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Posted Date: 20 May 2024

doi: 10.20944/preprints202405.1204.v1

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Fusarium Species Associated with Wood Canker, Root and Basal Rot in Turkish Grapevine Nurseries

Davut Soner Akgül [1], Serkan Önder [2], Nurdan Güngör Savaş [3], Murat Yıldız [4], İzzet Bülbül[5], and Mümine Özarslandan[5]

[1] Çukurova University, Agriculture Faculty, Dept. of Plant Protection, 01330, Adana, Türkiye; sakgul@cu.edu.tr
[2] Eskişehir Osmangazi University, Agriculture Faculty, Dept. of Plant Protection, 26040, Eskişehir, Türkiye; onderserkan@gmail.com
[3] Manisa Viticulture Research Institute, Turkish Ministry of Agriculture and Forestry, 45125, Manisa, Türkiye; nurdangngrsvs10@gmail.com
[4] Malatya Apricot Research Institute, Turkish Ministry of Agriculture and Forestry, 44090, Malatya, Türkiye; muratyildizbaem@gmail.com
[5] Biological Control Research Institute, Turkish Ministry of Agriculture and Forestry, 01321, Adana, Türkiye; izzetblbl@gmail.com,
[5] Biological Control Research Institute, Turkish Ministry of Agriculture and Forestry, 01321, Adana, Türkiye; mumine_deniz@hotmail.com
* Correspondence: sakgul@cu.edu.tr

Abstract: *Fusarium* species are agriculturally important fungi with a broad host range and can be found as endophytic, pathogenic, or opportunistic parasites in many crop plants. This study aimed to identify *Fusarium* species in bare-rooted, dormant plants in Turkish grapevine nurseries using molecular identification methods and assess their pathogenicity. Asymptomatic dormant plants were sampled from grapevine nurseries (43) in different regions of the country, and fungi were isolated from plant roots and internal basal tissues. The *Fusarium* isolates were identified by performing gene sequencing (*TEF1-α, RPB2*) and phylogenetic analyses. Pathogenicity tests were carried out by inoculating mycelial agar pieces of isolates onto the stem or conidial suspensions into the rhizosphere of vines (1103 Paulsen rootstock). Laboratory tests revealed that *Fusarium* species were highly prevalent in Turkish grapevine nurseries (41 out of 43). Gene sequencing and phylogenetic analyses unraveled that 12 *Fusarium* species (*F. annulatum, F. brachygibbosum, F. clavum, F. curvatum, F. falciforme, F. fredkrugeri, F. glycines, F. nanum, F. nematophilum, F. nirenbergiae, F. solani*, and *Fusarium* spp.) existed in the ready-to-sale plants. Some of these species (*F. annulatum, F. curvatum* and *F. nirenbergiae*) consistently caused wood necrosis of seedling stems, rotting of the basal zone and roots, and reduced root biomass. Although the other nine species also caused some root rot and root reduction, their virulence was not as severe as the pathogenic ones, and they were considered opportunistic parasites or endophytic species. This study is the first detailed investigation of *Fusarium* species in grapevine nurseries in Türkiye and emphasizes the need to consider pathogenic species in producing healthy grapevine seedlings.

Keywords: 1103 Paulsen, *Fusarium*, grape, identification, pathogenicity, rootstock, Türkiye

1. Introduction

Grapevine sapling production is one of the most important agricultural sectors in Türkiye, and many young vines (2.5 to 3 million plants) are produced yearly in different geographical regions in the country [1]. The need for grapevine seedlings in the domestic market is relatively high, and this production needs to be increased to meet the demand for grapevine seedlings in Türkiye.

In grapevine nurseries, abiotic factors (unfavorable weather conditions, nutritional disorders, use of poor quality production materials, rootstock-scion incompatibility, etc.), nematodes, insects, and fungal pathogens cause the death of plants, and these factors bring about low productivity and economic losses every year. Fungal grapevine trunk disease (GTD) pathogens, which often settle on young seedlings with infected propagation materials, belonging to *Botryosphaeriaceae*, *Diaporthaceae*,

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and *Diatrypaceae* families, *Cadophora, Cytospora, Phaeomoniella, Phaeoacremonium Seimatosporium* genus, and soilborne fungi (*Armillaria, Cylindrocarpon*-like anamorphs, *Fusarium, Macrophomina, Phytophthora, Rhizoctonia*, and *Verticillium* sp.) are considered to be the main actors of plant mortality in the nurseries [2,3].

The genus *Fusarium* has an exceptional place in plant pathology, medical mycology, and the food industry as they are both plant and human pathogens and threaten human-animal health by producing mycotoxins in foods. To date, more than 400 *Fusarium* species have been identified, which nested in 23 different species complexes [4]. Most *Fusarium* species are soil-borne and are also called one of the ubiquitous fungal genera in mycology due to their endophytic, saprophytic, hemibiotrophic, or parasitic forms and strong competitive ability. Plant pathogenic species may result in significant crop damage and economic losses in some years by causing root and basal rots, damping-off, seed-tuber-fruit rots, wilt, and head blight diseases. According to the American Phytopathological Society, 83 out of 108 plant species in the field and horticultural crops are affected by one or more *Fusarium* diseases [5]. Many species in the *Fusarium* genus are true plant pathogens, while others are opportunists waiting for soil and environmental conditions to turn unfavorable for plants.

It has been pointed out that root rot-associated fungi considerably reduce young vine health and marketable sapling yield; *Fusarium* and *Cylindrocarpon*-like fungi were the main actors affecting plant vigor, and quality in grapevine nurseries. These fungi cause necrosis in the roots and basal tissues, leading to a reduction in hairy roots, retarded growth, and, the death of seedlings or young vines in later stages [6]. Research on the pathogenic roles and diversity of *Fusarium* species on plant death in grapevine nurseries and young vineyards has intensified in recent years. Highet and Nair [7] proved the infection of grapevine hairy roots by *Fusarium oxysporum* through transmission electron microscopy and pathogenicity tests and suggested it would be considered one of the fungi associated with root rot and decline in the nurseries. Reveglia et al. [8] revealed that the phytotoxins of *Fusarium oxysporum* (such as fusaric acid) and other potential metabolites have a critical role in the occurrence of these symptoms in seedlings and young vines in Australia. Vilvert et al. [9] claimed that *Fusarium oxysporum* f.sp. *herbemontis* is an important species responsible for decline and plant death in Brazilian grapevine nurseries, and it would be possible to control this pathogen using mycorrhizal fungi. Úrbez-Torres et al. [10] stated that *Fusarium* species were common in British Columbia (Canada) vineyards, but the most frequently isolated species might be as secondary pathogens on grapevine rootstock 3309C. Similarly, Bustamente et al. [11] suggested that *Fusarium* species isolated from grapevine nurseries and young vineyards in California (USA) are opportunistic pathogens attacking plants under stress. In contrast, Li et al. [12] reported that when the *Fusarium* isolates were inoculated into grapevine seedlings, they caused necrosis in the xylem vessels and basal regions of the plants resembling the infections of *D. macrodidyma* (a black-foot disease pathogen). Zhang et al. [13] reported for the first time *Fusarium commune* was a pathogen in grapevines causing leaf yellowing, stunting and root rot in Beijing Region, China. These studies indicate that *Fusarium* species on grapevines are a potential threat to nurseries and newly established vineyards and should not be underestimated. Furthermore, since these species can be found in the latent phase in plants, it is possible to spread them over large areas with marketable grapevine seedlings. Akgül and Ahioğlu [14] detected some *Fusarium* species in young vineyards, along with fungal pathogens associated with grapevine trunk diseases, in the southern Türkiye and confirmed the pathogenicity of these species. However, a nationwide study on the diversity and pathogenicity of *Fusarium* species in marketable grapevine saplings is needed. Therefore, this study aimed to reveal *Fusarium* diversity in dormant marketable plants in Turkish grapevine nurseries and to assess their pathogenicity.

2. Materials and Methods

2.1. Survey and isolation of Fusarium species

The survey was conducted in January 2021 in 43 grapevine nurseries in different geographical regions of Türkiye (in Adıyaman, Bursa, Denizli, Manisa, Mersin, Mersin, Tekirdağ, Tokat, and Urfa

 doi:10.20944/preprints202405.1204.v1

provinces). Ten dormant, commercially ready-for-sale seedlings from each nursery were randomly sampled and transported to the laboratory. The root and basal parts of the seedlings were washed under tap water and disinfected superficially with sodium hypochlorite solution (including >5% active clorine) for 3 minutes. Root and internal basal tissues (3-4 mm) were placed onto PDA (Potato dextrose agar, CondaLab; Spain) containing streptomycin-sulfate (250 mg×L-1), and the Petri plates were kept at 25°C in dark, for ten days to promote fungal colony growth. According to the morphological and microscopic characteristics detailed by Leslie and Summerell [15], a single spore was taken from the *Fusarium* colonies and purified on PDA for further stages. Ten Petri plates (containing seven tissue fragments in each) were used, and the isolation frequency of *Fusarium* colonies was calculated by proportioning the tissue number (*Fusarium* detected) to the total number (n=70).

2.2. Molecular identification and phylogenetic analyses

A total of 60 *Fusarium* isolates were selected for molecular identification. They were grown on PDA at 25°C in the dark for seven days, and mycelium (56-60 mg) was harvested for DNA extraction. The genomic DNA was obtained following the CTAB protocol recommended by O'Donnell et al. [16] and diluted with 100 µl PCR grade water (Lonza) and stored at -18°C for further use. Translation elongation factor (*TEF1-α*) and second largest protein subunit of RNA polymerase II (*RPB2*) genes were amplified using the primers, EF1/EF2 and RPB2-5f2/fRPB2-7cr respectively [17]. The PCR reaction mixture contained 5 µl of buffer (10X Green Buffer, DreamTaq Green DNA Polymerase, Thermo-Scientific, USA), 2 µl of dNTPs mixture (10 mM each, Thermo Scientific, USA), 1 µl of forward and reverse primers (10 pmol·µl −1), 0.25 µl of Taq polymerase (DreamTaq Green DNA Polymerase, Thermo-Scientific), 39.75 µl PCR grade water and 1 µl genomic DNA (100 ng·µl −1). PCR amplifications were conducted in SimpliAmp A24811™ Thermal Cycler, Applied Biosystems, (USA) with the conditions detailed in the publications of O'Donnell et al. [16–18]. The PCR products were separated using gel electrophoresis in 1.5% agarose (Invitrogen) gel under 55V DC voltage, 250 mA current for 90 min. and were checked for DNA quality visually. After that, PCR products were sequenced bidirectionally via Sanger sequencing, derived chronogram files were trimmed from 3 and 5 prime with CLC main Workbench 5.5, and manual editing was done where necessary. Cleaned sequences were compared with those deposited in the NCBI GenBank database using the NCBIblastn suite (National Center for Biotechnology Information). Nucleotide sequences of *TEF1-α* and *RPB2* genes were submitted to the NCBI GenBank, and the accession numbers were obtained. According to the nucleotide BLAST search results of *TEF1-α* and *RPB2*, a representative sequence dataset was used from the NCBI nucleotide database (Table 1) to perform the phylogenetic study. Constructed datasets for *TEF1-α* and *RPB2* sequences were aligned individually via the ClustalW alignment tool in Geneious Prime 2019.1.3 software. After the alignment step, *TEF1-α* and *RPB2* sequences were concatenated from end to end via Geneious Prime 2019.1.3 software for the multi-gene phylogenetic tree. Phylogenetic analyses were based on maximum likelihood (ML). The ML analysis was performed with IQ-TREE on the Galaxy Europe platform [19]. Model Finder was used to determine the best-fit model for the ML tree [20]. ML tree construction was performed under the TIM2e model with equal base frequencies and Invariable+Gamma with four categories (TIM2e+I+G4) nucleotide substitution model according to the Bayesian information criterion scores and weights (BIC and w-BIC). For the pseudoreplications of the ML tree, a 1000 ultrafast bootstrap parameter was used [21]. The alignment and the phylogenetic tree were deposited in TreeBASE under the study number S31385 (http://purl.org/phylo/treebase/phylows/study/TB2:S31385).

 doi:10.20944/preprints202405.1204.v1

Table 1. GenBank accession numbers of partial sequence of *TEF1-α* and *RPB2* of references species used in the phylogenetic analyses.

Reference Species	Isolate	GenBank Accession Numbers	
		TEF1-α	RPB2
F. solani	CBS 138564	KT272100	KT272102
"	CBS 131775	JX118990	JX237778
"	KARE_221	MK077042	MK077080
"	MRC_2565	MH582420	MH582410
"	CBS 102429	KM231936	KM232376
F. crassum	CPC_37122	MW248760	MW446594
F. noneumartii	IPN-AP1	OP902594	OP902591
"	IPN-AP3	OP902596	OP902593
F. falciforme	CBS 135521	KU711733	KU604357
"	CBS 138971	KT716212	KT716187
"	CBS 138963	KT716213	KT716188
F. martii	CBS 115659	JX435156	JX435256
F. keratoplasticum	LDCF109	OP184958	OP186372
"	MMC59F11-1	MF069182	MF069181
F. suttonianum	CML3942	MK158921	MH709236
F. stericicola	N/A	LR583659	LR583888
F. quercinum	NRRL:22611	DQ246841	EU329518
F. bostrycoides	FUS C11A	PP105767	PP125181
F. parceramosum	CBS 115695	JX435149	JX435249
F. petroliphilum	CBS 135955	KU711768	KU604337
F. metavorans	CBS 135789	KU711773	KU604374
F. vanettenii	NRRL 45880	FJ240352	JX171655
F. breve	LC2116	MW620163	MW474688
F. waltergamsii	NRRL 32323	DQ246951	EU329576
F. nematophilum	NRRL_54600	N/A	JX171664
F. clavum	CBS 131255	MN170460	MN170393
"	CBS 131787	MN170461	MN170394
"	CBS 126202	MN170456	MN170389
"	CBS 140912	MN170462	MN170395
F. ipomoeae	CBS 140909	MN170479	MN170412
"	NRRL 34034	GQ505636	GQ505814
F. compactum	NRRL 36323	GQ505648	GQ505826
F. lacertarum	NRRL 20423	GQ505593	GQ505771
F. duofalcatisporum	NRRL 36448	GQ505652	GQ505830
F. equiseti	CBS 307.94	KR071777	KU604327
F. toxicum	CBS 406.86	MN170508	MN170441
F. nanum	CBS 131781	MN170487	MN170420
F. persicinum	CBS 479.83	MN170495	MN170428
F. incarnatum	CBS 132194	KF255470	KF255542
F. sulawense	NRRL 34004	GQ505628	GQ505806
F. luffae	CBS 131097	MN170482	MN170415
F. irregulare	CBS 132190	MN170480	MN170413
F. tanahbumbuense	CBS 131009	MN170506	MN170439
F. citri	CBS 678.77	MN170453	MN170386
F. mucidum	CBS 102394	MN170484	MN170417
F. brachygibbosum	CBS 131252	JQ429334	JX162526
F. pentaseptatum	LLC1022	OP487255	OP486819
F. subflagellisporum	COAD 2989	MT774486	MZ970426
F. transvaalense	FRC R7052	MW233161	MW233505
"	NRRL 31008	MW233102	MW233446
F. sambucinum	NRRL 13394	MW233064	MW233407
F. longipes	NRRL 13317	MW233058	MG282411
F. annulatum	CBS 115.97	MW401973	MW402785
"	CBS 135791	MW402054	MW402746
F. udum	CBS 177.31	MH484957	MH484866
F. fredkrugeri	CBS 408.97	MW402126	MW402814
F. proliferatum	CBS 480.96	MN534059	MN534272

F. foetens	CBS 120665	MH485009	MH484918
F. commune	BHBR5	OR900978	OR888540
F. odoratissimum	CBS 794.70	MH484969	MH484878
F. callistephi	CBS 187.53	MH484966	MH484875
F. fabacearum	CPC 25801	MH485029	MH484938
F. gossypinum	CBS 116611	MH484998	MH484907
F. elaeidis	CBS 255.52	MH484965	MH484874
F. cugenangense	CBS 131393	MH485019	MH484928
Fusarium sp.	CBS 128.81	MH484975	MH484884
F. carminascens	CPC 25800	MH485028	MH484937
F. glycines	CBS 200.89	MH484979	MH484888
F. duoseptatum	CBS 102026	MH484987	MH484896
F. tardichlamydosporum	CBS 102028	MH484988	MH484897
F. libertatis	CPC 25788	MH485024	MH484933
"	CPC 28465	MH485035	MH484944
F. hoodiae	CBS 132474	MH485020	MH484929
F. oxysporum	CBS 221.49	MH484963	MH484872
"	CPC 25822	MH485034	MH484943
"	CBS 144134	MH485044	MH484953
"	CBS 144135	MH485045	MH484954
F. languescens	CBS 413.90	MH484981	MH484890
"	CBS 646.78	MH484972	MH484881
"	CBS 645.78	MH484971	MH484880
F. contaminatum	CBS 114899	MH484992	MH484901
F. pharetrum	CPC 30822	MH485042	MH484951
F. veterinarium	CBS 109898	MH484990	MH484899
F. nirenbergiae	CBS 130301	MH485017	MH484926
"	CBS 196.87	MH484977	MH484886
"	CBS 127.81	MH484974	MH484883
F. curvatum	CBS 141.95	MH484985	MH484894
"	CBS 247.61	MH484967	MH484876
"	CBS 238.94	MH484984	MH484893
Dactylonectria torresensis	CBS 129086	JF735870	KM232347

2.3. Pathogenicity tests

Based on the identification results, 38 Fusarium isolates were selected for pathogenicity tests, and two types of inoculation methods were followed to evaluate from different aspects. In the first, the bark of the dormant cuttings was removed with a sterile cork-borer (3 mm), fresh mycelial agar discs of the isolates (10-day old) were placed on these wounds, and these points were wrapped with parafilm™. The cuttings were planted in the pots and grown in greenhouse conditions for four months. The inoculation points were scraped with a scalpel, and necrosis lengths in the wood tissues were measured and recorded [11]. Plants inoculated with an Ilyonectria liriodendri (black-foot disease pathogen) isolate (AFP115) were set as positive, and sterile agar-inoculated plants were set as healthy controls. In the second trial, 1103 Paulsen rootstock cuttings were planted in the plastic pots (0.85 L) containing sterile rooting mix (equal volumes of peat moss and perlite), and the pots were kept in lath house conditions (natural temperature, relative humidity and illumination). The Fusarium isolates were grown on PDA at 25°C for 15 days, and conidial suspensions (in sterile distilled water at 10^6 conidia·ml^{-1} concentration) were prepared using Thoma™ slides. Following root formation, conidial suspensions were poured into the root zone of the plants (20 ml per pot), and plants were grown lath house conditions for four months. The pathogenicity of the isolates was assessed based on root dry weight and necrosis length in the plants' basal zone (in wood tissues). The seedlings were uprooted from the pots, the roots were gently washed under tap water, and were harvested using a pruning shear. After briefly blotting with paper towels, the roots were held in a drying chamber at 65°C for 48 hours, then weighed using a precision balance, and weights were recorded [22]. Nevertheless, the bark of the cuttings was carefully peeled off with a knife, and the length of the necroses in the wood tissues was measured with a caliper. Pathogenicity tests were arranged according to the design of the randomized plot with six replications (two plants in a replicate), and twelve plant were used for each isolate. The trials were repeated once (2022 and 2023 years), and the data were subjected to statistical analysis. To clarify the virulence of each Fusarium species, an analysis of variance was performed again on the mean values of all isolates belonging to the same species.

2.4. Statistical analyses

Analysis of variance (ANOVA) was performed on lengths of wood necrosis in basal parts and internodes of the stems and root dry weights. The data were checked for normality, and root square transformation was applied. Means were compared using Fisher's least significant difference (LSD) test at the 5% significance level [23].

3. Results

3.1. Fungal isolation and prevalence of Fusarium species

The first Fusarium colonies were aroused on the internal basal tissues and hairy roots of marketable, dormant grapevine plants after 5-6 days of incubation (at 24°C in the dark) in PDA media. Through morphological and microscopic examinations, 779 Fusarium colonies were detected in 3010 plant tissues (in 430 Petri dishes) plated for 43 grapevine nurseries. The isolation frequency of Fusarium species in these nurseries is shown in Table 2.

Table 2. Location of surveyed grapevine nurseries, rootstock/cultivars, isolation frequency of Fusarium species in Türkiye.

Nursery	Location	Rootstock or Cultivar	Isolation Frequency (%)
1	Bursa	1103P-Trakya İlkeren	32.9
2	Mersin	1103P- Victoria	32.9
3	Salihli, Manisa	Thompson Seedless	-
4	Salihli, Manisa	Sultana Seedless	28.5
5	Salihli, Manisa	Sultana Seedless	-
6	Salihli, Manisa	Sultana Seedless	38.5
7	Salihli, Manisa	1103P / Sultana Seedless	40.0
8	Alaşehir, Manisa	Sultana Seedless	34.3
9	Alaşehir, Manisa	Sultana Seedless	4.3
10	Alaşehir, Manisa	Sultana Seedless	24.3
11	Sarıgöl, Manisa	Sultana Seedless	58.6
12	Salihli, Manisa	Sultana Seedless	17.1
13	Tekirdağ	Kober 5BB / Sultan 1	8.6
14	Tekirdağ	Kober 5BB / Bozbey	17.2
15	Tekirdağ	1103P-Tekirdağ Çekirdeksizi	27.1
16	Tekirdağ	110R-Yapıncak	2.9
17	Denizli	41B / Sultana Seedless	20.0
18	Denizli	41B / Sultana Seedless	40.0
19	Denizli	41B / Sultana Seedless	65.7
20	Denizli	41B / Sultana Seedless	50.0
21	Denizli	41B / Michele Palieri	45.7
22	Şanlıurfa	1103P - Ergin Çekirdeksizi	12.9
23	Şanlıurfa	110R - Horozkarası	11.5
24	Şanlıurfa	99R - Çiloreş	12.9
25	Şanlıurfa	1103P - Victoria	5.7
26	Manisa	41B / Red Globe	47.1
27	Manisa	Kober 5BB / Royal	20.0
28	Manisa	1103P - Sultana Seedless	20.0
29	Manisa	Kober 5BB - Sultana Seedless	21.4
30	Manisa	1103P - Crimson Seedless	17.1
31	Manisa	110R / Alicante Bouschet	26.3
32	Alaşehir, Manisa	1103P - Thompson Seedless	60.0
33	Manisa	Kober 5BB / Ata Sarısı	22.9

 doi:10.20944/preprints202405.1204.v1

34	Turgutlu, Manisa	Kober 5BB /Sultana Seedless	28.6
35	Manisa	Kober 5BB / Trakya İlkeren	8.6
36	Tokat	1103P - Narince	40.0
37	Tokat	1103P/Narince	21.4
38	Tokat	1103P/Narince	11.4
39	Tokat	1103P/Sultan7	12.9
40	Tokat	1103P/Narince	20.0
41	Tokat	Du Lot / Narince	12.9
42	Adıyaman	Kober 5BB / Hatun Parmağı	17.9
43	Mersin	1103P / Victoria	31.3
Mean			24.9

As shown in Table 1, Fusarium species were detected in 41 out of 43 grapevine nurseries, and the prevalence of these species in Turkish grapevine nurseries was calculated at 95.3%. The isolation frequency in nurseries ranged between 2.9 and 65.7%, while the overall average was 24.9%. Nevertheless, black foot, Petri disease pathogens, Botryosphaeriaceae fungi, Cytospora, Diaporthe, Truncatella species, and soil-borne plant pathogenic fungi (Macrophomina and Rhizoctonia) were also found (data not shown). Considering different geographical regions and morphological-microscopic characteristics, 121 Fusarium colonies were pre-selected for molecular-phylogenetic analyses.

3.2. Molecular identification and phylogenetic analyses

Using the primers EF1/EF2 and RPB2-5f2/fRPB2-7cr, TEF1-α and RPB2 gene regions of the Fusarium isolates were amplified by end point PCR, and agarose gel electrophoresis revealed DNA bands with sizes ranging from 680 to 1600 bp (respectively). The initial approach for the identification of isolates relied on a blastn search of partial sequences of the TEF1-α and RPB2 gene. NCBIblastn search was performed with the nucleotide sequences of these regions, and the isolates were 99.2-100% similar to other Fusarium species in the GenBank. Afterwards, these sequences were aligned with the closest matching and nearly closest references obtained from GenBank (Table 1) for resolve the ambiguities. However, the NCBIblastn search results from the two gene regions of all isolates were not parallel, and the second gene region in some isolates matched with different Fusarium species. Yet, phylogenetic analyses conducted with concatenated nucleotide sequences clarified the ambiguity observed in the blastn results. The final phylogenetic dataset was contained a total of 150 taxa with 3040 nucleotide sites (89 references and 60 isolates) and Dactylonectria torresensis CBS 129086 as an outgroup at taxon level. Number of constant sites were 1787 (= 58.7829% of all sites) whereas there was no ambiguous constant sites. Number of parsimony informative sites and distinct site patterns were 1001 and 1598, respectivelly. Determining to the best fit model for ML tree, BIC score was found 43876.5755 whereas w-BIC score was 0.785. Total length of ML tree (sum of branch lengths) were 2.5465 whereas sum of internal branch lengths were 1.2670 (49.7559% of tree length) (data not shown in Figure 1).

In this study, 60 Fusarium isolates (Table 3) included in the phylogenetic analyses were clustered in six different species complexes, of which 38.3% were F. oxysporum (FOSC), 20% F. fujikuroi (FFSC), 18.3% F. solani (FSSC), 13.3% F. incarnatum-equiseti (FIESC), 6.7% F. sambucinum (FSAMSC) and 3.3% F. albidum. The Fusarium species clustered into these species complexes distributed in 12 species (Figure 1): F. annulatum (11 isolates-18.3%), F. brachygibbosum (4 isolates-6.7%), F. clavum (7 isolates- 11.6%) F. curvatum (12 isolates-20%), F. falciforme (one isolate-1.6%), F. fredkrugeri (one isolate-1.6%), F. glycines (3 isolates-5.0%), F. nanum (one isolate-1.6%), F. nematophilum (2 isolates-3.3%), F. nirenbergiae (2 isolates-3.3%), F. solani (10 isolates-16.7%), and Fusarium sp. (6 isolates-10.0%).

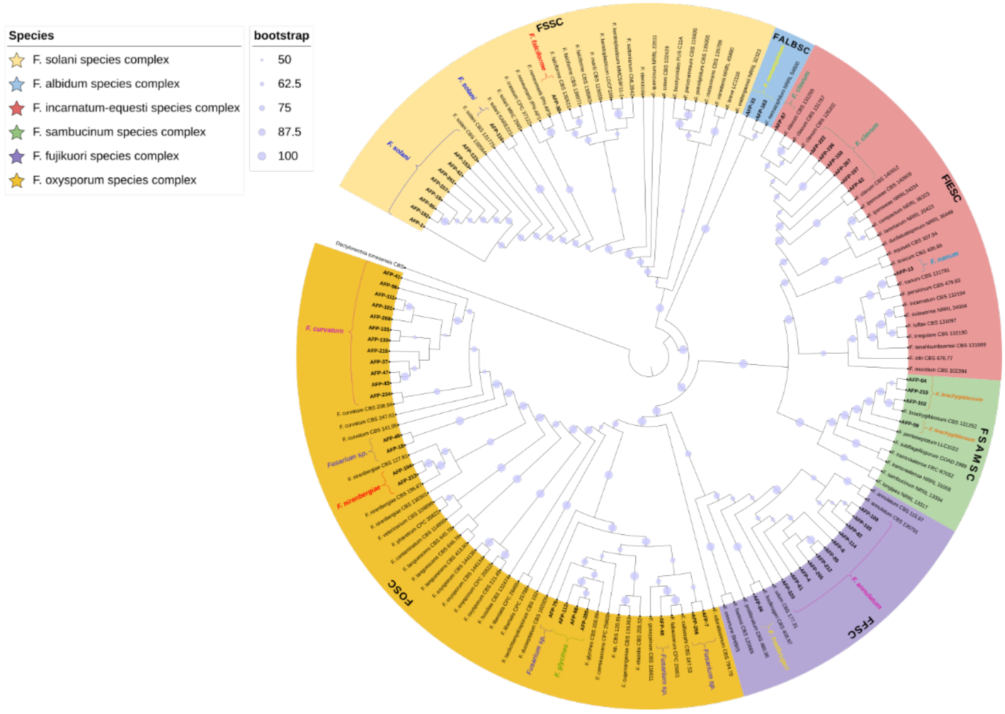

Figure 1. Multi gene (TEF1-α and RPB2) Maximum Likelihood tree of Fusarium isolates. Circles of different sizes show bootstrap support values from 1000 replicates are indicated at the nodes. Bootstrap values less than 50% are not shown. The bold characters represent the Turkish isolates. Dactylonectria torresensis CBS 129086 was used for rooting the ML tree.

Table 3. Location of surveyed grapevine nurseries, cultivars, and the species found with their TEF1-α and RPB2 gene sequence numbers.

Isolate	*Fusarium* Species	Location	Rootstock / Cultivar	GenBank Accession Numbers	
				TEF1-α	RPB2
AFP004	*Fusarium annulatum*	Bursa	1103 Paulsen	PP449277	PP449217
AFP006	"	Bursa	1103 Paulsen	PP449278	PP449218
AFP061	"	Manisa	Kober 5BB	PP449279	PP449219
AFP082	"	Tokat	1103 Paulsen	PP449280	PP449220
AFP085	"	Tokat	1103 Paulsen	PP449281	PP449221
AFP103	"	Manisa	Kober 5BB	PP449282	PP449222
AFP109	"	Manisa	110 Richter	PP449283	PP449223
AFP114	"	Manisa	Kober 5BB	PP449284	PP449224
AFP212	"	Tokat	1103 Paulsen	PP449285	PP449225
AFP265	"	Manisa	Sultana Seedless	PP449286	PP449226
AFP320	"	Tekirdağ	1103 Paulsen	PP449287	PP449227
AFP059	*Fusarium brachygibbosum*	Manisa	Kober 5BB	PP449288	PP449228
AFP064	"	Manisa	41B	PP449289	PP449229
AFP102	"	Manisa	Ramsey	PP449290	PP449230
AFP219	"	Şanlıurfa	1103 Paulsen	PP449291	PP449231
AFP062	*Fusarium clavum*	Manisa	Kober 5BB	PP449292	PP449232
AFP087	"	Tokat	1103 Paulsen	PP449293	PP449233
AFP107	"	Manisa	Ramsey	PP449294	PP449234
AFP150	"	Tokat	1103 Paulsen	PP449295	PP449235
AFP196	"	Manisa	Ramsey	PP449296	PP449236
AFP222	"	Tokat	1103 Paulsen	PP449297	PP449237
AFP267	"	Manisa	Sultana Seedless	PP449298	PP449238
AFP037	*Fusarium curvatum*	Denizli	140 Ruggeri	PP449299	PP449239
AFP041	"	Denizli	1103 Paulsen	PP449300	PP449240
AFP043	"	Denizli	140 Ruggeri	PP449301	PP449241
AFP047	"	Denizli	140 Ruggeri	PP449302	PP449242
AFP096	"	Manisa	Kober 5BB	PP449303	PP449243

AFP101	"	Manisa	Ramsey	PP449304	PP449244
AFP111	"	Manisa	110 Richter	PP449305	PP449245
AFP130	"	Mersin	1103 Paulsen	PP449306	PP449246
AFP191	"	Tokat	1103 Paulsen	PP449307	PP449247
AFP208	"	Tokat	1103 Paulsen	PP449308	PP449248
AFP215	"	Şanlıurfa	1103 Paulsen	PP449309	PP449249
AFP234	"	Şanlıurfa	1104 Paulsen	PP449310	PP449250
AFP038	*Fusarium falciforme*	Denizli	140 Ruggeri	PP449311	PP449251
AFP066	*Fusarium fredkrugeri*	Manisa	Kober 5BB	PP449312	PP449252
AFP098	*Fusarium glycines*	Manisa	Ramsey	PP449313	PP449253
AFP112	"	Manisa	110 Richter	PP449314	PP449254
AFP295	"	Manisa	Sultana Seedless	PP449315	PP449255
AFP013	*Fusarium nanum*	Mersin	140 Ruggeri	PP449316	PP449256
AFP033	*Fusarium nematophilum*	Manisa	Kober 5BB	PP449317	PP449257
AFP163	"	Tokat	1103 Paulsen	PP449318	PP449258
AFP194	*Fusarium nirenbergiae*	Manisa	Ramsey	PP449319	PP449259
AFP213	"	Tokat	1103 Paulsen	PP449320	PP449260
AFP001	*Fusarium solani*	Bursa	1103 Paulsen	PP449321	PP449261
AFP019	"	Manisa	1103 Paulsen	PP449322	PP449262
AFP042	"	Denizli	140 Ruggeri	PP449323	PP449263
AFP095	"	Manisa	Kober 5BB	PP449324	PP449264
AFP116	"	Manisa	Kober 5BB	PP449325	PP449265
AFP123	"	Mersin	1103 Paulsen	PP449326	PP449266
AFP153	"	Tokat	1103 Paulsen	PP449327	PP449267
AFP192	"	Manisa	Ramsey	PP449328	PP449268
AFP207	"	Mersin	1103 Paulsen	PP449329	PP449269
AFP261	"	Manisa	Sultana Seedless	PP449330	PP449270
AFP007	*Fusarium* sp.	Bursa	1103 Paulsen	PP449331	PP449271
AFP018	"	Manisa	1103 Paulsen	PP449332	PP449272
AFP040	"	Denizli	140 Ruggeri	PP449333	PP449273
AFP048	"	Denizli	1103 Paulsen	PP449334	PP449274
AFP075	"	Denizli	140 Ruggeri	PP449335	PP449275
AFP256	"	Manisa	Sultana Seedless	PP449336	PP449276

3.2. Pathogenicity of Fusarium isolates and species

In four-month pathogenicity tests, some Fusarium isolates inoculated on the plants' stems (considering the possibility of contamination to the vines through pruning wounds) produced lesions ranging from 5.9 to 12.0 mm (Figure 2). The lesion lengths of 10 of the 38 isolates inoculated on the plants in the first year were longer than those of the control and other isolates and statistically different.

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Figure 2. Wood necrosis in the stems of grapevine seedlings induced by Fusarium and Ilynonectria liriodendri after four months of inoculation. a) I. liriodendri, b) F. annulatum, c) F. brachygibbosum, d) F. nirenbergiae, e) F. curvatum, f) F. solani, g) F. glycines, h) F. falciforme, i) Non-inoculated control.

The majority of these isolates belonged to the following species: F. annulatum (seven isolates), F. brachygibbosum (one isolate), F. nirenbergiae (one isolate), and I. liriodendri. In the second year, only 9 isolates had lesions longer than other Fusarium isolates and control statistically. They were F. annulatum (five isolates), F. nirenbergiae (two isolates), F. curvatum (one isolate) and I. liriodendri (Table 4). In both years, lesions caused by other Fusarium isolates were not significantly longer than those of the control. However, these isolates could be re-isolated from the point of inoculation (except from the non-inoculated control) at rates ranging from 15.2% to 53.8%.

Table 4. Mean wood lesion lengths caused by Fusarium species in the inoculation points of 1103 Paulsen rootstock plants after four months.

Isolates	2022 Species	Lesion (mm)		Isolates	2023 Species	Lesion (mm)	
AFP006	*F. annulatum*	12.0	a*	AFP061	*F. annulatum*	8.9	a*
AFP114	*F. annulatum*	11.9	a	AFP115	*Ilyonectria liriodendri*	8.6	a
AFP109	*F. annulatum*	11.0	b	AFP213	*F. nirenbergiae*	8.4	a
AFP103	*F. annulatum*	10.9	bc	AFP103	*F. annulatum*	8.0	b
AFP059	*F. brachygibbosum*	10.4	bc	AFP265	*F. annulatum*	7.7	bc
AFP115	*Ilyonectria liriodendri*	10.3	c	AFP114	*F. annulatum*	7.5	c
AFP265	*F. annulatum*	7.9	d	AFP111	*F. curvatum*	7.5	c
AFP194	*F. nirenbergiae*	7.1	e	AFP194	*F. nirenbergiae*	7.5	c
AFP061	*F. annulatum*	6.9	e	AFP006	*F. annulatum*	6.3	d
AFP004	*F. annulatum*	6.9	e	AFP004	*F. annulatum*	5.9	de
AFP096	*F. curvatum*	5.3	f	AFP096	*F. curvatum*	5.2	e
AFP213	*F. nirenbergiae*	5.3	f	AFP109	*F. annulatum*	5.1	e
AFP256	*Fusarium sp.*	5.3	f	AFP059	*F. brachygibbosum*	5.1	e
AFP043	*F. curvatum*	5.2	f	AFP043	*F. curvatum*	5.1	e
AFP101	*F. curvatum*	5.2	f	AFP101	*F. curvatum*	5.0	e
AFP038	*F. falciforme*	5.2	f	AFP098	*F. glycines*	5.0	e
AFP040	*Fusarium* sp.	5.2	f	AFP019	*F. solani*	5.0	e
AFP013	*F. nanum*	5.2	f	AFP041	*F. curvatum*	5.0	e
AFP130	*F. curvatum*	5.1	f	AFP130	*F. curvatum*	5.0	e
AFP019	*F. solani*	5.1	f	AFP037	*F. curvatum*	5.0	e
AFP037	*F. curvatum*	5.1	f	AFP040	*Fusarium sp.*	5.0	e
AFP075	*Fusarium sp.*	5.1	f	AFP066	*F. fredkrugeri*	5.0	e
AFP095	*F. solani*	5.1	f	AFP123	*F. solani*	5.0	e
AFP111	*F. curvatum*	5.1	f	AFP256	*Fusarium sp.*	5.0	e
AFP191	*F. curvatum*	5.1	f	AFP191	*F. curvatum*	5.0	e
AFP041	*F. curvatum*	5.1	f	AFP038	*F. falciforme*	5.0	e
AFP001	*F. solani*	5.1	f	AFP001	*F. solani*	5.0	e
AFP007	*Fusarium sp.*	5.1	f	AFP018	*Fusarium sp.*	5.0	e
AFP222	*F. clavum*	5.1	f	AFP222	*F. clavum*	5.0	e
AFP261	*F. solani*	5.1	f	AFP261	*F. solani*	5.0	e
AFP066	*F. fredkrugeri*	5.1	f	AFP075	*Fusarium sp.*	5.0	e
AFP033	*F. nematophilum*	5.0	f	AFP033	*F. nematophilum*	5.0	e
AFP048	*Fusarium sp.*	5.0	f	AFP048	*Fusarium sp.*	5.0	e
AFP062	*F. clavum*	5.0	f	AFP062	*F. clavum*	5.0	e
AFP123	*F. solani*	5.0	f	AFP095	*F. solani*	5.0	e
AFP196	*F. clavum*	5.0	f	AFP196	*F. clavum*	5.0	e
AFP098	*F. glycines*	5.0	f	AFP013	*F. nanum*	5.0	e
AFP018	*Fusarium* sp.	5.0	f	AFP007	*Fusarium* sp.	5.0	e
Non-inoculated Control		5.0	f	Non-inoculated Control		5.0	e
LSD = 0.65				LSD = 0.92			

*Means accompanied by same letter are not significantly different (P = 0.05) according to LSD tests.

In the second type of pathogenicity test, 38 isolates increased basal rot and significantly reduced root dry weight compared to the non- inoculated control. Due to the large number of isolates and replicates, there was a large variance among the means of isolates, resulting in many statistical groups. The Fusarium isolates, and I. liriodenri caused basal rot of the wood tissues in the basal region of the seedlings (Figure 3), and their lengths ranged from 3.6 to 37.0 mm in the first year and from 3.7 to 7.8 mm in the second year (Table 5). As in the case of stem necrosis, F. annulatum, F. curvatum, and I. liriodendri species were found to cause the most extended necrose length in basal rot formation. However, the wood necrosis induced by the other species was not as severe and consistent as that of these three species. The Fusarium isolates and I. liriodendri could be re-isolated from the basal necroses (except from the non-inoculated control) at rates ranging from 10.3% to 36.8%.

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Figure 3. Basal wood necrosis in grapevine seedlings induced by Fusarium and Ilynonectria liriodendri after four months of inoculation. a) I. liriodendri, b) F. annulatum, c) F. nirenbergiae, d) F. curvatum, e) F. glycines, f) F. solani, g) F. fredkrugeri, h) F. falciforme, i) Non-inoculated control.

Table 5. Mean basal necrose lengths caused by *Fusarium* species in 1103 Paulsen rootstock plants after four months.

Isolates	2022 Fungal Species	Basal Necrosis (mm)		Isolates	2023 Fungal Species	Basal Necrosis (mm)	
AFP061	*F. annulatum*	37.0	a	AFP115	*Ilyonectria liriodendri*	7.8	a
AFP103	*F. annulatum*	34.6	ab	AFP004	*F. annulatum*	6.2	ab
AFP114	*F. annulatum*	30.8	a-c	AFP101	*F. curvatum*	6.2	ab
AFP041	*F. curvatum*	29.8	a-d	AFP194	*F. nirenbergiae*	6.2	ab
AFP115	*Ilyonectria liriodendri*	29.8	a-d	AFP103	*F. annulatum*	6.0	a-c
AFP004	*F. annulatum*	28.4	a-e	AFP111	*F. curvatum*	6.0	a-c
AFP109	*F. annulatum*	27.6	b-f	AFP114	*F. annulatum*	5.8	b-d
AFP098	*F. glycines*	27.4	b-f	AFP213	*F. nirenbergiae*	5.8	b-d
AFP006	*F. annulatum*	26.8	b-g	AFP037	*F. curvatum*	5.6	b-e
AFP265	*F. annulatum*	26.4	b-g	AFP109	*F. annulatum*	5.6	b-e
AFP019	*F. solani*	25.2	c-h	AFP256	*Fusarium sp.*	5.4	b-f
AFP111	*F. curvatum*	23.8	c-i	AFP006	*F. annulatum*	5.2	b-f
AFP018	*Fusarium sp.*	23.2	c-i	AFP013	*F. nanum*	5.2	b-f
AFP256	*Fusarium sp.*	22.8	c-i	AFP038	*F. falciforme*	5.2	b-f

AFP037	*F. curvatum*	21.2	d-j	AFP048	*Fusarium sp.*	5.2	b-f
AFP066	*F. fredkrugeri*	21.2	d-j	AFP061	*F. annulatum*	5.2	b-f
AFP075	*Fusarium sp.*	19.8	d-j	AFP191	*F. curvatum*	5.2	b-f
AFP096	*F. curvatum*	19.0	f-k	AFP040	*Fusarium sp.*	5.0	b-f
AFP123	*F. solani*	17.8	g-l	AFP098	*F. glycines*	5.0	b-f
AFP101	*F. curvatum*	17.0	h-m	AFP265	*F. annulatum*	5.0	b-f
AFP043	*F. curvatum*	16.0	i-m	AFP075	*Fusarium sp.*	4.8	b-g
AFP007	*Fusarium sp.*	15.4	i-n	AFP123	*F. solani*	4.8	b-g
AFP038	*F. falciforme*	15.4	i-n	AFP001	*F. solani*	4.6	b-g
AFP130	*F. curvatum*	15.0	i-n	AFP019	*F. solani*	4.6	b-g
AFP261	*F. solani*	15.0	i-n	AFP041	*F. curvatum*	4.6	b-g
AFP191	*F. curvatum*	13.6	j-o	AFP043	*F. curvatum*	4.6	b-g
AFP194	*F. nirenbergiae*	12.8	j-o	AFP222	*F. clavum*	4.6	b-g
AFP059	*F. brachygibbosum*	12.4	j-p	AFP007	*Fusarium sp.*	4.4	b-g
AFP213	*F. nirenbergiae*	12.2	j-p	AFP066	*F. fredkrugeri*	4.4	b-g
AFP095	*F. solani*	10.4	k-q	AFP096	*F. curvatum*	4.4	b-g
AFP033	*F. nematophilum*	10.2	k-q	AFP130	*F. curvatum*	4.2	c-g
AFP040	*Fusarium sp.*	9.8	l-q	AFP018	*Fusarium sp.*	4.0	d-g
AFP001	*F. solani*	8.6	m-q	AFP095	*F. solani*	4.0	d-g
AFP196	*F. clavum*	8.4	m-q	AFP261	*F. solani*	4.0	d-g
AFP062	*F. clavum*	6.6	n-q	AFP033	*F. nematophilum*	3.8	e-g
AFP048	*Fusarium sp.*	6.4	n-q	AFP062	*F. clavum*	3.8	e-g
AFP222	*F. clavum*	5.2	o-q	AFP196	*F. clavum*	3.8	e-g
AFP013	*F. nanum*	3.6	p-q	AFP059	*F. brachygibbosum*	3.7	f-g
Non-inoculated Control		2.6	q	Non-inoculated Control		3.0	g
LSD = 9.06				LSD = 1.87			

*Means accompanied by same letter are not significantly different (P = 0.05) according to LSD tests.

In parallel to basal rot, the Fusarium isolates and I. liriodendri decreased hair root formation and root dry weight in the inoculated plants compared with the non-inoculated control plants. In 30-35% of the plants inoculated with F. annulatum, F. curvatum, and I. liriodendri, shoots dried up, and plants died after one month. The average root dry weight recorded per plant in the first year varied between 0.022 and 0.344 g, while in the second year, these values were recorded between 0.599 and 1.463 g (Table 6).

Table 6. Mean root dry weights of 1103 Paulsen rootstock plants inoculated with Fusarium species after four months.

Isolates	2022 Species	Root Dry Weight (g)		Isolates	2023 Species	Root Dry Weight (g)	
Non-inoculated Control		0.344	a*	Non-inoculated Control		1.463	a
AFP041	*F. curvatum*	0.224	b	AFP222	*F. clavum*	1.436	b
AFP033	*F. nematophilum*	0.196	c	AFP048	*Fusarium* sp.	1.425	c
AFP256	*Fusarium* sp.	0.182	d	AFP062	*F. clavum*	1.417	d
AFP123	*F. solani*	0.150	e	AFP013	*F. nanum*	1.410	e
AFP098	*F. glycines*	0.146	f	AFP101	*F. curvatum*	1.399	f
AFP018	*Fusarium* sp.	0.134	g	AFP033	*F. nematophilum*	1.369	g
AFP062	*F. clavum*	0.125	h	AFP075	*Fusarium* sp.	1.353	h
AFP261	*F. solani*	0.125	h	AFP261	*F. solani*	1.349	i
AFP048	*Fusarium* sp.	0.119	i	AFP038	*F. falciforme*	1.336	j
AFP013	*F. nanum*	0.112	j	AFP001	*F. solani*	1.327	k
AFP130	*F. curvatum*	0.111	j	AFP095	*F. solani*	1.286	l
AFP196	*F. clavum*	0.109	jk	AFP007	*Fusarium* sp.	1.282	m
AFP019	*F. solani*	0.108	k	AFP019	*F. solani*	1.266	n
AFP109	*F. annulatum*	0.104	l	AFP040	*Fusarium* sp.	1.251	o
AFP222	*F. clavum*	0.103	l	AFP059	*F. brachygibbosum*	1.245	p
AFP038	*F. falciforme*	0.098	m	AFP098	*F. glycines*	1.232	q
AFP004	*F. annulatum*	0.087	n	AFP256	*Fusarium* sp.	1.231	q
AFP001	*F. solani*	0.083	o	AFP066	*F. fredkrugeri*	1.181	r
AFP040	*Fusarium* sp.	0.082	o	AFP191	*F. curvatum*	1.172	s

doi:10.20944/preprints202405.1204.v1

AFP007	*Fusarium* sp.	0.078	p	AFP103	*F. annulatum*	1.160	t
AFP194	*F. nirenbergiae*	0.072	q	AFP018	*Fusarium* sp.	1.118	u
AFP096	*F. curvatum*	0.070	q	AFP196	*F. clavum*	1.108	v
AFP213	*F. nirenbergiae*	0.065	r	AFP096	*F. curvatum*	1.101	w
AFP043	*F. curvatum*	0.063	r	AFP006	*F. annulatum*	1.098	x
AFP114	*F. annulatum*	0.058	s	AFP213	*F. nirenbergiae*	1.069	y
AFP059	*F. brachygibbosum*	0.054	t	AFP043	*F. curvatum*	1.056	z
AFP101	*F. curvatum*	0.054	t	AFP115	*Ilyonectria liriodendri*	1.047	a1
AFP103	*F. annulatum*	0.051	tu	AFP123	*F. solani*	1.026	b1
AFP006	*F. annulatum*	0.050	u	AFP111	*F. curvatum*	1.016	c1
AFP075	*Fusarium* sp.	0.049	uv	AFP109	*F. annulatum*	1.012	d1
AFP095	*F. solani*	0.047	vw	AFP037	*F. curvatum*	0.974	e1
AFP037	*F. curvatum*	0.045	wx	AFP041	*F. curvatum*	0.921	f1
AFP111	*F. curvatum*	0.044	x	AFP130	*F. curvatum*	0.919	f1
AFP115	*Ilyonectria liriodendri*	0.042	xy	AFP194	*F. nirenbergiae*	0.919	f1
AFP066	*F. fredkrugeri*	0.040	yz	AFP114	*F. annulatum*	0.901	g1
AFP191	*F. curvatum*	0.038	z	AFP004	*F. annulatum*	0.893	h1
AFP265	*F. annulatum*	0.026	a1	AFP265	*F. annulatum*	0.880	i1
AFP061	*F. annulatum*	0.022	b1	AFP061	*F. annulatum*	0.599	j1
LSD =	0.002			LSD =	0.003		

*Means accompanied by same letter are not significantly different (P = 0.05) according to LSD tests.

The average values (necrose lengths in stem and basal part, and dry root weights) of the isolates (belonging to the same species) were considered, and analysis of variance was performed to the means to clarify the pathogenicity of each Fusarium species. When the pathogenicity of Fusarium species was evaluated according to the length of necrosis at the inoculation point, it was found that F. annulatum, F. brachygibbosum, F. nirenbergiae and Ilyonectria liriodendri caused wood necrosis. In contrast, the others did not show the same influence (Figure 4). Regarding the effects of Fusarium species on basal rot formation, it was determined that the three species causing the most extended necrosis in the first year were F. annulatum, I. liriodendri, and F. glycines; in the second year, I. liriodendri, F. nirenbergiae, and F. annulatum, respectively (Figure 5).

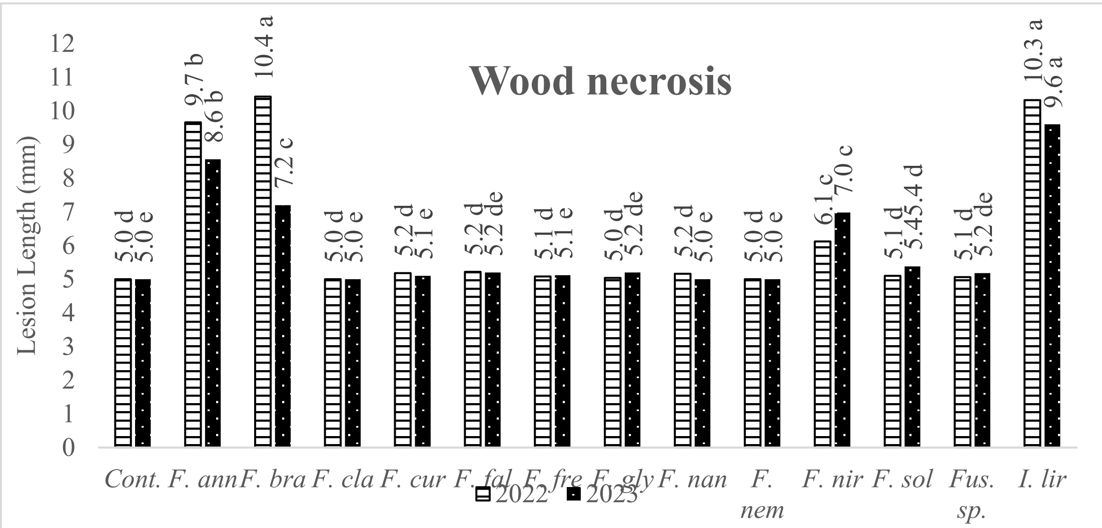

Figure 4. The average lesion lengths in the wood tissues of grapevine seedlings (1103 Paulsen rootstock) induced by Fusarium species and I. liriodendri.

 doi:10.20944/preprints202405.1204.v1

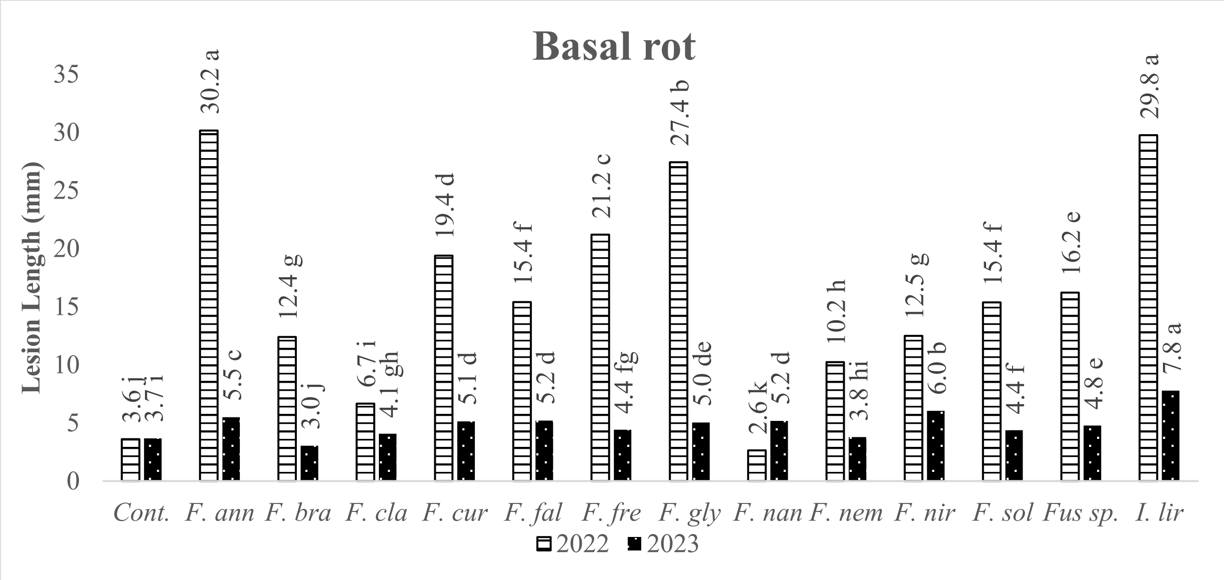

Figure 5. The average lesion lengths in the basal wood tissues of grapevine seedlings (1103 Paulsen rootstock) induced by Fusarium species and I. liriodendri.

The effect of Fusarium species on root dry weight reduction was almost parallel to basal rot; when the results of both years were generally evaluated, F. annulatum, I. liriodendri, and F. nirenbergiae were found to be the most effective species (Figure 6).

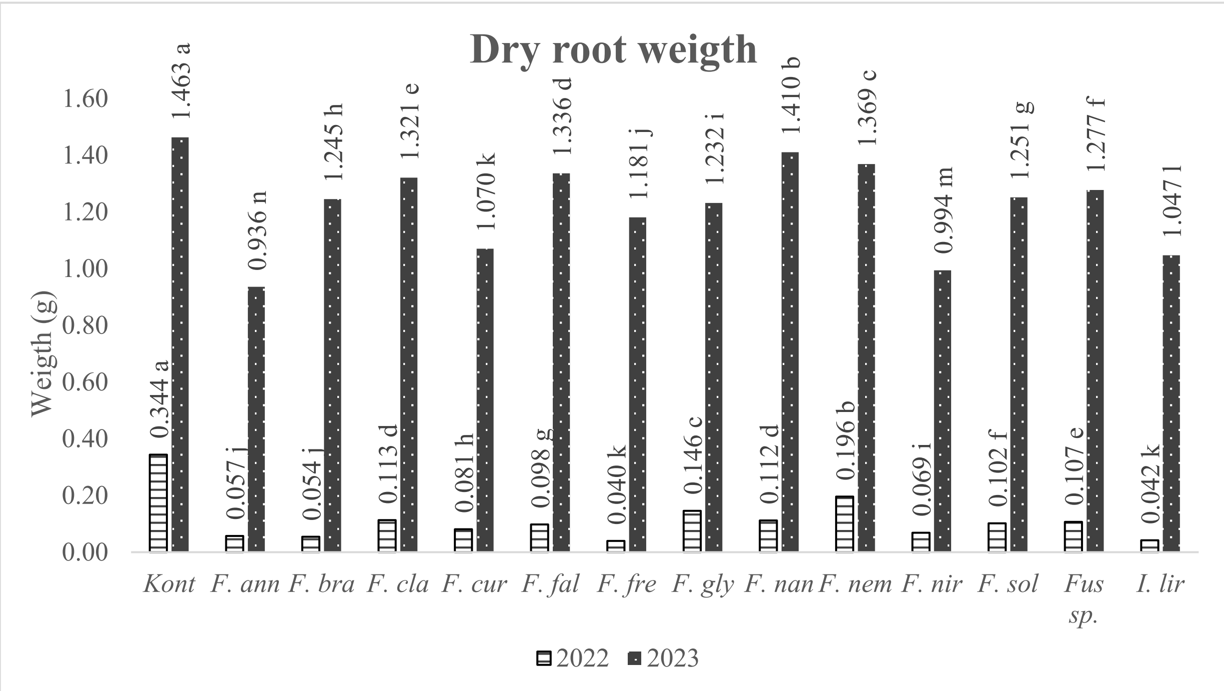

Figure 6. The average dry root weight of grapevine seedlings (1103 Paulsen rootstock) induced by Fusarium species and I. liriodendri.

4. Discussion

In this study, *Fusarium* fungi were found to be relatively common (95.3% of the nurseries) in bare-rooted plants ready for sale in Turkish grapevine nurseries. This rate is considerably higher than that found in North America and Canada, but it is close to that in the nurseries in France and Spain. Garnett et al. [24] investigated the fungal species associated with root rot of grapevines in two different vineyards in California and isolated a high proportion of *Fusarium* species (together with

 doi:10.20944/preprints202405.1204.v1

Rhizoctonia, *Pythium*, *Macrophomina*, *Phytophthora* fungi) in the sampled vines. Torres et al. [10] found *Fusarium* species in 43.9% of the seedlings ready for sale in four grapevine nurseries in Canada and determined that these species were isolated from the plants between 20.0 and 86.7%. Bustamente et al. [11] determined that the incidence of *Fusarium* species was 36.7% in young vineyards and 31.7% in nursery plants in California. However, Pintos et al., [25] detected 92% to 98% of *Fusarium* fungi, among other GTD pathogens, from plants sampled from two commercial grapevine nurseries in Spain and one in France.

In the current study, 12 distinct *Fusarium* species were found in six *Fusarium* species complexes in the grapevine nurseries, with the most common species complexes being *F. oxysporum* (38.3%), *F. fujikuroi* (20.0%), and *F. solani* (18.3%). The results revealed more diversity of *Fusarium* species than previous studies conducted in Canada and United States. Urbez-Torres et al. [10] reported that *Fusarium* species diversity was very low in four nurseries in British Columbia (Canada) and found only two species from two different species complexes (*F. oxysporum* and *F. fujikuroi*). However, Bustamente et al. [11] reported a high diversity of *Fusarium* in young vines in California (total, nine *Fusarium* species in six species complexes) and found five species (*F. annulatum, Fusarium* sp., F. *solani, F. keratoplasticum, F. nirenbergiae*) belonging to these complexes in the nurseries. The fungal isolation results in our study, the high *Fusarium* species diversity in ready-to-sale grapevine seedlings, and the presence of joint species *(F. annulatum, F. brachygibbosum, F. clavum, F. nirenbergiae, F. solani*) in the plants were consistent with the findings of Bustamente et al. [11]. However, in the abovementioned studies, *F. avenaceum, F. ramigenum, F. culmorum, F. keratoplasticum, F. oxysporum*, and *F. proliferatum* were not found in grapevine nurseries in Türkiye. Interestingly, although *F. oxysporum* is a large species complex, including 21 species [26], and *F. oxysporum* has an essential place in this complex, we could not detect *F. oxysporum* among the *Fusarium* species we isolated from vines. When phylogenetic analyses were performed, it was revealed that many isolates similar to this species were *F. curvatum, F. glycines*, and *F. nirenbergiae*. Similarly, although *F. proliferatum* has been reported as an important root rot pathogen in maize, soybean, tomato, and grapevine [10,27–29], we could not detect *F. proliferatum* among the available grapevine *Fusarium* isolates. These differences may have been made possible by detailed phylogenetic analyses using concatenated genes such as *TEF1-α* and *RPB2*, which are highly recommended to identify *Fusarium*. O'Donnell et al. [4] suggested that when identifying *Fusarium* species, the *TEF1α* and *RPB2* gene regions should be amplified and concatenated to perform phylogenetic analyses if possible, and in case of financial limitations, the sequence of the *TEF1α* region might be sufficient.

Based on the pathogenicity results of *Fusarium* isolates inoculated on grapevine stems, *F. annulatum, F. brachygibbosum, F. curvatum*, and *F. nirenbergiae* were found to produce more considerable wood necrosis in comparison to the control and other *Fusarium* species. Reveglia et al. [7] widely isolated *F. oxysporum* isolates from grapevines showing young vine decline symptoms (in Italy) and investigated their phytotoxic metabolites. The fusaric acid purified from these isolates caused severe necrosis when injected into tobacco leaves, and they suggested that this metabolite may also cause root and basal rot in grapevines. Akgül and Ahioğlu [14] inoculated *F. brachygibbosum* isolates (obtained from three-year-old young grapevines) on the stems of grapevine seedlings and determined it to be a highly virulent species in woody tissues. Rajput et al. [30] investigated the pathogenicity of *F. equiseti* isolates isolated from the trunks of grapevines in the Kunar province of Afghanistan and reported that tissue necrosis occurred when this species was inoculated on woody shoots of three-year-old plants. Bustamente et al. [11] inoculated *F. annulatum, F. nirenbergiae*, and *F. solani* isolates from young grapevines on the stems of one-year-old vines and revealed that after seven months; these species produced longer necroses in the wood tissues of plants when compared to non-inoculated controls. The results of these studies support the view that the wounds occurring via disbudding of cuttings or basal cuts in the seedlings or wounds by removing vine suckers on trunks (in the vineyards) may be susceptible to *Fusarium* infections and that *Fusarium* species may be involved in young vine decline or trunk diseases.

Another outcome from the pathogenicity tests was that some *Fusarium* species (*F. annulatum, F. curvatum, F. nirenbergiae, F. solani*) significantly increased basal rot and reduced root biomass in the inoculated plants. Highet and Nair [7] investigated the effect of *Fusarium oxysporum* infections on root rot development in grapevines (cv. Semillon 5-25 years old) in New Zealand. When plants were inoculated with *F. oxysporum*, they observed the disintegration of bark cells (by transmission electron microscopy) and determined that *Fusarium*-infected root cells lacked cytoplasm compared to uninfected cells. Vilvert et al. [9] stated that *F. oxysporum* f.sp. *herbemontis* was an important fungal pathogen in Brazilian grapevine nurseries, causing basal rot, reduction in root biomass, and root rot symptoms in infected vines. Zhang et al. [13] detected several *Fusarium* species from young grapevines (cv. Red Globe) showing decline and leaf yellowing in vineyards in Beijing, China and found that *F. commune* was pathogenic among these species and associated with these symptoms. Li et al. [12] revealed that *Fusarium* isolates inoculated on grapevine seedlings caused not only a reduction in root biomass, root rot but also interveinal discolorations and coalescent necrosis on the leaves of the plant. When these isolates were inoculated together with *Dactylonectria macrodidyma*, the severity of the disease was further increased.

Regarding the pathogenicity of other *Fusarium* species, *F. annulatum* has also been reported to cause fruit-corm and root rot in crop plants such as melon and onion, as well as medicinal-aromatic plants such as *Blettila striata* L. and saffron, in addition to grapevine. [31–34]. These studies indicate that *F. annulatum* is pathogenic in many hosts. *F. nirenbergiae* and *F. curvatum* were other virulent species in the pathogenicity tests on the grapevine seedlings. When we reviewed the studies on this subject, we found only one study [11] in which *F. nirenbergiae* was detected as a pathogen in grapevine. However, in other studies, it has been reported to be pathogenic in crop plants such as maize, passion fruit, almond (in Portugal and Spain), and maple. Sanna et al. [29] investigated *Fusarium* species associated with post-emergence damping-off and root rot in maize and found that *F. nirenbergiae* caused a disease index of over 50% in some maize areas of Italy, as did *F. verticilloides, F. annulatum* and *F. commune*. Aiello et al. [35] identified *F. nirenbergiae* as the cause of root rot and wilt in passion fruit plants. Zhao et al. [36] identified it as the cause of wilt in maple trees (in China), and Moral-Lopez et al., [37] in almonds (in Portugal and Spain). Another virulent species, *F. curvatum*, which we identified in pathogenicity tests, has previously been found to be associated with dieback disease in *Dendrobium officinale* [38] and leaf spot of cherry [39] in China.

In this study, although other *Fusarium* species (*F. clavum, F. falciforme, F. fredkrugeri, F. glycines, F. nanum, F. nematophilum, F. solani*) from Turkish grapevine nurseries reduced root biomass in grapevine seedlings compared to the control, their virulence was not as consistent as *F. annulatum, F. brachygibbosum, F. curvatum*, and *F. nirenbergiae*. These results support the view that other species may be present in grapevine seedlings as endophytic or opportunistic parasites. Some studies also suggest that *Fusarium* species provide various benefits to plants by enhancing plant growth, and triggering production of secondary metabolites [40]. The rhizosphere of plants contains diverse microbial communities, such as actinomycetes, bacteria, fungi, and protozoa, which interact with plant roots and each other. Climatic conditions, soil texture, chemistry, and the plant species or cultivars may closely influence the formation of these communities in the rhizosphere [41]. These factors may affect the resistance of plants to pathogens and may also play a role in the transition of *Fusarium* species, which were found to be opportunistic in this study, to the pathogenic form. Pathogenic *Fusarium* species should be considered in grapevine nurseries, and various biological and chemical control possibilities should be investigated in the future.

Author Contributions: Davut Soner AKGÜL designed this study, archived the fungal isolates, performed statistical analyses, molecular studies and pathogenicity tests, wrote the paper. Serkan ÖNDER identified the species performing phylogenetic analyses and obtained GenBank accession numbers from NCBI. Nurdan GÜNGÖR SAVAŞ, Murat YILDIZ, İzzet BÜLBÜL and Mümine ÖZRASLANDAN surveyed the grapevine nurseries, obtained-archived the fungal isolates. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Çukurova University, Scientific Research Projects Department in project FBA-2021-13533.

Acknowledgments: The authors thank The Rectorate of Çukurova University for its financial contribution. Additionally, we thank Assoc. Prof. Adem Yağcı (Tokat Gazi Osman Paşa University), Dr. Yüksel Savaş, Metin Kesgin (Manisa Viticulture Research Institute), Kürşat Alp Arslan (Pistachio Research Institute), Mehmet Ali Kiracı (Tekirdağ Viticulture Research Institute), and Assoc. Prof. Arif Atak (Bursa Uludağ University) for their valuable contributions to this study.

Conflicts of Interest: The authors declare no conflict of interest.

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