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Assessment of the Seedling Resistance of Spring Wheat Lines to *Fusarium culmorum*

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ABSTRACT

Wheat diseases are one of the constraints limiting wheat yields wherever the crop is grown. *Fusarium crown rot*, incited by *Fusarium culmorum*, is one of the most important diseases limiting wheat yields especially in dryland areas. Although there are no wheat varieties which are fully resistant to crown rot, the use of varieties showing some degree of resistance is the most reliable and cost effective method to control this disease. In this study, seedling reactions of 165 spring wheat breeding lines (*Triticum aestivum* L.) obtained from CIMMYT, Mexico were determined under growth room conditions using an aggressive isolate of *Fusarium culmorum*. Crown rot severity

was assessed using a 1-5 scale. The mean disease severity scores for the lines tested ranged from 1.4 to 4.4. Two out of the 165 lines tested (lines 147 and 158) were resistant (R) in their reaction and had scores of 1.4. Twenty lines showed moderately resistant (MR) reaction and had scores ranging from 1.6 to 2.4. The scores of both the R and MR lines were not significantly different from scores of MR control cultivars. Sixty-three percent of the lines were moderately susceptible (MS). Out of the 165 lines tested, 39 were susceptible (S) in their reaction. The promising wheat lines that showed some degree of resistance to *Fusarium culmorum* in the present study can serve as useful sources of genetic resistance in breeding for *Fusarium crown rot*.

Keywords: Wheat; *Triticum aestivum*; *Fusarium culmorum*; Disease resistance

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1. Introduction

Wheat (*Triticum* spp.) plays a tremendous role in human nutrition. It serves as a staple food for 40% of the world's population (Bockus et al 2010). Its high yield and nutrition, ease of grain storage and processing it into different food forms made wheat the major diet component (Curtis 2002; Shewry 2009). It provides essential amino acids, minerals, vitamins and dietary fiber (Shewry 2009; Bockus et al 2010). Wheat serves

as a source of more calories and protein to the world's diet than any other food crop. In Turkey, wheat contributes to more than half of the calories and protein in the diet (Hanson et al 1982).

Turkey is an important wheat producer in the world with annual production of around 22.1 million tons from a total wheat production area of 7.77 million ha in 2013 (Anonymous 2014). The average yield of wheat in Turkey is 2.8 tons ha⁻¹ (Anonymous 2014), however, the yield varies from 1 ton ha⁻¹ in the Eastern region to 3 tons ha⁻¹ in the European part of Marmara region (Braun et al 2001). In Turkey wheat accounts for 3.9% of total world wheat production, more than 32% of total cultivated land and 60% of cereal production (Geçit et al 2009).

Cereal diseases exist wherever the crops are grown. Soilborne diseases including crown rot are important diseases of cereals in the world, particularly in areas where cereal based rotations and marginal growing conditions are common. Despite their economic importance, some soilborne diseases are given less attention because of the difficulty in working with them (Wallwork 2000; Singleton 2002). Soilborne pathogens of cereals invade crown and root tissues and interfere with nutrient and water uptake which lead to economic yield losses (Singleton 2002). Damage caused by *Fusarium* species on small grain cereals include rotting of seeds, seedlings, crowns, roots, basal stems or heads (Paulitz et al 2002). *Fusarium* crown rot also causes damping-off, reduction in grain and straw yields and grain quality (Smiley et al 2005). Crown rot pathogens cause yield losses due to damaged seedlings, improper grain filling and lodging (Schilling et al 1996). The disease is of economic importance in dryland wheat producing regions including Turkey, Europe, North and South Africa, Australia, North and South America and West Asia (Smiley et al 2005; Chakraborty et al 2006; Bockus et al 2010; Gebremariam et al 2018a). World-wide losses exceeding 30% have been documented (Cook 1968, 1992; Mishra 1973; Klein et al 1991; Burgess et al 2001; Hekimhan et al 2004). Yield losses ranging from 24% to 43% caused by crown rot diseases have been recorded on common bread wheat cultivars in Turkey (Nicol et al 2001; Hekimhan et al 2004).

Management of crown rot has relied on cultural practices that only provide partial control and are not reliable for limiting damage caused by the disease (Cook 1981; Smiley & Patterson 1996; Paulitz et al 2002). Although there are no fully resistant wheat cultivars to crown rot disease (Pereyra et al 2004; Wisniewska & Kowalczyk 2005), use of genotypes that show some degree of resistance/tolerance is the most reliable and efficient approach to reduce yield losses due to *Fusarium* crown rot disease (Cook 2001).

The disease crown rot is also known by different common names including *Fusarium* crown rot, dryland foot rot, dryland root rot, *Fusarium* root rot and common root rot (Paulitz et al 2002). Crown rot is caused by a complex of fungal pathogens which include *F. culmorum* (W. G. Smith) Sacc. (Bockus et al 2010). These pathogens may occur singly, but they often exist together in the same fields and even within individual plants, and there may be difference in dominance of different pathogens at a specific location from year to year (Smiley & Patterson 1996). In Turkey and other parts of the world *F. culmorum* and *F. pseudograminearum* are the two most commonly reported damaging *Fusarium* species causing the disease (Cook 1992; Aktaş et al 1999; Burgess et al 2001; Tunali et al 2006). In Turkey, *F. pseudograminearum* is relatively common in the Marmara region while *F. culmorum* is more prevalent in the Central Anatolian Plateau (Burgess et al 2010).

In this study, reactions of 165 spring wheat lines obtained from CIMMYT were determined using an aggressive isolate of *Fusarium culmorum*. An abstract of this study has been published previously (Gebremariam et al 2018b).

2. Material and Methods

Fusarium culmorum isolate Fc2 determined as the most aggressive isolate in Gebremariam (2015) study was used in assessing reactions of 165 lines of spring wheat (*Triticum aestivum* L.) obtained from CIMMYT, Mexico. Monosporic cultures of *F. culmorum* isolate were grown on synthetic nutrient-poor agar (SNA) for 10-14 days at a temperature of 25 °C day/20 °C night, with 12 h photoperiod under cool white and black fluorescent light to initiate spore formation. Autoclavable plastic bags (25 cm x 38 cm) were filled with wheat bran (150 g) and moistened with distilled water (50 mL). It was then sterilized at a

temperature of 121 °C for 15 min. The sterilization was repeated two times at an interval of 24 h. The cultures were cut into pieces and put into plastic bags containing sterile wheat bran and incubated for 10-14 days under the same incubation conditions mentioned above. After 3 days, flasks and bags were shaken daily to provide uniform colonization of grains. The wheat bran colonized by spores of *F. culmorum* isolate was air dried under aseptic conditions before use. Spore suspensions were made by putting enough amount of wheat bran colonized by spores of the isolate in sterile distilled water, mixed well to let the spores become suspended in water, filtered using several layers of cheesecloth and the concentration adjusted to 1×10^6 spores/ml after counting spore number using a haemocytometer. The amount of suspension was adjusted according to the number of seedlings inoculated. One percent NaOCl solution was used for 3 min for surface disinfestation of wheat seeds. Surface disinfested seeds were rinsed twice in sterile distilled water. Seeds were then placed in Petri dishes with a stack of filter paper saturated with sterile distilled water and kept in an incubator at a temperature of 23 °C for 3-4 days for germination.

Single pre-germinated seed was placed in each plastic tube (2.5 cm in diam. x 16 cm in length) containing 55 g of sterile potting mixture of sand: soil: organic matter (50:40:10, v/v/v), covered with thin layers of same soil mixture and moistened. Plants were then kept in a growth chamber at a condition of 16 h photoperiod under artificial light, 25/15(±5) °C day and night temperatures and relative humidity of 60/80 (±10)% (Mitter et al 2006). Plants were supplied with water whenever necessary. One week after planting, plants were inoculated with 1 mL of spore suspension (1×10^6 spores mL⁻¹) amended with 0.1% v/v Tween 20 on stem bases (~ 0.5 cm above the soil) (Mitter et al 2006) using an aseptic pipette. Nine wheat cultivars (Table 1) were used as controls. The control cultivars were inoculated with the same amount and concentration of spore suspension. Each treatment (each wheat germplasm) was replicated 5 times. Treatments were arranged in Randomized Complete Block Design (RCBD) and plants were covered with plastic for 48 h to maintain high humidity and darkness required for fungal incubation (Mitter et al 2006). Plants were then placed at the same light, temperature and humidity conditions mentioned above. Plants were provided with an appropriate amount of water every day for the duration of the experiment. The experiment was repeated.

Nine weeks after inoculation, soil was washed off plants and leaf sheaths were removed. Scoring for the typical symptoms of browning on the crown and the main stem base was carried out using a 1-5 scale (1: 1-9%, 2: 10-29%, 3: 30-69%, 4: 70-89%, 5: 90-99%) modified from Wildermuth & McNamara (1994) according to Nicol et al (2001). Scale values were square root transformed and data were subjected to analysis of variance (ANOVA) using general linear models (GLM) procedure of SPSS (IBM SPSS Statistics 21) and means were compared using Tukey's HSD test (P= 0.05).

Table 1- Wheat genotypes used as controls in the screening experiment

<i>Wheat genotype</i>	<i>Accession no</i>	<i>CID</i> ¹	<i>Reaction</i> ²	<i>Wheat type</i> ³	<i>Sources</i> ⁴
Adana-99			MS	SW	TK
Altay-2000	010627		MR/MS	WW	TK
Carisma			MR	WW	IT
Suntop		200000963	MR	SW	AUS
Emu Rock		200000805	MS	SW	AUS
Janz	960370	4982215	MS	WW	AUS
Seri-82	951027		S	SW	MX
Kutluk-94	950660		S	WW	TK
Süzen-97	950283		S	WW	TK

¹CID, Cross Identification; ²MS, moderately susceptible; MR, moderately resistant; MS, moderately susceptible; S, susceptible
³SW, spring wheat; WW, winter wheat; ⁴TK, Turkey; IT, Italy; AUS, Australia; MX, Mexico

3. Results and Discussion

Based on the result of aggressiveness tests, the most aggressive *F. culmorum* isolate Fc2 was used to screen wheat germplasm for their reaction (Gebremariam 2015).

The mean disease severity scores for the lines tested ranged from 1.4 to 4.4 with an average of 3.1 (Table 2). Two lines out of the 165 lines tested (lines 147 and 158) were resistant (R) in their reaction and had scores of 1.4. Twenty lines (lines 5, 100, 143, 163, 32, 138, 86, 89, 104, 123, 153, 161, 8, 34, 142, 9, 15, 47, 116, 146) showed a moderately resistant (MR) reaction and had scores ranging from 1.6 to 2.4. The scores of both the R and MR lines were not significantly different from scores of MR control cultivars Suntop (1.6), Carisma (1.8) and Altay-2000 (2.4). Sixty-three percent of the lines were moderately susceptible (MS). The scores of MS lines ranged from 2.6 to 3.4 which were not significantly different from the MS control cultivars Adana-99 (2.6), Janz (2.6) and Emu Rock (2.6). Out of the 165 lines tested, 39 were susceptible (S) in their reaction. These S lines had scores ranging from 3.6 to 4.4 which were not significantly different from the score of the S control cultivars Süzen-97 (3.6) and Kutluk-94 (4.0).

Table 2- Mean disease severity scores and reactions of wheat lines and control cultivars tested against *Fusarium culmorum* isolate Fc2

Germplasm ^a	Score ¹	Reaction ^{2,3}
158, 147	1.4 ^{a*}	R
5, 100, 143	1.6 ^{ab}	MR
Suntop	1.6 ^{ab}	MR
163, 32, 138	1.8 ^{abc}	MR
Carisma	1.8 ^{abc}	MR
86, 89, 104, 123, 153, 161	2.0 ^{abcd}	MR
8, 34, 142	2.2 ^{abcde}	MR
9, 15, 47, 116, 146	2.4 ^{abcdef}	MR
Altay-2000	2.4 ^{abcdef}	MR
33, 54, 57, 76, 78, 87, 115, 120, 135, 137, 156, 157, 169	2.6 ^{abcdefg}	MS
Adana-99	2.6 ^{abcdefg}	MS
Janz	2.6 ^{abcdefg}	MS
Emu Rock	2.6 ^{abcdefg}	MS
67	2.8 ^{bcdefgh}	MS
22, 28, 30, 43, 75, 82, 85, 93, 95, 102, 103, 109, 114, 117, 118, 124, 127, 130, 148, 152, 155, 159, 160, 165, 168	3.0 ^{cdefghi}	MS
Seri-82	3.0 ^{cdefghi}	MS
12, 13, 16, 21, 40, 41, 44, 61, 65, 66, 74, 79, 80, 88, 96, 105, 111, 112, 119, 121, 122, 129, 132, 134, 139, 145, 154,	3.2 ^{defghij}	MS
2, 4, 10, 14, 17, 18, 23, 24, 26, 27, 29, 31, 35, 45, 46, 48, 55, 56, 63, 64, 77, 91, 94, 97, 98, 101, 107, 108, 113, 125, 126, 133, 141, 144, 149, 151, 162, 166,	3.4 ^{efghij}	MS
3, 68, 71, 73, 128, 136, 19, 25, 36, 42, 51, 52, 58, 62, 84, 92, 99, 106, 110, 164, 37, 39, 49, 60, 70, 72, 81, 131	3.6 ^{fghij}	S
Süzen-97	3.6 ^{fghij}	S
49, 60, 70, 72, 81, 131	3.8 ^{ghij}	S
6, 20, 38, 69, 83, 150, 167	4.0 ^{hij}	S
Kutluk-94	4.0 ^{hij}	S
7, 11, 53	4.2 ^{ij}	S
59	4.4 ^j	S

*, values that share a letter are not significantly different at 0.05 level, according to Tukey's HSD test; ¹, score of each germplasm is the mean of five replicates; ², R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible; ³, score ranges for corresponding reaction R=1-1.4, MR= 1.5-2.4, MS= 2.5-3.4, S= 3.5-4.4 and HS= 4.5-5. ^a, Adana-99, Altay-2000, Seri-82, Kutluk-94, Süzen 97, Carisma, Janz, Emu Rock and Suntop are control cultivars

Screening for resistance to *Fusarium* crown rot of wheat was started in Australia in 1960s (McKnight & Hart 1966; Purss 1966). Although most wheat varieties are susceptible, partial resistance to crown rot occurs. Screening for resistance and susceptibility can be carried out using seedling and adult plant tests and positive correlations between crown rot ratings in greenhouse and field trials have been documented (Klein et al 1985; Wildermuth & McNamara 1994; Mitter et al 2006; Li et al 2008). Therefore, the seedling

bioassay which is time saving and avoids effects of other seasonal or environmental factors can be used to screen large quantities of germplasms rapidly and promising materials can be taken to field testing. Wheat varieties vary in their reaction to crown rot, ranging from very susceptible to moderately resistant (Wallwork 2000). However, there are no fully resistant wheat cultivars to this disease (Pereyra et al 2004; Wisniewska & Kowalczyk 2005). The genotypes tested in our study showed differences in reaction ranging from resistant (R) to susceptible (S) to *F. culmorum* isolate Fc2. Two lines (147, 158) were resistant in their reaction and had scores of 1.4. Thirteen percent of the lines tested showed consistently resistant/moderately resistant (R/MR) reaction to *F. culmorum* isolate Fc2. Differences in reaction ranging from moderately resistant (MR) to susceptible (S) in wheat genotypes against *F. culmorum* have also been reported from Turkey (Demirci 2003). The search for resistance initially should focus upon only one species and expand later to include other species (Paulitz et al 2002; Miedaner et al 2012). For crown rot, a high correlation between the resistance to *F. graminearum* and *F. culmorum* in wheat and rye has been documented (Miedaner 1997). Therefore, the wheat lines that showed some degree of resistance to *F. culmorum* in our research can serve as useful sources of genetic resistance in breeding for *F. culmorum* in particular or can be expanded and used to search for resistance to other *Fusarium* species. These lines can also be used to reduce yield losses due to Fusarium crown rot and carryover of inoculum to the subsequent years.

The lines that showed consistent resistant/moderately resistant (R/MR) reactions can serve as useful sources of genetic resistance in breeding for Fusarium crown rot. Plant breeders attempting to incorporate resistance to crown rot into cereal crops in Turkey should focus on screening with *F. culmorum* isolates.

In areas where the damaging *F. culmorum* is prevalent, integrated management options should include crop rotation with at least 2 years break from wheat, use of varieties showing some degree of resistance to the disease, selecting proper nitrogen fertilization rates and irrigation management to maintain continuous moisture throughout the growing season.

4. Conclusions

Fusarium crown rot incited by *Fusarium culmorum* is one of the most important diseases limiting wheat yields. In this study, seedling reactions of 165 spring wheat breeding lines (*Triticum aestivum* L.) obtained from CIMMYT, Mexico were determined under growth room conditions using an aggressive isolate of *Fusarium culmorum*. Two out of the 165 lines tested (lines 147 and 158) were resistant (R) in their reaction and twenty lines (lines 5, 100, 143, 163, 32, 138, 86, 89, 104, 123, 153, 161, 8, 34, 142, 9, 15, 47, 116, 146) showed moderately resistant (MR) reaction. These promising wheat lines that showed some degree of resistance to *Fusarium culmorum* can serve as useful sources of genetic resistance in breeding for Fusarium crown rot.

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