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Occurrence and Diversity of Black-Foot Pathogens on Asymptomatic Nursery-Produced Grapevines in Turkiye

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Abstract

Black-foot (BF) disease of grapevines in nurseries and young vineyards is caused by soil-borne *Cylindrocarpon*-like asexual morphs. They can be found both in symptomatic and asymptomatic vines, being spread to new grape growing areas during vineyard establishment. In this study, 42 grapevine nurseries located in different geographical regions in Turkiye were surveyed in 2021 to determine the presence of BF pathogens on asymptomatic marketable plants. Black-foot fungi were isolated from the roots or basal ends of asymptomatic dormant vines in 39 of them (92.9%). The percentage of isolation of BF pathogens ranged from 1.4 to 51.4% (average 18.4%). Seven species: *Cylindrodendrum alicantinum, Cylindrocladiella peruviana, Dactylonectria macrodidyma, D. novozelandica, D. torresensis, Ilyonectria liriodendri*, and *I.*

robusta were identified based on DNA sequencing of histone H3 gene and phylogenetic analyses, *D. torresensis* being the most frequent. From these species *Ca. peruviana*, *D. novozelandica* and *I. robusta* were detected for the first time on grapevines in Turkiye. Pathogenicity tests on 1103P rootstock cuttings revealed that all species significantly decreased root biomass and increased root disease severity index, when compared with the non-inoculated control, *D. novozelandica* being the most virulent. Pathogenicity of *Cm. alicantinum* to grapevine was confirmed for the first time, thus this species should be included as causal agent of BF of grapevines. These findings point out that BF pathogens are highly prevalent in asymptomatic nursery produced grapevines and could represent a serious threat for Turkish viticulture.

Key words: *Cylindrocladiella, Cylindrodendrum, Dactylonectria, Ilyonectria,* Trunk pathogens, *Vitis vinifera*

Introduction

Black-foot (BF) is one of the most important trunk diseases of grapevine throughout the main grape producing countries worldwide (Agustí-Brisach & Armengol, 2013; Gramaje et al., 2018). This disease, which was first detected in 1961 in Sicily (Grasso & Magnano Di San Lio, 1975), is a common problem in nurseries causing root and crown rot, reduced root biomass and root hairs, poor plant development and plant death. Black-foot can also affect 2-5-year-old young vines after transplanting in commercial vineyards, with the following symptoms: reduced plant vigor, chlorotic small leaves, internal blackish wood discoloration of the rootstock and a general decline leading to plant death (Gramaje & Armengol, 2011; Reis et al., 2013; Gramaje et al., 2018).

Causal agents of BF are soil-borne fungi belonging to the genera *Campylocarpon* (*Cn.*), *Cylindrocladiella* (*Ca.*), *Dactylonectria, Ilyonectria, Neonectria, Pleiocarpon* and *Thelonectria* (Carlucci et al., 2017; Gramaje et al., 2018; Aigoun-Mouhous et al., 2019). These pathogens can produce abundant conidia and be present in nursery soils as mycelia in decaying plant tissues or

chlamydospores. Black-foot pathogens usually infect plants at the grapevine propagation process during hydration of cuttings and grafting operations or throughout bare-roots and cutting wounds in field conditions (Agustí-Brisach & Armengol, 2013).

Black-foot pathogens have been reported from different grapevine growing regions including Europe (Maluta & Larignon, 1991; Fischer & Kessemeyer, 2003; Alaniz et al., 2007; Cabral et al., 2012), America (Gatica et al., 2001; Abreo et al., 2010; Petit et al., 2011), South Africa (Halleen et al., 2004), Australia and New Zealand (Whitelaw-Weckert et al., 2007; Jones et al., 2012), China (Ye et al., 2021), Iran (Mohammadi et al., 2009) and Algeria (Aigoun-Mouhous et al., 2019).

To date, seven fungal species have been reported in Turkiye associated with BF of symptomatic grapevines, both in nurseries and vineyards. *Ilyonectria macrodidyma* was the first species reported from roots of 4-15 years-old vines exhibiting black foot symptoms in Ankara province (Özben et al., 2012). After that, Akgül et al., (2014) isolated *Cn. fasciculare* from grapevines (1103 Paulsen / Sultana Seedless) in a nursery located in Manisa. *Ilyonectria liriodendri* was found on young grapevines (Chardonnay / 110R) showing decline symptoms in Tekirdağ province with 27.3% disease incidence and 24.3% isolation rate (Savaş et al., 2015). Four years later, Özben et al., (2019) detected *Ca. parva* from rootstocks of symptomatic grapevines in Ankara and these authors proved its pathogenicity on Sultana Seedless plants in greenhouse conditions. More recently, *D. alcacerensis* and *D. torresensis* species were isolated from the roots of 2-3-year-old young vines (SO4 / Chardonnay and 1103 Paulsen / Victoria) in Tekirdağ and Mersin provinces, showing disease incidence rates of 2.5% and 3.0% in the surveyed vineyards, respectively (Savaş et al., 2020). *Cylindrodendrum (Cm.) alicantinum* was the last BF pathogen reported on grapevines in Turkiye. Özben (2020) detected this species from symptomatic nursery plants in Ankara, Bursa and Manisa provinces with 2.5%, 10.9% and 7.0% isolation rates respectively.

It is important to mention that BF pathogens can be present on asymptomatic grapevine plants as latent fungi. This is epidemiologically relevant, because they can potentially be transported to uncontaminated areas while establishing a new vineyard, contributing to the dissemination of BF pathogens (Langenhoven et al., 2018; Berlanas et al., 2020). Thus, screening of asymptomatic vines for the presence of BF pathogens and determining the species composition is very important in terms of disease management practices that should be applied during the nursery propagation process: for instance: hot-water treatments and fungicide or biological control agent application. However, to date no detailed study has been conducted in grapevine nurseries in Turkiye aiming at screening of BF pathogens in ready to go grafted and non-grafted grapevines.

Thus, the main goals of this study were to: (i) evaluate the presence of BF pathogens on asymptomatic ready to go plants produced in Turkish grapevine nurseries, (ii) identify the species composition based on molecular characterization and (iii) determine the virulence of selected isolates representative of the different species found through pathogenicity tests on dormant grapevine cuttings.

Materials and methods

Survey and isolation of black foot pathogens

In this study, 42 grapevine nurseries (located in different geographical regions of Turkiye) were surveyed (Fig. 1; Table 1). Five to ten dormant, externally asymptomatic bare-rooted and marketable plants (both grafted and non-grafted), from commonly grown cultivars were randomly taken in each nursery in January 2021. Fungal isolations were made from asymptomatic tissues of the endorhizosphere root and internal parts of rootstock, as described by Berlanas et al., (2020). For superficial disinfection, tissues were kept in sodium hypochlorite (>5% active chlorine) solution (diluted with sterile distilled water, in 1:1 v/v) for three minutes, then they were rinsed twice with sterile distilled water. After a brief blotting, the tissues were cut

in 3-5 mm-long fragments and placed (seven fragments per plate) onto PDA (Potato Dextrose Agar, Conda Lab., Spain) amended with streptomycin-sulphate (250 $mg \cdot L^{-1}$). Petri plates (10 plates per nursery, each with seven tissue fragments) were incubated at 25°C, for 10 days, in darkness. Fungal colonies resembling BF fungi were sub-cultured to PDA and further incubated for 15 days at 25°C in darkness. These colonies were confirmed as BF species based on morphological and microscopic examination (Cabral et al., 2012; Agustí-Brisach & Armengol, 2013). Then, total isolation frequency (%) of BF fungi in each nursery was calculated as a proportion of the total of 70 wood fragments plated. Single spore cultures from the sub-cultured colonies were obtained in order to provide genetic purity of isolates for further studies.

Molecular identification and phylogenetic analyses

Fifty-one obtained isolates were used for molecular identification (Table 1). These isolates were grown on PDA (at 25°C for 15 days) and aerial mycelia (approximately 50 mg) were harvested for DNA extraction. Fungal DNA was extracted following 2% CTAB protocol (O'Donnell et al., 1998) and genomic DNA was diluted with 50 µl of PCR grade water, then maintained at -20°C for further use. Part of histone H3 gene was amplified with PCR reactions using CYLH3F-CYLH3R primers (Crous et al., 2004). PCR reaction mixture contained 5 µl of buffer (10X Green Buffer, DreamTaq Green DNA Polymerase, Thermo Scientific, Massachusetts, USA), 2 µl of dNTPs mixture (10 mM each, Thermo Scientific, Massachusetts, USA), 1 µl of forward and reverse primers (stock concentration:10 $pmol \cdot \mu l^{-1}$), 0.25 µl of Taq polymerase (DreamTaq Green DNA Polymerase, Thermo Scientific), 39.75 µl PCR grade water and 1 µl genomic DNA (approx. 100 $ng \cdot \mu l^{-1}$). PCR amplifications were performed in Simpli-Amp A24811™ Thermal Cycler, Applied Biosystems, (USA) with the following conditions; 95°C for 3 min. (initial denaturation), followed by 35 cycles each of denaturation at 95°C for 1 min, annealing at 53°C for 1 min. and extension at 72°C for 1 min and final extension at 72°C for 10 min. PCR products were sequenced by Macrogen Co. (South Korea). Sequences were

edited to resolve ambiguities with Sequencher software version 5.0 (Gene Codes Corporation Ann Arbor, MI, USA) and were compared to those deposited in the NCBI GenBank database using the BLASTn software. The sequences generated in this study were deposited in NCBI GenBank (Table 1).

Based on the blast search results, sequence data set was set up to conduct phylogenetic inference using representative sequences (Table 2) from previous publications (Agustí-Brisach et al., 2016; Lawrence et al., 2019; Aiello et al., 2020). *Xenogliocladiopsis cypellocarpa* (CBS133814) was used as outgroup. The nucleotide alignment was performed with ClustalW algorithm (Thompson et al., 1994) contained within MEGA X software package (Kumar et al., 2018) and manual adjustments were done where necessary.

Phylogenetic analyses were based on maximum parsimony (MP) and maximum likelihood (ML). MP analysis was performed in MEGA X (Kumar et al., 2018) with the Tree Bisection and Reconnection (TBR) algorithm, where gaps were treated as missing data. The robustness of the topology was evaluated by 1,000 bootstrap replications (Felsenstein, 1985). Measures for the maximum parsimony as tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were also calculated. The ML analyses were done with the tool Randomized Axelerated Maximum Likelihood RAxML-HPC2 on XSEDE implemented on CIPRES Science Gateway v 3.3 (Stamatakis, 2014). ML tree searches were performed under the generalized time-reversible with gamma correction (GTR + Γ) nucleotide substitution model using 1000 pseudoreplicates. The other parameters were used as default settings. The alignment and the phylogenetic tree were deposited in TreeBASE under the study number S29180 (http://purl.org/phylo/treebase/phylows/study/TB2:S29180).

Pathogenicity Tests

1103 Paulsen rootstock cuttings were used in pathogenicity tests. Dormant cuttings were surface disinfected with sodium-hypochlorite solution (2.5%) for five minutes and rinsed with

sterile distilled water. Bottom of the cuttings were placed in plastic beakers (5 L) containing tap water (5-6 cm in depth) and beakers were kept in acclimatized room (at 25°C temperature, 85% relative humidity and 12-hours illumination) to induce root formation. Twenty BF isolates, belonging to seven different species, were used in pathogenicity tests (Table 3). They were grown on PDA at 25°C, in 12 h photoperiod for 20 days. Then, Petri plates were flooded with sterile distilled water, mycelia were scrapped with a sterile plastic needle and filtered through sterile cheesecloths to prepare conidial suspensions. Their concentration was adjusted at 10^6 conidia·ml^{-1} using Thoma® slides (haemocytometer). Root tips of the cuttings were slightly trimmed and immersed into conidial suspensions of the isolates for 24 hours and cuttings were planted in the 2.5 L-pots (one cutting per pot) containing peat moss, sawdust and perlite mixture (1:1:1 v/v/v).

Additionally, at the planting moment, one entire agar colony grown on a 90 mm diameter Petri plate was placed at the base of the rootstock and then the potting mixture was added to cover it. Twelve plants per isolate (one plant per pot and four replicates with three plants per replicate) were inoculated with BF isolates. Control plants were inoculated with sterile distilled water and a non-colonized agar plug. Pathogenicity tests were set up in a completely randomized design and cuttings were grown in greenhouse conditions (at 22-27°C, 60-80% RH), watering with Hoagland solution once a week during four months. The experiment was repeated.

At the end of the experiments, cuttings were uprooted from their pots and virulence of the isolates was determined with a root disease severity index (Alaniz et al., 2011), according to a 0-5 rating scale: 0= healthy roots without any lesions, 1= slight discoloration with 0-25% of root mass reduction, 2= slight to moderate discoloration with 26-50% of root mass reduction, 3= moderate discoloration with 51-75% of root mass reduction, 4= severe discoloration with >75% of root mass reduction and, 5= dead plant. Disease severity was calculated by using the formula of Towsend & Heuberger (1943): ($\sum$ (number of plants in a disease scale category × disease scale category) / (total number of plants × maximum disease scale category)) × 100). Moreover, root

biomass reduction was determined by drying the roots of the cuttings in an air circulated oven at 65°C for 48 hours. Root dry weights (RDW) were recorded by a digital scale and virulence (%) was determined by subtracting dry weights of infected roots from those weights of non-inoculated control [% Abbott formula: (RDW in non-inoculated plants– RDW in pathogen inoculated plants) / RDW in non-inoculated plants) x 100]. Koch's postulates were confirmed by re-isolation of BF isolates from the roots and basal ends of inoculated cuttings, but not from the controls.

Statistical Analyses

Analyses of variance (ANOVA) were performed on disease severity values and dry root weight. Means were compared using Fisher's least significant difference (LSD) test at the 5% significance level (Gomez & Gomez, 1984).

Results

Identification of BF pathogens on asymptomatic grapevines

Fungal isolations revealed that BF fungi were found in 39 out from the 42 Turkish nurseries surveyed (92.9%). Along with BF pathogens, *Fusarium, Rhizoctonia, Macrophomina, Botryosphaeriaceae*, Petri Disease and *Diaporthe* fungi were also isolated from the roots and basal tissues of seedlings (data not shown). The isolation frequency of BF fungi in each nursery ranged from 1.4 to 51.4%, being the overall average percentage of 18.4% (Table 1). Based on colony morphology and microscopic examination, 51 BF isolates were selected for molecular identification.

The first approximation to the identification of the isolates was based on the BLAST search of their partial sequence of histone H3 gene. Subsequently, these sequences were aligned with the most similar relative references downloaded from GenBank (Table 2). The resulting dataset contained a total of 96 ingroup taxa (45 references and 51 isolates) and one outgroup taxa (*X. cypellocarpa* CBS133814) resulting in a dataset of 477 characters including alignment gaps, of

which 270 characters were constant, 198 parsimony-informative and 9 were variable and parsimony-uninformative. The MP analysis yielded seven equally most parsimonious trees (TL = 680; CI = 0.553; RI = 0.903; RC = 0.499), one of them is presented (Fig. 2). The sequences of the isolates obtained in this study clustered into four genera distributed in seven species: *Cm. alicantinum* (3 isolates – 5.8%), *Ca. peruviana* (5 isolates – 9.8%), *D. torresensis* (20 isolates – 39.2%), *D. macrodidyma* and *D. novozelandica* (7 isolates – 13.7%, each one), *I. liriodendri* (8 isolates – 15.7%) and *I. robusta* (1 isolate – 1.9%).

Pathogenicity tests

At the end of the repeated experiments, all the *Cylindrocladiella, Cylindrodendrum, Dactylonectria* and *Ilyonectria* isolates were found to be pathogenic on 1103 Paulsen rootstock rooted cuttings. After the four-months-incubation period in greenhouse conditions, no visible symptoms were observed neither on leaves of the inoculated nor the non-inoculated controls. However, considerable reduction in root biomass and severe root discoloration were observed in the inoculated cuttings when compared to the non-inoculated controls. For each variable, ANOVA analyses indicated that the data between repeated pathogenicity experiments were similar ($P > 0.05$), thus data of all variables from both experiments were combined. The mean root disease severity index values and dry root weights caused by the BF isolates inoculated are shown in Table 3. The mean dry root weight values ranged between 0.130 g to 0.922 g, this latter value corresponding to the non-inoculated rootstock cuttings. According to root dry weight reduction, *D. novozelandica* (isolate AFP31) was the most virulent species followed by *I. robusta* (isolate AFP160), *D. macrodidyma* (AFP32, AFP74) and *I. liriodendri* (AFP30) (Table 3). Regarding the mean root disease severity index values, they ranged between 1.8 to 4.0. However, these scores were not in correspondence with the dry root weights showed previously. Severe colonization of the roots and basal ends was also confirmed by the re-isolation study and all the inoculated BF fungi were successfully recovered with isolation rates ranging from 61.9 to 88.1%.

Discussion

This is the first study conducted in Turkish nurseries to determine the presence of BF pathogens on asymptomatic grapevines. Our results revealed that BF fungi were highly prevalent (92.9%) in asymptomatic grapevines, despite of a low isolation frequency (average: 18.4%). Moreover, some Botryosphaeria and Diaporthe dieback and Petri disease pathogens were also detected in the investigated plants, but their isolation was less frequent than BF fungi (data not shown). Pintos et al., (2018) investigated the phytosanitary status of asymptomatic grapevines produced in French and Spanish nurseries, obtaining 449 fungal isolates, from which the species related to Botryosphaeria dieback were the most frequent (147 isolates) followed by Diaporthe dieback (126 isolates), BF (117 isolates), Petri disease (30 isolates) and Pestalotioid fungi (29 isolates). Nevertheless, Berlanas et al., (2020) inspected 15 grapevine nursery fields in Northern Spain for black-foot pathogens associated with asymptomatic dormant plants and detected 11 different species belonging to *Dactylonectria, Ilyonectria, Neonectria* and *Thelonectria* genera. Among the 1427 isolates obtained by these authors, *D. torresensis* was the most common species and counted for 75% of all the fungal isolates. Thus, our results are more similar to those obtained by Berlanas et al., (2020), indicating that BF is a prevalent disease when compared to other grapevine trunk disease fungi in marketable grapevine plants in Turkiye, and also *D. torresensis* being the most frequent species detected.

Soil-borne infections might be a primary cause of the high incidence of BF fungi in Turkish grapevine nurseries. Several authors have emphasized the importance of soil-borne inoculum in the occurrence of BF disease because of the capacity of this inoculum to naturally infect grapevines (Halleen et al., 2006; Agustí Brisach et al., 2013). This type of contamination has been indicated as very common in those nurseries where rootstock mother plants are buried under the soil to avoid frost injury during winter (Yu et al., 2021). Moreover, BF infections can have

their origin from the cuttings of mother plants used during the grafting process, being also detectable in marketable dormant vines (Gramaje & Armengol, 2011).

According to the results of the histone H3 gene sequencing and phylogenetic analyses, we identified seven BF species *Ca. peruviana, Cm. alicantinum, D. macrodidyma, D. novozelandica, D. torresensis, I. liriodendri*, and *I. robusta*. Among them, *Ca. peruviana, D. novozelandica* and *I. robusta* had never been reported associated with BF of grapevines in Turkiye, so this study is the first report of these species in this country. Regarding the species *Cm. alicantinum*, this species was originally described from loquat (*Eriobotrya japonica*) roots in Spain (Agustí-Brisach et al., 2016), and recently it was already reported on grapevines in Turkiye in the year 2020 (Özben, 2020).

Savaş et al., (2020) reported *D. alcacerensis* and *D. torresensis* for the first time in Turkiye, but we could not find *D. alcacerensis* in our analysis from the asymptomatic plants. Özben (2020) found six BF pathogens species in symptomatic grapevines sampled from 12 grapevine nurseries in Turkiye, being *I. liriodendri* and *D. macrodidyma* (identified with species-specific primers) the most frequent. However, our more extensive survey study, conducted in 42 nurseries, showed that *D. torresensis* was the predominant species in Turkish grapevine nurseries. Similarly, this situation was confirmed in the research conducted by Aigoun-Mouhous et al., (2019), in which these authors obtained 79 *Cylindrocarpon*-like isolates from one-year-old vineyards collected in different regions of Algeria. Three different species: *D. torresensis, D. macrodidyma* and *D. novozelandica* were identified and *D. torresensis* was found to be the most common in this country. Carlucci et al., (2017) surveyed young grapevine (12 to 18 month-old) and nursery stock plants in Apulia and Molise Regions (Italy) for the occurrence of BF pathogens. *Dactylonectria torresensis, I. liriodendri* and *Thelonectria* sp. species were the most common BF species detected among the 182 isolates *Cylindrocarpon*-like asexual morph isolates obtained.

In our pathogenicity tests, all selected BF isolates, representing the seven species found in this study, significantly decreased root biomass and increased root disease severity index, when compared to the non-inoculated control. From these isolates, *D. novozelandica* (isolate AFP31) was the most virulent. It is interesting to note that our study represents the first confirmation of the pathogenicity of *Cm. alicantinum* to grapevine, thus this species should be included as causal agent of BF of grapevines.

To date, many studies aiming to determine the pathogenicity of BF fungi have been conducted showing variable results regarding the most virulent species (Rego et al., 2001; Alaniz et al., 2012; Aigoun-Mouhous et al., 2019). Recently, Berlanas et al., (2020) determined the virulence of 24 isolates belonging to 13 BF species obtained in Spanish grapevine nurseries, and *D. novozelandica, D. alcacerensis, D. macrodidyma* and *I. vivaria* were found to be the most virulent species. Later, Yu et al., (2021) conducted pathogenicity tests of *Cylindrocladiella*, *Dactylonectria* and *Neonectria* isolates obtained from symptomatic young grapevines in China, being the virulence of *D. macrodidyma* higher than that of *Ca. lageniformis, D. torresensis, D. alcacerensis* and *Neonectria* sp..

Conclusion

The findings obtained in our study point out that BF pathogens are highly prevalent in asymptomatic nursery produced grapevines. This could represent a serious threat for Turkish viticulture in the following years, if these pathogens are disseminated to newly established vineyards, potentially increasing young vine decline and economic losses (Berlanas et al., 2020). Thus, soil-borne pathogen management practices such as crop rotation and soil solarization should be recommended to reduce BF pathogens inoculum density in nursery soils throughout the country, and also methods to reduce fungal infections on grapevine plants in the grafting process such as the use of biological control agents or hot-water treatments.

Conflict of interest

The authors declare that they have no conflict of interest

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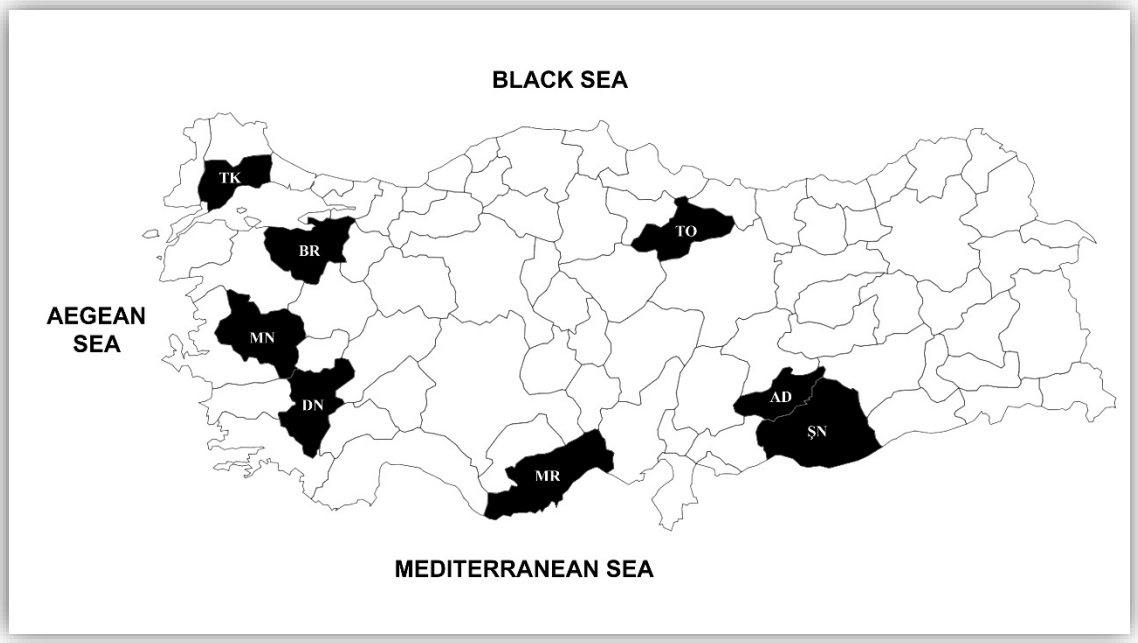

Fig. 1 Provinces in Turkiye from which grapevine nurseries were sampled in this study. AD: Adıyaman, BR: Bursa, DN: Denizli, MN: Manisa, MR: Mersin, ŞN: Şanlıurfa, TK: Tekirdağ, TO: Tokat

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Table 1 Location of surveyed grapevine nurseries, cultivars, isolation frequency of BF fungi and the species found with their histone H3 sequence numbers

Nursery	Isolate Code	Location	Rootstock / Cultivar	Isolation Frequency of BF Fungi (%)	Fungal Species	GenBank Accession Numbers
1	AFP2	Bursa	1103P-Trakya İlkeren	40.0	*Dactylonectria torresensis*	OM055886
2	AFP15	Mersin	1103P- Victoria	8.6	*Cylindrocladiella peruviana*	OM055869
3	AFP31	Salihli, Manisa	Thompson Seedless	45.7	*Dactylonectria novozelandica*	OM055906
4	AFP270	Salihli, Manisa	Sultana Seedless	12.9	*Dactylonectria torresensis*	OM055902
5	AFP32	Salihli, Manisa	Sultana Seedless	8.6	*Dactylonectria macrodidyma*	OM055913
	AFP235	Salihli, Manisa	Sultana Seedless		*Dactylonectria torresensis*	OM055901
6	AFP202	Salihli, Manisa	Sultana Seedless	20.0	*Dactylonectria novozelandica*	OM055907
	AFP272	Salihli, Manisa	Sultana Seedless		*Dactylonectria macrodidyma*	OM055916
7	AFP237	Salihli, Manisa	1103P / Sultana Seedless	21.4	*Dactylonectria macrodidyma*	OM055915
	AFP279	Salihli, Manisa	1103P / Sultana Seedless		*Dactylonectria macrodidyma*	OM055918
	AFP280	Salihli, Manisa	1103P / Sultana Seedless		*Dactylonectria novozelandica*	OM055911
	AFP281	Salihli, Manisa	1103P / Sultana Seedless		*Ilyonectria liriodendri*	OM055880
8	AFP278	Alaşehir, Manisa	Sultana Seedless	51.4	*Dactylonectria torresensis*	OM055903
	AFP289	Alaşehir, Manisa	Sultana Seedless		*Dactylonectria novozelandica*	OM055912
9	AFP30	Alaşehir, Manisa	Sultana Seedless	35.7	*Ilyonectria liriodendri*	OM055879
	AFP293	Alaşehir, Manisa	Sultana Seedless		*Dactylonectria macrodidyma*	OM055919
10	AFP227	Sarıgöl, Manisa	Sultana Seedless	8.6	*Dactylonectria torresensis*	OM055900
11	AFP29	Salihli, Manisa	Sultana Seedless	34.3	*Ilyonectria liriodendri*	OM055878
12	AFP308	Tekirdağ	Kober 5BB / Sultan 1	18.6	*Ilyonectria liriodendri*	OM055885
13	AFP290	Tekirdağ	Kober 5BB / Bozbey	17.1	*Dactylonectria torresensis*	OM055904
14	AFP318	Tekirdağ	1103P-Tekirdağ Çekirdeksizi	42.9	*Dactylonectria torresensis*	OM055905
15	AFP306	Tekirdağ	110R-Yapıncak	38.6	*Ilyonectria liriodendri*	OM055884
16	AFP36	Denizli	41B / Sultana Seedless	8.6	*Dactylonectria torresensis*	OM055887
17	AFP285	Denizli	41B / Sultana Seedless	4.3	*Ilyonectria liriodendri*	OM055881
18	AFP287	Denizli	41B / Sultana Seedless	10.0	*Ilyonectria liriodendri*	OM055882
19	AFP288	Denizli	41B / Sultana Seedless	7.1	*Ilyonectria liriodendri*	OM055883
20	NF	Denizli	41B / Michele Palieri	-	*NF*	
21	AFP220	Şanlıurfa	1103P - Ergin Çekirdeksizi	14.3	*Cylindrodendrum alicantinum*	OM055875

	AFP226	Şanlıurfa	1103P - Ergin Çekirdeksizi		*Cylindrocladiella peruviana*	OM055872
22	AFP228	Şanlıurfa	110R - Horozkarası	1.4	*Cylindrocladiella peruviana*	OM055873
23	AFP81	Şanlıurfa	99R - Çiloreş	22.9	*Dactylonectria torresensis*	OM055891
	AFP200	Şanlıurfa	99R - Çiloreş		*Cylindrodendrum alicantinum*	OM055874
24	AFP273	Şanlıurfa	1103P - Victoria	2.8	*Dactylonectria macrodidyma*	OM055917
25	AFP233	Manisa	41B / Red Globe	5.7	*Cylindrodendrum alicantinum*	OM055876
26	AFP253	Manisa	Kober 5BB / Royal	4.3	*Dactylonectria novozelandica*	OM055908
27	AFP161	Manisa	1103P - Sultana Seedless	11.4	*Dactylonectria torresensis*	OM055899
28	AFP74	Manisa	Kober 5BB - Sultana Seedless	27.1	*Dactylonectria macrodidyma*	OM055914
29	AFP117	Manisa	1103P - Crimson Seedless	24.3	*Dactylonectria torresensis*	OM055893
30	AFP76	Manisa	110R / Alicante Bouschet	25.7	*Dactylonectria torresensis*	OM055888
31	NF	Alaşehir, Manisa	1103P - Thompson Seedless	-	*NF*	
32	AFP77	Manisa	Kober 5BB / Ata Sarısı	4.3	*Dactylonectria torresensis*	OM055889
33	AFP254	Turgutlu, Manisa	Kober 5BB /Sultana Seedless	11.4	*Dactylonectria novozelandica*	OM055909
34	AFP255	Manisa	Kober 5BB / Trakya İlkeren	7.1	*Dactylonectria novozelandica*	OM055910
35	AFP141	Tokat	1103P - Narince	1.4	*Dactylonectria torresensis*	OM055895
36	AFP86	Tokat	1103P/Narince	32.9	*Dactylonectria torresensis*	OM055892
	AFP155	Tokat	1103P/Narince		*Dactylonectria torresensis*	OM055898
37	NF	Tokat	1103P/Narince	-	*NF*	
38	AFP149	Tokat	1103P/Sultan7	2.9	*Dactylonectria torresensis*	OM055897
39	AFP146	Tokat	1103P/Narince	22.9	*Dactylonectria torresensis*	OM055896
40	AFP80	Tokat	Du Lot / Narince	12.9	*Dactylonectria torresensis*	OM055890
	AFP160	Tokat	Du Lot / Narince		*Ilyonectria robusta*	OM055877
41	AFP133	Adıyaman	Kober 5BB / Hatun Parmağı	20.5	*Dactylonectria torresensis*	OM055894
	AFP205	Adıyaman	Kober 5BB / Hatun Parmağı		*Cylindrocladiella peruviana*	OM055871
42	AFP126	Mersin	1103P / Victoria	27.7	*Cylindrocladiella peruviana*	OM055870
			average	18.4		

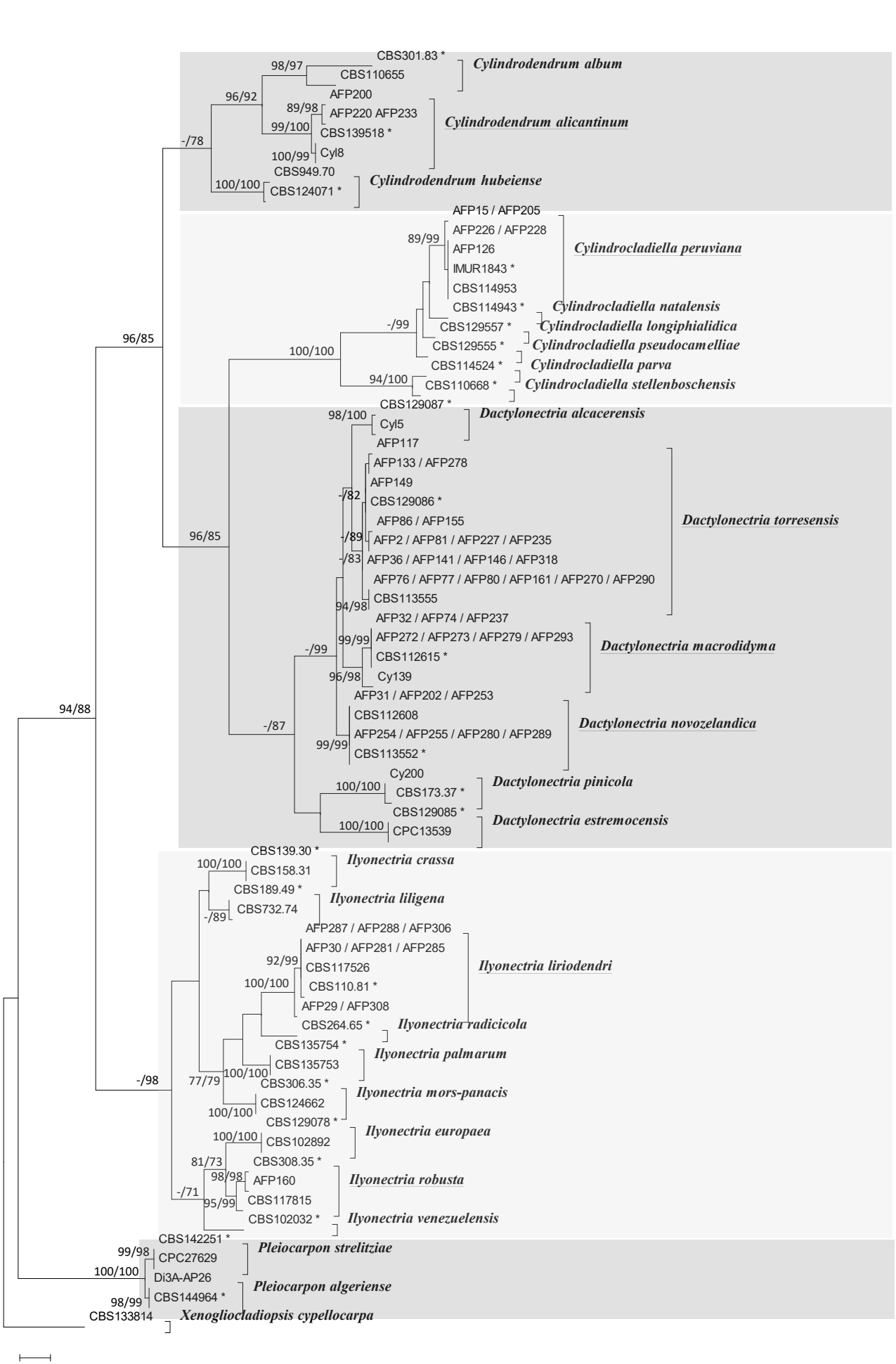
1 NF: Not found

Table 2 GenBank accession numbers of partial sequence of histone H3 of references species used in the phylogenetic analyses

Species	Isolate	GenBank Accession Nº
Cylindrocladiella longiphialidica	CBS 129557 *	JN098851
Cylindrocladiella natalensis	CBS 114943 *	JN098895
Cylindrocladiella parva	CBS 114524 *	AY793526
Cylindrocladiella peruviana	IMUR 1843 *	AY793540
	CBS 114953	JN098885
Cylindrocladiella pseudocamelliae	CBS 129555 *	JN098843
Cylindrocladiella stellenboschensis	CBS 110668 *	JN098922
Cylindrodendrum album	CBS 301.83 *	KM231484
	CBS 110655	KM231485
Cylindrodendrum alicantinum	CBS139518 *	KP639555
	Cyl8	KP639556
Cylindrodendrum hubeiense	CBS 949.70	KP639560
	CBS 124071 *	KR909093
Dactylonectria alcacerensis	CBS 129087 *	JF735630
	Cyl5	KC514071
Dactylonectria estremocensis	CBS 129085 *	JF735617
	CPC 13539	JF735627
Dactylonectria macrodidyma	CBS 112615 *	JF735647
	Cy139	JF735650
Dactylonectria novozelandica	CBS 112608	JF735632
	CBS 113552 *	JF735633
Dactylonectria pinicola	CBS 173.37 *	JF735614
	Cy200	JF735612
Dactylonectria torresensis	CBS 129086 *	JF735681
	CBS 113555	JF735661
Ilyonectria crassa	CBS 139.30 *	JF735534
	CBS 158.31	JF735535
Ilyonectria europaea	CBS 129078 *	JF735567
	CBS 102892	JF735569
Ilyonectria liligena	CBS 189.49 *	JF735573
	CBS 732.74	JF735574
Ilyonectria liriodendri	CBS 117526	JF735508
	CBS 110.81 *	JF735507
Ilyonectria mors-panacis	CBS 306.35 *	JF735557
	CBS 124662	JF735559
Ilyonectria palmarum	CBS 135754 *	HF922620
	CBS 135753	HF922621
Ilyonectria radicicola	CBS 264.65 *	JF735506
Ilyonectria robusta	CBS 308.35 *	JF735518
	CBS 117815	JF735522
Ilyonectria venezuelensis	CBS 102032 *	JF735571
Pleiocarpon algeriense	CBS 144964 *	MH587296
	Di3A-AP26	MT635011
Pleiocarpon strelitziae	CBS 142251 *	KY304616

	CPC 27629	KY304617	1
Xenogliocladiopsis cypellocarpa	CBS 133814	KM231479	2

* Ex-type culture


CBS301.83 *
98/97
CBS110655
AFP200
96/92
89/98
AFP220 AFP233
99/100
CBS139518 *
-/78
100/99
Cyl8
CBS949.70
100/100
CBS124071 *
Cylindrodendrum album
Cylindrodendrum alicantinum
Cylindrodendrum hubeiense
AFP15 / AFP205
89/99
AFP226 / AFP228
AFP126
IMUR1843 *
CBS114953
CBS114943 *
-/99
CBS129557 *
CBS129555 *
100/100
CBS114524 *
94/100
CBS110668 *
Cylindrocladiella peruviana
Cylindrocladiella natalensis
Cylindrocladiella longiphialidica
Cylindrocladiella pseudocamelliae
Cylindrocladiella parva
Cylindrocladiella stellenboschensis
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96/85
-/89
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-/83
AFP36 / AFP141 / AFP146 / AFP318
AFP76 / AFP77 / AFP80 / AFP161 / AFP270 / AFP290
94/98
CBS113555
Dactylonectria torresensis
AFP32 / AFP74 / AFP237
99/99
AFP272 / AFP273 / AFP279 / AFP293
-/99
CBS112615 *
96/98
Cy139
Dactylonectria macrodidyma
94/88
AFP31 / AFP202 / AFP253
CBS112608
-/87
AFP254 / AFP255 / AFP280 / AFP289
99/99
CBS113552 *
Dactylonectria novozelandica
Cy200
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CBS173.37 *
Dactylonectria pinicola
CBS129085 *
100/100
CPC13539
Dactylonectria estremocensis
CBS139.30 *
100/100
CBS158.31
Ilyonectria crassa
CBS189.49 *
Ilyonectria liligena
-/89
CBS732.74
AFP287 / AFP288 / AFP306
AFP30 / AFP281 / AFP285
92/99
CBS117526
100/100
CBS110.81 *
AFP29 / AFP308
Ilyonectria liriodendri
CBS264.65 *
Ilyonectria radicicola
CBS135754 *
Ilyonectria palmarum
CBS135753
100/100
77/79
CBS306.35 *
-/98
Ilyonectria mors-panacis
CBS124662
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Ilyonectria europaea
100/100
CBS102892
81/73
CBS308.35 *
98/98
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Ilyonectria robusta
-/71
95/99
CBS117815
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Ilyonectria venezuelensis
CBS142251 *
99/98
CPC27629
Pleiocarpon strelitziae
100/100
Di3A-AP26
CBS144964 *
Pleiocarpon algeriense
98/99
CBS133814
Xenogliocladiopsis cypellocarpa
10.00

Fig. 2 One of seven most parsimonious trees obtained from the analysis of the alignment of partial sequences of the histone H3 gene. Scale bar shows 10 changes and bootstrap support values from 1,000 replicates of maximum likelihood and maximum parsimony are indicated at the nodes. Bootstrap values less than 70% are indicated with "- ". Ex-type strains are indicated with an asterisk and blocks indicate the genera included in the phylogeny. Underlined species contain the Turkish isolates. The tree was rooted to Xenogliocladiopsis cypellocarpa (CBS 133814).

Table 3 Pathogenicity of selected BF isolates on 1103 Paulsen rootstock cuttings

Fungal species	Isolate	Dry root weights (g)	Root Disease Severity Index	Reisolation (%)
Dactylonectria novozelandica	AFP31	0.130 a	4.0 e	88.1
Ilyonectria robusta	AFP160	0.184 ab	3.0 bcde	88.0
D. macrodidyma	AFP32	0.189 ab	3.7 de	80.9
D. macrodidyma	AFP74	0.189 ab	3.5 cde	76.2
I. liriodendri	AFP30	0.193 ab	3.7 de	80.9
D. novozelandica	AFP255	0.246 abc	2.5 bcd	76.2
I. liriodendri	AFP29	0.261 abc	3.3 cde	66.6
Cylindrocladiella peruviana	AFP15	0.268 abc	3.0 bcde	85.7
D. torresensis	AFP36	0.269 abc	3.3 cde	78.5
Cylindrodendrum alicantinum	AFP233	0.285 abc	3.2 cde	73.8
I. liriodendri	AFP115	0.295 abc	3.3 cde	71.4
D. torresensis	AFP80	0.332 abcd	3.0 bcde	73.8
D. macrodidyma	AFP279	0.368 abcde	2.8 bcde	83.3
D. torresensis	AFP2	0.384 abcde	2.8 bcde	80.9
D. macrodidyma	AFP293	0.388 abcde	2.8 bcde	69.0
D. macrodidyma	AFP272	0.400 bcde	2.7 bcd	61.9
C. peruviana	AFP228	0.440 bcde	2.8 bcde	81.0
D. novozelandica	AFP253	0.479 cde	2.3 bc	78.6
D. novozelandica	AFP289	0.579 de	1.8 b	64.3
C. alicantinum	AFP200	0.605 e	1.8 b	66.6
Non-inoculated control	-	0.922 f	0.0 a	-

*Means with the same letter are not significantly different (P=0.05) according to LSD test. They are the average of twenty-four cuttings (twelve per experiment).