Phytopathology® • 2023 • 113:1967-1978 • https://doi.org/10.1094/PHYTO-02-23-0043-R

Genetics and Genomics of Resistance

Association Mapping of Resistance to Tan Spot in the Global Durum Panel

Agnes Szabo-Hever,[1] Gurminder Singh,[2] Amanda R. Peters Haugrud,[1] Katherine L. D. Running,[2] Sudeshi Seneviratne,[2] Zengcui Zhang,[1] Gongjun Shi,[3] Filippo M. Bassi,[4] Marco Maccaferri,[5] Luigi Cattivelli,[6] Roberto Tuberosa,[5] Timothy L. Friesen,[1] Zhaohui Liu,[3] Steven S. Xu,[7] and Justin D. Faris[1,†]

[1] U.S. Department of Agriculture-Agricultural Research Service, Cereal Crops Research Unit, Edward T. Schafer Agricultural Research Center, Fargo, ND 58102
[2] Department of Plant Sciences, North Dakota State University, Fargo, ND 58102
[3] Department of Plant Pathology, North Dakota State University, Fargo, ND 58102
[4] International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat Institutes, Rabat 10101, Morocco
[5] Department of Agricultural and Food Sciences, University of Bologna, Bologna 40127, Italy
[6] Council for Agricultural Research and Economics-Research Center for Genomics and Bioinformatics, Fiorenzuola d'Arda 29017, Italy
[7] U.S. Department of Agriculture-Agricultural Research Service, Western Regional Research Center, Albany, CA 94710
Accepted for publication 15 May 2023.

Abstract

Tan spot, caused by the necrotrophic fungal pathogen *Pyrenophora tritici-repentis* (Ptr), is an important disease of durum and common wheat worldwide. Compared with common wheat, less is known about the genetics and molecular basis of tan spot resistance in durum wheat. We evaluated 510 durum lines from the Global Durum Wheat Panel (GDP) for sensitivity to the necrotrophic effectors (NEs) Ptr ToxA and Ptr ToxB and for reaction to Ptr isolates representing races 1 to 5. Overall, susceptible durum lines were most prevalent in South Asia, the Middle East, and North Africa. Genome-wide association analysis showed that the resistance locus *Tsr7* was significantly associated with tan spot caused by races 2 and 3, but not races 1, 4, or 5. The NE sensitivity genes *Tsc1* and *Tsc2* were associated with susceptibility to Ptr ToxC- and Ptr ToxB-producing isolates, respectively, but *Tsn1* was not associated with tan spot caused by Ptr ToxA-producing isolates, which further validates that the *Tsn1*−Ptr ToxA interaction does not play a significant role in tan spot development in durum. A unique locus on chromosome arm 2AS was associated with tan spot caused by race 4, a race once considered avirulent. A novel trait characterized by expanding chlorosis leading to increased disease severity caused by the Ptr ToxB-producing race 5 isolate DW5 was identified, and this trait was governed by a locus on chromosome 5B. We recommend that durum breeders select resistance alleles at the *Tsr7*, *Tsc1*, *Tsc2*, and the chromosome 2AS loci to obtain broad resistance to tan spot.

Keywords: chlorosis, durum, *Pyrenophora tritici-repentis*, QTL, resistance, tan spot, tetraploid wheat, *Tsc1*, *Tsc2*, *Tsr7*

Wheat is the second most important grain crop after corn with the worldwide production of 778.6 million ton in 2021/2022 (Shahbandeh 2022). About 5% of the total global wheat production (35 to 40 million t) is durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husnot., $2n = 4x = 28$, AABB genomes), which is well adapted to the hot, dry conditions surrounding the Mediterranean Sea and similar climates in other regions of the world. Durum is mainly used for making pasta, couscous, and other semolina-based products (reviewed in Giraldo et al. 2019).

†Corresponding author: J. D. Faris; justin.faris@usda.gov

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. USDA is an equal opportunity provider and employer.

Funding: This research was supported in part by an appointment to the Agricultural Research Service (ARS) Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA). ORISE is managed by ORAU under DOE contract number DE-SC0014664. All opinions expressed in this paper are the authors' and do not necessarily reflect the policies and views of USDA, DOE, or ORAU/ORISE. This work was supported in part by the USDA-ARS through project 3060-21000-038-000D.

***e*-Xtra:** Supplementary material is available online.

The author(s) declare no conflict of interest.

Tan spot, or yellow leaf spot, caused by the necrotrophic fungal pathogen *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* [Died.] Shoem.) (Ptr), is a severe foliar disease of both durum and bread wheat (*T. aestivum* L., $2n = 6x = 42$, AABBDD genomes). Average yield losses due to tan spot can reach 50% under extreme conditions (Rees et al. 1982; Shabeer and Bockus 1988). The practice of no-till farming can create favorable conditions for increased disease severity because infections very early in the season are caused by ascospores (primary inoculum) ejected from pseudothecia that mature on stubble residue over the fall and winter (Bockus and Claassen 1992; Shabeer and Bockus 1988). Fungal conidia (secondary inoculum) from the previous year's wheat residue cause increased disease incidence, especially in moist weather conditions (Shabeer and Bockus 1988).

Tan spot symptoms include necrotic lesions surrounded by chlorotic borders on the leaf tissue, and they are associated with the production of necrotrophic effectors (NEs), previously referred to as host-selective toxins (Strelkov and Lamari 2003). Ptr produces three NEs, including Ptr ToxA, Ptr ToxB, and Ptr ToxC (Ciuffetti et al. 1998), and Ptr isolates are classified into eight races based on the NEs they produce (reviewed in Faris et al. 2013). These NEs are recognized by corresponding host sensitivity genes in an inverse gene-for-gene manner to elicit cell death, which allows the pathogen to gain nutrients, sporulate, and ultimately cause disease (reviewed in Ciuffetti et al. 2010). In the nomenclature, sensitivity genes associated with reaction to culture filtrates containing NEs are designated as "*Tsc*" or "*Tsn*" depending on the chlorosis or necrosis symptoms exhibited on sensitive wheat plants. Other qualitative resistance genes identified only through conidial inoculations

are designated as "*Tsr*" (Faris et al. 2020; McIntosh et al. 2013, 2017). Previous reports suggest that the wheat-Ptr pathosystem also includes broad-spectrum, race-non-specific resistance quantitative trait loci (QTLs) in some materials (reviewed in Faris et al. 2013).

The sensitivity genes *Tsn1*, *Tsc1*, and *Tsc2* recognized the Ptr-produced NEs Ptr ToxA, Ptr ToxC, and Ptr ToxB and are located on wheat chromosome arms 5BL, 1AS, and 2BS, respectively (Effertz et al. 2001; Faris et al. 1996; Friesen and Faris 2004). *Tsn1* was cloned and found to encode serine/threonine protein kinase (S/TPK), nucleotide binding (NB), and leucine-rich repeat (LRR) domains (Faris et al. 2010). Recently, the *Tsc1* genomic region was delimited to a 1.4-centimorgan (cM) genetic interval spanning 184 kb on the short arm of chromosome 1A (Running et al. 2022). The *Tsc2* gene was delineated to a 3.3-cM interval and cosegregated with marker *XBE444541* (Abeysekara et al. 2010). Corsi et al. (2020) showed that the *Tsc2* locus on chromosome 2BS corresponds to a physical interval of 1.921 mega basepairs (Mbp) (23.106 to 25.027 Mbp) and containing 104 candidate genes in the reference genome of Chinese Spring.

Tan spot resistance in durum wheat has been less studied compared with that of hexaploid wheat. It has been shown in both natural and biparental populations that the *Tsn1*−Ptr ToxA interaction is not associated with the development of tan spot in durum wheat (Chu et al. 2010; Galagedara et al. 2020; Virdi et al. 2016). On the contrary, the *Tsc2*−Ptr ToxB interaction has been shown to play a significant role in disease development in various durum materials (Galagedara et al. 2020). Galagedara et al. (2020) conducted a genome-wide association study (GWAS) using a worldwide durum wheat collection inoculated with race 1 and race 3 isolates, which both produce Ptr ToxC. The results indicated significant *Tsc1*-Ptr ToxC association with tan spot caused by the race 1 isolate, but not with disease caused by the race 3 isolate.

Recently, a race-non-specific QTL region on chromosome 3BL that was first identified in the Brazilian spring wheat cultivar BR34 (Faris and Friesen 2005), and later in the U.S. spring wheat cultivar Penawawa (Kariyawasam et al. 2016), was defined as a single dominant gene in wild emmer wheat and designated as *Tsr7* (Faris et al. 2020). This QTL has been confirmed in durum wheat materials having broad-spectrum effects on resistance to different tan spot races (Chu et al. 2010; Galagedara et al. 2020). Furthermore, it was reported to be epistatic to the *Tsn1*−Ptr ToxA interaction in common wheat (Kariyawasam et al. 2016).

Although race 4 isolates have been collected from bread wheat (Abdullah et al. 2017a; Ali and Francl 2003; Singh et al. 2007) and occasionally from durum wheat (Benslimane et al. 2011; Kamel et al. 2019; Laribi et al. 2019), most have been collected from alternative hosts (Abdullah et al. 2017b; Ali and Francl 2003; Friesen et al. 2005; Šárová et al. 2005; Wei et al. 2021). Guo et al. (2020) identified several North Dakota Ptr isolates that were classified as race 4. These isolates were avirulent on common wheat lines, but they were virulent on some durum cultivars. Using two segregating tetraploid wheat populations, Guo et al. (2020) identified six QTLs on chromosomes 1A, 4B, and 5A associated with disease caused by these isolates.

The prevalence of Ptr races has been studied in several wheat-growing areas worldwide. Ptr ToxA-producing isolates are dominant in Canada, the United States, South America, Australia, and South Asia (Aboukhaddour et al. 2013; Ali and Francl 2003; Ali et al. 2010; Antoni et al. 2010; Engle et al. 2006; Kamel et al. 2019; Momeni et al. 2014; Singh et al. 2007; Wei et al. 2021). Ptr ToxB-producing isolates are rare in the Americas and Australia (Aboukhaddour et al. 2013; Ali and Francl 2003; Antoni et al. 2010; Wei et al. 2021), but they are common on durum wheat in North Africa and in some Caucasian countries (Gamba et al. 2017; Kamel et al. 2019; Lamari et al. 1995). Race 3 has been reported only in low frequencies in some areas in the United States and Canada (Ali et al. 2010; Engle et al. 2006; Singh et al. 2007; Wei et al. 2021). On durum wheat, races 5 and 7 were recovered from North African and European samples, and in the Americas, races 1, 2, 3, 4, and 5 have been reported (Ali and Francl 2003; Guo et al. 2020; Kamel et al. 2019; Laribi et al. 2019; Singh et al. 2007; Wei et al. 2021).

In 2015, the international durum wheat research community initiated a project with the goal to identify beneficial alleles among the global durum wheat germplasm and make them available for breeding programs. In this international call to action, the Global Durum Wheat Panel (GDP) was developed, which is a worldwide representation of *T. turgidum* ssp. *durum* modern germplasm and landraces, along with a selection of emmer and primitive tetraploid wheats (Mazzucotelli et al. 2020). Here, we selected durum wheat cultivars and landraces from the GDP and evaluated them for reaction to tan spot. Our objectives were to identify tan-spot-resistant durum lines and genetic loci associated with tan spot resistance.

Materials and Methods

Plant materials

The original set of lines making up the GDP consisted of 987 tetraploid lines including *T. turgidum* spp. *aethiopicum*, *carthlicum*, *dicoccoides*, *dicoccum*, *durum*, *polonicum*, *turanicum*, and *turgidum* (Mazzucotelli et al. 2020) was imported from the International Center for Agricultural Research in the Dry Areas (ICARDA) (Beirut, Lebanon). All the lines were grown under quarantine in 2018 in a greenhouse at the USDA-ARS Edward T. Shafer Agricultural Research Center, Fargo, North Dakota. Of this initial set, a total of 510 lines including 332 modern and 178 landrace accessions that exhibited feasible growth habits and produced adequate amounts of seeds were selected for further experiments. The selected lines represented 13 geographic regions and were obtained from 41 countries, the International Maize and Wheat Improvement Center (CIMMYT), and ICARDA (Supplementary Table S1). The four commonly used tan spot differential lines were included in each inoculation experiment including Salamouni (universal resistant), 'Glenlea' (sensitive to Ptr ToxA), 6B662 (sensitive to Ptr ToxB), and 6B365 (sensitive to Ptr ToxC). In addition to the differential lines, the durum variety 'Kronos' was included in the Ptr ToxB infiltration experiments as a Ptr ToxB-sensitive tetraploid check. In the Ptr ToxA infiltration experiments, the durum variety 'Langdon' and the common wheat differential line BG223 were planted as sensitive and insensitive checks, respectively.

Fungal inoculations and necrotrophic effector infiltrations

Seeds were planted in RL98 trays holding cones (Stuewe & Sons, Inc., Corvallis, OR) filled with Sunshine SB100 soil (Sun Grow Horticulture, Bellevue, WA). For each experiment, three seeds were planted per cone from all genotypes and checks. About 1 g of Osmocote Plus 15-19-12 fertilizer (Scotts Sierra Horticultural Product Company, Maysville, OH) was added to the soil in each cone. Plants were grown in a randomized complete block design consisting of one replicate per experiment, and each experiment was repeated three times. In Ptr spore inoculation experiments, the highly susceptible North Dakota State University winter wheat cultivar 'Jerry' was planted in cones along the borders in each RL98 tray to reduce edge effects. The trays were placed in a cold room (4°C, 12-h photoperiod) for 7 days to synchronize germination between genotypes. The plants were grown in a greenhouse with the temperature ranging from 20 to 25°C. For NE infiltrations, plants were grown in growth chambers at 21°C under a 12-h photoperiod. When the plants reached the two- to three-leaf stage (around 14 days after being planted under greenhouse/growth chamber conditions), the plants were used for spore inoculations or NE infiltrations.

The GDP was evaluated for reaction to tan spot using isolates Pti2 (race 1), 86-124 (race 2), 331-9 (race 3), L13-192 (race 4), and DW5 (race 5), which were all collected in the Upper Great Plains of North America (Friesen et al. 2005; Guo et al. 2020, and references therein). Pti2 produces the NEs Ptr ToxA and Ptr ToxC, 86-124 produces Ptr ToxA, 331-9 produces Ptr ToxC, L13-192 produces none

of the known NEs, and DW5 produces Ptr ToxB. Fungal spore suspensions were prepared following the standard procedure described by Lamari and Bernier (1989). The spore suspension was adjusted to approximately 2,000 spores/ml, and two drops of Tween-20 (surfactant agent) were added per 100 ml of inoculum prior to inoculation. Inoculum was applied using a paint sprayer (Husky; Home Depot) attached to an air supply with an air pressure setting of 1.0 bar. The plants were sprayed until the inoculum drops uniformly covered the leaf. Following inoculation, the plants were placed in misting chambers with 100% relative humidity at 21°C for 24 h and then placed in a growth chamber under a 12-h photoperiod at 21°C. Disease reactions were scored 7 days after inoculation following the 1 to 5 scale described by Lamari and Bernier (1989). The scale was expanded for isolate L13-192 (race 4), where lines exhibiting immunity were scored as 0. The accessions were categorized by lesion types in the following ranges: <1.0 (immune), 1.0 to 2.0 (highly resistant), 2.1 to 3.0 (moderately resistant to moderately susceptible), 3.1 to 4.0 (susceptible), and 4.1 to 5.0 (highly susceptible).

The *PtrToxA* and *PtrToxB* genes were expressed separately using the yeast *Pichia pastoris* expression system. The corresponding genetically modified X33 strains were prepared using the methods described by Friesen and Faris (2012), Liu et al. (2009), and Abeysekara et al. (2010). The effectors were expressed from the yeast cultures as follows: First, a small amount of frozen yeast culture was transferred and streaked onto a Petri plate (15 mm) with yeast peptone dextrose sorbitol (YPDS) and 100 µg/ml Zeocin media using a sterile toothpick. The plates were placed upside down in an incubator at 30°C for 3 days. Next, a sterile toothpick was used to transfer a single colony from the plate to 2 ml of yeast peptone dextrose (YPD) broth and the colony was incubated at 30°C for 48 h with constant shaking (220 rpm). The culture was then diluted 1:1,000 in 50 ml of new YPD media and incubated at 30°C for 48 h with constant shaking (220 rpm). Finally, the culture was centrifuged at 3,000 × *g* for 10 min, and the supernatant was filtered through a Millipore Durapore 0.45-µm low protein binding filter (HVLP04700). The purified culture was stored at –20°C until use.

To evaluate the GDP for reaction to Ptr ToxA and Ptr ToxB, approximately 20 µl of NE-containing culture filtrate (CF) was infiltrated into two plants (one leaf per plant) in each cone using a needleless 1-ml syringe. The infiltrated area (~3 cm) was marked with permanent marker on the leaves, and the plants were placed in a growth chamber (21°C, 12-h photoperiod) for 5 days. To evaluate reactions to Ptr ToxA and Ptr ToxB, a 0 to 3 scale was used as described by Friesen and Faris (2012). The expansion of chlorosis beyond the boundaries of Ptr ToxB infiltration to the distal end of the leaf was scored separately using 0 or 1, meaning the absence or presence of the symptom, respectively (Fig. 1).

Statistical analysis

Descriptive statistics were calculated using the software JMP Genomics 7 (SAS Institute, Cary, NC). A normality test for distribution of disease reaction data of each isolate was performed using Shapiro-Wilk under the "Goodness of Fit" option using the same program. Both Levene's and Bartlett's tests under the general linear model (GLM) procedure were used to test homogeneity of variances among replicates of each isolate and NE using SAS program version 9.4 (SAS Institute 2013). The least significant difference (LSD) value was also calculated under the same procedure. The responses of durum lines to tan spot were analyzed by macro areas and countries. The data were geographically plotted on a map using the "Graph Builder" option of the software JMP Genomics 7 (SAS Institute).

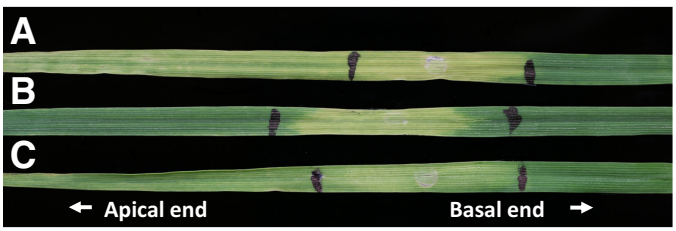

Fig. 1. Types of reaction to *Pyrenophora tritici-repentis* (Ptr) ToxB infiltration in durum lines. **A,** Durum line with scores 2 and 1 for infiltration area and expansion of chlorosis, respectively. **B,** Durum line with scores 2 and 0 for infiltration area and expansion of chlorosis, respectively. **C,** Durum line with scores 1.5 and 1 for infiltration area and expansion of chlorosis, respectively.

Plant genotyping and association mapping

Lines of the GDP were genotyped using the Illumina iSelect 90K single-nucleotide polymorphism (SNP) array (Wang et al. 2014) at the USDA-ARS Small Grains Genotyping Laboratory, Fargo, ND. DNA extraction, SNP data analysis, and the steps of marker filtering were as described by Mazzucotelli et al. (2020). Markers were filtered using previously published genetic maps (Maccaferri et al. 2015, 2019), and marker sequences were aligned to the Svevo.v1 reference genome (Maccaferri et al. 2019). SNP imputation was performed, and the redundant markers were pruned based on genome-wide linkage disequilibrium (LD) as explained by Mazzucotelli et al. (2020), which resulted in a data set with no missing SNP calls. The hapmap information of 855 genotypes and 13,373 SNPs developed by M. Maccaferri is located on the GrainGenes website (https://wheat.pw.usda.gov/GG3/global_durum_genomic_resources). The haplotype map of the 855 genotypes was reduced to 510 genotypes that were selected for phenotypic tests and for GWAS, as explained above. The SNP data set was selected according to the set filtering for minor allele frequency (MAF) >5% that resulted in a marker set containing 12,222 SNPs. Calculation of LD as r^2 values between markers (all possible marker pairs within chromosomes) and principal component analysis (PCA) were performed in R (https://www.r-project.org/) using the GAPIT v.3 toolpack (https://zzlab.net/GAPIT) (Wang and Zhang 2021). Population structure was analyzed in Structure v.2.3.4 (Pritchard et al. 2000) using a Bayesian clustering method. Structure analysis was implemented using a subset of 264 SNPs with LD at $r^2 < 0.2$ and setting the number of subpopulations (k) from 1 to 15 with each run repeated four times. The length of burn-in period was set at 5,000, followed by a simulation run length set at 10,000 using admixture model and correlated allele frequency. To estimate the optimal number of subpopulations, Δk values were calculated using the log probability (LnP[D]) values according to Evanno et al. (2005). Visualization of clusters was performed using JMP Genomics 7 (SAS Institute) under the "Clustering" and "Graph Builder" options.

Marker-trait association analysis was performed in R (https://www.r-project.org/) using the GAPIT v.3 toolpack (https://zzlab.net/GAPIT) (Wang and Zhang 2021). Three statistical models were tested in R: general linear model (GLM) (Patterson et al. 2006), mixed linear model (MLM) (Zhao et al. 2007), and fixed and random model circulating probability unification (FarmCPU) (Liu et al. 2016). The best model was determined using the quantile-quantile (QQ)-plot, where the negative logarithms of the *P* values from the models fitted in GWAS are plotted against their expected value under the null hypothesis of no association with the trait. Significant marker-trait associations were determined by false discovery rate (FDR) adjusted *P* values smaller than 0.05.

Identification of candidate genes

The closest flanking markers to the QTLs on chromosome arms 2AS and 5BL and at the *Tsr7* locus were used to identify candidate genes in the Svevo.v1 reference genome (Maccaferri et al. 2019). Annotated genes were identified by using the BioMart data mining tool on the EnsemblPlants website (https://plants.ensembl.org/). Attribute settings included "Gene stable ID," "Gene start (bp)," "Gene end (bp)," "Gene description," "GO term accession," and

"GO term name" to describe candidate genes. Attempts to identify candidate genes for other significant QTLs were not performed because the underlying genes have already been cloned.

Results

Reactions to tan spot inoculations and NE infiltrations

Normality tests revealed that disease reactions of the GDP did not fit a normal distribution for any of the five isolates ($P < 0.01$). Levene's test revealed that the three replicates for each of the isolates Pti2, 86-124, 331-9, and DW5 were homogeneous ($P = 0.0137$, $P = 0.2519$, $P = 0.0769$, $P = 0.0625$, respectively), whereas the three experiments of L13-192 were heterogeneous ($P < 0.0001$). Therefore, the disease scores of L13-192 are presented both for individual experiments and for average disease scores (Fig. 2; Supplementary Table S2).

Most GDP accessions were susceptible to isolates Pti2, 86-124, and 331-9, with overall average disease means of 3.41, 3.18, and 3.73, respectively (Fig. 2; Supplementary Table S2). About half of the panel was moderately susceptible to isolate DW5, resulting in an average lesion type of 2.76. Forty-one lines were susceptible to the race 4 isolate L13-192, with two of the lines being highly susceptible. L13-192 had an average disease score of 1.98, which was the lowest among all isolates (Fig. 2; Supplementary Table S2). Twelve durum accessions and two landraces had average disease scores of 2.5 or less for all five isolates (Table 1).

A total of 368 lines were insensitive and 142 lines (28%) were sensitive to Ptr ToxA, and all three replicates were homogeneous ($P < 0.9038$) (Supplementary Table S2). For the Ptr ToxA-producing isolates, Pti2 produced average tan spot reaction scores of 3.34 and 3.50 for the Ptr ToxA-insensitive and -sensitive lines, respectively, and 86-124 averaged 3.13 and 3.28 for insensitive and sensitive lines, respectively. The differences were not significant at the 0.01 level of probability ($t(507) = 2.25$, $P = 0.0248$ and $t(508) = 1.88$, $P = 0.0604$, respectively).

A total of 351 lines were insensitive to Ptr ToxB, and 159 lines (31%) were sensitive, and all three replicates were homogeneous ($P = 0.4689$). The Ptr ToxB-sensitive and -insensitive lines had average disease reactions of 3.29 and 2.51 to the Ptr ToxB-producing isolate DW5, showing a significant difference between the two groups ($t(507) = 13.39$, $P < 0.0001$). Among the Ptr ToxB-sensitive lines, a previously unreported phenotype consisting of chlorosis extending beyond the boundaries of infiltration to the distal end of the infiltrated leaf was observed in 101 (64%) of the 159 sensitive lines (Fig. 1). Ptr ToxB-sensitive lines that exhibited this expansion of chlorosis were more susceptible to disease caused by DW5 (average

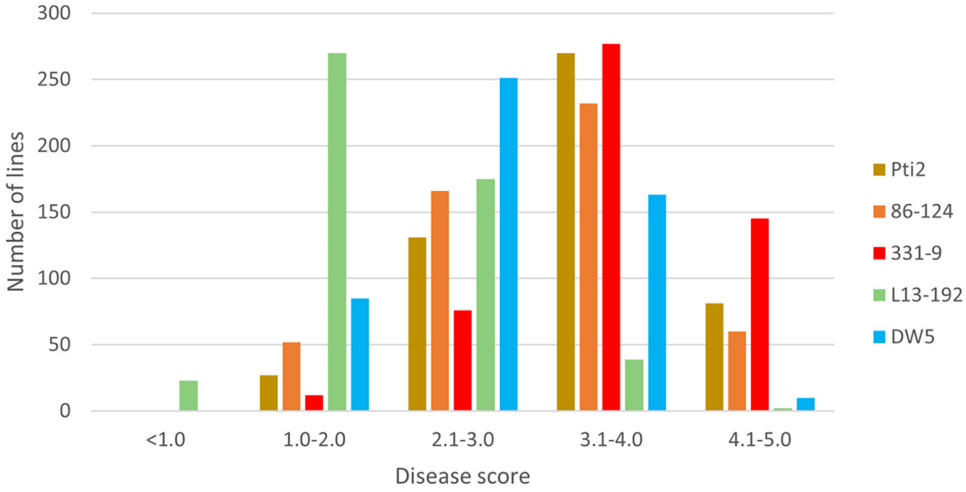

Fig. 2. Distribution of disease response to tan spot inoculations in the durum panel. Lines were evaluated on a 1 to 5 scale for Pti2 (race 1), 86-124 (race 2), 331-9 (race 3), and DW5 (race 5) and on a 0 to 5 scale for L13-192 (race 4).

TABLE 1. Durum lines resistant to all tan spot races and their reactions to necrotrophic effectors[a]

GDP accession code	Accession name	Improvement status	Country of collection/ development[b]	Pti2 Ave	86-124 Ave	331-9 Ave	L13-192 Ave	DW5 Ave	Ptr ToxA Ave	Ptr ToxB Ave	Ptr ToxB Chlorosis expansion Ave	SNB[c]
DWRC-0759	1A.1D5 + 106[d]	Modern	CIMMYT	1.50	1.33	1.50	1.50	1.00	0.08	0.83	0.00	R
DWRC-1435	CORE	Modern	Italy	1.50	1.67	2.17	1.00	1.17	0.00	0.75	0.00	R
DWRC-1393	SSD_531	Landrace	Italy	1.50	1.50	1.67	0.83	2.00	0.00	0.00	0.00	S
DWRC-1439	Odisseo	Modern	Italy	1.75	1.67	2.33	1.50	2.25	0.00	0.75	0.00	S
DWRC-1442	Tirex	Modern	Italy	2.00	1.17	2.50	0.83	1.00	0.08	0.00	0.00	R
DWRC-0779	Nefer	Modern	France	2.00	1.83	2.33	0.67	1.33	0.25	0.00	0.00	R
DWRC-0968	Miki1	Modern	ICARDA	2.00	1.50	2.17	1.00	1.33	0.00	0.33	0.00	R
DWRC-0639	Miki3	Modern	ICARDA	2.00	1.83	1.83	1.00	1.67	0.17	0.08	0.00	R
DWRC-0198	CBW_08131	Modern	Argentina	2.00	2.50	2.50	1.67	2.33	0.33	0.92	0.00	R
DWRC-0237	DAKTER	Modern	France	2.17	1.25	1.75	1.00	1.33	0.00	0.00	0.00	R
DWRC-0717	Tamaroi	Modern	Australia	2.33	1.83	2.50	1.00	1.00	3.00	0.17	0.00	R
DWRC-1024	Lebrija	Landrace	Spain	2.50	1.50	2.00	1.17	1.17	0.08	0.00	0.00	S
DWRC-0736	AAC_Raymore	Modern	Canada	2.50	1.17	1.83	1.33	1.50	3.00	0.08	0.00	R
DWRC-0255	ASTIGI	Modern	Spain	2.50	2.17	2.17	1.00	2.17	0.00	0.00	0.00	R

[a] Accession names, improvement status (accession history), and country of collection/development were obtained from Mazzucotelli et al. (2020).
[b] CIMMYT: International Maize and Wheat Improvement Center; ICARDA: International Center for Agricultural Research in the Dry Areas.
[c] Reaction to Septoria nodorum blotch (SNB) using *Parastagonspora nodorum* isolates SN4, Sn2000, and Sn2000KO6-1 (A. Szabo-Hever and J. D. Faris, *unpublished data*). R: resistant to all three isolates; S: susceptible at least to one of the isolates.
[d] Full accession name: 1A.1D5 + 106/2*WB881//1A.1D5 + 106/3*MOJO/3/BISU1/PATKA3.

disease reaction type = 3.54) than were Ptr ToxB-sensitive lines that did not show the spreading chlorosis phenomenon (average disease reaction type = 2.86), and the significant difference between the two groups ($t(157) = 9.52$, $P < 0.0001$) indicated that this trait is associated with enhanced virulence.

Geographically, the durum lines in South Asia, the Horn of Africa, the Middle East, the Balkans, and North Africa showed the highest susceptibility to all races with average tan spot reactions of 3.71, 3.63, 3.51, 3.44, and 3.12, respectively (Fig. 3; Supplementary Fig. S1; Supplementary Tables S3 and S4). In addition to the lines from these areas, accessions from Central Asia and North America were susceptible to Pti2 (race 1) and 331-9 (race 3), with average tan spot reactions of 3.63, 3.94, 3.48, and 3.64, respectively. Durum accessions from France, Australia, and Argentina showed the highest level of resistance to all races, with average disease scores of 2.51, 2.61, and 2.73, respectively. A high number of accessions from South Asia, Ukraine, and Canada showed sensitivity to Ptr ToxA, whereas lines from the Middle East, Eastern and Southern Europe, North Africa, the Horn of Africa, Australia, and Argentina showed sensitivity to ToxB (Fig. 3; Supplementary Fig. S1; Supplementary Tables S3 and S4). A low number of accessions does not demonstrate the true status of a country; therefore those represented by only one line are not mentioned above. It is also important to note that generally the durum landraces showed higher average disease severity (3.43) than the modern varieties (2.77). Therefore, the ratio between the two types of accessions might have influenced the classification of countries in some cases. For example, Iran and Iraq were represented only by durum landraces and were ranked as areas of highly susceptible lines, whereas France and Australia were represented only by modern durum wheat

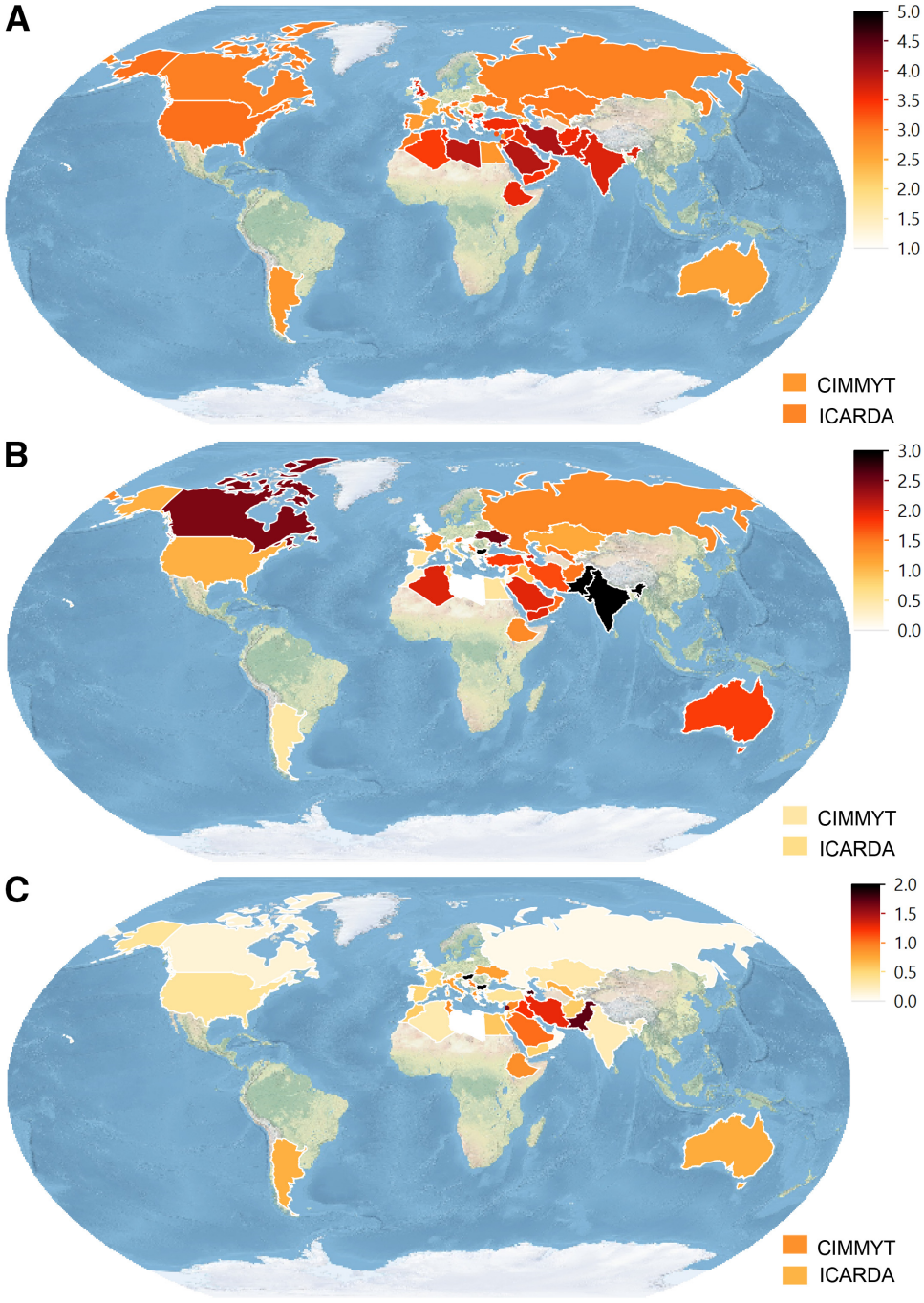

Fig. 3. Tan spot reactions on durum lines from the International Maize and Wheat Improvement Center (CIMMYT), the International Center for Agricultural Research in Dry Areas (ICARDA), and the countries included in this study. Color code indicates the severity of reaction. **A,** Average of all five isolates. **B,** Reaction to *Pyrenophora tritici-repentis* (Ptr) ToxA infiltration. **C**, Reaction to Ptr ToxB infiltration.

varieties and were ranked as regions of lines with moderate tan spot resistance.

Population structure and LD analysis

Mazzucotelli et al. (2020) developed a haplotype map consisting of 855 genotypes and 13,373 SNPs, and here we analyzed population structure, LD, and marker-trait associations, using a total of 12,222 SNP markers on the selected 510 genotypes. Population structure analyses using both the model-based clustering method implemented in the Structure program and the PCA from the program R revealed that the accessions of the GDP were grouped into two main clusters (Fig. 4; Supplementary Table S5). The first cluster (Cluster 1) consisted of 331 durum lines, including 95.2% of the modern durum accessions in the GDP. The second cluster (Cluster 2) consisted of 179 accessions, including 91.6% of the landraces in the GDP. Accessions from all geographic regions were represented in both clusters except landraces from South Asia, which were present only in Cluster 2, and all modern durum lines from CIMMYT were in Cluster 1. Accessions from different geographical regions were dispersed between subgroups within each cluster. The mean of LD decayed to 0.73 between markers with distance <0.1 Mb and 0.55 with distance <0.2 Mb (Fig. 5).

GWAS mapping

GWAS analysis was performed using GLM, MLM, and FarmCPU for each isolate and NE. The analysis of QQ plots of each method suggested that MLM was the best model to use for marker-trait association analysis. Only one significant QTL associated with tan spot caused by the race 1 isolate Pti2 was identified (Fig. 6; Supplementary Table S6). This QTL included two SNP markers (*Jagger_c1888_277* and *IACX2941*) on chromosome arm 1AS at positions 4,886,507 and 4,886,172 bp, respectively ($-\log_{10}(p)$ value = 5.1).

For the race 2 isolate 86-124, one QTL on chromosome 3B containing six SNPs was significantly associated with disease severity (Fig. 6; Supplementary Table S6). These markers spanned a physical segment from 469,885,090 to 478,521,702 bp with the most significant marker being *BobWhite_c5968_868* ($-\log_{10}(p)$ value = 10.6). The same QTL on 3B was significantly associated with tan spot caused by isolate 331-9 (race 3). This QTL included the same six SNPs that were associated with disease caused by isolate 86-124 and showed the strongest association with marker *BobWhite_c5968_868* ($-\log_{10}(p)$ value = 14.6).

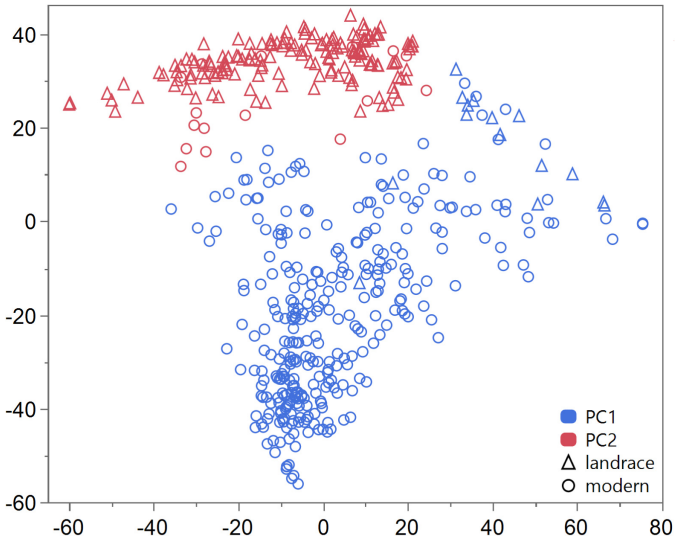

Fig. 4. Scatter plot of principal component (PC)1 and PC2 based on principal component analysis for the 510 durum wheat lines with the accession history categories.

The GWAS results of isolate L13-192 are presented both for individual experiments (Supplementary Table S6) and for average disease scores (Fig. 6; Supplementary Table S6) due to the heterogeneity of disease scores from the three replicates. There were no significant marker-trait associations detected with the disease scores from the first replicate. Three SNPs on chromosome 2A (*Tdurum_contig49928_728*, *Tdurum_contig10785_816*, and *Tdurum_contig10785_103*) were significantly associated with the disease scores from the second and third replicates, as well as with the average disease scores derived from all three replicates. This QTL spanned from 11,295,195 to 12,100,896 bp, with the most significant marker being *Tdurum_contig49928_728* ($-\log_{10}(p)$ value = 8.1). Another SNP marker (*Kukri_c102346_668*) near the same region of chromosome 2A at position 16,488,615 bp was also significant, but only in the second replicate.

Two QTLs significantly associated with tan spot caused by the race 5 isolate DW5 were identified (Fig. 6; Supplementary Table S6). Twelve SNPs between positions 22,398,197 and 24,313,744 bp on chromosome 2B showed significant associations. In this region, markers *BS00010318_51* and *BS00070900_51* showed the strongest association, with a $-\log_{10}(p)$ value of 18.7. In addition, nine SNPs between positions 696,814,300 and 698,038,471 bp on chromosome 5B were also significantly associated with disease, including the most significant makers *BobWhite_c31_3667* and *Excalibur_c31364_298* ($-\log_{10}(p)$ value = 5.6).

As expected for reaction to infiltration with Ptr ToxA, markers within the known location of the *Tsn1* gene were significant (Fig. 7; Supplementary Table S6). A total of 33 SNPs within this region of chromosome 5B were significantly associated with reaction to Ptr ToxA, and these markers spanned from 539,047,066 to 547,323,443 bp, including the most significant markers *BS00010590_51* and *IACX9261*, which both had a $-\log_{10}(p)$ value of 24.8.

Similarly, markers near the *Tsc2* locus were significantly associated with reaction to Ptr ToxB infiltrations (Fig. 7; Supplementary Table S6). Twenty-eight SNPs were significant and spanned a region from 21,589,974 to 31,661,087 bp on chromosome 2B. SNP markers with the strongest association were *BS00010318_51* and *BS00070900_51*, with a $-\log_{10}(p)$ value of 40.0.

With the expansion of chlorosis scores of Ptr ToxB infiltrations, three chromosome regions showed significant associations (Fig. 7; Supplementary Table S6). The QTL near the *Tsc2* locus on chromosome 2B contained 28 SNPs. This region spanned from 19,023,444 to 31,661,087 bp, including the most significant markers *BS00010318_51* and *BS00070900_51*, with a $-\log_{10}(p)$ value of 22.4. Two proximate chromosome regions on chromosome 5B were also associated with the expansion of chlorosis scores. The QTL spanning between 607,822,975 and 608,019,198 bp included

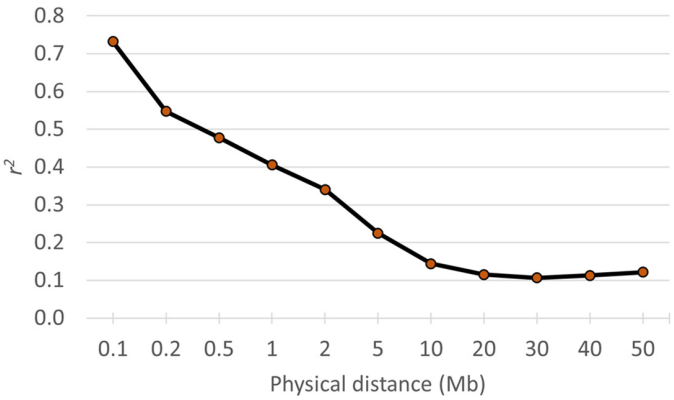

Fig. 5. Distribution of the mean pairwise linkage measure r^2 depending on the map distance between single-nucleotide polymorphism (SNP) markers.

the most significant marker, *Tdurum_contig68741_494* ($-\log_{10}(p)$ value = 4.3). The second QTL on chromosome 5B spanned between 696,814,300 and 698,194,557 bp, including the most significant markers *BobWhite_c31_3667* and *Excalibur_c31364_298* ($-\log_{10}(p)$ value = 7.7).

To further analyze the genetic background of the trait causing expansion of chlorosis, GWAS was performed on the 159 Ptr ToxB-sensitive lines, which included 101 lines displaying the chlorosis expansion trait and 58 lines lacking it. Results of the association mapping indicated that only one region on chromosome 5B, between positions 696,814,300 and 698,194,557 bp, containing 12 SNPs, was associated with the expansion of chlorosis phenomenon (Fig. 7; Supplementary Table S6). This QTL overlapped with the QTL identified with isolate DW5 and similarly showed the strongest association at markers *BobWhite_c31_3667* and *Excalibur_c31364_298* with a $-\log_{10}(p)$ value of 13.6.

The allele information of the SNPs identified in the QTL regions is given in Supplementary Table S7. The same information

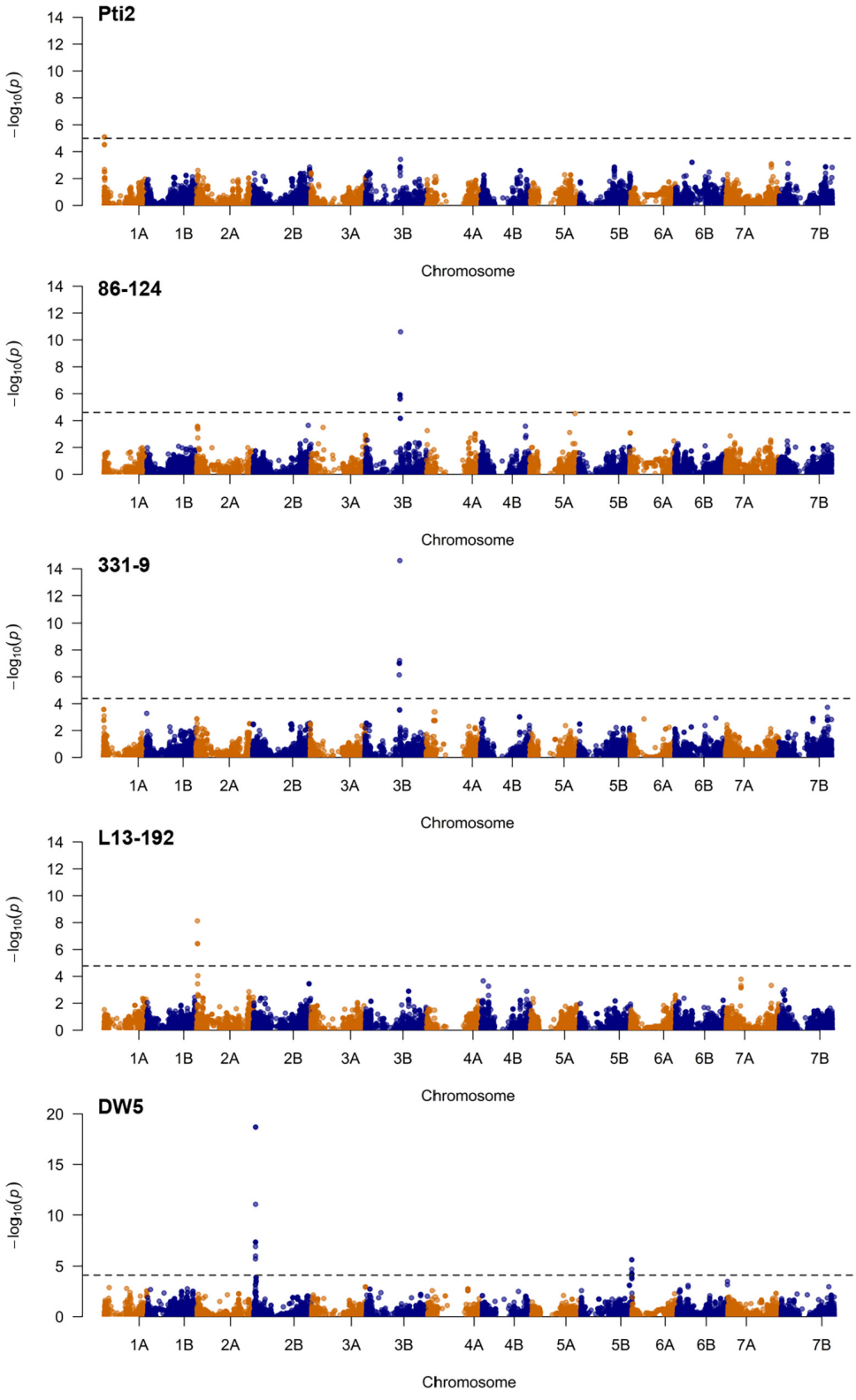

Fig. 6. Manhattan plots for the association mapping of reaction to the *Pyrenophora tritici-repentis* isolates including Pti2 (race1), 86-124 (race 2), 331-9 (race 3), L13-192 (race 4), and DW5 (race 5).

of the 14 durum lines that showed resistance to all races is shown in Supplementary Table S8.

Identification of candidate genes

Using the Svevo.v1 reference genome, totals of 76, 76, and 57 high-confidence annotated genes within 2.86-, 5.06-, and 12.12-Mb intervals were identified in the regions of QTLs on chromosome arms 2AS, 5BL, and the *Tsr7* locus, respectively (Supplementary Tables S9, S10, and S11). Each list contains several putative genes that encode proteins with disease-resistance-like features, making them the most plausible candidates.

Discussion

Geographical diversity of tan spot susceptibility in the GDP

Although the prevalence of Ptr races has been studied in several wheat-growing areas worldwide, less is known about the geographical distribution of prevalent susceptibility/sensitivity genes in wheat, especially in durum. Aboukhaddour et al. (2011) stated that the host type appears to influence the race structure of the pathogen population. This suggests that the frequency of sensitivity genes in wheat varieties is determined by the prevalent Ptr races in a geographical region. The GDP represents global diversity among durum varieties, and the overall reaction of the GDP to tan spot indicated that durum lines (including both landraces and durum varieties) from countries in South Asia, the Middle East, and North Africa are generally the most susceptible when averaging disease reaction over all races. This was also true for each individual Ptr race and for sensitivity to Ptr ToxA and Ptr ToxB; i.e., durum lines from these regions had more susceptibility to Ptr and more NE sensitivity. This is not unexpected given that the Middle East is considered the center of origin and diversity for Ptr (Vavilov 2009). It was found that Ptr race structure in the wheat center of origin (e.g., the Middle East, North Africa, and Caucasian regions) is more complex, which can be explained by the wide spectrum of host plants of Ptr in these regions (Aboukhaddour et al. 2011; Kamel et al. 2019).

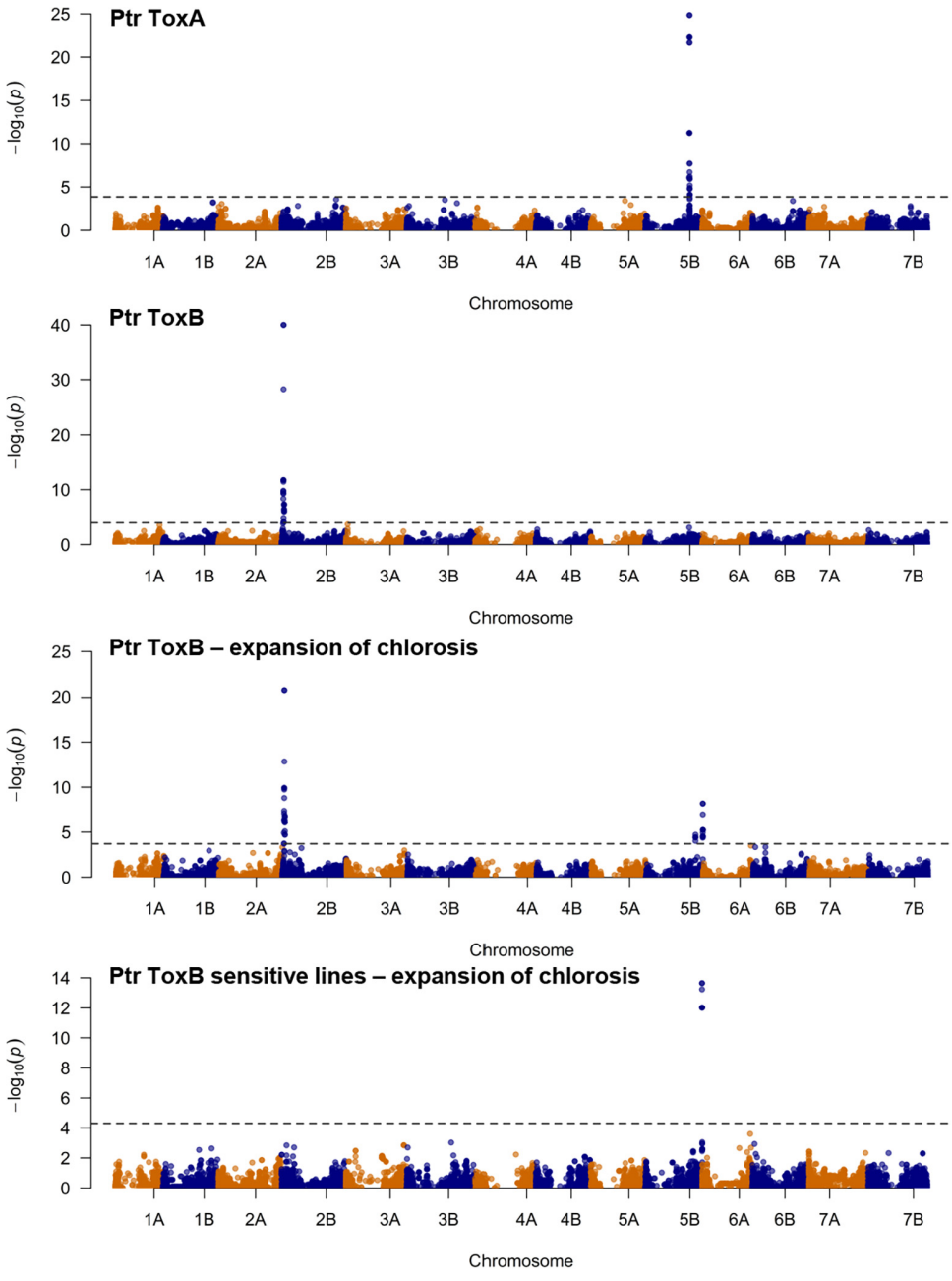

Fig. 7. Manhattan plots for the association mapping of sensitivity to *Pyrenophora tritici-repentis* (Ptr) ToxA, Ptr ToxB, and expansion of chlorosis in Ptr ToxB infiltrations for the whole population and for the Ptr ToxB-sensitive lines, respectively.

It is also interesting to note that Ptr ToxA sensitivity (i.e., presence of *Tsn1*) among durum lines from Canada, Australia, South Asia, the Middle East, and North Africa was highly prevalent, and, with the exception of Canada and Australia, the frequency of durum lines with susceptibility to race 2, which produces Ptr ToxA, was also high in these regions. Although Ptr ToxA-producing isolates are prevalent in these regions (Ali and Francl 2003; Singh et al. 2007), we would not expect the high frequency of *Tsn1* among durum varieties to be the reason because a compatible *Tsn1*−Ptr ToxA interaction is not associated with the development of tan spot in durum (Chu et al. 2010; Faris et al. 2020; Galagedara et al. 2020; Virdi et al. 2016). A more likely explanation is that common wheat varieties in these areas also have a high frequency of *Tsn1*, and Ptr ToxA sensitivity does play a role in disease development in some common wheat−Ptr interactions. The relatively low level of race 2 susceptibility (lesion type ~3.0) observed among Canadian and Australian durum lines compared with the high level of Ptr ToxA sensitivity in those same lines supports this notion.

Race 3 isolates have been reported in low frequencies on wheat in the United States and Canada (Ali et al. 2010; Engle et al. 2006; Singh et al. 2007; Wei et al. 2021), whereas race 1 isolates were prevalent in Canada, the United States, South Asia, the Middle East, and some European countries (Abdullah et al. 2017a; Ali and Francl 2003; Ali et al. 2010; Engle et al. 2006; Kamel et al. 2019; Šárová et al. 2005; Singh et al. 2007; Wei et al. 2021). Here, we observed the highest overall levels of susceptibility to races 1 and 3. The frequency of lines with susceptibility to race 1 would provide an explanation for the observed global frequency of race 1 Ptr isolates, but the same explanation does not apply to race 3 isolates, which are relatively rare, at least in the United States and Canada. The race 3 isolate used in this study (331-9) was isolated from durum wheat in Canada (Friesen et al. 2005); therefore it may be more adapted to durum wheat grown in North America. Because most studies conducted to evaluate the frequency of Ptr races used isolates collected from common wheat, more studies on the frequencies of Ptr isolates collected specifically from durum wheat lines may be needed to provide further insights on the relationships between the frequencies of races 1 and 3 and the susceptibility of durum varieties to these races. Infiltration assays with Ptr ToxC, once available, might also shed more light on the importance of a compatible *Tsc1*−Ptr ToxC interaction in disease caused by races 1 and 3, because by definition, both races produce Ptr ToxC.

Race 4 isolates have been reported on wheat in Tunisia, Morocco, Algeria, Canada, and the United States (Ali and Francl 2003; Benslimane et al. 2011; Gamba et al. 2017; Kamel et al. 2019; Laribi et al. 2019; Singh et al. 2007). Durum lines from these areas were moderately susceptible to the race 4 isolate L13-192. However, durum lines from West Asia (Saudi Arabia, Iran, and Israel) and the Horn of Africa showed the highest disease scores in our experiments, suggesting that virulent race 4 isolates may exist in these regions as well.

Regarding race 5, durum lines in the Middle East and North Africa exhibited the most susceptibility. This agrees with the finding that durum lines from these areas also showed the highest frequency of sensitivity to Ptr ToxB, indicating the frequency of the dominant *Tsc2* allele among the lines. As mentioned above, the higher levels of NE sensitivity and tan spot susceptibility observed in this region are not unexpected given that the Middle East is considered the center of origin for Ptr (Vavilov 2009).

Known genes governing resistance and susceptibility to tan spot in the GDP

The *Tsc1*−Ptr ToxC interaction was significantly associated with tan spot caused by isolate Pti2, but the effects of the *Tsc1* locus were relatively minor. This is in contrast to the results of Galagedara et al. (2020), who identified a stronger association between the *Tsc1* locus and isolate Pti2 in a GWAS using a set of durum lines from the USDA National Small Grains Collection (NSGC). It is possible that epistatic interactions involving genetic loci with relatively minor effects or insufficient marker coverage are reasons for the relatively minor association between *Tsc1* and tan spot caused by Pti2 in this panel. SNP loci on chromosome 3B near the *Tsr7* locus did not reach the significance threshold for association with tan spot caused by Pti2. Galagedara et al. (2020) found the *Tsr7* locus to be significantly associated with resistance to isolate Pti2 in the NSGC, but its effects were relatively minor compared with those of the *Tsc1* locus, which agrees with the findings of the current study.

The *Tsr7* locus on chromosome arm 3BL was associated with resistance to both race 2 and race 3 isolates used in this study. This finding agrees with others in that *Tsr7* plays a strong role in conferring resistance to these races in tetraploid wheat (Faris et al. 2020; Galagedara et al. 2020). One might expect to observe a significant effect of the *Tsc1* locus associated with the Ptr ToxC-producing race 3 isolate because *Tsr7* is not epistatic to *Tsc1* (Kariyawasam et al. 2016), and the latter was significantly associated with disease caused by Pti2. However, our results agree with those of Galagedara et al. (2020), who used the same isolates and found *Tsr7* but not *Tsc1* to be associated disease caused by the race 3 isolate 331-9 in the NSGC durum panel. The reason for significance of *Tsc1* in disease caused by Pti2 but not 331-9 is unknown, but perhaps *PtrToxC* is expressed at higher levels in the former compared with the latter. Once *PtrToxC* is cloned, expression analysis using these and other race 1 and race 3 isolates can address this question.

As expected, the *Tsn1*−Ptr ToxA interaction was not significantly associated with disease caused by the two Ptr ToxA-producing isolates, Pti2 and 86-124, in the GDP. It has been confirmed in multiple studies that this interaction does not play a role in tan spot development in durum wheat, but it is sometimes an important factor in disease development in hexaploid wheat (Chu et al. 2010; Faris et al. 2013, 2020; Galagedara et al. 2020; Virdi et al. 2016). In contrast, the *Tsn1*−ToxA interaction consistently plays a major role in septoria nodorum blotch (SNB) development caused by the fungus *Parastagonospora nodorum* in both durum and common wheat. The reason remains unknown, but Virdi et al. (2016) showed that the *SnToxA* gene from *P. nodorum* is expressed at much higher levels upon infection of durum lines compared with the *PtrToxA* gene from Ptr, which suggests that differences in gene regulation in the pathogen may affect the role of the *Tsn1*−ToxA interaction in contributing to disease susceptibility.

Identification of novel loci governing tan spot in the GDP

The disease data of race 4 isolate L13-192 showed association with SNPs located on chromosome arm 2AS. The same SNPs were near the significance threshold with isolate 86-124, indicating that the same QTL may be involved in governing reaction to both isolates. Guo et al. (2020) evaluated two tetraploid wheat populations using the race 4 isolates L13-192 and L13-14. However, it is interesting to note that even though Guo et al. (2020) used the same isolate as we used in the current study, no significant QTL on 2A was identified in their study, and instead they identified QTLs on chromosomes 1A, 4B, and 5A. Other studies have reported QTLs on chromosome arm 2AS associated with races 1 to 3 and 5 in hexaploid wheat (Chu et al. 2008; Friesen and Faris 2004; Gurung et al. 2014; Liu et al. 2017, 2020; Lozano-Ramírez et al. 2022; Shankar et al. 2017; Stadlmeier et al. 2019), and it is possible that some may be the same as the QTL reported here. Regardless, this is the first report of a QTL for resistance to tan spot caused by a Ptr race 4 isolate on durum chromosome 2A. Follow-up genetic studies are needed to determine whether the gene underlying the 2AS QTL is a dominant susceptibility gene like *Tsn1*, *Tsc1*, and *Tsc2* that recognizes an NE, or whether it confers dominant resistance like *Tsr7*.

As expected, infiltration of the GDP with Ptr ToxB and subsequent association analysis revealed a major QTL at the known position of the *Tsc2* locus. Inoculation experiments with fungal spores of the race 5 isolate DW5 revealed the same QTL associated with tan spot resistance caused by this isolate, indicating that the

Tsc2−Ptr ToxB interaction plays a major role in governing susceptibility to Ptr race 5 isolates in durum wheat. Again, this was not an unexpected result because the same has been observed in previous studies (Virdi et al. 2016). However, the GWAS involving tan spot caused by DW5 inoculations also revealed a second QTL on the long arm of chromosome 5B that has not previously been reported. Subsequent analysis revealed that the 5BL QTL observed in the DW5 GWAS was the same as the QTL associated with chlorosis expansion observed under Ptr ToxB infiltrations. Comparison of average disease lesion types among Ptr ToxB-sensitive lines with and without chlorosis expansion indicated that this phenotype, i.e., the ability for the NE to move beyond the site of infiltration to the distal end of the leaf, was associated with the development of larger lesions and increased disease caused by DW5 inoculations. The chlorosis expansion trait could therefore be considered a pathogen virulence factor, and response to that factor is governed by a single genetic locus on chromosome 5B of durum wheat.

To our knowledge, this factor has not previously been reported in either durum or common wheat. However, using crude culture filtrates from Ptr isolates of unknown races, Tomás and Bockus (1987) observed chlorosis extending toward the leaf tip but not toward the leaf base when large areas of the leaf were infiltrated, and they therefore suggested that toxic compounds are translocated through the vascular system. Indeed, it is logical to conclude that movement of toxic components, in this case Ptr ToxB, occurs through the vascular system. It is also possible that Ptr ToxB may activate a cell signaling pathway, resulting in expansion of chlorosis toward the leaf tip. However, it is intriguing that a genetic component governed by a single locus on chromosome 5B in the host can preclude movement of the NE in some sensitive lines. Furthermore, to date, we have not observed the translocation of any other NE including Ptr ToxA produced by Ptr or NEs such as SnTox1, SnTox3, and others produced by the necrotrophic fungus *P. nodorum*, which causes SNB in wheat. This would suggest that translocation of NEs is not universal or general and that specific recognition by the host via the 5B locus may be required for translocation of specific NEs. Clearly, more work is needed to understand the genetic and molecular mechanisms associated with Ptr ToxB translocation.

Identification of candidate genes

The sensitivity gene *Tsn1* has been cloned (Faris et al. 2010). We have also recently cloned *Tsc1* and *Tsc2,* and we are in the process of functionally characterizing both genes (K. L. D. Running, G. Singh, and J. Faris, *personal communication*). Here we list candidate genes providing susceptibility/resistance in the QTL regions on chromosome arms 2AS, 5BL, and at the *Tsr7* locus. The candidate gene analysis revealed several genes with LRR, NB, and PK domains, which are common among resistance genes. These resistance genes are often hijacked by necrotrophic pathogens, resulting in programmed cell death allowing the pathogen to cause disease (Faris and Friesen 2020). Development of new genetic markers and additional experiments are necessary to further reduce the number of candidate genes at these loci.

Conclusions

In conclusion, the results of this study indicate that *Tsr7* is a major resistance factor, *Tsc1* and *Tsc2* play significant roles in conferring susceptibility to tan spot among durum wheat varieties representing global diversity, and *Tsn1* is not a relevant factor associated with tan spot in durum. These results support those of multiple related studies evaluating the genetics of resistance to tan spot in durum wheat using both biparental and GWAS populations. However, unique findings of the current study include the factor on chromosome arm 2AS associated with tan spot caused by race 4, which was previously considered avirulent, and the novel trait characterized by expanding chlorosis leading to increased disease severity caused by the Ptr ToxB-producing race 5 isolate DW5. Taking the data together, we recommend that durum breeders strive to incorporate resistance alleles at the *Tsr7*, *Tsc1*, *Tsc2*, and the chromosome 2AS loci to obtain broad resistance to tan spot. And, although *Tsn1* is not associated with tan spot in durum, we strongly recommend the elimination of dominant *Tsn1* alleles from breeding material as well to enhance resistance to other ToxA-producing pathogens such as *P. nodorum* and *Bipolaris sorokiniana*. The 14 durum lines in Table 1 and Supplementary Table S7 provide breeders with a list of germplasm resistant to all races of tan spot that can be used in durum breeding programs to enhance resistance in local germplasm. It is also worthy to note that, in related research, we have found that all but three of these 14 lines (DWRC-1024, DWRC-1393, and DWRC-1439) are also resistant to SNB (Table 1) (A. Szabo-Hever and J. D. Faris, *unpublished data*). Therefore, 11 of the lines can be used to enhance resistance to both leaf blotch diseases.

Acknowledgments

We thank Danielle Holmes, Cayley Steen, Erika Shay Baer, Molly Holt, Jonathan Schwartz, Brandon Rasmusson, Lydia Lyons, Megan Overlander, and Stephanie McCoy for their technical assistance.

Literature Cited

Abdullah, S., Sehgal, S. K., Ali, S., Liatukas, Z., Ittu, M., and Kaur, N. 2017a. Characterization of *Pyrenophora tritici-repentis* (tan spot of wheat) races in Baltic States and Romania. Plant Pathol. J. 33:133-139.

Abdullah, S., Sehgal, S. K., Glover, K. D., and Ali, S. 2017b. Reaction of global collection of rye (*Secale cereale* L.) to tan spot and *Pyrenophora tritici-repentis* races in South Dakota. Plant Pathol. J. 33:229-237.

Abeysekara, N. S., Friesen, T. L., Liu, Z., McClean, P. E., and Faris, J. D. 2010. Marker development and saturation mapping of the tan spot Ptr ToxB sensitivity locus *Tsc2* in hexaploid wheat. Plant Genome 3:179-189.

Aboukhaddour, R., Cloutier, S., Lamari, L., and Strelkov, S. E. 2011. Simple sequence repeats and diversity of globally distributed populations of *Pyrenophora tritici-repentis*. Can. J. Plant Pathol. 33:389-399.

Aboukhaddour, R., Turkington, T. K., and Strelkov, S. E. 2013. Race structure of *Pyrenophora triciti-repentis* (tan spot of wheat) in Alberta, Canada. Can. J. Plant Pathol. 35:256-268.

Ali, S., and Francl, L. J. 2003. Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and nonce real grasses in the Great Plains. Plant Dis. 87:418-422.

Ali, S., Gurung, S., and Adhikari, T. B. 2010. Identification and characterization of novel isolates of *Pyrenophora tritici-repentis* from Arkansas. Plant Dis. 94:229-235.

Antoni, E. A., Rybak, K., Tucker, M. P., Hane, J. K., Solomon, P. S., Drenth, A., Shankar, M., and Oliver, R. P. 2010. Ubiquity of ToxA and absence of ToxB in Australian populations of *Pyrenophora tritici-repentis*. Australas. Plant Pathol. 39:63-68.

Benslimane, H., Lamari, L., Benbelkacem, A., Sayoud, R., and Bouzand, Z. 2011. Distribution of races of *Pyrenophora tritici-repentis* in Algeria and identification of a new virulence type. Phytopathol. Mediterr. 50:203-211.

Bockus, W. W., and Claassen, M. M. 1992. Effects of crop rotation and residue management practices on severity of tan spot of winter wheat. Plant Dis. 76:633-636.

Chu, C. G., Chao, S., Friesen, T. L., Faris, J. D., Zhong, S., and Xu, S. S. 2010. Identification of novel tan spot resistance QTLs using an SSR-based linkage map of tetraploid wheat. Mol. Breed. 25:327-338.

Chu, C.-G., Friesen, T. L., Xu, S. S., and Faris, J. D. 2008. Identification of novel tan spot resistance loci beyond the known host-selective toxin insensitivity genes in wheat. Theor. Appl. Genet. 117:873-881.

Ciuffetti, L. M., Francl, L. J., Ballance, G. M., Bockus, W. W., Lamari, L., Meinhardt, S. W., and Rasmussen, J. B. 1998. Standardization of toxin nomenclature in the *Pyrenophora tritici-repentis*/wheat interaction. Can. J. Plant Pathol. 20:421-424.

Ciuffetti, L. M., Manning, V. A., Pandelova, I., Betts, M. F., and Martinez, J. P. 2010. Host-selective toxins, Ptr ToxA and Ptr ToxB, as necrotrophic effectors in the *Pyrenophora tritici-repentis*—wheat interaction. New Phytol. 187:911-919.

Corsi, B., Percival-Alwyn, L., Downie, R. C., Venturini, L., Iagallo, E. M., Mantello, C. C., McCormick-Barnes, C., See, P. T., Oliver, R. P., Moffat, C. S., and Cockram, J. 2020. Genetic analysis of wheat sensitivity to the ToxB fungal effector from *Pyrenophora tritici-repentis*, the causal agent of tan spot. Theor. Appl. Genet. 133:935-950.

Effertz, R. J., Anderson, J. A., and Francl, L. J. 2001. Restriction fragment length polymorphism mapping of resistance to two races of *Pyrenophora tritici-repentis* in adult and seedling wheat. Phytopathology 91:572-578.

Engle, J. S., Madden, L. V., and Lipps, P. E. 2006. Distribution and pathogenic characterization of *Pyrenophora tritici-repentis* and *Stagonospora nodorum* in Ohio. Phytopathology 96:1355-1362.

Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol. Ecol. 14:2611-2620.

Faris, J. D., Anderson, J. A., Francl, L. J., and Jordahl, J. G. 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. Phytopathology 86: 459-463.

Faris, J. D., and Friesen, T. L. 2005. Identification of quantitative trait loci for race-nonspecific resistance to tan spot of wheat. Theor. Appl. Genet. 111:386-392.

Faris, J. D., and Friesen, T. L. 2020. Plant genes hijacked by necrotrophic fungal pathogens. Curr. Opin. Plant Biol. 56:74-80.

Faris, J. D., Liu, Z., and Xu, S. S. 2013. Genetics of tan spot resistance in wheat. Theor. Appl. Genet. 126:2197-2217.

Faris, J. D., Overlander, M. E., Kariyawasam, G. K., Carter, A., Xu, S. S., and Liu, Z. 2020. Identification of a major dominant gene for race-nonspecific tan spot resistance in wild emmer wheat. Theor. Appl. Genet. 133: 829-841.

Faris, J. D., Zhang, Z., Lu, H., Lu, S., Reddy, L., Cloutier, S., Fellers, J. P., Meinhardt, S. W., Rasmussen, J. B., Xu, S. S., Oliver, R. P., Simons, K. J., and Friesen, T. L. 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proc. Natl. Acad. Sci. U.S.A. 107:13544-13549.

Friesen, T. L., Ali, S., Klein, K. K., and Rasmussen, J. B. 2005. Population genetic analysis of a global collection of *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. Phytopathology 95:1144-1150.

Friesen, T. L., and Faris, J. D. 2004. Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. Theor. Appl. Genet. 109:464-471.

Friesen, T. L., and Faris, J. D. 2012. Characterization of plant−fungal interactions involving necrotrophic effector-producing plant pathogens. Methods Mol. Biol. 835:191-207.

Galagedara, N., Liu, Y., Fiedler, J., Shi, G., Chiao, S., Xu, S. S., Faris, J. D., Li, X., and Liu, Z. 2020. Genome-wide association mapping of tan spot resistance in a worldwide collection of durum wheat. Theor. Appl. Genet. 133: 2227-2237.

Gamba, F. M., Bassi, F. M., and Finckh, M. R. 2017. Race structure of *Pyrenophora tritici-repentis* in Morocco. Phytopathol. Mediterr. 56:119-126.

Giraldo, P., Benavente, E., Manzano-Agugliaro, F., and Gimenez, E. 2019. Worldwide research trends on wheat and barley: A bibliometric comparative analysis. Agronomy 9:352.

Guo, J., Shi, G., Kalil, A., Friskop, A., Elias, E., Xu, S. S., Faris, J. D., and Liu, Z. 2020. *Pyrenophora tritici-repentis* race 4 isolates cause disease on tetraploid wheat. Phytopathology 110:1781-1790.

Gurung, S., Mamidi, S., Bonman, J. M., Xiong, M., Brown-Guedira, G., and Adhikari, T. B. 2014. Genome-wide association study reveals novel quantitative trait loci associated with resistance to multiple leaf spot diseases of spring wheat. PLoS One 9:e108179.

Kamel, S., Cherif, M., Hafez, M., Despins, T., and Aboukhaddour, R. 2019. *Pyrenophora tritici-repentis* in Tunisia: Race structure and effector genes. Front. Plant Sci. 10:1562.

Kariyawasam, G. K., Carter, A. H., Rasmussen, J. B., Faris, J., Xu, S. S., Mergoum, M., and Liu, Z. 2016. Genetic relationships between race-nonspecific and race-specific interactions in the wheat−*Pyrenophora tritici-repentis* pathosystem. Theor. Appl. Genet. 129:897-908.

Lamari, L., and Bernier, C. C. 1989. Evaluation of wheat lines and cultivars to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. Can. J. Plant Pathol. 11:49-56.

Lamari, L., Sayoud, R., Boulif, M., and Bernier, C. C. 1995. Identification of a new race in *Pyrenophora tritici-repentis*: Implications for the current pathotype classification system. Can. J. Plant Pathol. 17:312-318.

Laribi, M., Gamba, F. M., Hassine, M., Singh, P. K., Yahyaoui, A., and Sassi, K. 2019. Race structure and distribution of *Pyrenophora tritici-repentis* in Tunisia. Phytopathol. Mediterr. 58:473-483.

Liu, X., Huang, M., Fan, B., Buckler, E. S., and Zhang, Z. 2016. Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. PLoS Genet. 12:e1005767.

Liu, Y., Salsman, E., Wang, R., Galagedara, N., Zhang, Q., Fiedler, J. D., Liu, Z., Xu, S., Faris, J. D., and Li, X. 2020. Meta-QTL analysis of tan spot resistance in wheat. Theor. Appl. Genet. 133:2363-2375.

Liu, Z., Faris, J. D., Oliver, R. P., Tan, K.-C., Solomon, P. S., McDonald, M. C., McDonald, B. A., Nunez, A., Lu, S., Rasmussen, J. B., and Friesen, T. L. 2009. SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the *Snn3* gene. PLoS Pathog. 5:e1000581.

Liu, Z., Zurn, J. D., Kariyawasam, G., Faris, J. D., Shi, G., Hansen, J., Rasmussen, J. B., and Acevedo, M. 2017. Inverse gene-for-gene interactions contribute additively to tan spot susceptibility in wheat. Theor. Appl. Genet. 130:1267-1276.

Lozano-Ramírez, N., Dreisigacker, S., Sansaloni, C. P., He, X., Islas, S. S., Pérez-Rodríguez, P., Carballo, A. C., Nava-Díaz, C., Kishii, M., and Singh, P. K. 2022. Genome-wide association study for resistance to tan spot in synthetic hexaploid wheat. Plants 11:433.

Maccaferri, M., Harris, N. S., Twardziok, S. O., Pasam, P. K., Gundlach, H., Spannagl, M., Ormanbekova, D., Lux, T., Prade, V. M., Milner, S. G., Himmelbach, A., Mascher, M., Bagnaresi, P., Faccioli, P., Cozzi, P., Lauria, M., Lazzari, B., Stella, A., Manconi, A., Gnocchi, M., Moscatelli, M., Avni, R., Deek, J., Biyiklioglu, S., Frascaroli, E., Corneti, S., Salvi, S., Sonnante, G., Desiderio, F., Marè, C., Crosatti, C., Mica, E., Özkan, H., Kilian, B., Vita, P. D., Marone, D., Joukhadar, R., Mazzucotelli, E., Nigro, D., Gadaleta, A., Chao, S., Faris, J. D., Melo, A. T. O., Pumphrey, M., Pecchioni, N., Milanesi, L., Wiebe, K., Ens, J., MacLachlan, R. P., Clarke, J. M., Sharpe, A. G., Koh, C. S., Liang, K. Y. H., Taylor, G. J., Knox, R., Budak, H., Mastrangelo, A. M., Xu, S. S., Stein, N., Hale, I., Distelfeld, A., Hayden, M. J., Tuberosa, R., Walkowiak, S., Mayer, K. F. X., Ceriotti, A., Pozniak, C. J., and Cattivelli, L. 2019. Durum wheat genome highlights past domestication signatures and future improvement targets. Nat. Genet. 51:885-895.

Maccaferri, M., Ricci, A., Salvi, S., Milner, S. G., Noli, E., Martelli, P. L., Casadio, R., Akhunov, E., Scalabrin, S., Vendramin, V., Ammar, K., Blanco, A., Desiderio, F., Distelfeld, A., Dubcovsky, J., Fahima, T., Faris, J., Korol, A., Massi, A., Mastrangelo, A. M., Morgante, M., Pozniak, C., N'Diaye, A., Xu, S., and Tuberosa, R. 2015. A high-density, SNP based consensus map of tetraploid wheat as a bridge to integrate durum and bread wheat genomics and breeding. Plant Biotechnol. J. 13:648-663.

Mazzucotelli, E., Sciara, G., Mastrangelo, A. M., Desiderio, F., Xu, S. S., Faris, J., Hayden, M. J., Tricker, P. J., Ozkan, H., Echenique, V., Steffenson, B. J., Knox, R., Niane, A. A., Udupa, S. M., Longin, F. C. H., Marone, D., Petruzzino, G., Corneti, S., Ormanbekova, D., Pozniak, C., Roncallo, P. F., Mather, D., Able, J. A., Amri, A., Braun, H., Ammar, K., Baum, M., Cattivelli, L., Maccaferri, M., Tuberosa, R., and Bassi, F. M. 2020. The Global Durum Wheat Panel (GDP): An international platform to identify and exchange beneficial alleles. Front. Plant Sci. 11:569905.

McIntosh, R. A., Dubcovsky, J., Rogers, W. J., Morris, C. F., Appels, R., and Xia, X. C. 2017. Catalogue of gene symbols for wheat, supplement. National BioResource Project. https://shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp

McIntosh, R. A., Yamazaki, Y., Dubcovsky, J., Rogers, J., Morris, C., Appels, R., and Xia, X. C. 2013. Catalogue of gene symbols for wheat. National BioResource Project. http://www.shigen.nig.ac.jp/wheat/komugi/genes/download.jsp

Momeni, H., Aboukhaddour, R., Javan-Nikkhah, M., Razavi, M., Naghavi, M. R., Akhavan, A., and Strelkov, S. E. 2014. Race identification of *Pyrenophora tritici-repentis* in Iran. J. Plant Pathol. 96:287-294.

Patterson, N., Price, A. L., and Reich, D. 2006. Population structure and eigenanalysis. PLoS Genet. 2:e190.

Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Rees, R. G., Platz, G. J., and Mayer, R. J. 1982. Yield losses in wheat from yellow spot: Comparison of estimates derived from single tillers and plots. Austr. J. Agric. Res. 33:899-908.

Running, K. L. D., Momotaz, A., Kariyawasam, G. K., Zurn, J. D., Acevedo, M., Carter, A. H., Liu, Z., and Faris, J. D. 2022. Genomic analysis and delineation of the tan spot susceptibility locus *Tsc1* in wheat. Front. Plant Sci. 13:793925.

Šárová, J., Hanzalová, A., and Bartoš, P. 2005. Races of *Pyrenophora tritici-repentis* in the Czech Republic. Acta Agrobot. 58:73-78.

Shabeer, A., and Bockus, W. W. 1988. Tan spot effects on yield and yield components relative to growth stage in winter wheat. Plant Dis. 72:599-602.

Shahbandeh, M. 2022. Worldwide production of grain in 2021/22, by type. Statista. https://www.statista.com/statistics/263977/world-grain-production-by-type/ (accessed September 23, 2022).

Shankar, M., Jorgensen, D., Taylor, J., Chalmers, K. J., Fox, R., Hollaway, G. J., Neate, S. M., McLean, M. S., Vassos, E., Golzar, H., Loughman, R., and Mather, D. E. 2017. Loci on chromosomes 1A and 2A affect resistance to tan (yellow) spot in wheat populations not segregating for *tsn1*. Theor. Appl. Genet. 130:2637-2654.

Singh, P. K., Mergoum, M., and Hughes, G. R. 2007. Variation in virulence to wheat in *Pyrenophora tritici-repentis* population from Saskatchewan, Canada, from 2000 to 2002. Can. J. Plant Pathol. 29:166-171.

Stadlmeier, M., Jørgensen, L. N., Corsi, B., Cockram, J., Hartl, L., and Mohler, V. 2019. Genetic dissection of resistance to the three fungal plant pathogens *Blumeria graminis*, *Zymoseptoria tritici*, and *Pyrenophora tritici-repentis* using a multiparental winter wheat population. G3 9:1745-1757.

Strelkov, S. E., and Lamari, L. 2003. Host parasite interactions in tan spot (*Pyrenophora tritici-repentis*) of wheat. Can. J. Plant Pathol. 25:339-349.

Tomás, A., and Bockus, W. W. 1987. Cultivar-specific toxicity of culture filtrates of *Pyrenophora tritici-repentis*. Phytopathology 77:1337-1340.

Vavilov, N. I. 2009. Origin and Geography of Cultivated Plants. Cambridge University Press, Cambridge, U.K.

Virdi, S. K., Liu, Z., Overlander, M. E., Zhang, Z., Xu, S. S., Friesen, T. L., and Faris, J. D. 2016. New insights into the roles of host gene-necrotrophic effector interactions in governing susceptibility of durum wheat to tan spot and Septoria nodorum blotch. G3 6:4139-4150.

Wang, J., and Zhang, Z. 2021. GAPIT Version 3: Boosting power and accuracy for genomic association and prediction. Genom. Proteom. Bioinform. 19: 629-640.

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S., Milner, S. G., Cattivelli, L., Mastrangelo, A. M., Whan, A., Stephen, S., Barker, G., Wieseke, R., Plieske, J., International Wheat Genome Sequencing Consortium, Lillemo, M., Mather, D., Appels, R., Dolferus, R., Brown-Guedira, G., Korol, A., Akhunova, A. R., Feuillet, C., Salse, J., Morgante, M., Pozniak, C., Luo, M.-C., Dvorak, J., Morell, M., Dubcovsky, J., Ganal, M., Tuberosa, R., Lawley, C., Mikoulitch, I., Cavanagh, C., Edwards, K. J., Hayden, M., and Akhunov, E. 2014. Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. Plant Biotechnol. J. 12: 787-796.

Wei, B., Despins, T., Fernandez, M. R., Strelkov, S. E., Ruan, Y., Graf, R., and Aboukhaddour, R. 2021. Race distribution of *Pyrenophora tritici-repentis* in relation to ploidy level and susceptibility of durum and winter bread wheat. Can. J. Plant Pathol. 43:582-598.

Zhao, K., Aranzana, M. J., Kim, S., Lister, C., Shindo, C., Tang, C., Toomajian, C., Zheng, H., Dean, C., Marjoram, P., and Nordborg, M. 2007. An *Arabidopsis* example of association mapping in structured samples. PLoS Genet. 3:e4.