International winter/facultative wheat elite germplasm: screening for resistance to soil borne pathogens in CIMMYT-Türkiye

AKIN B.[1,*], OZDEMIR F.[2], KESER M.[3], DABABAT A. A.[1], KOC E.[1], KOZVEREN F.[1], ERGINBAS ORAKCI G.[1]

[1] International Maize and Wheat Improvement Center (CIMMYT), Ankara, Türkiye, [3] International Center for Agricultural Research in Dry Areas (ICARDA), Ankara, Türkiye, [2] Bahri Dagdas International Agricultural Research Institute, Konya, Türkiye. * Correspondence: b.akin@cgiar.org

Summary

The International Winter Wheat Improvement Program (www.iwwip.org) develops germplasm adapted to Central and West Asia combining broad adaptation with resistance to prevailing abiotic and biotic stresses. IWWIP also plays a key role in facilitating global germplasm exchange among the winter wheat breeding programs. Germplasm developed by IWWIP as well as the material received from cooperators is distributed as Observation Nurseries and Yield Trials to more than 100 cooperators in 50 countries.

In this study, 982 wheat lines from Candidate International Winter Wheat Yield Trial Nurseries Irrigated & Semi-arid (CAND-IWWYT-IRR&SA), representing a broad geographical spectrum of breeding lines and varieties from Türkiye, Europe, USA, CIMMYT-Mexico, Central Asia, Iran, and the International Winter Wheat Improvement Program (IWWIP), have been screened against Cereal Cyst Nematodes (*Heterodera filipjevi)* and Crown Rot (*Fusarium culmorum*), under controlled conditions (growth room and greenhouse) by the Soil Borne Pathogen Program (SBP) team in Türkiye. One hundred thirty and 136 lines were scored resistant and moderately resistant to *H. filipjevi*, representing 13.2% and 13.8% of the screened germplasm, respectively. Out of the 446 wheat germplasm, 4 (0.9%) ranked as resistant and 18 (4%) moderately resistant (MR) to a local isolate of *Fusarium culmorum* under controlled greenhouse and growth room conditions. The best lines in terms of resistance for CCN-*H. filipjevi* and Crown rot-*F. culmorum* have been distributed to cooperators in 50 countries.

Introduction

Wheat is grown on 20% of the cultivated land area of the world and is the main food resource for 40% of the world's population (Braun et al. 2010). Among the biotic stress factors, *Fusarium species* causing foot, crown, and root rots occur in winter cereals worldwide virtually wherever cereal-based farming systems predominate (Burgess et al., 2001). In the drylands of Central and West Asia and North Africa (CWANA) soil-borne diseases (like crown rot) and various nematode species reduce the grain yield by up to 40%. These diseases and pests benefit from higher temperatures when they become more active (Colagiero and Ciancio 2011). The International Winter Wheat Improvement Program (IWWIP) 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). IWWIP aims to develop broadly adapted lines for irrigated and semi-arid areas of CWANA. More than 120 winter wheat varieties originating from IWWIP germplasm have been released in the target region and are grown on more than 2.5 mln ha. IWWIP-derived varieties are characterized by higher yield potential, drought tolerance, and disease resistance. Substantial additional income to farmers and the environment comes from reduced fungicide application on disease-resistant varieties (Morgounov et al. 2019).

A program for screening, validating, and disseminating SBPs resistant/tolerant germplasm has been established linking CIMMYT Mexico and IWWIP in Türkiye. One of the major components of the SBPs program work at CIMMYT-Türkiye involves screening of IWWIP developed elite lines and/or germplasm originating from different countries. The main objective is to work with plant pathologists to transfer SBPs resistance into adapted wheat backgrounds. For this purpose, IWWIP annually provides around 500 winter wheat lines comprised of facultative winter wheat observation nurseries (FAWWON) to screen winter wheat germplasm against SBPs.

Methods

Candidate lines from the 15Cand.IWWYT-IRR&SA to the 21Cand. IWWYT-IRR&SA nurseries, distributed between 2015 to 2021. Nine hundred eighty-two lines from 18 countries were evaluated for resistance to *H. filipjevi* and 446 lines for resistance to *F. culmorum*.

Screening for Nematodes: Screening tests were carried out using a population of *H. Filipjevi* collected from the naturally infested field in Kırsehir, Türkiye (39º 39′ 709″ N, 32º 37′ 14″ E) molecularly identified according to the Peng et al. 2013.

Growth Room Screening: Single pregerminated wheat seed was planted in standard small tubes (16 cm in height × 2.5 cm in diameter) containing a sterilized mixture of sand, field soil, and organic matter (70:29:1 v/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. At the sowing date freshly hatched 300 juvenile/ml were inoculated on the soil around the stem base. 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 (Erginbas et al. 2018).

Screening for Fusarium: A local *Fusarium species* isolated from a naturally infested field in Kırsehir, Türkiye (39º 39′ 709″ N, 32º 37′ 14″ E), molecularly identified as *F. culmorum* according to Nicholson et al. (1998) was used in all tests (Erginbas et al. 2018).

Greenhouse Screening: A single wheat seed was sown in each plastic tube (Stuewe and Sons, Corvallis, OR) (21 cm height x 3.8 cm diam.) filled with the same potting mixture as mentioned above and was inoculated with 0.25 g of wheat bran *F. culmorum* consisted of about $5x10^5$ spore per ml. Wheat bran was soaked in 1 ml water to estimate relative spore concentration by using a hemocytometer. The plants were left to grow under the greenhouse from October to June (winter wheat growing season) and harvested at maturity as lined with natural field conditions (Erginbas et al. 2018).

Experimental Design: Each treatment was replicated 3 times and tubes were placed in a completely randomized block design (CRBD).

Harvest: *Nematode-*; 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. *Crown Rot-*; The germplasma were ranked according to the browning/rotting severity on the crown and were compared to the controls used in this study (Erginbas et al. 2013a).

Assessment: *Nematode-*; The grouping was performed based on cyst number per plant compared to the cyst numbers on the known checks. The following ranking was followed; 1= resistant (R), 2= moderately resistant (MR), 3= moderately susceptible (MS), 4= susceptible (S) and 5= highly susceptible (HS) (Dababat et al. 2019). *Crown Rot-*; Infested plants were harvested and assessed based on the browning/rotting percentage on the crown which describes the stem (1 cm above soil level) according to the modified 1-5 scale: 1= 1-9% Resistant (R), 2= 10- 29% Moderately Resistant (MR), 3= 30-69% Moderately Susceptible (MS), 4= 70-89% Susceptible (S), 5= 90-100% Highly Susceptible (HS) (Erginbas et al. 2013a).

Results

In this report, all SBPs screening data of the CAND-IWWYT-IRR&SA generated between 2015-2021 provided by the SBP team is collated and presented. The cyst number ranged from 1 to 41. The tested lines showed a disease severity ranging between 1% and 89%. The lines were categorized into 5 groups based on their reaction ranging from Resistant (R) to Highly Susceptible (HS). The screening of 982 winter wheat germplasm resulted in identifying 13.2% as resistant, and 13.8% as moderately resistant to *H. filipjevi* in the growth room (Table 1). The screening of 446 winter wheat revealed that 0.9% of germplasm were resistant, and 4% were moderately resistant to *F. culmorum* in the greenhouse (Table 2). A total of 262 and 22 germplasm selected for CCN-*H. filipjevi* and CR-*F. culmorum* were distributed to cooperators (Table 3).

Table 1. Percentage of resistant and moderately resistant lines to Cereal Cyst Nematodes, *Heterodera filipjevi* in Candidates International Winter Wheat Yield Trials Nurseries

Nursery Name	Total tested	Group 1- Resistant				Group 2- Moderately Resistance			
		IRR/SA Germ-plasm	%	Globally distribut-ed germ-plasm	%	IRR/SA Germ-plasm	%	Globally distribut-ed germ-plasm	%
21CIWWYT-IRR&SA	137	8	6	3	2	12	9	3	2
20CIWWYT-IRR&SA	162	13	8	8	5	13	8	4	2
19CIWWYT-IRR&SA	157	18	11	11	7	29	18	20	13
18CIWWYT-IRR&SA	150	26	17	13	9	13	9	5	3
17CIWWYT-IRR&SA	147	22	15	11	7	43	29	21	14
16CIWWYT-IRR&SA	98	23	23	13	13	8	8	4	4
15CIWWYT-IRR&SA	131	20	15	11	8	18	14	10	8
TOTAL	**982**	**130**	**13.2**	**70**	**7.1**	**136**	**13.8**	**67**	**6.8**

Table 2. Percentage of resistant and moderately resistant lines to Crown Rot, *Fusarium culmorum* in the Candidates International Winter Wheat Yield Trials Nurseries

Nursery Name	Total tested	Group 1- Resistant in GR&GH				Group 2- Moderately Resistance in GR&GH			
		IRR/SA Germplasm	%	Globally distributed germplasm	%	IRR/SA Germ-plasm	%	Globally distributed germplasm	%
21CIWWYT-IRR&SA	138	1	0.7	1	0.7	5	3.6	2	1.4
19CIWWYT-IRR&SA	158	1	0.6	1	0.6	6	3.8	4	2.5
18CIWWYT-IRR&SA	150	2	1.3	2	1.3	7	4.7	3	2.0
TOTAL	**446**	**4**	0.9	**4**	0.9	**18**	4.0	**9**	2.0

Table 3. The number of screened and globally distributed germplasms selected resistance against to the Cereal Cyst Nematode *Heterodera filipjevi* and Crown Rot *Fusarium culmorum*

Country	Total num-bers of entries	CCN-number of resistant (group 1&2) entries	%		Total num-bers of entries	CR-GH number of resistant (group 1&2) entries	%
Azerbaijan	3	0	0		3	0	0
Belarus	1	0	0		1	0	0
Bulgaria	4	0	0		-	-	-
Switzerland	5	0	0		5	0	0
Croatia	2	1	50		-	-	-
Germany	5	2	40		5	0	0
Georgia	1	1	100		1	0	0
Hungary	11	5	45		4	0	0
Iran	25	4			13	1	7.7
Kazakhstan	14	4	29		6	0	0
Morocco	3	1	33		3	0	0
Romania	20	2	10		11	1	9.1
Tajikistan	3	0	0		3	0	0
Russia	26	3	12		17	0	0
Ukraine	5	0	0		5	0	0
USA	140	45	32		70	4	5.7
Turkiye	58	13	22		14	0	0
IWWIP-USA	75	23	31		39	4	10.3
IWWIP-Mexico	71	23	32		9	0	0
IWWIP	510	139	27		237	12	5.1
TOTAL	**982**	**266**	**27**		**446**	**22**	**4.9**

Discussion

Using resistant lines with high yield is the most effective and economical way to control SBPs, especially in drought-affected areas where cereals are cultivated and monoculture cropping systems exist (Erginbas-Orakci et al. 2013b). The performances of wheat lines tested in field trials in Türkiye and CWANA have indicated that some of them combine resistance to CCN and Crown Rot with competitive grain yield and resistance to yellow and stem rust. These lines have been extensively used as parental lines the most competitive lines may be released cultivars. Particular attention must be paid to managing SBPs through the introduction of resistance genes in widely grown cultivars, since an evaluation is needed regarding the persistence of the genetic pool introduced, on a scale of decades or years. Breeding for SBPs is extremely still a bottleneck and should be given more attention. More information about breeding challenges can be found in Braun's paper in this book.

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