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Fusarium species and assessments of mycotoxin (deoxynivalenol), in wheat seeds from different regions of Türkiye

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Summary. Wheat cultivation is important in Turkish agriculture, which ranks 10th among international wheat producers, and is an important wheat exporter, particularly to Europe. *Fusarium*-related threats, such as Fusarium Head Blight (FHB) and Fusarium Crown and Root Rots (FCR, FRR), and related mycotoxin seed contamination, jeopardize product quality. This study analysed 65 wheat seed samples for presence of *Fusarium* species, from cultivars of *Triticum aestivum* (bread wheat) and *T. durum* (durum wheat) collected from seven regions of Türkiye. PCR with specific primers, and phylogenetic analyses of TEF1-*α* segments, discriminated *Fusarium* species. Levels of the mycotoxin deoxynivalenol (DON) in flour samples were also evaluated. Out of 195 *Fusarium* isolates, the prominent species included *F. graminearum* (32% of isolates), *F. proliferatum* (16%), *F. avenaceum* (11%), *F. clavum* (11%), and *F. verticillioides* (7%). Less frequently isolated species were *F. oxysporum* (6%), *F. acuminatum* (3%), *F. ramigenum* (3%), *F. culmorum* (3%), *F. poae* (2%), *F. sambucinum* (2%), *F. tricinctum* (2%), *Fusarium* sp. FTSC12 (2%), *F. andiyazi* (1%), and *F. equiseti*, *F. incarnatum*, and *F. fasciculatum* (each 0.5%). Five of the 65 samples tested positive for DON, with two exceeding the European Commission threshold for mycotoxin contamination; one bread wheat from the Black Sea region, known for its annual rainfall, and a durum wheat sample from southeastern Anatolia, which had the highest detected DON level of 1730 μg kg^{-1}. Among these samples *F. graminearum* was the predominant species. As *F. andiyazi* and *F. ramigenum* are not normally associated with wheat plants, a pathogenicity test was conducted with two isolates of each of these species, revealing no pathogenicity on the durum wheat cultivar 'San Carlo'. These results provide a basis for managing fungal threats and mycotoxin contamination, safeguarding the quality of wheat grain as an essential agricultural product.

Keywords. Species complex, DON, wheat kernels, molecular characterisation, phylogenetic analyses.

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INTRODUCTION

The wheat species *Triticum aestivum* L. and *Triticum durum* Desf. are important cereals produced in Türkiye, where wheat production in 2021 was 17,650,000 tonnes from 6,623,061 ha, placing the country as 10th among world producers (FAOSTAT, 2021). Wheat production in Türkiye is 80% for food, 11% for feed, and 6% for seed, and wheat is produced across all regions of Türkiye, with most in Central Anatolia, Marmara, and Southeastern Anatolia regions (TÜİK, 2021). In this country, 82% of wheat production is from 495 varieties of bread wheat, and 128 high-quality durum wheat varieties are registered or approved for cultivation (BÜGEM, 2021; TÜİK, 2021).

The diseases Fusarium Head Blight (FHB) and Fusarium Crown/Root Rots (FCR, FRR), caused by different *Fusarium* species, are among major contributors to large international economic losses in wheat production (Savary *et al*., 2019). FCR and FRR cause pre- and post-emergence death of wheat seedlings, particularly in areas with dry weather and minimal tillage. In contrast, FHB reduces grain yield and quality by generating small, weak, and poor-quality seeds, and hollow, white spikes that occur during anthesis under humid conditions (Smiley *et al*., 2005; Scherm *et al*., 2013). Seeds obtained from diseased plants have reduced viability, are smaller than those from healthy plants, are wrinkled and pale, and occasionally have black spots on the surfaces (Busman *et al*., 2012; Shah *et al*., 2018; Hassani *et al*., 2019).

FHB, FCR and FRR are usually caused by multiple *Fusarium* species complex groups. *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum* are the predominant species in wheat production areas (Shikur Gebremariam, 2015; Zhou *et al*., 2019; Abdallah-Nekache *et al*., 2019; Dehghanpour-Farashah *et al*., 2019; Rigorth *et al*., 2021; Leyva-Mir *et al*., 2022). *Fusarium acuminatum*, *F. avenaceum*, and *F. tricinctum*, in the *Fusarium tricinctum* species complex (FTSC), are emerging as wheat pathogens in Europe, especially related to FHB (Senatore *et al*., 2021). These species are difficult to distinguish morphologically, and require use of molecular identification techniques for discrimination. *Fusarium* sp. FTSC 1 to FTSC 11, *F. flocciferum*, *F. torolosum*, *F. iranicum* and *F. gamsii* are often reported on wheat, although they have minor impacts on production (O'Donnell *et al*., 2018; Summerell, 2019; Torbati *et al*., 2019).

Fusarium species can occur singly or coexist in the different organs of wheat plants, varying from year to year as different pathogens dominate. Identification of *Fusarium* species in wheat seeds is important, as these pathogens can disseminate across regions or countries. This is facilitated by use of seeds for crop establishment, and can cause diseases with far-reaching consequences.

Fusarium species are also responsible for production and accumulation of mycotoxins in wheat grains (Isebaert *et al*., 2009). The most important mycotoxins in wheat are trichothecenes and zearalenone, including deoxynivalenol (DON), in the trichothecenes B group and which is synthesized by several *Fusarium* species (Xu and Nicholson, 2009; Mishra *et al*., 2019; Wang *et al*., 2019; Mousavi Khaneghah *et al*., 2020). DON has harmful effects on human and animal health, including oxidative and endoplasmic reticulum stress, ribosomal toxicity, hepatotoxicity, immunotoxicity, gastrointestinal toxicity, and neurotoxicity (Tian *et al*., 2022). This mycotoxin is stable and heat-resistant, making it difficult to prevent DON contamination in food products for humans and animals (Guo *et al*., 2020). European Commission Regulation (EU) 2024/1022 of 8th April 2024, amending Regulation (EU) 2023/915 on maximum levels of deoxynivalenol (DON) in foodstuffs, stipulates that the maximum limits for DON in unprocessed wheat as 1000 μg kg^{-1} for bread wheat and 1500 μg kg^{-1} for durum wheat. Considering the prevalence of DON-producing *F. graminearum* and *F. culmorum* in European wheat crops, DON has emerged as the dominant mycotoxin associated with FHB in European food and feed wheat (Waalwijk *et al*., 2003; Gruber-Dorninger *et al*., 2019; Eskola *et al*., 2020). Species such as *F. pseudograminearum* and *F. cerealis* (syn. *F. crookwellence*) have also been reported on wheat as DON producers (Ji *et al*., 2019; Haidukowski *et al*., 2022). In Türkiye, changes in climatic conditions between regions have promoted the spread of several foodborne mycotoxins, especially in cereal crops and cereal products; based on a literature review, Ünüsan (2019) showed that mycotoxin levels exceeding EU limits had been found in 2% of cereals and their derivatives.

In recent years, global climate changes have been altering the profiles of typical *Fusarium* species on wheat, which may also cause variability of associated mycotoxins. Vigilant monitoring and differentiation of *Fusarium* species, along with the associated mycotoxins in wheat, are therefore important. This proactive approach is required for early-stage assessment of mycotoxin risks, enabling formulation and implementation of effective control strategies (Tunali *et al*., 2008; Haidukowski *et al*., 2022). In Türkiye, which is a leading bread and durum wheat producing country, identification of *Fusarium* species in wheat seeds may affect production quality and sanitation.

The main purpose of the present study was to collect samples of bread and durum wheat seed cultivars from

across the Turkish regions, to assess by molecular characterization the contaminating *Fusarium* species, and to determine their DON production. This information would provide a starting point for implementing management strategies for wheat diseases and mycotoxin contamination caused by *Fusarium* in Türkiye, and for regulating DON through increased understanding of *Fusarium* populations in wheat seeds grown in Türkiye.

MATERIALS AND METHODS

Investigation areas, wheat sampling and fungal isolation

In 2020, 2021, and 2022 a total of 65 samples of wheat seeds (250 g each) were obtained of which 45 were of bread wheat and 20 of durum wheat. The samples represented the different wheat production in Türkiye (Figure 1). One hundred seeds from each sample, randomly selected out of the 250 g, were disinfected in a sodium hypochlorite solution for 4 min and then rinsed twice in sterile water. For each sample, ten Petri plates containing Potato Dextrose Agar (PDA, Biolife Italiana) supplemented with streptomycin sulfate (0.16 g L^{-1}; Sigma Aldrich) were prepared with ten seeds added to each plate. The cultures were then incubated at 24°C in the dark, and fungal growth was observed from the 5th day of incubation. Colonies resembling *Fusarium* species, according to morphological characteristics on PDA (Leslie and Summerell, 2006), were selected and each transferred to a new PDA plate and then incubated for 7 d at 22°C in darkness. A single conidium procedure (Leslie and Summerell, 2006) was used to ensure reliable isolate characterization. Incidence of *Fusarium* colonies was determined by calculating the percentage relative to the total number of seeds from which the isolations were made. Morphologically identified isolates of *F. graminearum*, *F. culmorum*, *F. proliferatum*, *F. verticillioides* and *F. poae* were subjected to molecular analyses with species-specific primers, while colonies belonging to the *Fusarium tricinctum* species complex (FTSC) and *Fusarium incarnatum-equiseti* species complex (FIESC) were subjected to phylogenetic analyses with the TEF1-α gene.

PCR-based diagnoses with specific primers, and phylogenetic analyses of FIESC and FTSC groups

Pure cultures of representative colonies displaying morphological characteristics of *F. graminearum*, *F. culmorum*, *F. proliferatum*, *F. verticillioides* or *F. poae* were transferred onto fresh PDA and were then incubated at 22°C for 1 week to obtain enough mycelium for genomic DNA isolation using the protocol described by Prodi *et*

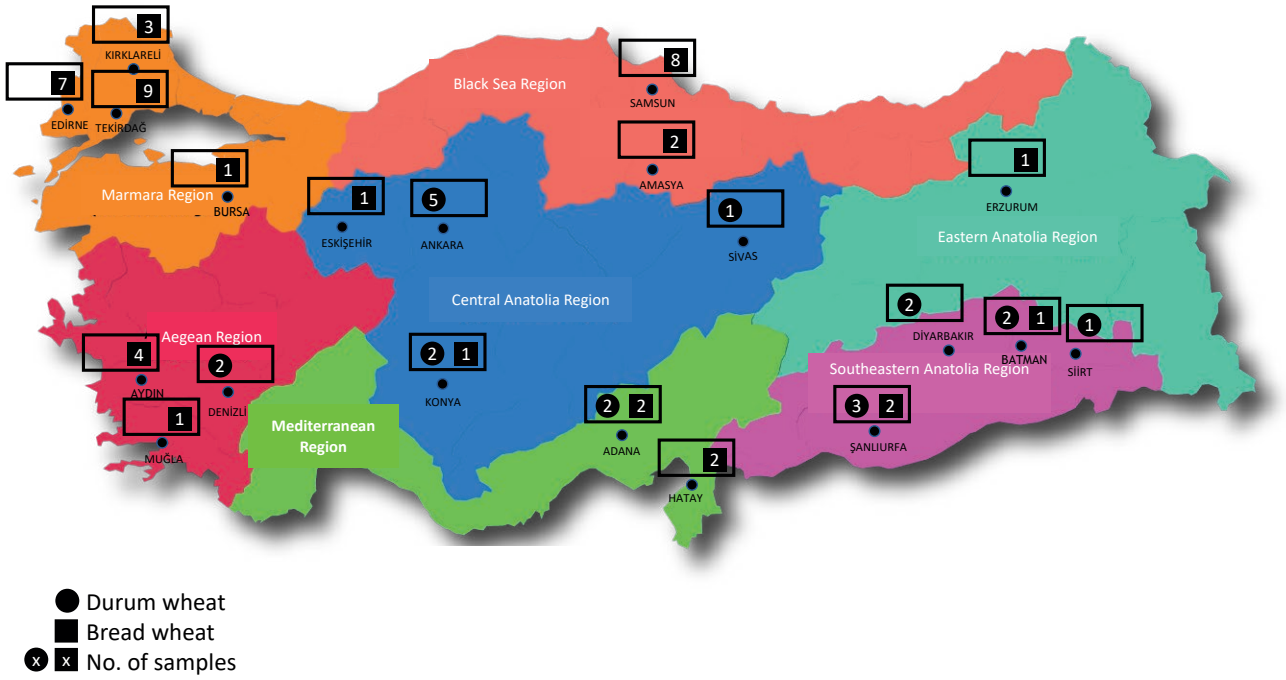

Figure 1. Map of Türkiye showing the regions from which samples were collected for this study.

Table 1. Data on the *Fusarium* isolates obtained from wheat seed samples in 2020, 2021, and 2022, and the corresponding quantities of DON mycotoxin detected in each sample.

Sample No.	Wheat cultivar	Wheat type	Turkish provinces	Region	Year	Isolate code	*Fusarium* species	DON (μg kg^{-1})
1	Kızıltan-91	Durum wheat	Denizli	Aegean	2022	1311a	*F. ramigenum*	20
						1312a	*F. culmorum*	<LOD
						1312b	*F. culmorum*	
						1312c	*F. graminearum*	
						1312d	*F. graminearum*	
2	Çeşit-1252	Durum wheat	Denizli	Aegean	2022	-	-	0
3	Cumhuriyet-75	Bread wheat	Muğla	Aegean	2020	281	*F. proliferatum*	0
						277a	*F. oxysporum*	
						277b	*F. oxysporum*	
						278	*F. oxysporum*	
4	Masaccio	Bread wheat	Aydın	Aegean	2021	982a**	*F. incarnatum*	0
5	Sagittario	Bread wheat	Aydın	Aegean	2021	995	*F. proliferatum*	10
								<LOD
6	Adana 99	Bread wheat	Aydın	Aegean	2021	112	*F. proliferatum*	10
						115	*F. proliferatum*	<LOD
						116 (a=b)	*F. acuminatum**	
						116 (a=b)	*F. acuminatum**	
						119	*F. clavum*	
7	Negev	Bread wheat	Aydın	Aegean	2021	-	-	0
8	Kate-A1	Bread wheat	Amasya	Black Sea	2020	-	-	0
9	Flamura-85	Bread wheat	Amasya	Black Sea	2020	-	-	0
10	Sakin	Bread wheat	Samsun	Black Sea	2021	1230b	*F. graminearum*	1150
						1230c	*F. graminearum*	
						1230d	*F. graminearum*	
						1232c	*F. graminearum*	
						1232d	*F. graminearum*	
						1233b	*F. graminearum*	
						1234b	*F. avenaceum*	
						1235B	*F. avenaceum**	
						1236A	*F. avenaceum**	
						1236c	*F. graminearum*	
						1236d	*F. graminearum*	
						1237a	*F. graminearum*	
						1237b	*F. graminearum*	
						1237BmonoA	*F. avenaceum**	
						1237c	*F. graminearum*	
11	Pandas	Bread wheat	Samsun	Black Sea	2021	328a	*F. graminearum*	40
						328b	*F. graminearum*	<LOD
						328c	*F. graminearum*	
						330B	*F. avenaceum**	
						331	*F. poae*	
						332a	*F. clavum*	
						332B	*F. avenaceum**	
						335	*F. clavum*	
						336a	*F. graminearum*	
						336b	*F. graminearum*	
						336c	*F. graminearum*	
						337	*F. clavum*	

(Continued)

Table 1. (Continued).

Sample No.	Wheat cultivar	Wheat type	Turkish provinces	Region	Year	Isolate code	*Fusarium* species	DON (μg kg^{-1})
12	Canik	Bread wheat	Samsun	Black Sea	2021	81	*F. graminearum*	110
						82	*F. graminearum*	<LOD
						86A	*F. acuminatum**	
						86e	*F. graminearum*	
13	Altındane	Bread wheat	Samsun	Black Sea	2021	169a	*F. clavum*	320
						169B	*F. ramigenum*	
						171a	*F. oxysporum*	
						171b	*F. proliferatum*	
						173a	*F. proliferatum*	
						173B	*F. avenaceum**	
						174a	*F. proliferatum*	
						174B	*F. acuminatum**	
						175a	*F. verticillioides*	
						175b	*F. oxysporum*	
						176A	*F. avenaceum**	
						176b	*F. ramigenum*	
						177a	*F. oxysporum*	
						177b	*F. verticillioides*	
						177c	*F. verticillioides*	
						177d	*F. ramigenum*	
						178B	*F. andiyazi*	
						178a	*F. graminearum*	
						178c	*F. graminearum*	
						178d	*F. graminearum*	
14	Bafra	Bread wheat	Samsun	Black Sea	2021	91A**	*F. fasciculatum*	110
						91B	*F. avenaceum**	<LOD
						92a	*F. ramigenum*	
						92B	*F. ramigenum*	
						93A	*F. avenaceum**	
						95A**	*F. clavum*	
						95b	*F. clavum*	
						95C	*F. avenaceum**	
						95D	*F. avenaceum**	
						95x	*F. proliferatum*	
						95Z	*F. andiyazi*	
						98a	*F. graminearum*	
						98b	*F. graminearum*	
						98c	*F. graminearum*	
						98d	*F. graminearum*	
						98e	*F. proliferatum*	
						99	*F. verticillioides*	
						100C	*F. acuminatum*	
15	Nevzatbey	Bread wheat	Samsun	Black Sea	2021	190a	*F. poae*	710
						190b	*F. poae*	
						191	*F. poae*	
						195	*F. avenaceum*	
						197A	*F. avenaceum*	
						198A	*F. avenaceum**	
						198B	*F. avenaceum**	

(Continued)

Table 1. (Continued).

Sample No.	Wheat cultivar	Wheat type	Turkish provinces	Region	Year	Isolate code	*Fusarium* species	DON (μg kg^{-1})
16	Kirve	Bread wheat	Samsun	Black Sea	2021	102a	*F. proliferatum*	170
						102b	*F. sambucinum*	<LOD
						105B	*F. avenaceum**	
						105C	*F. avenaceum**	
						107a	*F. sambucinum*	
						107b	*F. sambucinum*	
						109a	*F. oxysporum*	
						109b	*F. graminearum*	
						110a	*F. graminearum*	
						110b	*F. graminearum*	
						110c	*F. graminearum*	
						110d	*F. graminearum*	
						117C	*F. avenaceum**	
17	Efe	Bread wheat	Samsun	Black Sea	2021	179a	*F. proliferatum*	380
						179b	*F. graminearum*	
						180C	*F. avenaceum**	
						182	*F. graminearum*	
						183a	*F. graminearum*	
						183b	*F. proliferatum*	
						186	*F. avenaceum*	
						187a	*F. proliferatum*	
						187b	*F. graminearum*	
18	Kızıltan-91	Durum wheat	Konya	Central Anatolia	2022	1265a	*F. clavum*	0
						1266a	*F. culmorum*	
						1266b	*F. culmorum*	
						1266d	*F. culmorum*	
						1272a	*F. graminearum*	
						1272b	*F. graminearum*	
19	Vehbibey	Durum wheat	Ankara	Central Anatolia	2022	-		0
20	Ankara98	Durum wheat	Ankara	Central Anatolia	2022	1324a	*F. clavum*	0
						1324b	*F. clavum*	
						1329a	*F. clavum*	
						1329b	*F. clavum*	
21	Mirzabey	Durum wheat	Ankara	Central Anatolia	2022	-		0
22	Eminbey	Durum wheat	Ankara	Central Anatolia	2022	1354a	*F. graminearum*	40
						1354b	*F. clavum*	<LOD
						1356	*F. avenaceum*	
						1356a	*F. proliferatum*	
						1360	*F. proliferatum*	
23	Kızıltan-91	Durum wheat	Sivas	Central Anatolia	2022	1364	*F. clavum*	0
24	Kızıltan-91	Durum wheat	Konya	Central Anatolia	2022	-		0
25	İmren	Durum wheat	Ankara	Central Anatolia	2022	-		0
26	Albachiara	Bread wheat	Konya	Central Anatolia	2020	215	*F. verticillioides*	0
						217	*F. verticillioides*	
						219	*F. verticillioides*	
27	Flamura-85	Bread wheat	Eskişehir	Central Anatolia	2020	-		0
28	Flamura-85	Bread wheat	Kırklareli	Marmara	2020	-		10
								<LOD

(Continued)

Table 1. (Continued).

Sample No.	Wheat cultivar	Wheat type	Turkish provinces	Region	Year	Isolate code	*Fusarium* species	DON (μg kg^{-1})
29	Rumeli	Bread wheat	Tekirdağ	Marmara	2020	-		10 <LOD
30	Kağan	Bread wheat	Tekirdağ	Marmara	2020	1689	*F. proliferatum*	0
						1691a	*F. proliferatum*	
						1691b	*F. proliferatum*	
						1696	*F. proliferatum*	
						1697	*F. proliferatum*	
31	Kobra-59	Bread wheat	Tekirdağ	Marmara	2020	-		0
32	Turkuaz	Bread wheat	Tekirdağ	Marmara	2020	919	*F. verticillioides*	0
						927	*Fusarium* sp. FTSC12*	
33	Akmira	Bread wheat	Tekirdağ	Marmara	2020	-		0
34	Maden	Bread wheat	Tekirdağ	Marmara	2020	1315	*F. acuminatum*	0
						1318a	*F. verticillioides*	
						1318b	*F. verticillioides*	
						1319a	*F. proliferatum*	
						1319b	*F. verticillioides*	
						1320	*F. proliferatum*	
						1321	*Fusarium* sp. FTSC12*	
35	Hakan	Bread wheat	Tekirdağ	Marmara	2020	931	*Fusarium* sp. FTSC12*	0
						933	*F. proliferatum*	
36	Tekir	Bread wheat	Tekirdağ	Marmara	2020	-		0
37	TT601	Bread wheat	Tekirdağ	Marmara	2020	1334	*F. verticillioides*	0
						1335	*F. proliferatum*	
						1337**	*F. clavum*	
						1338	*F. proliferatum*	
38	Kayra	Bread wheat	Bursa	Marmara	2020	-		220 <LOQ
39	Tekirdağ	Bread wheat	Kırklareli	Marmara	2020	-		0
40	Yüksel	Bread wheat	Kırklareli	Marmara	2020	-		0
41	Kate A-1	Bread wheat	Edirne	Marmara	2020	-		0
42	Esperia	Bread wheat	Edirne	Marmara	2020	131	*F. clavum*	0
						134	*F. clavum*	
						2030	*F. proliferatum*	
43	Rumeli	Bread wheat	Edirne	Marmara	2020	144a	*F. oxysporum*	10 <LOD
						144b	*F. oxysporum*	
						148	*F. clavum*	
						2042	*F. graminearum*	
44	Eylül	Bread wheat	Edirne	Marmara	2020	1142a**	*F. clavum*	10
						1145	*F. oxysporum*	
						1147	*F. oxysporum*	
45	Saraybosna	Bread wheat	Edirne	Marmara	2020	-		0
46	Dropia	Bread wheat	Edirne	Marmara	2020	959	*F. clavum*	0
47	Glosa	Bread wheat	Edirne	Marmara	2020	1346	*F. proliferatum*	10 <LOD
						1352**	*F. clavum*	
48	Masaccio	Bread wheat	Hatay	Mediterranea	2021	-		10
49	Sarıbaşak	Durum wheat	Adana	Mediterranea	2022	-		0

(Continued)

Table 1. (Continued).

Sample No.	Wheat cultivar	Wheat type	Turkish provinces	Region	Year	Isolate code	*Fusarium* species	DON (μg kg^{-1})
50	Ayzer	Durum wheat	Adana	Mediterranea	2022	-		0
51	Adana-99	Bread wheat	Adana	Mediterranea	2021	-		0
52	Tekira	Bread wheat	Adana	Mediterranea	2021	-		0
53	Sagittario	Bread wheat	Hatay	Mediterranea	2021	-		0
54	Kızıltan-91	Durum wheat	Şanlıurfa	Southeast Anatolia	2021	-		0
55	Ağabey	Durum wheat	Şanlıurfa	Southeast Anatolia	2020	-		0
56	Fırat-93	Durum wheat	Şanlıurfa	Southeast Anatolia	2020	-		0
57	Fırat-93	Durum wheat	Siirt	Southeast Anatolia	2021	1019	*F. proliferatum*	0
						1024	*F. graminearum*	
						1025	*F. proliferatum*	
58	Svevo (Zivago)	Durum wheat	Diyarbakır	Southeast Anatolia	2021	-		0
59	Burgos	Durum wheat	Batman	Southeast Anatolia	2021	1363a	*F. graminearum*	10
						1363b	*F. graminearum*	<LOD
						1363c	*F. graminearum*	
						1363d	*F. verticillioides*	
						1365a	*F. graminearum*	
						1365b	*F. graminearum*	
						1365c	*F. graminearum*	
						1365d	*F. graminearum*	
						1370	*F. verticillioides*	
60	Tosunbey	Bread wheat	Batman	Southeast Anatolia	2021	-		0
61	Fırat-93	Durum wheat	Diyarbakır	Southeast Anatolia	2021	1164a	*F. graminearum*	1730
						1164b	*F. graminearum*	
						1165a	*F. graminearum*	
						1165b	*F. graminearum*	
						1166a	*F. graminearum*	
						1166b	*F. graminearum*	
						1169	*F. graminearum*	
						1170a	*F. proliferatum*	
						1170b	*F. proliferatum*	
						1171a**	*F. equiseti*	
						1172a	*F. graminearum*	
						1172b	*F. graminearum*	
						1172c	*F. graminearum*	
						1173a	*F. graminearum*	
						1173b	*F. graminearum*	
						1173c	*F. graminearum*	
62	Bayraktar2000	Bread wheat	Şanlıurfa	Southeast Anatolia	2021	-		0
63	Çeşit-1252	Durum wheat	Şanlıurfa	Southeast Anatolia	2021	213	*F. proliferatum*	10
								<LOD
64	Ceyhan-99	Bread wheat	Batman	Southeast Anatolia	2021	-		0
65	Nota	Bread wheat	Erzurum	East Anatolia	2022	-		0

#Isolates marked * and **, belonging, respectively, to FTSC and FIESC groups, were selected for phylogenetic analysis.

al. (2011). The quality and quantities of extracted DNA were evaluated using a Qubit 3.0 fluorometer (ThermoFisher Scientific) and a NanoDrop-2000 (ThermoFisher Scientific). In addition, DNA samples were analysed by 0.7% agarose gel electrophoresis to assess molecular integrity, size, concentrations, and to detect any traces of RNA contamination. PCR with specific primers was carried out with GoTaq® 5× green reaction buffer, sup-

plied with GoTaq® DNA Polymerase ($MgCl_2$ concentration 7.5 mM, for a final concentration of 1.5 mM in the 1× reaction; Promega Corporation). Each PCR reaction contained 7 µL of buffer, 0.7 µL of dNTPS, 0.2 µL of Taq DNA Polymerase, 1.4 µL of each forward and reverse primer (10 µM), and 17.5 µL of sterile water. The final volume of the PCR reaction, 30 µL, was obtained by adding 2 µL of DNA template. The specific primer names, sequences, amplicon sizes and reference sources are listed in Table 2. Each PCR analysis included a well-characterised DNA sample (positive controls) of *F. graminearum*, *F. culmorum*, *F. proliferatum*, *F. verticillioides* and *F. poae* from the in-house collection of the plant pathology group of the Department of Food Science and Technology, University of Bologna (Italy). PCR products were separated and visualised on 1× Tris-borate-EDTA buffer agarose (1%) gels, stained with ethidium bromide. Amplicons showing defined bands of expected sizes were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega Corporation), and were sent for Sanger sequencing with forward primers to an external molecular diagnostics laboratory (Eurofin Genomics, Ebersberg, Germany). Raw ABI sequence files were controlled and edited using Geneious Prime ® v2023.1.1 (Biomatters Ltd., Auckland, New Zealand). All FASTA sequences were then subjected to nucleotide basic local alignment search tool (BLASTn) analyses (National Center for Biotechnology Information, Bethesda, Maryland, United States of America) (Boratyn *et al*., 2013) and the *Fusarium* MLST database to confirm identifications.

Identification and discrimination of species belonging to the FTSC and FIESC groups were carried out by amplifying partial portions of the TEF1-*α* gene. The standard EF-1 and EF-2 primers (O'Donnell *et al*., 1998) gave suboptimal amplification results, characterized by nonspecific outcomes when applied to the majority of the FTSC isolates. To address this limitation, novel primers TEF1-F (5'-ATGGGTAAGGAGAAGAC-3') and TEF1-R (5'-GGAAGTACCAGTRATCATG-3') were designed by leveraging the genomic sequences of *F. acuminatum* strain F829, *F. avenaceum* strain S18/60, and *F. tricinctum* strain T6. These genomes were previously employed as references for phylogenetic and comparative analyses of species within the *Fusarium tricinctum* species complex by Turco *et al.* (2021). This strategic redesign aimed to enhance the efficiency of amplification for increased analysis accuracy of FTSC isolates. The PCR reaction, purification and sequencing protocols used were as described above. The PCR conditions were as follows: an initial denaturation phase of 2 min at 95°C, followed by 35 cycles each of 94°C for 30 s, 53 C for 30 s and 72°C for 45 s, and a final extension phase of 5 min at 72°C. Each forward DNA sequence was assembled with the corresponding reverse to generate a single consensus sequence, defined as the calculated order of the most frequent nucleotides found at each position in a sequence assembly. TEF1-*α* sequences of species in the FTSC group were submitted to GenBank (see Table S1 for accession numbers). The partial TEF1-*α* sequences obtained in the present study were aligned with those of 94 isolates from the reference FIESC group and 139 isolates from the reference FTSC group, in the FUSARIUM-ID v.3.0 database (https://github.com/fusariumid/fusariumid; Torres-Cruz *et al*., 2022), to perform phylogenetic analyses. The sequences were edited in Geneious Prime® v2023.1.1, and were aligned with MAFFT v7.450 (Katoh and Standley, 2013). Once the best fit molecular evolution model was determined (K2 + G) using MEGA 11 (Tamura *et al*., 2021) based on Bayesian information criterion (BIC) scores (Chernomor *et al*., 2016), the Markov chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian posterior probabilities for alignment. Four MCMC chains were run simultaneously for random trees for 5,000,000 generations and were sampled every 500 generations (obtained with MrBayes 3.2.6; Ronquist *et al*., 2012). The

Table 2. Species-specific primers used to identify *Fusarium* species.

Species	Primer name	Primer sequence (5'-3')	Product size	Reference
F. proliferatum	PRO1 PRO2	CTTTCCGCCAAGTTTCTTC TGTCAGTAACTCGACGTTGTTG	585 bp	Mulè *et al.* (2004)
F. verticillioides	VER1 VER2	CTTCCTGCGATGTTTCTCC AATTGGCCATTGGTATTATATATCTA	578 bp	Mulè *et al.* (2004)
F. graminearum	Fg16F Fg16R	CTCCGGATATGTTGCGTCAA GGTAGGTATCCGACATGGCAA	410 bp	Nicholson *et al.* (1998)
F. poae	Fp82F Fp82R	CAAGCAAACAGGCTCTTCACC TGTTCCACCTCAGTGACAGGTT	220 bp	Parry and Nicholson (1996)
F. culmorum	Fc01F Fc01R	ATGGTGAACTCGTCGTGGC CCCTTCTTACGCCAATCTCG	570 bp	Nicholson *et al.* (1998)

sequences of *Fusarium nurragi* NRRL 36452 (lineages most closely related to the FTSC), and *Fusarium camptoceras* CBS 193.65 (most closely related to FIESC), were used as outgroups.

Pathogenicity assay

Pathogenicity tests were carried out using two isolates each of *F. andiyazi* (isolates 95Z and 178B) and *F. ramigenum* (isolates 92B and 169B), the identities of which were confirmed by TEF1-*α* portion sequencing. The inoculum for each isolate was prepared by placing three pieces of 7-d-old culture from PDA in liquid V8 juice (Campbell) medium according to the protocol described by Singleton *et al.* (1992). The medium was then placed in an orbital shaker (Thermo Scientific) under 120 rpm at 25 ± 1°C for approx. 7 d. Conidia concentrations were estimated by haemocytometer and adjusted to 10^6 mL^{-1}. In a greenhouse, pots each containing 600 g of plant growth medium (1:2:2:4 (v/v/v/v) ratio of sand, perlite, vermiculite and soil) were prepared, and 120 mL of isolate inoculum was added and mixed with the medium, with 120 mL of sterile water poured into medium as a negative experimental control. Three pots with the inoculated soil per isolate were each planted with 30 seeds per pot of the *Fusarium*-susceptible durum wheat cultivar 'San Carlo'. Seedling emergence was first observed at 7 d post-inoculation, and was recorded again at 21 d post-inoculation. Plants were removed at the 21st day, were rinsed with water and then cut with a scalpel to inspect for symptoms attributable to diseases caused by *Fusarium* (FCR and FRR). Symptom severity was assessed according to Parry *et al.* (1985) (Table 3), based on the number of plants grown and calculated using the formula (McKinney's, 1923): [Infection rating = (sum of all numerical ratings × number of plants examined) ÷ (total number of observation × maximum disease rating) × 100], and these data were statistically analyzed using the software Statgraphics 18 (Statpoint Technologies, Inc.).

Table 3. Categories of symptoms (Parry *et al.*, 1985), used in this study to assess disease severity on wheat plantlets.

Category	Plantlet symptoms
0	healthy, symptom-free with normal development
1	symptom-free, but underdeveloped or slightly necrotic at neck level
2	neck necrosis
3	obvious neck necrosis, or death following necrosis

DON detection in wheat flour

Analyses were conducted by grinding 50 g of wheat seeds from each assessed sample. From each resulting flour, 20 g was weighed into a clean Erlenmeyer flask and 100 mL of distilled water was added. The flasks were then shaken for 5 min with a mechanical stirrer 5 min (ThermoFisher Scientific). The resulting liquid fractions were each transferred to a 50 mL capacity falcon tube, and then centrifuged at 4000 rpm for 4 min. Subsequently, 500 µL of solution was aliquoted and diluted to 1:4 with distilled water. Quantification of DON was accomplished on a total of 65 samples, using the AgraQuant® Deoxynivalenol Plus kit (0.25 - 5.0 ppm), following the manufacturer's instructions. Optical densities of samples and standard curves were read at 450 nm wavelength using a spectrophotometer (OpsysMR, Dynex Technologies), assisted by Agraquant Assay Spreadsheet software (Romer Labs Division Holding GmbH).

RESULTS

Fusarium *species distributions*

In the years 2020, 2021 and 2022, a total of 195 *Fusarium* isolates were obtained from 32 of 65 wheat seed bag samples collected in the seven regions of Türkiye. All the isolates characterized in the present study are shown in Table 1. Samples from the Black Sea region were those with the most (50.2%) *Fusarium* isolates obtained, and these isolates included 11 different *Fusarium* species. The 16.9% of isolates were from samples from the Marmara region, 14.8% were from Southeastern Anatolia, 9.7% were from Central Anatolia, and 8.2% were Aegean, No *Fusarium* isolates were identified in samples from the East Anatolia and Mediterranean regions.

Fusarium graminearum was the dominant species isolated, accounting for 32.3% of the isolates obtained, followed by *F. proliferatum* (16.4%), *F. avenaceum* (11.2%), *F. clavum* (FIESC 5) (10.7%) and *F. verticillioides* (7.1%). Less frequently isolates were *F. oxysporum* (5.6%), *F. acuminatum* (3%), *F. ramigenum* (3%), *F. culmorum* (2.5%), *F. poae* (2%), *F. sambucinum* (1.5%), *F. tricinctum* (1.5%), *Fusarium* sp. FTSC12 (1.5%), *F. andiyazi* (1%), and one isolate (0.5%) each of *F. equiseti*, *F. incarnatum* and *F. fasciculatum*.

A total of 50 *Fusarium* isolates were obtained from durum wheat seed samples, of which 26 (52%) were identified as *F. graminearum*. In comparison from bread wheat, this species accounted for 25.5% of the 145 *Fusarium* isolates (Figure 2). Seven representative isolates of the *Fusarium incarnatum-equiseti* species complex

(FIESC), selected according to morphological characteristics, were discriminated by partial sequence analysis of the TEF1-α gene. The sequences generated in this study (final length ≈ 600 bp) were aligned with those of 94 reference isolates. The phylogenetic tree based on maximum likelihood and Bayesian posterior probability (BPP) analyses showed that *F. clavum* was the most abundant species (21 of 24) within the FIESC group (Figure 3). For *F. clavum*, *F. equiseti*, *F. incarnatum* and *F. fasciculatum* as FIESC members, these accounted for 16.0% (eight of 50) of the isolates in durum wheat and 11.0% (16 of 145) in bread wheat seeds. *Fusarium proliferatum* was detected in 25 (17%) of the 145 bread wheat samples, while *F. verticillioides* was present in 12 (8%) of these samples. In durum wheat, seven (14%) of 50 isolates were *F. proliferatum* and 4% (two of 50 samples) were *F. verticillioides*. Similarly, five isolates (3.4%) of *F. ramigenum* were detected in bread wheat and one isolate (2%) was obtained from durum wheat. *Fusarium culmorum* was only isolated from durum wheat, with 5 isolates (10%) detected out of the 50 samples. *Fusarium poae* (2.7% of isolates), *F. sambucinum* (2.1%), and *F. andiya-*

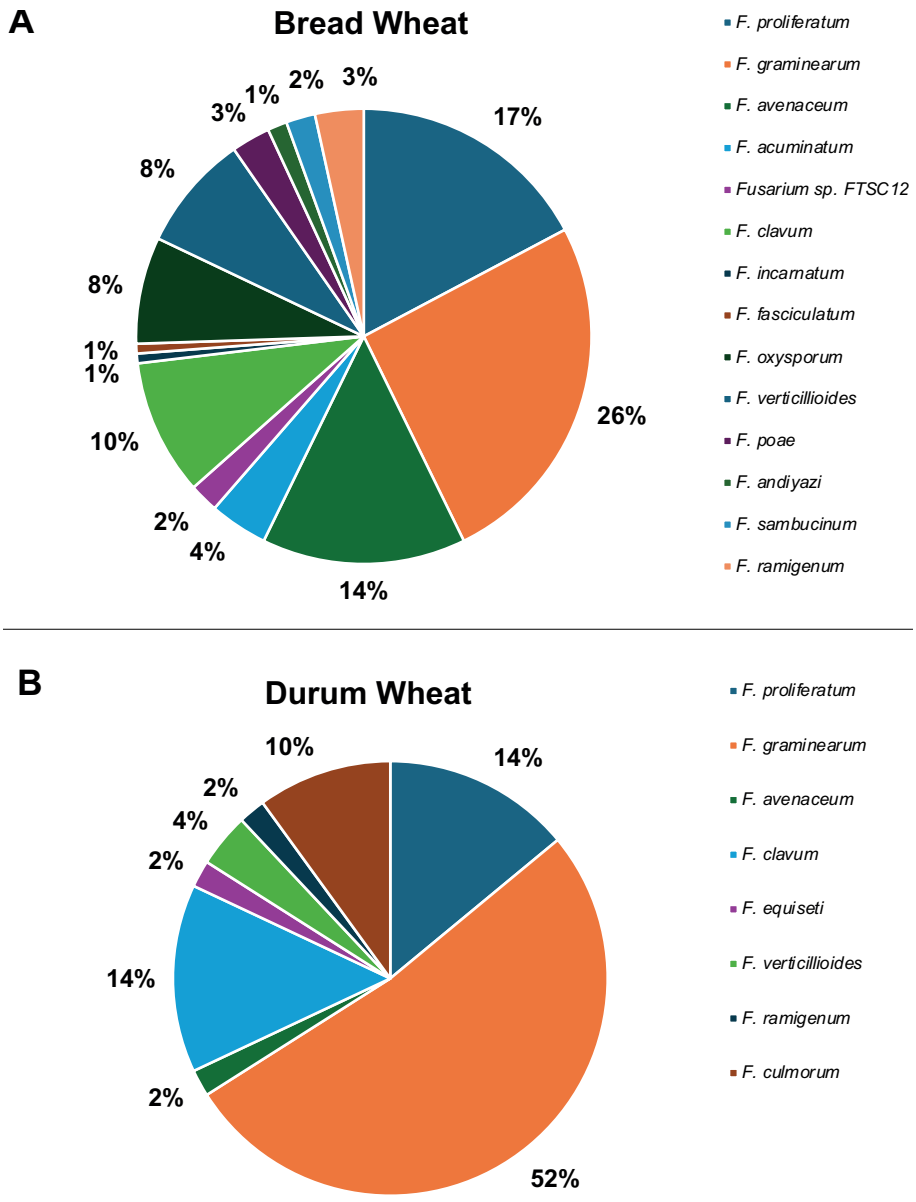

Figure 2. Occurrence (% of total) of different *Fusarium* species isolated from 45 bread wheat seed samples (A) and 20 durum wheat seed samples (B) seeds samples from Türkiye in 2020, 2021 and 2022.

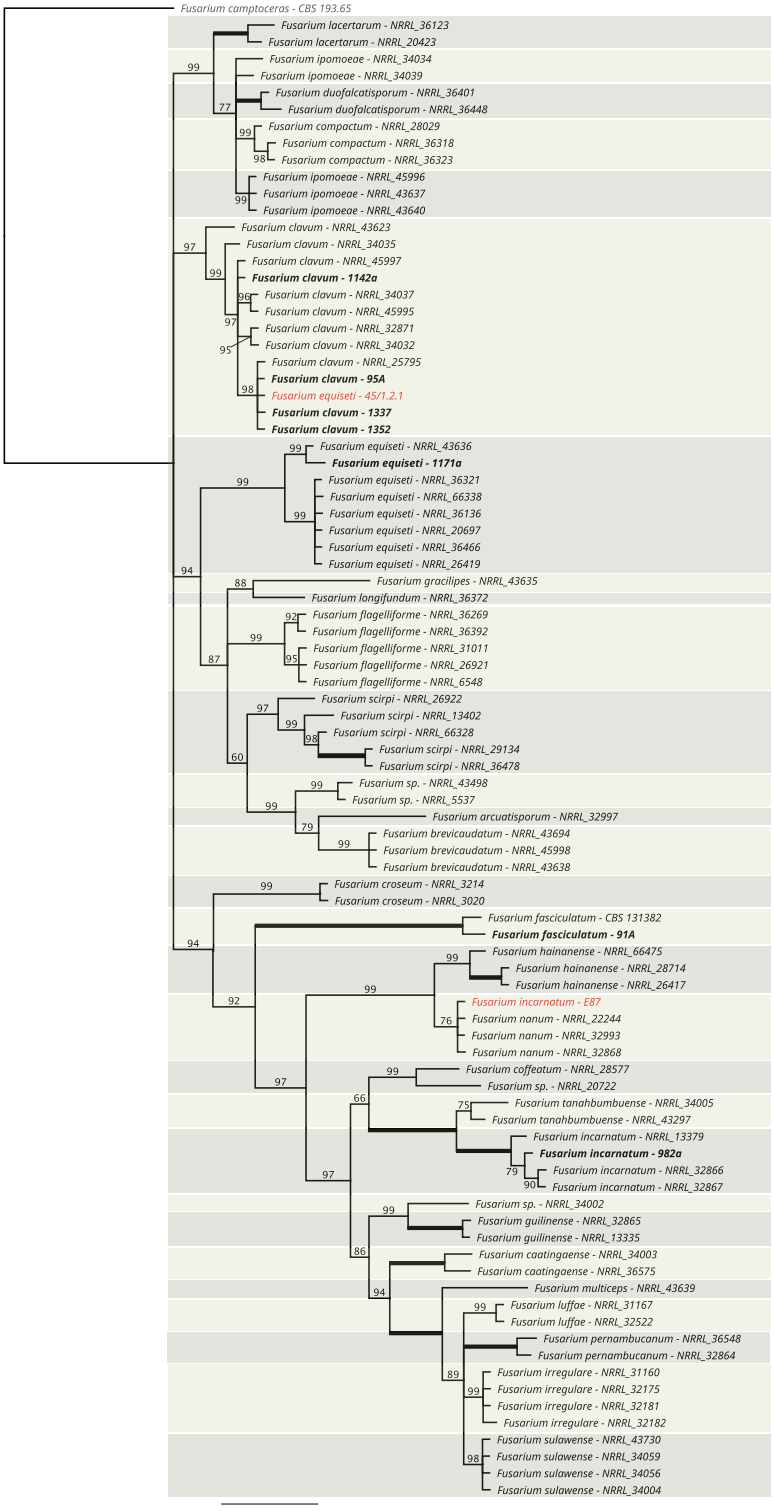

Figure 3. Bayesian inference phylogenetic tree of the *Fusarium incarnatum-equiseti* species complex (FIESC), which was constructed by aligning the sequences of the partial region of the TEF1-*α* gene produced in this study with those of the reference isolates available in the FUSARIUM ID database. The isolates obtained in this study are highlighted in bold font. *Fusarium camptoceras* CBS 193.65 was used as the outgroup. Bayesian posterior probabilities (BPP) are shown next to the nodes. The scale bar represents the number of expected changes per site. The isolate labelled "45/1.2.1" are taxonomic references for *F. equiseti*, and those labelled "E87," are taxonomic references for *F. incarnatum* (Shikur Gebremariam *et al.*, 2018). These isolates clustered with, respectively, *F. clavum* and *F. nanum*.

zi (1.3% of isolates) were only identified from the bread wheat samples.

The 23 isolates of the FTSC group were obtained only from bread wheat samples (16%), and theses isolates were further identified at species level based on partial sequence analyses of the TEF1-α gene. The sequences generated were aligned with those of 139 reference isolates, and were trimmed to final length of approx. 600 bp. The phylogenetic tree based on maximum likelihood and BPP analyses (Figure 4) showed that most of the FTSC group isolated in Türkiye from bread wheat seeds (16 of 23 samples) clustered with *F. avenaceum*. Fewer isolates (four of 23) clustered in the monophyletic group of *F. acuminatum*. Three of the 23 were assigned to FTSC group 12, which has not yet been described at species level. All clades comprising distinct isolates of the three species *F. avenaceum*, *F. acuminatum* and *Fusarium* sp. FTSC12 identified on wheat seeds from Türkiye were supported by bootstrap proportions close to 100% and had BPP values ranging from 0.91 to 0.97.

Pathogenicity assays

Pathogenicity evaluation of isolates 95Z and 178B (*F. andiyazi*) and 92B and 169B (*F. ramigenum*) on 30 seeds of the cultivar 'San Carlo' gave germination of 80% for 95Z, 76% for 178B, 72% for 92B, and 85% for 169B, at the 7 d for inoculated plants. Mean germination was similar (82%) for seeds treated with sterile water (Table 4). Disease severity, calculated at 21 d post-inoculation, was 5.9% from isolate 95Z and 3.4% from and 178B (both *F. andiyazi* isolates), and 4.4% isolate 92B and 3.3% from isolate 169B (both *F. ramigenum* isolates). No statically significant differences ($P > 0.05$) were found among the tests; germination of inoculated plants and disease severity were comparable to the negative experimental controls.

DON contamination in Turkish wheat grain

Based on the results of ELISA tests for DON in grain samples, numbers of wheat samples positive for the mycotoxin were: ten samples of 'Sakin' (mean DON concentration = 1150 µg kg^{-1}), 13 of 'Altındane' (mean = 320 µg kg^{-1}), 15 of 'Nevzatbey' (mean = 710 µg kg^{-1}), 17 of 'Efe' (mean = 380 mg kg^{-1}), and 61 samples of 'Fırat-93' (mean = 1730 mg kg^{-1}). Four of the five positive samples are bread wheats from the Black Sea region (see Table 1) among which sample No. 10 'Sakin' exceed the maximum limits for DON in unprocessed bread wheat. In sample No. 61 ('Fırat-93', from Southeastern Anatolia), DON concentration (1730 µg kg^{-1}) was the greatest amount detected, up to the European Commission threshold for mycotoxin contamination in durum wheat. The greatest amount of *F. graminerarum* was isolated from this sample (13 isolates from 100 seeds plated. Similarly, for sample No. 10, with 1150 µg kg^{-1} of DON, ten *F. graminearum* isolates were found from 100 seeds plated. In contrast, 710 µg kg^{-1} of DON were detected from sample No. 15, from which *F. avenaceum* and *F. poae* were the only isolated species.

DISCUSSION

The present study research is first to provide an overview of *Fusarium* species obtained from wheat seeds sampled in the Aegean, Black Sea, Central Anatolia, Eastern Anatolia, Marmara, Mediterranean and South-Eastern Anatolia regions of Türkiye. Although this country is a major wheat producer (FAOSTAT, 2021), detailed information is lacking on the *Fusarium* species associated with wheat seeds produced and harvested in Türkiye. Bentley *et al*. (2006) investigated the presence of *Fusarium* species obtained from diseased plants with crown and sub-crown rot symptoms in the Black Sea, Central Anatolia, and Marmara regions. They isolated 36 *Fusarium* colonies from 160 wheat plants based on morphological identifications, of which 13 isolates (36%) were identified as *F. culmorum*. In contrast, Shikur Gebremariam *et al.* (2018) identified 339 isolates of *Fusarium* from crown rot-affected plants from the Aegean, Black Sea, Central Anatolia and South-Eastern Anatolia regions of Türkiye, and, based on translation elongation factor 1-alpha (TEF1-α) segment sequencing, determined that *F. equiseti* was the predominant species. Both of these studies evaluated diseased wheat plants with crown or sub-crown rot symptoms and conducted pathogenicity tests on wheat plants, showing that *F. culmorum*, *F. graminearum* and *F. pseudograminearum* were the most pathogenic, but *F. equiseti* isolates were not pathogenic.

In the present study, 195 *Fusarium* strains isolated from wheat seeds were molecularly identified as *F. culmorum*, *F. graminearum*, *F. poae*, *F. proliferatum,* or belonging to FTSC or FIESC. In 2009 and 2010, in the neighboring country Syria, Alkadri *et al*., (2013) carried out mycological analyses on 48 wheat grain samples from diverse areas with different climates. The predominant *Fusarium* species detected were *F. tricinctum* (30%), *F. culmorum* (18%), *F. equiseti* (14%), and *F. graminearum* (13%). In Iran, which also borders Türkiye, prevalent species in pre-base wheat seeds collected in 2016 and 2017 in northern regions were *F. graminearum*, *F. cul-*

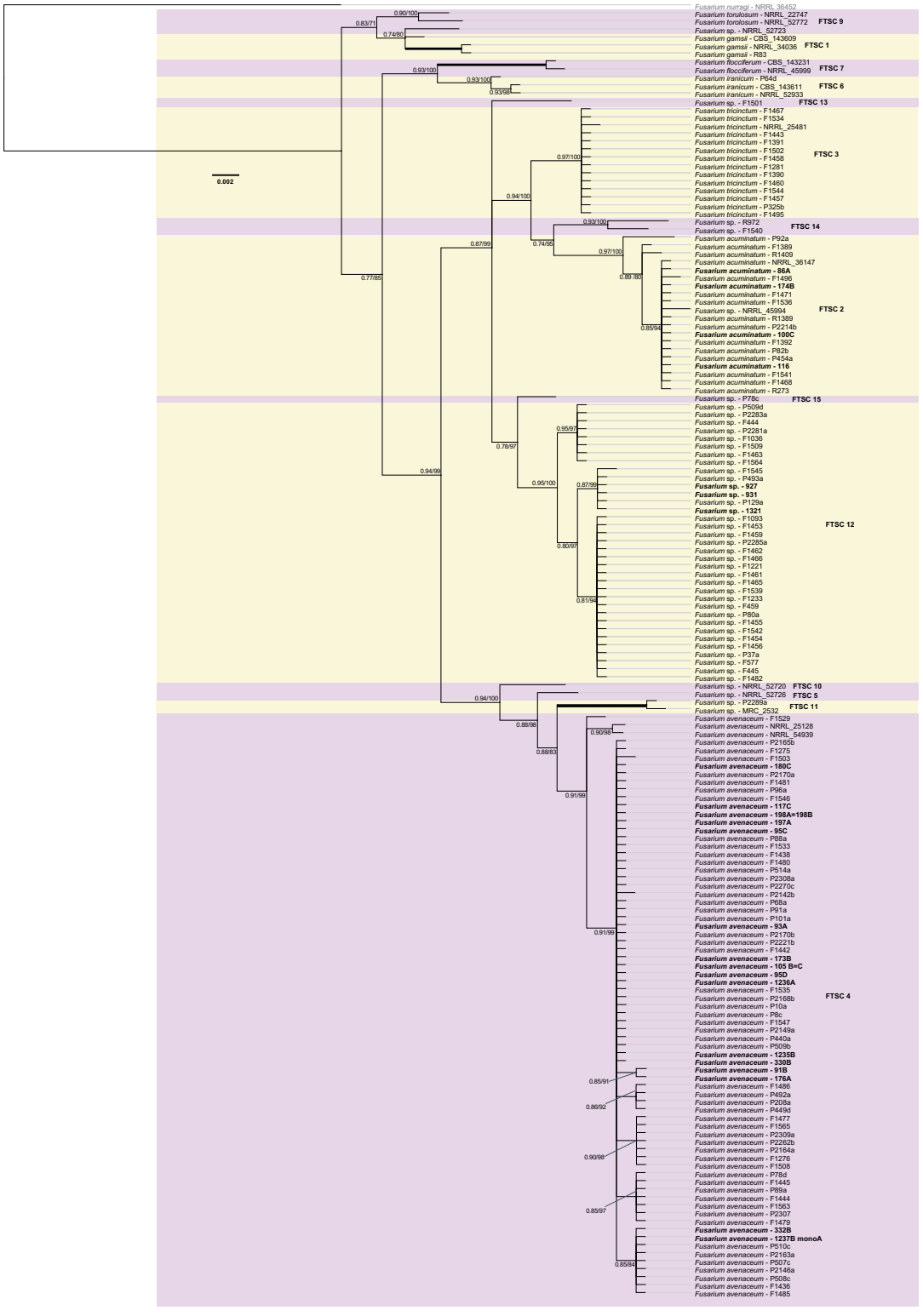

Figure 4. Bayesian inference phylogenetic tree of the *Fusarium tricinctum* species complex (FTSC), which was constructed by aligning the sequences of the partial region of the TEF1-*α* gene produced in this study with those of the reference isolates available in the FUSARIUM ID database. The isolates obtained in this study are highlighted in bold font. *Fusarium nurragi* (NLR 135819) was used as the outgroup. Bayesian posterior probabilities (BPP) are shown next to the nodes. The scale bar represents the number of expected changes per site. The different FTSC groups are highlighted by light violet and yellow boxes and are named on the right.

Table 4. Average germination rates and disease severities at the 7 and 21 d after inoculation of seeds of wheat 'San Carlo' with isolates of *F. andiyazi* or *F. ramigenum*.

Isolate code	Average germination (%)		Mean disease severity (%)	Tukey/Duncan test ($P < 0.05$)
	7th day	21st day		
169B, *F. ramigenum*	85	91.1	3.3	a
92B, *F. ramigenum*	72	80.0	4.4	a
95Z, *F. andiyazi*	80	85.6	5.9	a
178B, *F. andiyazi*	76	81.1	3.4	a
Control	82	78.9	0.7	a

morum, *F. avenaceum*, and *F. poae* (Hassani *et al*., 2019). However, in 2023, only *F. graminearum* and *F. culmorum* were detected in samples collected from northern and southwestern regions of the same country (Khaledi *et al*., 2023).

In the different geographical context of Western Australia, which is characterized by climates ranging from arid, semi-arid, Mediterranean, to tropical, Wright *et al*. (2010) found that *F. graminearum* was the predominant species in wheat grains, while *F. avenaceum*, *F. acuminatum*, and *F. culmorum* were identified in four grain samples. In Italy, Senatore *et al*. (2023) assessed fungal on durum wheat seeds across 13 regions. They identified *F. avenaceum* and *F. graminearum* as predominant species using culturing and TEF1-α assessments, and *F. proliferatum* using deep-freezing blotter (DFB) and morphological traits. In Norway, which has severe winters and high precipitation, Kosiak *et al*. (2003) surveyed wheat grain samples from different regions for *Fusarium* species. They ranked the most common species in order of *F. avenaceum*, *F. poae*, *F. tricinctum*, *F. culmorum,* and *F. graminearum*.

Across several studies in different countries and over different years, there is uniformity in the predominant *Fusarium* species that have been associated with wheat grains. However, there has been variability in the relative abundance of particular *Fusarium* species recorded across different countries and survey periods.

Fusarium graminearum, acknowledged internationally as a major contributor to FHB (Pecoraro *et al*., 2018), was shown to prevail in Türkiye in the present study. The absence of statistically significant differences in the *t*-test analyses of incidence of *F. graminearum* isolates in the bread and durum wheat samples is noteworthy. These results indicate the broad genetic differences for FHB resistance in bread wheat, while durum wheat, acknowledged as highly susceptible to this disease, lacks identified resistance sources (Haile *et al*., 2019). *Fusarium graminearum* was prevalent in bread wheat samples from the Black Sea region, where no durum wheat samples were obtained. This region, characterized by high and consistent rainfall and mild temperatures, provides optimal conditions for FHB development (Tunali *et al*., 2008). The Black Sea region has substantial maize production, which also applies in southeastern Anatolia, from which the second greatest numbers of *F. graminearum* isolates were detected. This indicates that increased susceptibility to FHB infection and mycotoxin contamination in wheat and barley crops could result from previous maize cultivation, and the adoption of diminished or nil tillage techniques (Drakopoulos *et al*., 2020).

Fusarium culmorum, which can cause FHB and FCR (Pecoraro *et al*., 2018), was identified in 2.5% of seed samples examined in this study. This contrasts with results from 2006 and 2018 in Türkiye (Bentley *et al*., 2006; Shikur Gebremariam *et al*., 2018), where *F. culmorum* predominated on wheat plants affected by crown rot, and *F. graminearum* was absent or negligible. Similar prevalence was observed for *F. poae*, detected in only 2% of the samples in the present study. Despite being categorized as a weak pathogen, monitoring for *F. poae* is important, due to its association with FHB and its capability to produce trichothecenes, which are regulated by EU Commission limits (Nazari *et al*., 2018). *Fusarium sambucinum* is closely related to *F. graminearum*, *F. culmorum* and *F. poae*. In this study, it was found in 1.5% of samples assessed. Therefore, this fungus probably does not pose threats to wheat cultivation (Kosiak *et al*., 2003; Pereyra and Dill-Macky, 2021).

The frequency of isolation of *F. avenaceum* in this study is noteworthy, as this fungus accounted for 11.2% of the isolates obtained. During the last two decades, *F. avenaceaum* has become a major contributor to FHB in cereals grown in Europe, including Denmark (Nielsen *et al*., 2011), France (Ioos *et al*., 2004), Italy (Beccari *et al*., 2018; Senatore *et al*., 2021), Norway (Kosiak *et al*., 2003), and Poland (Golinsky *et al*., 1996). *F. avenaceum* isolates were mainly obtained in the present study from bread wheat (21 of 22 isolates compared to one of 22 isolates from durum wheat), although durum wheat has been

reported as highly susceptible to this fungus (Beccari *et al*., 2018; Senatore *et al*., 2021). Two additional species of the FTSC group were found in the present study: *F. acuminatum* (3% of isolates) and the unnamed taxon *Fusarium* sp. FTSC12 (1.5% of the isolates). *F. acuminatum* has been reported as a minor contaminant in wheat crops in Spain and Canada (Marín *et al*., 2012; Grafenhan *et al*., 2013), while in North Carolina, United States of America, incidence of this fungus was approx. 49%, suggesting that it could become a significant contaminant under favourable conditions (Cowger *et al*., 2020). *Fusarium* sp. FTSC 12, in contrast, was the second most common FTSC species recovered from Italian wheat and barley by Senatore *et al*. (2021). They suggested that this species could contribute to mycotoxin contamination of Italian cereals, given its ability to produce mycotoxins such as enniatins (ENNs) and moniliformin (MON).

Incidence of FIESC was high (12.3%) in the present study. In 2018, *F. equiseti* was the predominant species from wheat plants with crown rot symptoms, constituting 36% of the isolates, while Tunali *et al*. (2006) reported lower incidence of this fungus (two isolates out of 32). In both studies, however, the isolates tested for pathogenicity gave no disease-causing effects on cultivars 'Pehlivan' and 'Kızıltan 91' (durum wheat) (Tunali *et al*., 2006; Shikur Gebremariam *et al*., 2018). In the present study, *F. clavum* was the most prevalent within the FIESC group. The phylogenetic analysis of Jedidi *et al*. (2021), examining TEF1-*α* sequences of three FIESC isolates from barley and wheat in Tunisia in comparison with isolates from northern and southern Europe, showed that the Tunisian southern European isolates were grouped in *F. clavum*, whereas isolates from northern Europe were assigned to *F. equiseti*.

Shikur Gebremariam *et al*. (2018) identified *F. equiseti* using sequencing of TEF1-*α* (amplicon sizes 243 to 655) for 123 isolates, but the sequences have not been deposited in databases, which prevents comparative analyses. The sequence comparisons performed by Shikur Gebremariam *et al*. (2018) using the BLASTn algorithm on NCBI demonstrated 97% to 100% similarity with accession number DQ854855. This refers to '*Fusarium equiseti* isolates 45/1.2.1', deposited in the GenBank database in 2006, from fungi isolated from *Lygeum spartum* in the Mediterranean region. However, a phylogenetic analysis carried out in the present study indicated that isolate '45/1.2.1', previously identified as *F. equiseti*, is closely related to isolates 95A, 1337 and 1352 of *F. clavum* obtained in the present study, as well as to the reference isolate *F. clavum* - NRRL_25795 from the FUSARIUM-ID database v.3.0 (Torres-Cruz *et al*., 2022). *F. clavum* represented the phylo-species FIESC 5 (O'Donnell *et al*., 2009), to which the Latin binomials were attributed in the 2019 taxonomic revision of this group by Xia *et al*. (2019). Three additional FIESC species were found in the present study: *F. equiseti*, *F. incarnatum* and *F. fasciculatum*. The first two were previously reported on wheat (Tunali *et al*., 2006; Shikur Gebremariam *et al*., 2018), and *F. incarnatum* was shown to be pathogenic causing foot rot in wheat plants (Er and Akgül, 2021). For *F. fasciculatum*, however, the only currently available report is for three isolates from a wild rice species (*Oryza australiensis*) cultivated in Australia, so it is uncertain whether *F. fasciculatum* species is a pathogen or an endophyte (Xia *et al*., 2019). Species belonging to the *Fusarium fujikuroi* species complex (FFSC) were relevant in this the present study: *F. proliferatum* was the second most frequently isolated (16.4%), while *F. verticillioides* was found at 7.1% incidence. These two species have been mainly associated with severe maize kernel rot (Desjardins *et al*., 2007; Amato *et al*., 2015). In wheat, *F. proliferatum* can cause the disease kernel black point, characterised by dark discolouration of the embryonic faces of grains. This disease can reduce germination of wheat seeds and alter seedling development (Busman *et al*., 2012), which can contribute to reductions in wheat crop yields and grain quality (El-Gremi *et al*., 2017; Busman *et al*., 2012; Stanković *et al*., 2012). *F. verticillioides* is a minor threat for wheat, and was found at low frequency on wheat plants with root rot in Türkiye in surveys by Arıcı *et al*. (2013) and Ünal *et al*. (2017). However, *F. proliferatum* and *F. verticillioides* should continue to be monitored, as these two species have potential to produce fumonisin, leading to economic losses in for maize and wheat cultivation (Leslie and Summerell, 2006; Niehaus *et al*., 2014). This also applies in Türkiye, where wheat is a primary crop and maize is secondary (Demirdogen *et al*., 2023).

Fusarium andiyazi and *F. ramigenum,* both members of the FFSC, were observed at incidence of 1% and 3%, respectively. These two species were reported as part of the *Fusarium* community associated with the 'Bakanae' disease of rice in Türkiye (Eğerci, 2019). The two fungi have also been reported in Italy: *F. andiyazi* as a pathogen causing 'Bakanae' (Dal Prà *et al*., 2010), and *F. ramigenum* responsible for endosepsis of fig (Moretti *et al*., 2010). The present study is the first to report *F. andiyazi* and *F. ramigenum* in association with wheat plants but, based on the pathogenicity tests, both fungi were non-pathogenic on the durum wheat 'San Carlo'.

DON mycotoxin detection was carried out for all 65 wheat seed samples examined, with four DON-positive bread wheat samples (from Samsun province in the central Black Sea area) out of five positive samples.

This region is in the northern transition zone with high annual rainfall (Morgounov *et al*., 2016). The presence of *F. graminearum* was detected in three of the four samples from the Black Sea region, with the highest amount in sample No. 10, which reached a DON concentration of 1150 μg kg^{-1}, surpassing the latest European Commission threshold for mycotoxin contamination. In contrast sample No. 15, in which only *F. avenaceum* and *F. poae* were isolated showed DON quantities of 710 μg kg^{-1}. These fungi are not renowned for producing DON, but DON-producing *Fusarium* species could be present in this sample, which were not detected during the isolation procedures. The greatest DON concentration (1730 μg kg^{-1}) was detected in sample No. 61 from Diyarbakır, exceed the maximum limits for DON in unprocessed durum wheat, where greatest contamination by *F. graminearum* was also observed. This area of Türkiye is characterized by hot and dry Mediterranean summers and mild and rainy winters (Tunali *et al*., 2008; Morgounov *et al*., 2016). Investigations are few on *Fusarium* species and their genetic variability in wheat seeds collected from different regions of Türkiye. The present study survey, identified the predominant species responsible for FHB, FCR and FRR. Notable findings include detection of *F. andiyazi*, *F. ramigenum*, and *F. fasciculatum*, heretofore unreported in wheat seeds. Research is required to assess the pathogenicity of these species; continued monitoring of the *Fusarium* community is necessary. This will be important for evaluating potential shifts in the *Fusarium* community associated with wheat, which may change, with species defined as weak pathogens becoming more dominant and destructive. The outcomes of the present study suggest that, although only two samples exceed the most recently updated European Union thresholds for DON content in unprocessed cereal grains, the Black Sea and southeastern Anatolia regions should be monitored, and because of the important export of wheat from all parts of Türkiye (FAOSTAT, 2021). Monitoring the presence of mycotoxins, including DON, ENN, MON and others, is important, as these compounds can be virulence factors for *Fusarium* species. This surveillance is important for understanding qualitative damage to wheat production and for implementing measures to mitigate these emerging threats to human health.

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