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Resistance of winter wheat to *Heterodera filipjevi* in Turkey

Abdelfattah A. DABABAT[1,*], Gül ERGİNBAŞ-ORAKCI[1], Halil TOKTAY[2], Mustafa İMREN[3], Beyhan AKIN[1], Hans-Joachim BRAUN[4], Susanne DREISIGACKER[4], İbrahim Halil ELEKCİOĞLU[5], Alexei MORGOUNOV[1]

[1]International Maize and Wheat Improvement Center (CIMMYT), Emek, Ankara, Turkey
[2]Department of Plant Production Sciences and Technology, Faculty of Agricultural Sciences and Technology, Niğde University, Niğde, Turkey
[3]Biological Control Research Station, Adana, Turkey
[4]International Maize and Wheat Improvement Center (CIMMYT), Mexico City, Mexico
[5]Department of Plant Protection, Faculty of Agriculture, Çukurova University, Adana, Turkey

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Abstract: Cereal cyst nematodes (CCNs) are plant parasites that significantly limit global cereal production. The most frequently reported pathogenic species are *Heterodera avenae*, *H. filipjevi*, and *H. latipons*. One of the most cost-effective, environmentally friendly, and easily adopted control measures is the use of genetic host resistance, which maintains nematode populations below the economic damage threshold level. Many effective sources of resistance to CCNs have been identified in cereals; however, their effectiveness and usefulness is dependent on the interaction of the specific putative resistant accession and the CCN pathotype found in a specific region. In this study, 719 wheat lines from the Facultative and Winter Wheat Observation Nurseries, representing a broad geographical spectrum of breeding lines and varieties from Europe, Central Asia, and the International Winter Wheat Improvement Program, were screened against *H. filipjevi* under controlled conditions. The results indicated that 114 and 90 genotypes were ranked resistant and moderately resistant, representing 15.8% and 12.5% of the screened genotypes, respectively. The frequency of resistant genotypes observed in the germplasms varied significantly among the different original countries and was the highest for genotypes that originated from Bulgaria (59.3%). From those phenotyped germplasms, a set of 289 lines was genotyped to understand if resistance sources are located at the same site or originate from different locations in the genome.

Key words: Cereal cyst nematodes, *Cre*, International Winter Wheat Improvement Program, screening, tolerance

1. Introduction

Nematodes occur worldwide in nearly all environments and result in losses of approximately 10% of world crop production (Whitehead, 1998). Cereal nematodes are considered the most important group of plant parasitic nematodes on wheat and barley on a worldwide basis (Sikora, 1988). The cereal cyst nematodes (CCNs) are a group of closely related species that have been documented to cause economic yield losses, especially in nonirrigated wheat production systems in North Africa, West Asia, China, India, Australia, the United States, and parts of Europe (Nicol and Rivoal, 2008). The most common species of CCNs are *Heterodera avenae*, *H. filipjevi*, and *H. latipons* (Rivoal and Cook, 1993). Each of these species can have numerous pathotypes, and at least 12 pathotypes have been described for *H. avenae* (Wouts et al., 1995). Their worldwide distribution, predominance in areas where cereals are grown, and devastating negative impact on yields make CCNs major pests affecting the world's food supply. The effects of nematodes on plant growth and yield are commonly underestimated by farmers, agronomists, and pest management advisors because of difficulties in field detection (Bridge et al., 2002).

Many attempts have been made to control CCNs around the world, including cultural practices, chemical control, biological control, and development of resistant wheat varieties (Smiley and Nicol, 2009; Dababat et al., 2011). The use of resistant crop cultivars is considered the most effective and economical method for managing nematodes in both high and low value cropping systems. The effectiveness of resistance to CCNs depends on the effectiveness and durability of the sources of resistance, and on the correct identification of the nematode species and/or pathotype(s) present in the system.

Heterodera filipjevi has been increasingly reported worldwide ever since it was distinguished from *H. avenae*

* Correspondence: a.dababat@cgiar.org

in Tajikistan by Madzhidow (1981). It has been reported in the United States (Smiley et al., 2008), Germany (Grosse and Kohlmüller, 2004), Iran (Tanha Maafi et al., 2003), Sweden (Cook and Noel, 2002), India (Bishnoi and Bajaj, 2002), Russia (Balakhnina, 1989), and most recently China (Li et al., 2010). Sturhan and Rumpenhorst (1996) suggested that *H. filipjevi* species are distributed mainly in the East European-Oriental region. In Norway, *H. filipjevi* has been reported on oat, barley, summer wheat, and winter wheat (Holgado et al., 2004). Its pathogenicity on summer cereals was reported as similar to that of *H. avenae* (Andersson, 2005).

Turkey is considered one of the top 10 wheat-producing countries worldwide, with production of 19.7 $\times 10^6$ t and an average yield of 2.4 t/ha in 2010 (http://faostat.fao.org/). On the Central Anatolian Plateau (CAP), where drought stress is common, 3.15×10^6 ha (35% of the total land area) is used to produce rain-fed winter wheat. *H. filipjevi* is found throughout 87% of the CAP wheat-growing area and transition zone in Turkey (Riley et al., 2009). Most recently, *H. filipjevi* was extracted from soils in Southeast Turkey, where spring wheat types are grown (İmren et al., 2012b). In the rain-fed winter wheat areas of Turkey, *H. filipjevi* is the predominant CCN species and causes high losses in yield (Rumpenhorst et al., 1996; İmren et al., 2012b; Toktay et al., 2012). Since many cereal-growing regions have conditions similar to those in Turkey, this study can be considered representative of many geographical locations worldwide. The International Winter Wheat Improvement Program (IWWIP; www.iwwip.org) is a cooperative breeding program of the Turkish Ministry of Food, Agriculture, and Livestock; the International Maize and Wheat Improvement Center (CIMMYT); and the International Center for Agricultural Research in the Dry Areas (ICARDA). It develops broadly adapted germplasms for irrigated and semiarid areas of Central and West Asia and serves as a vehicle for global germplasm exchange. New advanced lines from IWWIP and germplasms submitted by cooperators are distributed annually to around 130 breeding programs in 50 countries through the international Facultative and Winter Wheat Observation Nursery (FAWWON). The IWWIP is located in the same facilities as the CIMMYT-Turkey Soil-Borne Pathogens Program, and these programs work in close cooperation to develop winter/facultative germplasms with resistance to soil-borne pathogens. The objective of this study was to identify new sources of resistance to *H. filipjevi* in wheat germplasm.

2. Materials and methods

2.1. Plant material

Each year, the Soil-Borne Pathogens Program receives germplasms from cooperating breeding programs in Turkey and from the IWWIP for screening against soil-borne pathogens including CCNs. In 2009–2011, candidates from the 18th and the 19th FAWWON nurseries, a total of 719 genotypes from 25 countries, were evaluated for resistance to *H. filipjevi*. This set of germplasms represents the global diversity of modern winter wheat across the major regions where this crop is grown, with the exception of China.

2.2. Molecular analysis

The DNA of the plant material was isolated from 10 to 15 seedlings grown in the greenhouse according to a modified CTAB method described in the CIMMYT laboratory handbook (repository.cimmyt.org/xmlui/bitstream/handle/10883/1333 /91195.pdf?sequence=1). The presence of an *Aegilops ventricosa*-derived gene *Cre5* on chromosome 2AS was verified using the gene specific marker reported by Huelgera et al. (2003). The 2 primer pairs VENTRIUP (5'- AGG GGC TAC TGA CCA AGG CT -3') and LN2 (5'- TGC AGC TAC AGC AGT ATG TAC ACA AAA -3') were used to amplify a 259-bp fragment in plants carrying the 2NS translocation. The polymerase chain reaction was carried out using an ABI GeneAmp 9700 thermocycler (Applied Biosystems, CA, USA) with the same running conditions as described at http://maswheat.ucdavis.edu/protocols/Lr37/index.htm but adopted to a final volume of 10 µL. Samples were separated by electrophoresis in a 2% agarose gel and visualized using ethidium bromide under UV light.

2.3. Nematode inoculum

Soil samples were collected from a field in Haymana, Ankara, Turkey (39°24′13″N, 32°37′14″E), and *H. filipjevi* cysts were extracted according to Cobb's decanting and sieving method (Cobb, 1918). The cysts were collected and surface sterilized with 0.5% NaOCl for 10 min and rinsed several times in distilled water. The cysts were kept in a refrigerator at a temperature of 4 °C and then were transferred to room temperature (10–15 °C) to enhance hatching. The hatched J2 larvae were used as inoculants in screening tests.

2.4. Growth chamber experiment

Single wheat seeds were planted in standard small tubes (16 cm in height × 2.5 cm in diameter) filled with a sterilized mixture of sand, field soil, and organic matter (70:29:1 v/v). The field soil and sand were sieved and sterilized at 110 °C for 2 h for 2 successive days, and the organic matter was kept at 70 °C for 5 h. After plant emergence, 3 tubes were selected per genotype and were inoculated with 300 freshly hatched J2 in 3 holes around the stem base. In the week after nematode inoculation, the plants were gently watered to increase the efficiency of nematode penetration. The plants were grown in a growth chamber with a 16-h artificial photoperiod and maintained at a temperature of

22 ± 3 °C with 70% relative humidity. Experimental units were arranged in a randomized complete block design with 3 replicates. Based on the period of time needed for cyst formation, the plants were harvested 9 weeks after juvenile inoculation. Soil from each tube was collected in a 2-L pot filled with water for cyst extraction, while roots were washed on nested sieves with 850 µm and 250 µm mesh sizes to free cysts from the root system. Cysts from both root and soil extractions were collected on the 250-µm sieve and counted under a stereomicroscope. These trials were replicated.

The genotypes were divided into 5 groups based on the number of cysts per plant, taking into account the reaction of check varieties with known resistance to CCN. The groups were: resistant (R) = as few as or fewer cysts than in the known resistant check; moderately resistant (MR) = slightly more cysts than in the resistant check; moderately susceptible (MS) = significantly more cysts than in the resistant check, but not as many as in the susceptible check; susceptible (S) = as many cysts as in the susceptible check and the number of cysts per root system considered damaging; and highly susceptible (HS) = more cysts than in the susceptible check. The 4 widely grown winter wheat check lines Katea (MR), Sonmez (MR), Kutluk (S), and Bezostaya (S) were used in this study. Throughout the trials, the number of cysts formed on the root systems ranged from 5 to 30 in the MR and S germplasms, respectively.

2.5. Statistical analysis

The data were analyzed according to standard analysis of variance procedures with SPSS 14. The Tukey test was used to explore the differences between treatments, with statistical differences considered significant at $P \leq 0.05$.

3. Results

The evaluation of 719 varieties and breeding lines from 25 countries resulted in the identification of 114 R genotypes (15.8%) and 90 MR genotypes (12.5%), as shown in Table 1. The highest frequency of R genotypes was observed in the germplasms from Bulgaria (59.3%), Russia (48.5%), and South Africa (44.9%). Among the countries with sizable screening sets, the HR and R genotypes were frequently recorded for Iran and Turkey.

Table 2 lists the R genotypes (varieties and breeding lines) based on 2 years of testing, along with the presence of the *Cre*5 gene identified through molecular markers. The germplasm with the *Cre*5 gene had equal probability to be R or S to CCN. Entries from the 18th FAWWON nursery were evaluated by IWWIP-cooperating programs in numerous locations worldwide during the 2010 and 2011 seasons. Data for agronomic traits from these evaluations are available at www.iwwip.org.

IWWIP International Winter Wheat Yield Trials (IWWYTs) for irrigated and rain-fed conditions are conducted by cooperators across the region to evaluate germplasm performance and select the best breeding lines or varieties. The availability of the data of IWWYT germplasms showing resistance to *H. filipjevi* allowed for calculation of correlations between the number of cysts per plant in the growth chamber in Turkey and grain yield in several locations participating in the trial (Table 3). However, there was no clear relationship between CCN resistance and grain yield, and most correlations were insignificant.

4. Discussion

So far, 9 resistance genes have been identified as controlling CCNs in bread wheat: *Cre*1 and *Cre*8 from *Triticum aestivum*; *Cre*2, *Cre*5, and *Cre*6 from *Aegilops ventricosa*; *Cre*3 and *Cre*4 from *Triticum tauschii*; *Cre*7 from *Aegilops triuncialis*; and *Cre*R from *Secale cereale* (Barloy et al., 2007). In a recent study conducted by İmren et al. (2012a) to identify genetic resistance to the cereal cyst nematodes *H. avenae*, *H. filipjevi*, and *H. latipons*, 6 *Cre* genes were used in some international bread wheat germplasms. The obtained results showed that the resistance genes *Cre*1, *Cre*3, and *Cre*7 provided a resistance reaction against both *H. avenae* and *H. latipons*, whereas the *Cre*8 and *Cre*R genes gave a resistance reaction to *H. filipjevi* only. There was no complete resistance by any of the 6 studied *Cre* genes to the 3 species. Toktay et al. (2012) screened a germplasm with *Cre*1 genes against *H. filipjevi* and the root lesion nematode (RLN) *Pratylenchus thornei*. Their results indicated no complete resistance and no relationship between *H. filipjevi* and *P. thornei* resistance. These most likely have quantitative resistance genes against both CCNs and RLNs (Toktay et al., 2006; Sheedy et al., 2012). However, it is clear that while the *Cre* genes provide resistance to *H. avenae*, they do not confirm resistance to the *H. filipjevi* Turkish population.

Of the wheat germplasms screened for resistance to CCNs in the Soil-Borne Pathogens Program, just 20% were identified as having at least a moderate level of resistance. The most promising wheat germplasm lines with acceptable resistance levels (R and MR) are subsequently crossed with high-yielding cultivars. This is important in that many locally adapted wheat varieties are susceptible to CCNs and having new resistant wheat germplasms available allows collaborators to create new crosses with their local varieties, therefore improving their genetic resistance to CCNs. In general, a relatively high frequency of CCN-resistant genotypes are identified in the diverse germplasms screened by IWWIP, but the genetic nature of the resistance and genetic diversity of these germplasms are not yet known.

In this study, the highest frequency of resistance genotypes was observed in the germplasms from Bulgaria and Russia and this is most likely due to recycling of resistant genes from Katea and its derivatives.

Table 1. Distribution of winter wheat germplasm accessions originating from different countries into 3 groups according to their resistance to *Heterodera filipjevi*.

Country of origin	Total number of entries	Group 1: Highly resistant		Group 2: Resistant		Group 5: Highly susceptible	
		Number of entries	%	Number of entries	%	Number of entries	%
Australia	7	1	14.3	2	28.6	2	28.6
Austria	5	0	0	0	0	3	60.0
Bulgaria	27	16	59.3	1	3.7	5	18.5
Canada	29	4	13.8	0	0	6	20.7
Georgia	4	0	0	1	25.0	0	0
Hungary	9	0	0	0	0	3	33.3
Iran	49	12	24.5	11	22.4	6	12.2
Kazakhstan	12	1	8.3	3	25.0	7	58.3
Mexico	12	0	0	2	16.7	4	33.3
Moldova	9	0	0	2	22.2	3	33.3
People's Republic of China	10	2	20.0	2	20.0	1	10.0
Romania	12	0	0	0	0	3	25.0
Russia	33	16	48.5	4	12.1	2	6.1
South Africa	49	22	44.9	3	6.1	2	4.1
Spain	3	0	0	2	66.7	0	0
Switzerland	5	0	0	0	0	2	40.0
Syria	14	0	0	0	0	11	78.6
Tajikistan	7	2	28.6	2	28.6	1	14.3
Turkey	82	17	20.7	9	11.0	15	18.3
IWWIP (TCI)	184	9	4.9	30	16.3	56	30.4
Ukraine	37	5	13.5	5	13.5	7	18.9
United Kingdom	6	0	0	0	0	2	33.3
United States	99	4	4.0	8	8.1	28	28.3
USA-IWWIP	10	3	30.0	3	30.0	2	20.0
Uzbekistan	5	0	0	0	0	0	0
Total	719	114	15.8	90	12.5	171	23.8

In many wheat-producing countries, very little attention is given to soil-borne diseases, especially plant parasitic nematodes, due to a shortage of experts for conducting surveys on nematode distribution, occurrence, and damage to wheat. This flow of international germplasms to such countries will benefit the wheat-producing community and ultimately farmers who have no experience with or knowledge of soil-borne diseases.

Diverse collections of wheat germplasms are important in understanding the genetic basis for resistance, determining the gene(s) responsible, and identifying which may be of great benefit to breeding programs by pyramiding different resistance genes into single lines. Following this study, a total of 289 lines representing the 5 different levels of nematode resistance were selected to identify possible molecular markers for resistance to CCNs through association mapping.

In conclusion, based on our earlier results in Turkey (İmren et al., 2012a), none of the known *Cre* genes in winter and spring wheat types provided complete resistance against the Turkish population of *H. filipjevi* based on varieties carrying *Cre* genes. In this study, only the *Cre5* gene was identified and there was no association with CCN resistance. New sources of resistance are involved in resistant germplasms, and this should be further investigated. Furthermore, a correlation between resistance to CCNs and grain yield at several key regional locations has not yet been established. In some locations,

Table 2. Winter wheat germplasms resistant to cereal cyst nematode *Heterodera filipjevi* (groups 1 and 2) based on 2 years of testing.

Nursery	Entry	Cross name	Country of Origin	Presence of *Cre5*
11CBWF	1	Katea1	Bulgaria	-
11CBWF	24	8023.16.1.1/KAUZ	Mexico-TCI[1]	n.a.
11ELITE-IRR	38	TX69A509.2//BBY/FOX/3/GRK//NO64/PEX/4/CER/5/ CHIL/2*STAR	TCI	n.a.
11ELITE-SA	7	LFN/VOGAF//LIRA/5/K134(60)/4/TOB/BMAN//BB/3/ CAL/6/F339P1.2	TCI	n.a.
11ELITE-SA	11	ORKINOS-2	Turkey-TCI	n.a.
11ELITE-SA	13	777TWWON87/3/F12.71/SKA//CA8055	TCI	n.a.
11ELITE-SA	15	1D13.1/MLT//TUI	Mexico-TCI	n.a.
18FAWWON-IRR	83	OWL/SOISSONS//ZARRIN	Iran	-
18FAWWON-IRR	84	SPB"S"//K134(60)/VEE"S"/3/DRUCHAMPS/4/ALVAND	Iran	-
18FAWWON-SA	209	BUC/PVN//MILAN/3/TX96V2427	TCI	+
18FAWWON-SA	222	CM98-112/4/HAWK/81PYI9641//MESA MOTHER LINE/3/ KS82W418/SPN	US-TCI	+
18FAWWON-SA	225	2180*K/2163//?/3/W1062A*HVA114/W3416	USA	-
18FAWWON-SA	263	HBK1064-3/KS84063-9-39-3-4W//X960103	USA	-
19FAWWON-IRR	21	BSP01/18 (DUZI)	S. Africa	-
19FAWWON-IRR	22	PAMYAT	Russia	-
19FAWWON-IRR	27	55.1744/MEX67.1//NO57/3/KAUZ/4/SHARK/F4105W2.1/5/ TX96V2427	TCI	-
19FAWWON-IRR	38	HBA142A/HBZ621A//ABILENE/3/CAMPION/4/ F6038W12.1	TCI	-
19FAWWON-IRR	65	ECONOMKA	Ukraine	-
19FAWWON-IRR	73	T03/17	S. Africa	-
19FAWWON-IRR	74	OLIFANTS	S. Africa	-
19FAWWON-IRR	77	BSP06/17	S. Africa	-
19FAWWON-IRR	85	NUDAKOTA	USA	-
19FAWWON-IRR	87	JAGARENE	USA	+
19FAWWON-IRR	129	ALAMOOT/SIDS8	Iran	-
19FAWWON-IRR	131	ALAMOOT/SIDS8	Iran	-
19FAWWON-IRR	134	OWL/SHIROODI/3/OWL//OPATA*2/WULP	Iran	-
19FAWWON-IRR	142	1-68-120/1-68-22/4/KAL/BB//CJ"S"/3/HORK"S"	Iran	-
19FAWWON-SA	303	SONMEZ	Turkey-TCI	-
19FAWWON-SA	357	EC - P	S. Africa	-
19FAWWON-SA	358	ART	USA	+
19FAWWON-SA	365	BEZENCHUKSKAYA380	Russia	-
19FAWWON-SA	375	ZARRIN*2/SHIROODI/3/ZARRIN//VEE/NAC	Iran	-

[1] TCI: Turkey-CIMMYT-ICARDA.

Table 3. Coefficients of correlations between *Heterodera filipjevi* resistance (number of cysts per plant) and grain yield in international winter wheat yield trials across several regional locations. *: P < 0.05, **: P < 0.01.

Country	Location	12th IWWYT-Irrigated	13th IWWYT-Irrigated	14th IWWYT-Irrigated	10th IWWYT-Semiarid	12th IWWYT-Semiarid	13th IWWYT-Semiarid
		2008–9	2009–10	2010–11	2007–08	2009–10	2010–11
		17 entries	29 entries	30 entries	22 entries	26 entries	19 entries
Afghanistan	Kabul	0.30	–0.24	–0.17	-	-	-
	Jalalabad	0.13	0.07	-	-	-	-
	Balh	0.31	-	-	-	0.53**	0.23
	Bamyan	0.33	-	-	-	0.58**	-
Armenia	Yerevan	–0.55*	-	-	0.22	-	-
Azerbaijan	Baku	-	–0.06	0.27	-	0.13	–0.41
	Gobustan	-	-	-	–0.04	–0.16	0.11
	Terter	0.22	0.02	-	-	-	-
Bulgaria	Dobrich	-	-	–0.30	0.41	-	–0.15
Iran	Karadj	–0.05	0.22	0.16	-	-	-
	Ardebil	–0.06	0.26	0.30	-	-	0.29
	Mashad	–0.20	-	–0.01	-	-	-
	Maragheh	-	-	-	0.47*	0.03	0.03
	Gamloo	-	-	-	-	0.17	0.24
Kazakhstan	Red Fall	-	-	–0.05	-	-	0.02
Moldova	Beltsy	–0.28	-	-	-	0.27	-
Romania	Fundulea	-	0.21	–0.44*	-	0.11	–0.12
Russia	Krasnodar	0.02	0.33	–0.09	0.24	0.30	–0.33
Serbia	Novi Sad	-	0.02	-	0.23	0.36	-
Syria	Aleppo	–0.08	-	-	–0.30	-	-
Tajikistan	Hissar	-	0.26	-	-	0.28	0.37
Turkey	Sakarya	–0.33	-	0.02	-	-	-
	Edirne	-	0.03	0.14	-	-	-
	Erzurum	–0.38	-	-	-	-	-
	Eskişehir	0.25	–0.03	0.07	0.29	0.01	–0.40
	Konya	0.06	0.22	0.41	-	–0.17	0.10
	Ankara	-	-	-	0	–0.19	0.18
Ukraine	Odessa	–0.20	-	-	0.02	-	-
Uzbekistan	Tashkent	0.10	0.05	-	0.08	-	-
	Karshi	-	0.43	0.04	-	0.25	-

such as Armenia in 2009 and Romania in 2011, CCN-resistant lines have had a yield advantage, while in other locations their yield is lower compared to CCN-susceptible germplasms (personal communication based on data at www.iwwip.org). Theoretically, resistant genotypes should have better yields when compared to nonresistant genotypes in fields where CCNs are present. More detailed experiments with isogenic lines of a balanced set of germplasms are needed to establish the effect of CCN resistance on grain yield.

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