | - | - |
| 11 | CSC 11 | *C. fasciculare* | Bashagard | 26º 45 30' N | 55º 46' 48' E | - | - | - |
| 12 | CSC 12 | C. fasciculare | Hashtbandi | 27°8'41"N | 57°27'38"E | - | - | - |
| 13 | CSC 13 | *C. fasciculare* | Hashtbandi | 27°8'41"N | 57°27'38"E | - | - | - |
| 14 | CSC 14 | *C. fasciculare* | Hashtbandi | 27°8'41"N | 57°27'38"E | - | - | - |
| 15 | FDSC 15 | *F. decemcellulare* | Bandar Abbas | 27º 10' 59' N | 56º 16' 59' E | KT935560 | KT935548 | KT935544 |
| 16 | FDSC 16 | *F. decemcellulare* | Bashagard | 26º 45 30' N | 55º 46' 48' E | KT935561 | KT935549 | KT935545 |
| 17 | FDSC 17 | *F. decemcellulare* | Hashtbandi | 27°8'41"N | 57°27'38"E | - | - | - |
| 18 | FDSC 18 | *F. decemcellulare* | Hashtbandi | 27°8'41"N | 57°27'38"E | - | - | - |
| 19 | SI-FSSC 20A | FSSC 20 | Bandar Abbas | 27º 10' 59' N | 56º 16' 59' E | KT935556 | KT935550 | - |
| 20 | SI-FSSC 20B | FSSC 20 | Bashagard | 26º 45 30' N | 55º 46' 48' E | KT935557 | KT935551 | - |
| 21 | SI-FSSC 20C | FSSC 20 | Hashtbandi | 27°8'41"N | 57°27'38"E | KT935558 | KT935552 | - |
| 22 | SI-FSSC 20D | FSSC 20 | Hashtbandi | 27°8'41"N | 57°27'38"E | KT935559 | KT935553 | - |
| 23 | SI-FSSC 20E | FSSC 20 | Bandar Abbas | 27º 10' 59' N | 56º 16' 59' E | - | - | - |
| 24 | SI-FSSC 20F | FSSC 20 | Bandar Abbas | 27º 10' 59' N | 56º 16' 59' E | - | - | - |
| 25 | SI-FSSC 20G | FSSC 20 | Bandar Abbas | 27º 10' 59' N | 56º 16' 59' E | - | - | - |

In this research, weak grapevines with small and necrotic leaves, shortened internodes and uneven wood maturity were selected. Infected roots, crown and trunks of diseased grapevines were cut into small disks (1.5 cm). Necrotic wood were surface sterilised with 1% sodium hypochlorite solution for 1 min and then washed twice with sterilised distilled water. The tissues were placed on peptone-pentachloronitrobenzene agar (PPA) and potato dextrose agar (PDA) plates. All the plates were incubated under 12 h alternating light (black/white) at 25±2°C for 1 week and the resulting fungal colonies were transferred to fresh PDA plates and then purified using the single spore isolation technique.

The species were identified on the basis of macroscopic characteristics such as pigmentations and growth rate of the colony on PDA plates, as well as their microscopic features including shape and size of macroconidia, presence of microconidia and chlamydospores. For microscopic observations, all isolates were transferred to carnation leaf agar (CLA) (Fisher et al., 1982) medium. Thirty randomly selected macroconidia and microconidia were measured and analysed by 2-Sample T-Test by using MINITAB® 15. Identification to the species level was based on the descriptions of Halleen et al. (2004) for *Campylocarpon* species, and Leslie and Summerell (2006), Nalim et al. (2011) and Short et al. (2013) for *Fusarium* species.

**DNA Isolation, Sequencing and Phylogenetic Analyses**

Based on morphological identification, fungal mycelium of selected isolates was grown on PDA with sterile dialysis membranes (Lui et al., 2000) for one week and freeze-dried, and then the DNeasy® Plant Mini Kit (Qiagen) was used to extract total genomic DNA. Portions of the translation elongation factor-1**α** [*tef1*], internal transcribed spacers [ITS rDNA] and β-tubulin genes were amplified by PCR as described previously (Chehri, 2015; Chehri et al., 2015). PCR was performed in a Peltier Thermal Cycler, PTC-100® (MJ Research, Inc. USA). All PCR and sequencing primers used in this study are listed in Table 2. DNA amplification of the *tef1* was performed using the following programme: one cycle of 60 s at 94°C followed by 35 cycles of 30 s at 95°C, 55 s at 59°C, 90 s at 72°C, and a final extension of 10 min at 72°C. The PCR for ITS region was performed at 95°C (one cycle of 120 s) for a hot start, followed by 35 cycles of 60 s at 94°C, 30 s at 56°C, 120 s at 72°C, and a final extension of 72 °C (10 min). The PCR for β-tubulin gene was performed at 94°C (1 cycle of 60 s), followed by 39 cycles of 30 s at 94°C, 30 s at 58°C and 1 min at 72°C, and a final extension of 5 min at 72°C. PCR products were purified using Qiagen columns (QIAquick PCR Purification Kit (250)) according to the manufacturer's protocol and sent for sequencing to a service provider. The maximum parsimony (MP) method, using MEGA4.0 program (Molecular Evolutionary Genetic Analysis software, ver. 4.0; http://www.megasoftware.net) (Tamura et al., 2007), was used to assess the phylogenetic diversity of all three-locus sequences (*tef1*, ITS and β-tubulin) of selected isolates included in the present study. Bootstrap values for the maximum parsimony tree were calculated for 1000 replicates. In order to assess the relationships between the major taxa, ambiguous parts of the *tef1*, ITS and β-tubulin were removed from further analysis and more conserved and alignable parts of the region and gene were used to generate phylogenetic trees containing representative taxa from major groups. The edited *tef1* and ITS (CSC 1,

CSC 2, FDSC 15, FDSC 16, SI-FSSC 20A, SI-FSSC 20B, SI-FSSC 20C, SI-FSSC 20D) and β-tubulin (CSC 1, CSC 2, FDSC 15, FDSC 16) sequences were compared with other available fungal species sequences in the GenBank.

Table 2
*Primer sequences used for PCR amplification*

| | | |
|---|---|---|
| ITS1 | 5'-TCCGTAGGTGAACCTGCGG-3' | White et al. (1990) |
| ITS4 | 5'-TCCTCCGCTTATTGATATGC-3' | White et al. (1990) |
| EF1 | 5'-ATGGGTAAGGAGGACAAGAC-3' | O'Donnell et al. (1998) |
| EF2 | 5'-GGAAGTACCAGTGATCATGTT-3' | O'Donnell et al. (1998) |
| T1 | 5'-AACATGCGTGAGATTGTAAGT-3' | O'Donnell and Cigelnik (1997) |
| T2 | 5'-TAGTGACCCTTGGCCCAGTTG-3' | O'Donnell and Cigelnik (1997) |

### Pathogenicity Tests

All fungal isolates were used for pathogenicity assays on one-year-old rooted grapevine. The healthy grapevine cultivar, Askari, was used in this experiment. The experiments were carried out in greenhouse conditions maintained at 22 to 28°C, 60-70% RH. The experiments were arranged in a completely randomised design with three replications. The plants were inoculated by dipping the roots in a $1 \times 10^6$ conidial suspension for 60 min (Cabral et al., 2012). The control plants were dipped in sterile water. Inoculated plants were planted individually in pots containing sterilised soil:peat moss:vermiculite mixture (2:1:1) and placed in a glasshouse at 22 to 28°C for 90 days. Thirty days after the beginning of the pathogenicity test, the plants were re-inoculated using 50 mL conidial suspension including $10^6$ conidia per plant (Alaniz et al*.,* 2007). Starting 90 days after inoculation, the plants were immediately transferred to a mycology laboratory. The roots were washed under running tap water to eliminate soil and debris. Root symptoms of plants were evaluated on the following scale: 0 = healthy, with no lesions; and 1 = discolouration, 1 = discolouration with lesions.

## RESULTS

### Morpho-Cultural Characteristics

A total of 25 fungal isolates were obtained from two- to four-year old grapevine vineyards located in southern Iran that showed severe symptoms of black disease including necrosis, brown and black streaking on the outside of the foot and roots. Based on their morphological characteristics, all the isolates belonged to *Campylocarpon fasciculare* (14), *F. decemcellulare* (4) and *F. solani* (7). Morphological characteristics including means and ranges of spore dimensions of all the fungal species are summarised in Table 3.

Table 3
*Morphological characteristics of selected fungal species isolated from black foot disease of grapevine vineyards collected from southern Iran*

| Culture No. | Species Identified | Chlamydospores | Types of Conidigenious Cells | Sporodochia Colour | Shape of Basal Cell and Apical Cell | Length x Width of Macroconidia (µm)[a] | |
|---|---|---|---|---|---|---|---|
| | | | | | | 3- and 4-septate | 5-septate |
| CSC 1 | *C. fasciculare* | + | Monophialidic | Light brown | Rounded, tapered and curved | 40.5 ± 1.5 × 5.2 ± 0.2 | 44.5 ± 2.5 × 5.2 ± 0.2 |
| CSC 2 | *C. fasciculare* | + | Monophialidic | Light brown | Rounded, tapered and curved | 44.5 ± 2.5 × 4.0 ± 0.5 | 48.5 ± 2.5 × 5.4 ± 0.2 |
| CSC 3 | *C. fasciculare* | + | Monophialidic | White | Rounded, tapered and curved | 43.5 ± 1.5 × 5.3 ± 0.2 | 47.5 ± 2.5 × 5.3 ± 0.2 |
| CSC 4 | *C. fasciculare* | + | Monophialidic | Light brown | Rounded, tapered and curved | 42.5 ± 1.5 × 5.3 ± 0.2 | 45.5 ± 2.5 × 5.3 ± 0.2 |
| CSC 5 | *C. fasciculare* | + | Monophialidic | White | Rounded, tapered and curved | 40.5 ± 1.5 × 5.3 ± 0.2 | 44.5 ± 2.5 × 5.1 ± 0.2 |
| CSC 6 | *C. fasciculare* | + | Monophialidic | Light brown | Rounded, tapered and curved | 43.5 ± 1.5 × 5.3 ± 0.2 | 47.5 ± 2.5 × 5.3 ± 0.2 |
| CSC 7 | *C. fasciculare* | + | Monophialidic | Light brown | Rounded, tapered and curved | 43.5 ± 1.5 × 5.3 ± 0.2 | 47.5 ± 2.5 × 5.3 ± 0.2 |
| CSC 8 | *C. fasciculare* | + | Monophialidic | Light brown | Rounded, tapered and curved | 42.5 ± 1.5 × 5.3 ± 0.2 | 46.5 ± 2.5 × 5.3 ± 0.2 |
| CSC 9 | *C. fasciculare* | + | Monophialidic | Light brown | Rounded, tapered and curved | 39.5 ± 1.5 × 5.3 ± 0.2 | 45.5 ± 2.5 × 5.2 ± 0.2 |
| CSC 10 | *C. fasciculare* | + | Monophialidic | Light brown | Rounded, tapered and curved | 44.5 ± 1.5 × 5.3 ± 0.2 | 48.5 ± 2.5 × 5.3 ± 0.2 |
| CSC 11 | *C. fasciculare* | + | Monophialidic | Light brown | Rounded, tapered and curved | 43.5 ± 1.5 × 5.3 ± 0.2 | 47.5 ± 2.5 × 5.3 ± 0.2 |
| CSC 12 | *C. fasciculare* | + | Monophialidic | White | Rounded, tapered and curved | 41.5 ± 1.5 × 5.3 ± 0.2 | 46.5 ± 2.5 × 5.3 ± 0.2 |
| CSC 13 | *C. fasciculare* | + | Monophialidic | Light brown | Rounded, tapered and curved | 43.5 ± 1.5 × 5.3 ± 0.2 | 47.5 ± 2.5 × 5.3 ± 0.2 |
| CSC 14 | *C. fasciculare* | + | Monophialidic | Light brown | Rounded, tapered and curved | 44.5 ± 1.5 × 5.3 ± 0.2 | 48.5 ± 2.5 × 5.3 ± 0.2 |
| FDSC 15 | *F. decemcellulare* | - | Monophialidic | Yellow | Foot shaped, blunt | - | 61.5 ± 2.5 × 5.6 ± 0.2 |
| FDSC 16 | *F. decemcellulare* | - | Monophialidic | Yellow | Foot shaped, blunt | - | 65.5 ± 2.5 × 5.6 ± 0.2 |
| FDSC 17 | *F. decemcellulare* | - | Monophialidic | Yellow | Foot shaped, blunt | - | 66.5 ± 2.5 × 5.7 ± 0.2 |
| FDSC 17 | *F. decemcellulare* | - | Monophialidic | Yellow | Foot shaped, blunt | - | 66.5 ± 2.5 × 5.7 ± 0.2 |
| SI-FSSC 20A | FSSC 20 | + | Monophialidic | Cream | Foot cell and tapered, curved | 47.5 ± 2.5 × 5.8 ± 0.5 | 52.5 ± 2.5 × 5.6 ± 0.5 |
| SI-FSSC 20B | FSSC 20 | + | Monophialidic | Cream | Foot cell and tapered, curved | 49.2 ± 2.5 × 5.8 ± 0.5 | 54.5 ± 2.5 × 6 ± 0.5 |
| SI-FSSC 20C | FSSC 20 | + | Monophialidic | Cream | Foot cell and tapered, curved | 51.2 ± 2.5 × 5.8 ± 0.5 | 52.5 ± 2.5 × 6 ± 0.5 |
| SI-FSSC 20D | FSSC 20 | + | Monophialidic | Cream | Foot cell and tapered, curved | 50.2 ± 2.5 × 5.8 ± 0.5 | 51.5 ± 2.5 × 6 ± 0.5 |
| SI-FSSC 20E | FSSC 20 | + | Monophialidic | Cream | Foot cell and tapered, curved | 49.2 ± 2.5 × 5.8 ± 0.5 | 51.5 ± 2.5 × 6 ± 0.5 |
| SI-FSSC 20F | FSSC 20 | + | Monophialidic | Cream | Foot cell and tapered, curved | 48.2 ± 2.5 × 5.8 ± 0.5 | 50.5 ± 2.5 × 6 ± 0.5 |
| SI-FSSC 20G | FSSC 20 | + | Monophialidic | Cream | Foot cell and tapered, curved | 51.2 ± 2.5 × 5.8 ± 0.5 | 52.5 ± 2.5 × 6 ± 0.5 |

[a]Mean values of 30 random conidia ± standard deviation

All the studied isolates of *C. fasciculare* produced a cottony surface texture of colonies. The cultures grew slowly, and the growth rate on PDA at 25°C in intermittent light ranged from 4.3 to 4.7 mm/day. All the isolates formed white to light brown sporodochia on the surface of the leaves. Macroconidia arising from sporodochia were straight or slightly curved, with 3-5-septate and mostly 3-septate, tapered and curved apical cells and barely notched or rounded basal cells (Figure 1). All the *C. fasciculare* isolates produced branched conidiophores.

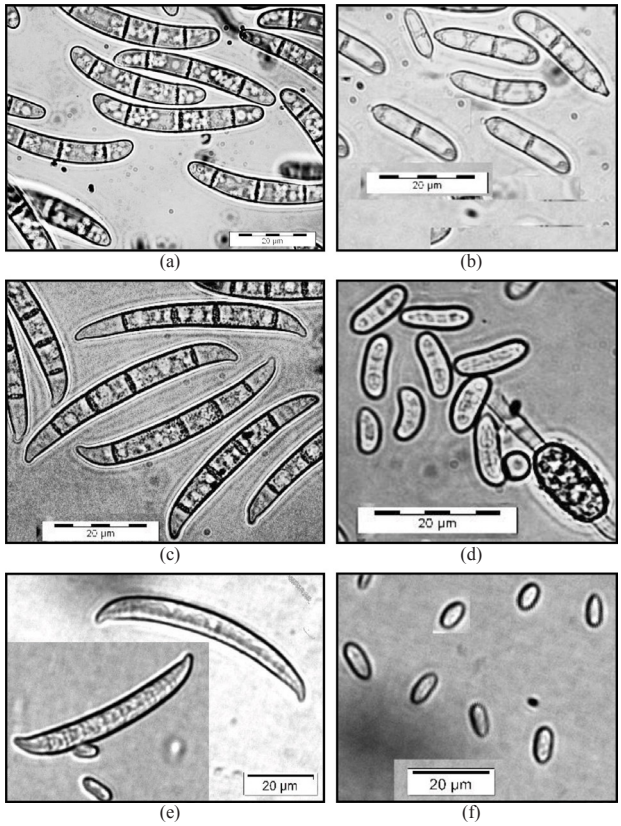


*Figure 1.* (a) and (b) Campylocarpon fasciculare, (a) macroconidia produced from sporodochia and (b) mesoconidia; (c) and (d) Fusarium solani species complex (FSSC 20), (c) macroconidia produced from sporodochia and (d) microconidia formed on conidiophores in hyphae; and (e) and (f) F. decemcellulare, (e) macroconidia produced from sporodochia and (f) microconidia formed on conidiophores in hyphae. Bar = 20 µm for all pictures

All studied isolates of *F. decemcellulare* produced a cottony surface texture of colonies. The cultures grew slowly, and the growth rate on PDA at 25°C in intermittent light ranged from 3.2 to 3.8 mm/day. All the isolates formed white to yellow sporodochia on the surface of the leaves. Macroconidia arising from sporodochia were very long and thick-walled relative to many other species with even curvature on both sides

of the macroconidia, 5-9-septate with rounded apical cells and foot-shaped basal cells (Figure 1). All the *F. decemcellulare* isolates produced branched monophialidic conidiophores. Oval microconidia were observed in long chains produced from monophialides in branched conidiophores.

All the studied isolates of *F. solani* produced a cottony surface texture of colonies. The cultures grew fast, and the growth rate on PDA at 25°C in intermittent light ranged from 7.8 to 8.6 mm/day. The macroconidia arising from sporodochia were typically falcate and mostly 5-septate with papillate, tapered and curved apical cells and well developed foot cells (Figure 1). Microconidia reniform, elongated oval to sometimes obovoid with a truncate base, were formed in false heads on long monophialides. All the *F. solani* isolates produced chlamydospores.

**Molecular Characterisation**

Multilocus DNA sequence data were used to assess the phylogenetic relationships and species identification of fungal isolates obtained from diseased grapevines in southern Iran. Based on morphological characteristics, eight isolates were selected for molecular studies (Tables 1 and 3). The aligned partial nuclear ITS regions, *tef1* and β-tubulin gene partitions consisted of 450, 500 and 450 characters, respectively, totalling 1400 bp of aligned DNA sequence per isolates. The edited *tef1*, ITS and β-tubulin sequences were compared with other available fungal species sequences in the GenBank. From similarities searched at the NCBI database, identification of all fungal species was confirmed with statistical significance. Also, this was confirmed by a phylogenetic analysis of the combined dataset (Figure 2). The edited *tef1* and ITS datasets were combined and analysed phylogenetically using MEGA4.0 software for all identified fungal species (Tamura et al., 2007). However, due to highly divergent paralogs, it was thought that the use of β-tubulin gene sequences for phylogeny reconstruction within the members of FSSC could be problematic. So, the individual β-tubulin dataset was used for phylogeny reconstruction for the species of *C. fasciculare* and *F. decemcellulare*.

The phylogenetic trees generated from the combined ITS regions and *tef1* sequences and the individual β-tubulin dataset revealed a monophyly among two isolates (CSC 1 and CSC 2) and *C. fasciculare* (CBS 112613) with strongly supported relationship (Figures 2 and 3). The trees also showed two isolates of FDSC 15 and FDSC 16 with strong bootstrap support placed in distinct lineage of *F. decemcellulare* (Figures 2 and 3). The phylogenetic tree generated from the combined ITS regions and *tef1* dataset showed a monophyly between FSSC 20-c (NRRL 32316) and FSSC 20-a (NRRL 22608) and isolates SI-FSSC 20A, SI-FSSC 20B, SI-FSSC 20C and SI-FSSC 20D (92% MP).

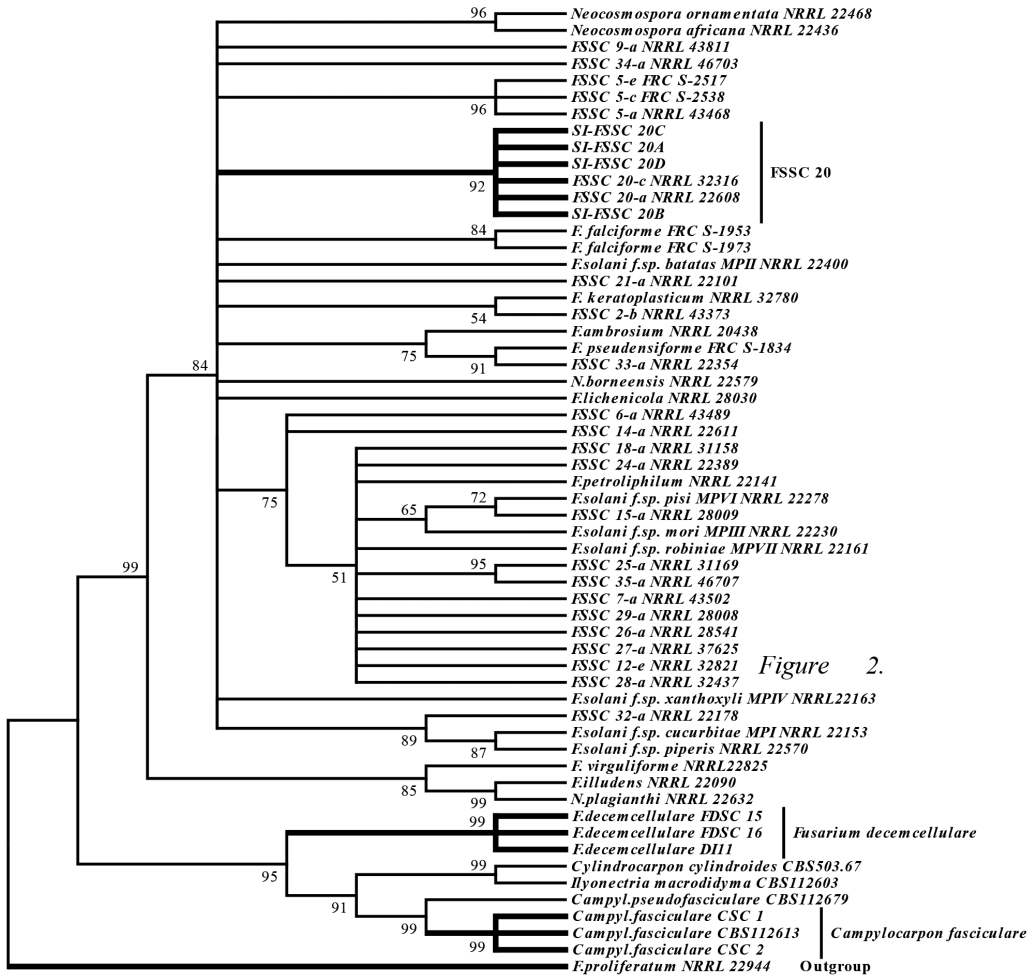


*Figure 2*. A maximum parsimony phylogeny for 79 taxa of the fungal species inferred from combined ITS and *tef1* sequences. Bootstrap tests were performed with 1000 replications. *Fusarium proliferatum* (NRRL 22944) obtained from GenBank was treated as the outgroup.

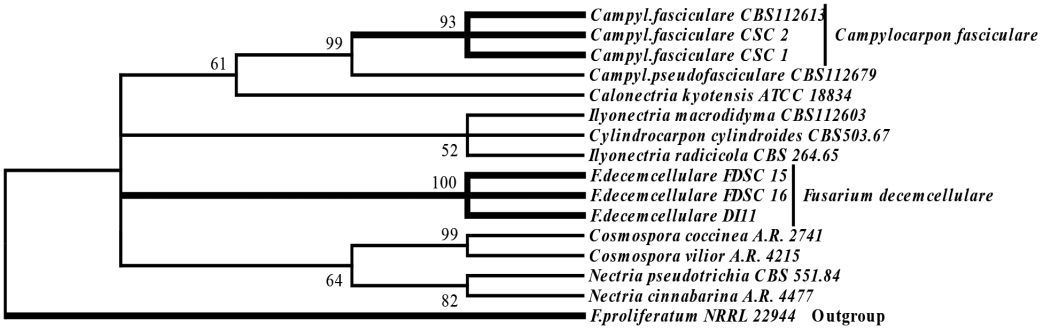


*Figure 3*. A maximum parsimony phylogeny for eight taxa of the fungal species inferred from partial sequence of β-tubulin gene. Bootstrap tests were performed with 1000 replications. *Fusarium proliferatum* (NRRL 22944) obtained from GenBank was treated as the outgroup.

[a]GenBank numbers for *tef1* and *tub2* genes sequences

**Pathogenicity Test**

In this experiment, 25 isolates were used for pathogenicity assays on one-year-old rooted grapevine cultivar, Askari. None of the control plants died. Of the 11 isolates belonged to *F. solani* (7) and *F. decemcellulare* (4), isolate SI-FSSC 20D and FDSC 16 showed small lesions on grapevine roots that are considered hypovirulent to grapevine plants. The results of the pathogenicity test also showed that isolates CSC 1, CSC 2 and CSC 13 belong to *C. fasciculare*; the isolates showed external symptoms (long lesions) on the grapevine roots and were considered a virulent group. Re-isolations on PDA medium were attempted for all the isolates.

**DISCUSSION**

The *Campylocarpon* species are considered the causal agents of black disease of grapevine in different regions of the world (Santos et al., 2014). From the genus *Campylocarpon*, two species were included as the causal agents of black disease in this study i.e. *C. pseudofasciculare*, which has been reported in South Africa (Halleen et al., 2004), Uruguay (Abreo et al., 2010), Brazil (Santos et al., 2014; Correia et al., 2012) and Perú (Álvarez et al., 2012) and *C. fasciculare*, which has been reported in Spain (Alaniz et al., 2011), Turkey (Akgül et al., 2014) and South Africa (Halleen et al., 2004); all the studies were in agreement regarding the result. *Phaeoacremonium (Pm.), Pm. parasiticum*, *Pm. inflatipes*, *Pm. cinereum, Pm. aleophilum, Phaeomoniella chlamydospore, Cylindrocarpon liriodendri*, *Diplodia seriata* and *Neofusicoccum parvum* have been identified to be associated with grapevines, but from other locations in Iran (Mohammadi et al., 2009, 2013). This is the first study reporting on the presence of *C. fasciculare* and *F. decemcellulare* and an unnamed phylogenetic species of FSSC 20 in vineyards in southern Iran.

Phylogenetic studies are very useful in differentiating closely related strains, and they allow clear separation of some morphologically similar species (Leslie & Summerell, 2006; Chaverri et al., 2011; Lombard et al., 2012). Therefore, in this research, in order to define fungal taxa, the combination of different methods namely, morphological and phylogenetic studies based on combined ITS regions and *tef1* datasets, are consistently applied and they support each other. It seems that based on the β-tubulin dataset, highly divergent paralogs were discovered within members of FSSC (O'Donnell, 2000). So, molecular phylogeny based on the β-tubulin dataset was used for phylogeny reconstruction for the species of *C. fasciculare* and *F. decemcellulare*.

*Fusarium decemcellulare* is usually found in tropical and sub-tropical regions, and has been consistently associated with branch canker and die back of a range of tropical fruit trees (Ploetz et al., 1996). It seems that weather conditions and climate and types of agricultural crop in southern Iran similar to those in subtropical countries

make correct identification of this species very important for management and control of tropical fruit tree diseases in Iran.

Molecular phylogeny demonstrated that *F. solani* isolates in this research formed a monophyletic group with typical strains, FSSC 20-c (NRRL 32316) and FSSC 20-a (NRRL 22608), obtained from GenBank. These strains undoubtedly represent a new species within Clade 3. However, this species should be further studied (in terms of ecological and biological aspects) before its taxonomic status can be drawn.

## CONCLUSION

The present study will serve as a basis for future studies of the epidemiology of black disease of grapevine in southern Iran and possibilities for effective management of black foot in Iranian vineyards.

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