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Host Susceptibility of CIMMYT's International Spring Wheat Lines to Crown and Root Rot Caused by *Fusarium culmorum* and *F. pseudograminearum*

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Abstract: The destructive soilborne *Fusarium* species is one of the most serious challenges facing agriculture. Mycotoxins produced by *Fusarium* spp. can induce both acute and chronic toxic effects on humans and animals. Massive investments have been made in the last few decades to develop an appropriate management strategy to control *Fusarium* species in cereals, particularly in wheat, using genetic resistance and other practices, with varied outcomes. The purpose of this research was to find new sources of resistance to both *Fusarium culmorum* and *F. pseudograminearum*, which are wheat's most destructive pathogens in seedlings and adult plants stages. In this study, 26 lines were selected and promoted from a total of 200 spring wheat germplasm received from CIMMYT Mexico plus 6 local check lines. The 32 lines were screened for their resistance reactions to both *Fusarium* species under different environmental conditions. The discriminant factorial analysis indicated that 7, 12, and 5 were resistant lines against *F. culmorum* under field, greenhouse, and growth room conditions, respectively. Four lines, L12, L19, L21, and L26, were found to be jointly resistant at the adult and seedling stages in the field and greenhouse. On the other hand, only moderately resistant lines were found for *F. pseudograminearum* but not completely resistant, which was limited to growth room conditions. Interestingly, five lines (L10, L13, L17, L25, and L28) have shown resistant properties to both *Fusarium* species. To further evaluate the yield performance of the best-selected 26 lines plus 6 check lines, field trials were conducted under ± *F. culmorum* inoculum. The highest yield values were obtained from three check lines, as well as the L26, which showed consistency in its reaction to *F. culmorum* under both field and greenhouse conditions, and produced a high yield (5342 kg/ha). Based on the result obtained, L26 showed a high potential to improve wheat yield and resistance to *F. culmorum*-caused root and crown rot; therefore, it should be used in wheat crossing programs. Having *Fusarium*-resistant varieties will ultimately reduce crown rot symptoms and increase grain quality by reducing mycotoxin levels.

Keywords: *Fusarium* crown and root rot; *Triticum*; resistance screening

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1. Introduction

Wheat (*Triticum aestivum* L. and *T. durum* L.) is a major staple food crop that provides sustenance to around 40% of the world's population [1]. It offers 55% of the carbohydrates and 20% of the dietary calories consumed on a global scale [1,2]. In 2020, a total of 760.9 million tons (MT) of grain were globally produced over an area of 219 million hectares (Mha) (FAOSTAT 2022). Turkey is the tenth-largest wheat producer, with an average annual production of 20 MT produced over 7 Mha [3].

By 2050, the world's population is predicted to reach 9–10 billion people; as a result, grain output will need to increase by 50% by 2030 to meet the growing demand [4]. Wheat consumption is rising as the world's population grows, but wheat production has been dropping in recent years due to abiotic and biotic factors [5]. For instance, approximately 90% of wheat production is in rainy areas or where semi-supplemental irrigation is applied.

The majority of wheat-producing areas face drought challenges, especially at the post-anthesis stages [6]. At the same time, political instability, drought, other weather extremes, and persistent pest and disease pressure have exacerbated volatility in wheat yields, exports, and prices [7]. Above the yield damage that microorganisms cause, they also affect wheat crop quality [1]. *Fusarium* species cause severe and chronic diseases in cereals in many parts of the world. Disease symptoms known as foot rot (FFR), crown rot (FCR), and head blight (FHB) are caused primarily by *Fusarium culmorum*, *F. pseudograminearum* (formerly *F. graminearum* group 1), and *F. graminearum* (formerly *F. graminearum* group 2) are of high economic importance in wheat crops globally [8]. These three species have been reported to be associated with crown rot in wheat and cause significant yield damage in West Asia (Azerbaijan and Kazakhstan), North Africa (Egypt, Tunisia, and Morocco), the USA, Canada, Australia, and Turkey [8–12]. The same species have been reported for FHB epidemics in Asia, Canada, China, Europe, and South America [13].

Studies have demonstrated that the isolates of FCR and FHB pathogens can cause both diseases under appropriate climatic conditions. FCR occurs especially under hot and drought conditions [14] in rainfed and wheat monoculture systems, whereas FHB occurs in wet and warm environments. The pathogens cause necrosis and dry root, crown, and basal stem, known as FCR, whereas the same species infect floral tissue and cause blighting of grains, known as FHB.

The devastating and widespread disease FHB results in yield and quality loss in most wheat-growing regions [15]. Apart from reductions in grain yield and seed quality, the major risk due to FHB is the contamination of the crop with toxic fungal secondary metabolites known as mycotoxins [16,17]. They pose a chronic health risk; prolonged exposure through diet has been linked to cancer, diseases of the kidney and liver, and suppression of the immune system. In addition, mycotoxins can be present in livestock feed, reducing productivity in meat and dairy production. When these toxins find their way from feed into milk or meat (carry-over), they become a food safety hazard in these products as well [18]. Mycotoxins may be produced by *Fusarium* species during various growth stages in the field as well as during the storage of grains and other products.

Many cereal-infecting *Fusarium* species produce trichothecenes, including deoxynivalenol (DON) and nivalenol (NIV), and other mycotoxins in plant tissue that are harmful to animal and human health. Deoxynivalenol is the most frequent mycotoxin reaching the highest concentration levels also under the conditions of Central Europe [16]. DON accumulation in the grain is an acute problem as it is toxic to humans and livestock [18]. DON concentration varies with *Fusarium* species, weather conditions, and the plant organ affected. As FHB pathogens, *F. culmorum* and *F. graminearum* produce more DON in grains than *F. pseudograminearum*, but as FCR pathogens, *F. graminearum* and *F. pseudograminearum* produce similar amounts of DON in straw. When DON is produced in the infected stem base tissue, the water-soluble metabolite can be translocated into other plant parts. FCR infection by all three species, *F. culmorum*, *F. graminearum*, and *F. pseudograminearum* can lead to DON contamination of grains [10].

Crown and root rot, caused primarily by *Fusarium* species, are severe soilborne diseases that limit productivity in dryland wheat production areas across the world's major wheat-producing countries, as well as in Turkey [1,10–12,19,20]. Rotted seeds, seedlings, roots, crowns, and basal stems are among the cereal damage caused by these *Fusarium* species [21]. The etiology of *Fusarium* crown and root rot (FCRR) is complex, with multiple species frequently isolated from infected plants [22–24]. *Fusarium acuminatum*, *F. algeriense*, *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. hostae*, *F. graminearum*, *F. oxysporum*, *F. pseudograminearum*, *F. redolens*, etc. are the most common species which have been reported causing FCRR [10,23,25–28]. Infections by *Fusarium* species may exist independently but might tend to co-exist in the same locations and even in the same plants [23].

Several studies on *Fusarium* species causing FCRR revealed that *F. culmorum* and *F. pseudograminearum* are the most common and destructive species [22,25,29,30]. Both species share a number of physiological, genetic, and pathological similarities [31,32] as

well as the prevalence of both pathogens increased by hotter temperatures in dryland areas. However, *F. culmorum* prefers cooler temperatures than *F. pseudograminearum* [14,33].

Yield losses of up to 35% have been recorded from crown rot in the Pacific Northwest (PNW) of America, 25–58% in Australia [8,9,34], and up to 49% in Tunisia [35]. In Turkey, losses in winter wheat reached up to 43% [36] and 54% in durum wheat in the Central Anatolian Plateau (CAP) [37]. Aktaş et al. (1999) reported a disease intensity of 36.2% in winter cereals as a result of root and crown rots. Production losses have been estimated at $13 million in PNW, and the potential loss due to the reduced grain yield and quality in Australia is $80 million [24,35,38].

Several disease management strategies are already in use to reduce the burden of *Fusarium* species. Crop debris or contaminated plant stubble harbors the inocula [39]; therefore, managing stubble can have a significant impact on disease control [40]. Crop rotation is another agronomic strategy for reducing FCRR growth. In Turkey, the winter wheat–summer fallow rotation is the most common cropping system; however, wheat rotation with legumes is practiced in some areas. A further agronomic approach that can reduce disease incidence is the proper control of weed grasses that harbor *Fusarium* species. Seed treatment with fungicides or the application of fungicides to stem bases does not appear to provide adequate protection from *Fusarium* infections [41,42], particularly in winter crops, where *Fusarium* infections are more noticeable in the later stages when drought is present at maturity. However, more recently, successful use of fungicides against FCRR caused by *F. culmorum* has been reported, either as a seed dressing or as a foliar spray (treated twice at Zadoks development stages 31 and 45 with fluquinconazole, tebuconazole, or epoxiconazole with carbendazim) [43–45]. As can be evident, agronomic and chemical interventions aimed at reducing FCRR incidence are not always compatible with economic and practical concerns.

After all the above mentioned, increasing the genetic resistance of wheat cultivars against FCRR diseases is a prime priority. Controlling FCRR is difficult due to the scarcity of commercial cultivars resistant to all *Fusarium* species., No complete resistance exists even against a single pathogen, and the term "resistance" in this sense only refers to "partial resistance", which is a measurement of disease symptom development and/or fungal biomass. However, despite having a high fungal load, it is likely that some grain genotypes are tolerant and thus can maintain their yield potential or show reduced symptom development when infected. In the beginning, the accurate identification of the causative agents is critical for resistant cultivar breeding studies. In inbreeding programs and field studies, where diverse genotypes are examined for their reaction to a given pathogen, a mixture of isolates from the same species is typically utilized. The use of different field ranking procedures by different workers makes finding glasshouse inoculation procedures that correlate with field rankings more difficult [46]. It is also likely that different inoculation procedures used to detect partial resistance mechanisms operate differently at different stages of development [47].

The International Wheat and Maize Improvement Centre (CIMMYT) in Turkey receives approximately 1000 accessions of wheat each year from the CIMMYT Mexico spring wheat program and the International Winter Wheat Program (IWWIP, www.iwwip.org (accessed on 1 September 2021)). Accessions received are screened against various pathogens, including cereal nematodes and different *Fusarium* species in various geographical regions throughout Turkey, growth rooms, and greenhouses, to have single germplasm resistant to multiple soilborne diseases. The germplasm with multiple resistance is then distributed to international collaborators to employ in their breeding programs. This is a critical strategy to ensure that a chosen resistant wheat line can target as many distinct species/isolates as possible.

Therefore, the objectives of this study were to (i) screen spring wheat germplasm provided by CIMMYT with good quality characteristics against *F. culmorum* and *F. pseudograminearum* under controlled growth room conditions, (ii) validate the resistance situation of the best-selected lines to *F. culmorum* under the greenhouse and field conditions (iii) study yield perfor-

mances of the best-selected lines under *F. culmorum*-infested field. The best resistant/tolerant line will then be recommended to be used in international breeding programs.

2. Materials and Methods

2.1. Germplasm Selection

Out of 200 spring wheat germplasm obtained from CIMMYT Mexico, a set of 26 lines with good quality characteristics were chosen based on their resistance performance to be further validated for their resistance potential to *F. culmorum* (isolate FC14) and *F. pseudograminearum* (isolate FPG03). Three standard moderate resistant check lines: 249, Altay, and Yelken, as well as three susceptible check lines, Kızıltan, Gerek, and Kutluk, were included as controls due to their known reactions (Table 1). This set of 26 lines plus 6 checks (total 32), was tested for *F. culmorum* resistance in three different field conditions in Eskişehir, Yozgat, and Konya, as well as in Eskişehir's greenhouse and growth room facilities. The meteorological conditions for each region are displayed according to the Turkish State Meteorological Service (TSMS) in Table 1. To screen for *F. pseudograminearum* resistance, only growth room facilities were used. Furthermore, the same set was tested for yield performance in Konya under plus/minus *F. culmorum* in field conditions (Table 2).

Table 1. Meteorological characteristics of each region during the 2017–2018 growing season.

Locality	Average Annual Temperature	Annual Precipitation	Humidity
Eskişehir	12.7 °C	200–390 mm	67.1%
Yozgat	13.3 °C	450–570 mm	54.6%
Konya	23.6 °C	124–300 mm	57.9%

Table 2. The list of the spring wheat germplasm used in the field studies and the six check lines.

Ent	CNAME	CID
1	PRL/2*PASTOR//WAXWING*2/KRONSTAD F2004/4/PBW343*2/KUKUNA//KRONSTAD F2004/3/PBW343*2/KUKUNA	546,349
2	DANPHE/2*BAJ #1	546,357
3	PICAFLOR #1/5/FRET2/KUKUNA//FRET2/3/YANAC/4/FRET2/KIRITATI	553,138
4	DANPHE/3/PBW343*2/KUKUNA//PBW343*2/KUKUNA	553,204
5	FRANCOLIN #1/BAJ #1	553,377
6	SOKOLL/3/PASTOR//HXL7573/2*BAU*2/4/PASTOR//MILAN/KAUZ/3/BAV92	554,318
7	TUKURU//BAV92/RAYON/6/NG8201/KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/5/MILAN/KAUZ*2/7/KINGBIRD #1	559,533
8	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1*2/6/WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/KACHU #1	559,752
9	PBW343*2/KUKUNA//PBW343*2/KUKUNA/3/2*BAJ #1	559,939
10	PAURAQ/4/HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07	545,670
11	ATTILA*2/PBW65/5/PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI/6/PFUNYE #1	546,353
12	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/8/VEE#8//JUP/BJY/3/F3.71/TRM/4/BCN/5/KAUZ/6/MILAN/KAUZ/7/SKAUZ/PARUS//PARUS/9/KACHU	546,418
13	KACHU*2/BECARD	546,469
14	BABAX/LR42//BABAX/3/ER2000/4/2*MUNAL	549,129
15	ROBIN,KEN	448,396
16	BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ*2/5/MUNAL #1	546,537
17	MERCATO/4/FRAME//MILAN/KAUZ/3/PASTOR/5/WHEAR/SOKOLL	548,932
18	KENYA SUNBIRD/2*KACHU	541,193
19	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/NAVJ07	549,534

Table 2. *Cont.*

Ent	CNAME	CID
20	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/HUW234+LR34/ PRINIA//PBW343*2/KUKUNA/3/ROLF07	549,549
21	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/MASSIV/PPR47.89C	549,913
22	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1/5/SOKOLL/3/ PASTOR//HXL7573/2*BAU	552,587
23	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/GLADIUS	552,597
24	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1*2/5/WHEAR/ SOKOLL	554,181
25	TRCH/SRTU//KACHU*2/3/PVN	559,568
26	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/2*PASTOR//HXL7573/2*BAU/3/SOKOLL/WBLL1	560,562
27	249 (Check-CR-Mr)	
28	Altay (Check-CR-Mr)	
29	Yelken (Check-CR-Mr)	
30	Kızıltan (Check-CR-S)	
31	Gerek (Check-CR-S)	
32	Kutluk (Check-CR-S)	

Ent: Entity; CNAME: Cross Name; CID: Cross Identification Number; * means crosses

2.2. Inoculum Preparation

Monosporic isolates of *F. culmorum* and *F. pseudograminearum* were plated on the Spezieller Nährstoffarmer Agar (SNA) medium (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \times 7H_2O$, 0.5 g KCl, 0.2 dextrose, 0.2 sucrose, 20 g agar, distilled water to 1 L) and cultured for 10 days at 23 °C ± 1 °C with a 12 h photoperiod. Oven bags (35 cm × 48 cm), quarter filled with wheat bran, were humidified and sealed with cotton. Separate wheat brans were allocated for each inoculum. The bags were autoclaved at 121 °C for 20 min for 3 successive days. The spore suspension was prepared by adding sterilized distilled water to each Petri dish containing *Fusarium* cultures. Autoclaved wheat bran bags were allowed to cool before being inoculated with the spore suspension under sterilized conditions. Inoculated wheat bran was mixed by shaking the bags and incubated at 23 °C ± 1 °C for 2–3 weeks at a 12 h photoperiod or until the fungus sufficiently colonized the bran. Finally, the fungus-infested wheat bran was allowed to dry at room temperature. The fungus-colonized bran was used as a source of inoculum in experiments conducted in the growth room, greenhouse, and field.

2.3. Growth Room Experiment

Infested wheat bran was suspended in distilled water before being filtered through two layers of cheesecloth. Before use, the spore concentration was adjusted to 10^6 conidia mL^{-1} of water, and methylcellulose (0.1% v/v) was added to the conidial suspension. To achieve sufficient plantlets with a similar phenological stage, ten wheat seeds were placed on moist blotting paper in sterilized Petri dishes for germination at 22 °C for 2–3 days. Each pre-germinated seed was sown in a separate plastic tube (2.5 cm in diameter × 16 cm in height) filled with potting mix and covered in the same substrate. A sterile potting mix of sand, soil, and organic manure (50:40:10; $v/v/v$) was used for growth room and greenhouse trials. One week after sowing, the stem base of each seedling (0.5–1 cm above the soil level, including the coleoptile) was inoculated with 1 mL (1×10^6 conidia mL^{-1}) of the abovementioned spore suspension. Following incubation, the seedlings were kept in a growth room for 42 days (early tillering, Zadoks growth stage 14), with a day/night photoperiod of 16/18 h at a temperature of 23 °C ± 1 °C and relative humidity of 60/80% (±5%). A randomized complete block design with five replications (1 plant per replicate) was used, and the experiment was repeated twice.

2.4. Greenhouse

Each tube was sown with two seeds of each wheat accession, and received 0.5 g of fungal colonized wheat bran (as inoculum source). To facilitate root growth, these tubes

were set on a platform of sand in a greenhouse characterized by a range of temperatures from 16 °C to 35 °C with relative humidity (RH) of 25–90%. During the growing seasons, the experiments were watered as needed. Plants were exposed to water stress at maturity stages to promote disease development. A randomized complete block design was used to set up the experiment, which had six replications (two plants per replicate).

2.5. Field Conditions

During the 2017/18 growing season (October to June), plant materials and check cultivars were planted in field conditions at the ILCI Çiçekdağı Agricultural Enterprise (ICAE) in Yozgat (Latitude 39.63806; Longitude 34.46722), at the Transitional Zone Agricultural Research Institute (TZARI) in Eskişehir (Latitude 39.76670; Longitude 30.40518), and the Bahri Dağdaş International Agricultural Research Institute (BDIARI) in Konya (Latitude 37.85789; Longitude 32.556), Turkey. Each 5 g seed sample of entries was sown in a one-meter row and inoculated with 2 g of fungus-colonized wheat bran of *F. culmorum*. Experiments with three replications were set up using a randomized complete block design. Disease symptoms were scored by picking up 15 individual plants from each row.

2.6. Disease Assessment and Data Analysis

Plants were harvested, and stems were collected at the end of the growing season. Seedling resistance was tested on plants that were grown in a growth room (Zadoks growth stage 14). At the end of the maturity stage, plants grown in greenhouses and fields were examined, and adult plant resistance was tested. Plants were scored on a numeric scale of 1–5 for the typical symptoms of browning percentage on the crown (by observing the disease on the crown) and the main stem (by measuring the disease symptoms on the stem). The scale was modified from Wildermuth et al. [40] (1: 1–9% as resistant, 2: 10–29% moderately resistant, 3: 30–69% moderately susceptible, 4: 70–89% susceptible, and 5: 90–99% highly susceptible). Plants grown under field conditions were also scored for whitehead symptoms at the ripening stage using the same 1–5 scale.

2.7. Yield Performance

The selected spring wheat germplasm was evaluated for yield performance under field conditions in Konya, both with and without *F. culmorum* inoculation. Each entry was replicated three times and planted in a 6 m^2 plot of six rows in a randomized complete block design with plus or minus artificial inoculum. For inoculated plots, a 140 g seed sample from each entry was inoculated with 5 g of fungus-colonized wheat bran, as described in the 'Field conditions' section. Grain yield was weighted and recorded in kg per ha per plot for all plots.

2.8. Statistical Analysis

The analysis of variance was used to analyze all of the data (ANOVA). Protected least significant difference at $p < 0.001$ was used to detect significant differences between studied lines using SPSS statistical software V 17.0 (SPSS Inc., Chicago, IL, USA). A linear discriminant analysis (AFD) was performed using R 3.4.3 software to distinguish the mainline groups based on their disease index. Linear regression analyses were also performed to uncover relationships between each line's plant height and grain weight. All other analyses were carried out with the XLSTAT software (2016.02.28451) (Addinsoft Inc, New York, NY, USA).

3. Results

The selected 26 spring wheat lines were tested against the crown rot disease caused by *F. culmorum* and precisely evaluated via the disease index for each condition (Figure 1), and the discriminant factorial analysis (AFD) revealed four groups of lines: Group 1, Group 2, Group 3, and Group 4, comprising resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S) lines, respectively (Figure 2A). In all field conditions, eight lines, L1, L9, L16, L22, L29, L30, L31, and L32, had an index of > 3.1, indicating a significantly higher severity (Figure 1A) and corresponding to S Group 4 (Figure 2A). MS

Group 3 comprised three lines: L3, L7, and L14, with disease indices of 2.8, 2.9, and 2.8, respectively. Fourteen lines (L2, L4, L5, L6, L8, L10, L13, L15, L17, L20, L23, L24, L25, and L28) had mean indices of between 2.4 and 2.7 and belonged to MR Group 2. Seven lines, L11, L12, L18, L19, L21, L26, and L27, had the lowest severity between indices of 2.2 and 2.3 and belonged to R Group 1.

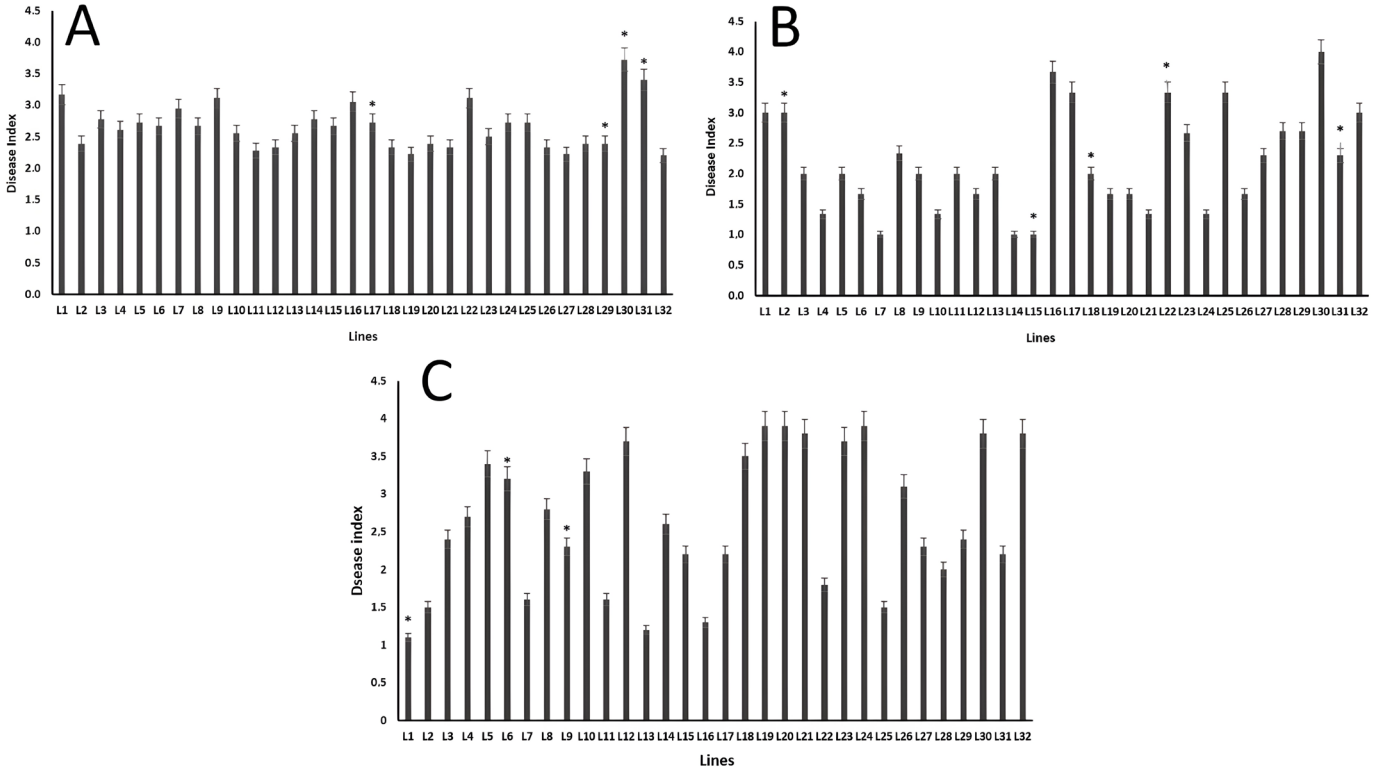

Figure 1. Crown rot disease index of 32 spring wheat lines (including 6 check lines) to *Fusarium culmorum*. (**A**) Disease index in field conditions of three locations (Eskişehir, Yozgat, and Konya). (**B**) Greenhouse conditions in Eskişehir. (**C**) Growth room conditions in Eskişehir. Asterisks (*) represent homogeneous groups based on the protected least significant difference test for each variable at $p < 0.001$. Error lines on bars represent the standard error (n = 6).

In Eskişehir greenhouse conditions (Figure 2B), the same number of groups were observed. The *F. culmorum* disease index ranged from 1 (L7, L14, and L15) to 4 (L30) (Figure 1B). Line 16, with an index of 3.7, exhibited the highest severity and was included in the S Group 4 together with the susceptible check line 30. Nine lines: L1, L2, L17, L22, L23, L25, L28, L31, and L32, also exhibited high disease severity with indices between 2.7 and 3.3 and were included in MS Group 3, while nine other lines: L3, L5, L8, L9, L11, L13, L18, L27, and L29, belonged to MR Group 2 and had significantly lower values between 2.0 and 2.3. The lowest severity indices between 1.0 and 1.7 were found in 12 lines: L4, L6, L7, L10, L12, L14, L15, L19, L20, L21, L24, and L26, which were included in R Group 1.

Within the analyzed lines, *F. culmorum*-induced disease index values were increased significantly in growth chamber settings (Figure 1C), resulting in five discrete resistance categories (Figure 2C). Aside from the other conditions, the fifth group, Group 5, included three highly susceptible (HS) lines, L19, L20, and L24, each with an index value of 3.9. Six lines, L5, L10, L12, L18, L21, and L23, as well as check lines L30 and L32, had a significantly high severity that exceeded the mean index value of 3.3 and were included in S Group 4. Five lines, L4, L6, L8, L14, and L26, had lower indices ranging from 2.6 to 3.2 and were assigned to MS Group 3. MR Group 4 included seven lines (L3, L7, L9, L11, L15, L17, and L22) as well as four check lines (L27, L28, L29, and L31). The lowest disease index values,

ranging from 1.1 to 1.5 were observed in five lines, L1, L2, L13, L16, and L25, all of which were part of the R Group 1.

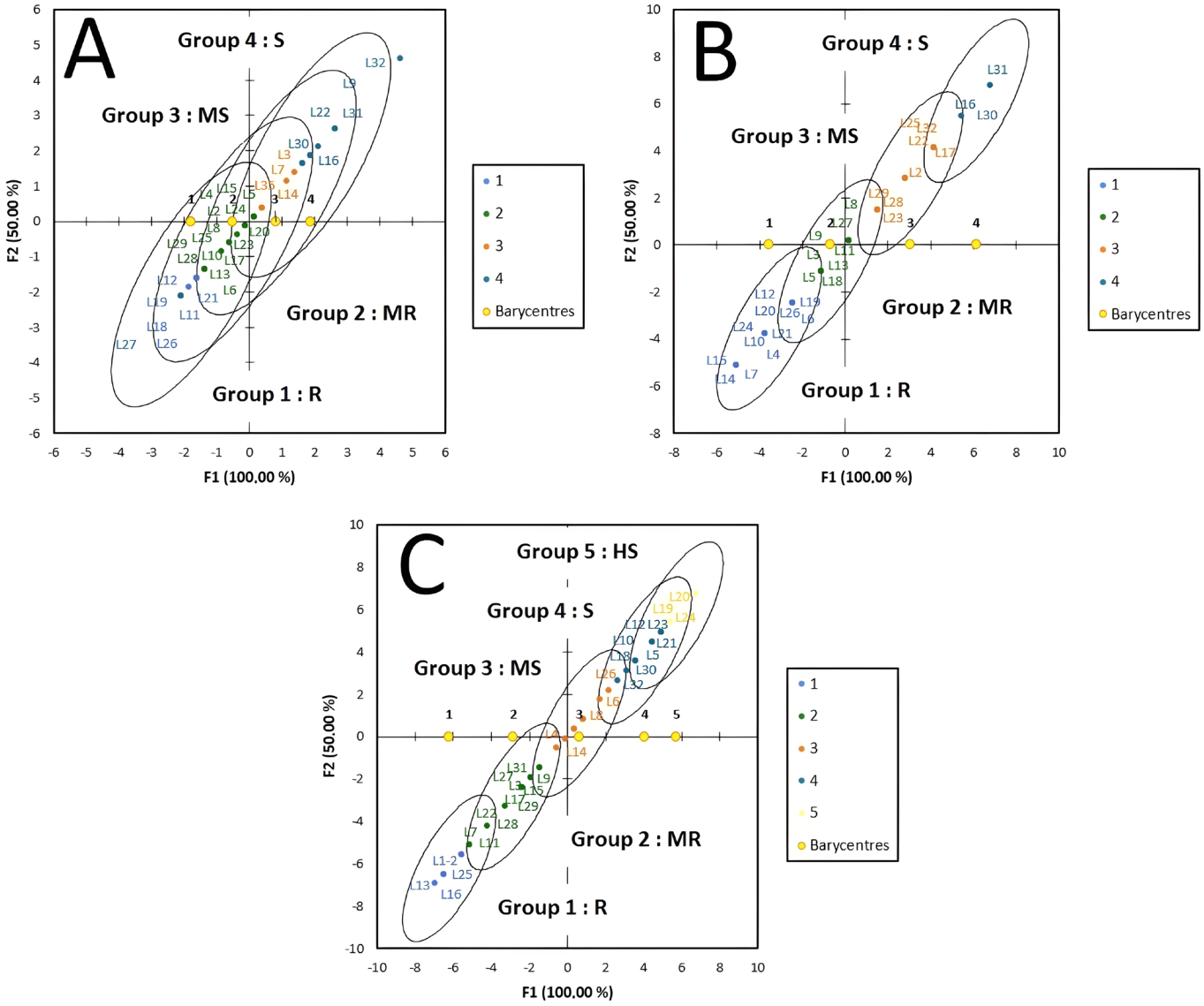

Figure 2. Discriminant factorial analysis (AFD) showing the population structure for a set of 32 lines from CIMMYT's spring wheat nursery based on their resistance reaction against *Fusarium culmorum*. (**A**) Field conditions of three locations (Eskişehir, Yozgat, and Konya). (**B**) Greenhouse conditions in Eskişehir. (**C**) Growth room conditions in Eskişehir. Numbers represent resistance reaction: 1: resistant; 2: moderately resistant; 3: moderately susceptible; 4: susceptible; 5: highly susceptible. Yellow points represent the barycenter's defining groups.

There were significant differences ($p < 0.001$) across the evaluated 32 lines in terms of the crown rot disease index for *F. pseudograminearum* (Figure 3A). The AFD analysis revealed that there are four distinct groups of lines based on their resistance reaction to *F. pseudograminearum* (Figure 3B). Among them, 12 lines were shown to have a high disease index that exceeded 3, and just 2(L20 and L26) in HS Group 5 were able to attain the maximum 4.5 and 5 index values, respectively. The remaining eight lines: L4, L5, L6, L12, L15, L18, L19, and L23, and two check lines (L30 and L32), were in the S Group 4. Among the 15 moderately resistant lines (MR) in the first group, L11 and L16 gave the lowest index value (1.8). L1, L2, L3, L8, and L24 were moderately susceptible (MS) lines in Group 3. L7,

L9, L10, L11, L13, L14, L16, L17, L21, L22, L25, L27, and L28, as well as 2 check lines (L29 and L31), were other members of MR Group 2.

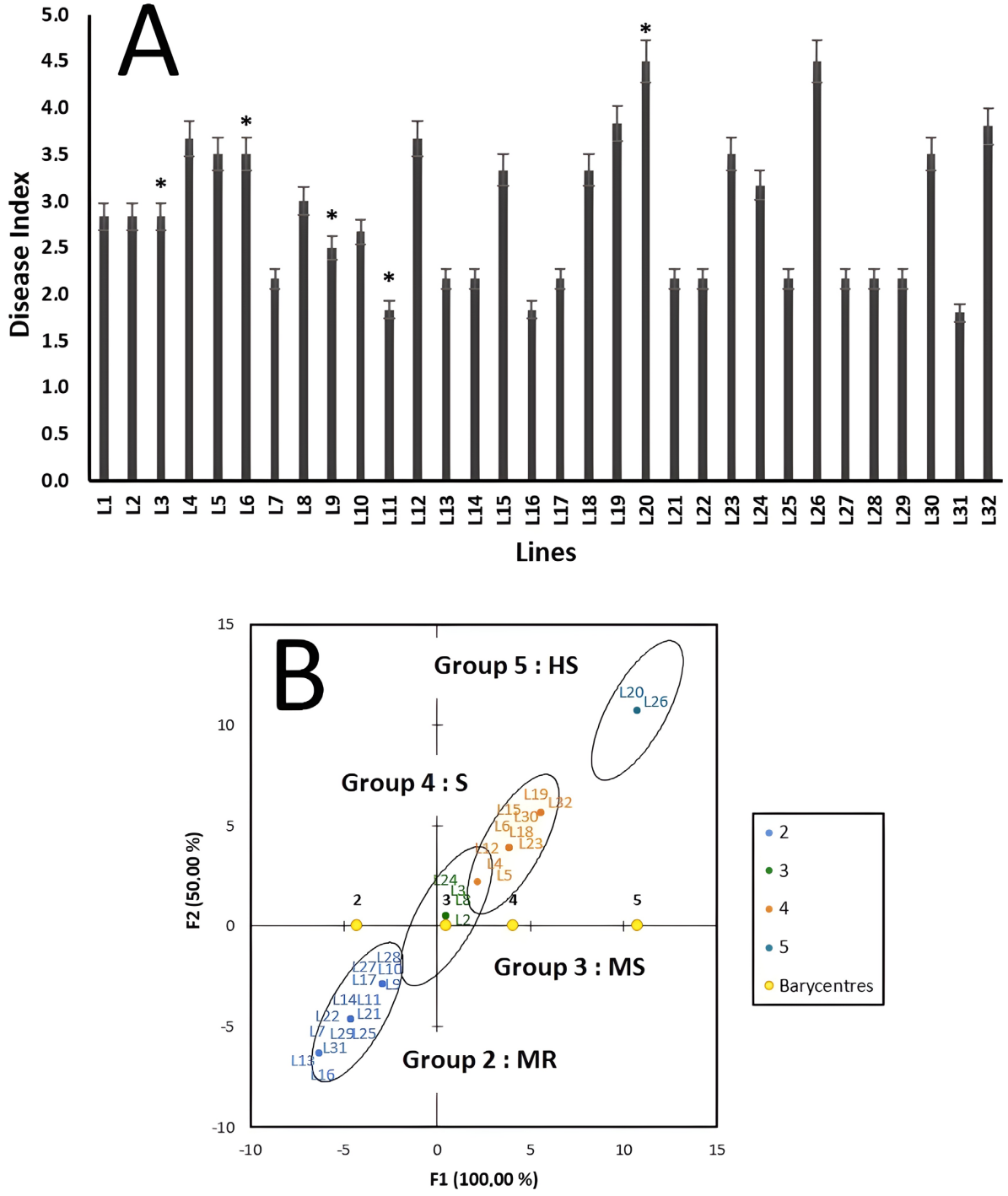

Figure 3. (**A**) Crown rot disease index of 32 spring wheat lines (including six check lines) of *Fusarium pseudograminearum* in Eskişehir. Asterisks (*) represent homogeneous groups based on the protected least significant difference test for each variable at $p < 0.001$. Error lines on bars represent the standard error (n = 6). (**B**) Discriminant Factorial Analysis (AFD) showed the population structure for a set of 32 lines from CIMMYT's spring wheat nursery based on their resistance reaction against *F. pseudograminearum* in growth room conditions. Numbers represent resistance reaction: 2: moderately resistant; 3: moderately susceptible; 4: susceptible; 5: highly susceptible. Yellow points represent the barycenter's defining groups.

The grain yield of the 32 lines was evaluated in big plots of 6 m^2 with or without *F. culmorum* inoculum. It is indicated that fungal inoculation showed various grain yield responses (Figure 4). L1, L2, L3, L4, L5, L11, L12, L19, L20, L21, L23, L26, L27, L28, L29, and L30, for example, were negatively affected by *Fusarium* inoculation and yielded less, demonstrating these lines' susceptibility. However, 14 lines, namely L7, L8, L9, L10, L13, L14, L15, L16, L17, L18, L22, L24, L25, and L31, increased their yielding potential, whereas two lines, L6 and L32, were unaffected by inoculation, indicating resistance to *F. culmorum*. Those lines might have a tolerant reaction as well. Indeed, the inoculation appeared to increase rather than decrease the productivity of these lines. Except for check lines, L26 had the highest yield (5342 kg/ha), while L1 had the lowest (2623 kg/ha). The yield values of three reference lines (L30, L31, and L32) were exceptionally high. The other three, L27, L28, and L29, had a moderate yield associated with pathogenic fungal damage.

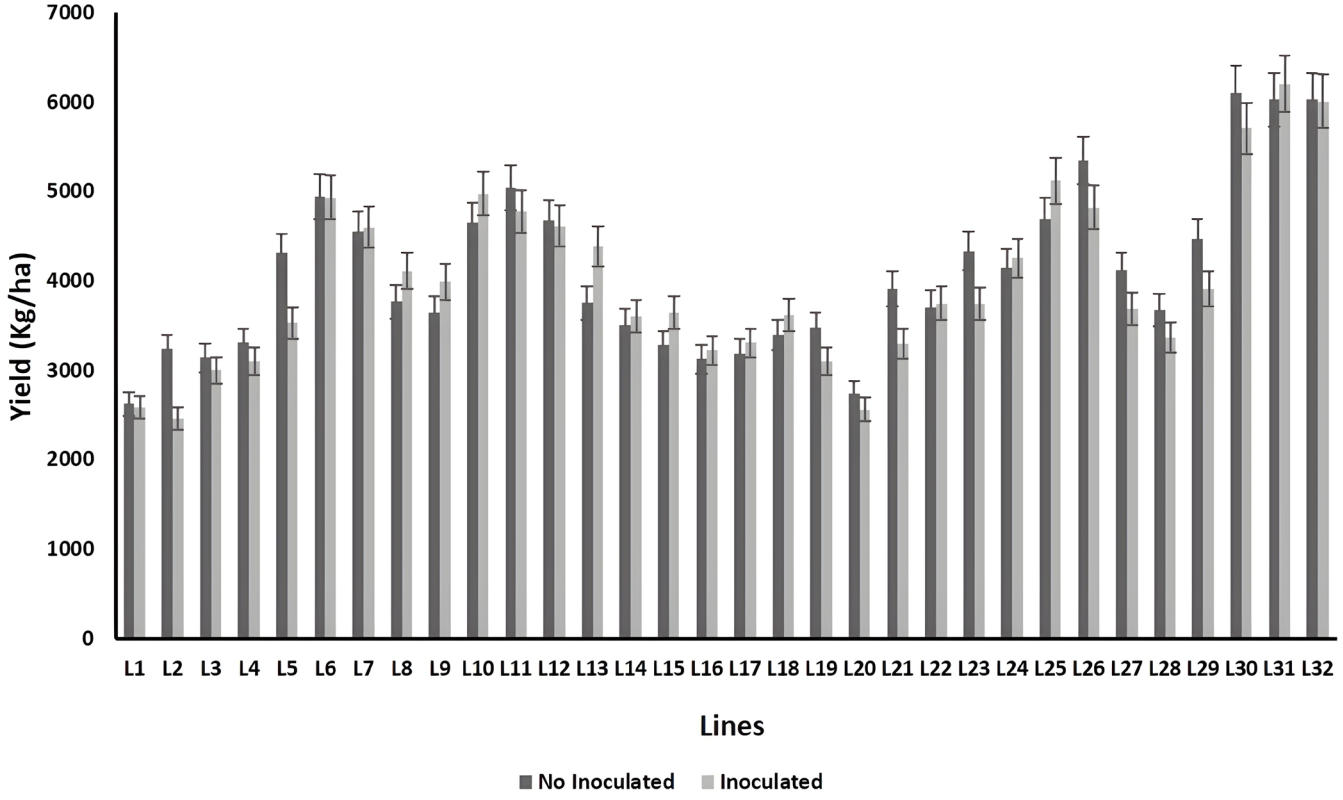

Figure 4. Grain yield of 32 CIMMYT's spring wheat lines, including 6 check lines, inoculated and noninoculated with *Fusarium culmorum*, in Konya, Turkey.

4. Discussion

Fusarium root and crown rot of wheat are most commonly caused by the infection of a diverse species of *Fusarium* genus, including *F. pseudograminearum*, *F. culmorum*, *F. avenaceum*, and *F. graminearum*, which rots small-grain cereal seeds, seedlings, roots, crowns, basal stems, and heads. On wheat plants, these fungi cause browning and decay by infecting the coleoptile, leaf sheath, and stem base of seedlings.

The most effective technique to manage crown and root rot infections is through wheat breeding. Wheat plants generally lack genetic resistance against these pathogens, and these diseases are more frequently caused by multiple pathogens in the field; thus, the goal is to find genetic resistance to these pathogens in wheat germplasm that offer resistance to as many pathogens as possible. However, few wheat germplasms have moderate levels of resistance to *F. culmorum* or *F. pseudograminearum*. Because most wheat varieties are susceptible to these pathogens, identifying new resistance sources and crossing them with

high-yielding cultivars is essential. Therefore, for effective wheat breeding applications, large-scale screenings of resistant wheat germplasm are still required.

The primary goal of this study was to assess the resistance in 32 wheat germplasm to *F. culmorum* or *F. pseudograminearum* at the adult plant stage under field conditions in the provinces of Yozgat, Eskişehir, and Konya in Turkey, as well as at the seedling stage in greenhouse and growth chamber conditions in Eskişehir. Because investigating resistant or tolerant lines necessitates a thorough understanding of the pathogenicity spectrum and pathotypes of the target species, highly virulent and aggressive isolates of both species were used in all experiments. Approximately 65.6% of the lines (21 lines) were resistant (7 lines) to moderately resistant (14 lines) against FCRR caused by *F. culmorum* at the adult plant stage. Seedling resistance screening in the greenhouse identified two, nine, nine, and 12 lines as S, MS, MR, and R against *F. culmorum*, respectively. In the field and greenhouse, four lines (L12, L19, L21, and L26) were found to be jointly R, and three lines (L5, L8, and L13) were jointly MR at the adult and seedling stages, respectively. At the seedling stage, six other lines that were MR at the adult plant stage (L4, L6, L10, L15, L20, and L24) were R in the greenhouse. In that manner, Özdemir et al. [48] have screened many wheat varieties from Turkey, Australia, and the Pacific Northwest of the USA for their resistance to *F. culmorum* and *F. pseudograminearum* using the Real-Time PCR method. Three types of lines were detected, Resistant-Tolerant, Resistant-Intolerant, and Susceptible-Intolerant. Good correlations between the results of specific resistant lines under different conditions suggest that these lines could be useful for pyramiding genetic variants for long-term resistance. Controlled (growth room), greenhouse, and field-based experiments sometimes produced diverse results among lines, which is understandable. However, it is assumed that controlled conditions are more suitable and reliable for disease screening due to their reduced variation error. For instance, *F. culmorum*-induced disease index values increased significantly in growth chamber settings within the analyzed lines, indicating that *F. culmorum* found optimal conditions to emerge on the studied spring wheat lines. Furthermore, some studies [49–51] have found that earlier inoculations result in more severe disease infection, which can lead to higher disease levels, and that the early response to FCRR is not always related to adult plant responses. Three check lines (L30, L31, and L32) and L26 had the highest yield values. *Fusarium* inoculation had a negative effect on 16 lines, causing them to yield less, demonstrating their susceptibility. However, inoculation did not affect 16 lines, indicating resistance/tolerance to *F. culmorum*. Indeed, the inoculation appeared to boost rather than reduce the productivity of these lines. This has to do with tolerance attributes of spring wheat lines related to the decent yielding proprieties despite the pathogen's inoculum. In the case of *F. pseudograminearum*, screening was limited to growth room conditions, and no R lines were identified, but 15 MR (four of which were checked) lines were observed. On the other hand, only five lines (L10, L13, L17, L25, and L28) have shown resistant status to both *Fusarium* species which is an important perspective for breeding.

Genetic wheat resistance against *F. pseudograminearum* has been highlighted in many studies. However, no completely resistant (R) lines currently exist that support our findings in this context [52]. A few lines of bread wheat were reported to offer partial resistance to *F. pseudograminearum* under field conditions [53]. The mechanism of this resistance is related to the colonization and growth characteristics of *F. pseudograminearum* in wheat seedlings. Therefore, partially resistant cultivars were shown to have slow mycelium growth (in both plant's xylem and phloem) compared to the susceptible ones [54]. Interestingly, the resistance pattern against *Fusarium* pathogens could exhibit eventual variations in the field conditions due to the associated meteorological factors. For instance, Birr et al. [55] emphasized a positive correlation between climatic variables (e.g., precipitation and relative humidity) and *F. graminearum*'s abundance. In addition, mycotoxin concentrations fluctuated across wheat grains due to the high moisture factors.

Deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), nivalenol (NIV), and zearalenone (ZEA) toxins are some of the most important mycotoxins found in harvested

wheat grains all of which are produced by *Fusarium* species. Earlier studies showed that disease severity caused by *Fusarium* species and mycotoxin contamination in harvested wheat grain were remarkably correlated [56]. It is considered that the determination of resistant wheat varieties against *Fusarium* species will also help to reduce the contamination level of the mycotoxins in harvested wheat grains. This part of the research will be given a high priority by the International Soil Borne Pathogens Platform in Turkey to evaluate R vs. S to accumulate mycotoxins in wheat plants at different growing stages.

A major impediment to FCRR resistance breeding is the lack of research and understanding of the genetic basis of resistance [57]. Furthermore, there is a scarcity of dependable, reproducible, and high-throughput phenotyping procedures for screening large numbers of genotypes, leading to the discovery of novel sources of FCRR resistance and the underlying genetic factors [52,58]. In Turkey, the main *Fusarium* species involved in wheat fields is *F. culmorum* and, since research collaborations are made with other *Fusarium*-infested countries (mainly with *F. pseudograminearum*), lines with multiple resistance traits to both species are extremely needed for breeding programs.

In conclusion, the present study has revealed new sources of resistance to *F. culmorum* and *F. pseudograminearum* derived from experiments in the growth chamber, greenhouse, and field conditions. New R or MR germplasm resources found in the present research may therefore hold a lot of promise for improving wheat resistance to these fungi that cause root and crown rot, as there is currently no durable resistant cultivar for both species and they can further be exploited in breeding programs for the development of disease-resistant commercial cultivars. Internationally, it is recommended to perform a rank test of wheat lines based on their resistance to *Fusarium* spp. This test could involve many scientists from main *Fusarium*-infested countries, and it could be an innovative way to produce innovative management solutions to this disease. However, screening procedures must be improved in the sense that all resistance/tolerance aspects will be well covered from breeding perspectives. For instance, using new technologies-based approaches (e.g., QTL and GWAS studies) could be extremely doable to enforce resistance discovery in wheat cultivars. Additionally, it could be useful to adopt these conceptional genetics to promote high grain yield despite *Fusarium* infestations through investing in tolerance traits.

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