GENETIC DISSECTION OF TAN SPOT RESISTANCE IN WHEAT

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Tan spot, caused by the necrotrophic fungal pathogen *Pyrenophora tritici-repentis* (*Ptr*), is a major foliar disease in wheat. QTL mapping and meta-QTL analysis are effective methods to understand genetic basis of tan spot resistance, which can further facilitate resistant variety development. A number of QTL mapping studies have been conducted in hexaploid bread wheat whereas few mapping studies have been carried out in tetraploid wheat. Four interconnected tetraploid wheat mapping populations were evaluated for resistance to race 2 isolate 86-124. Twelve QTL were identified in three of the four mapping populations. To further extend understanding of tan spot resistance, meta-QTL analysis was conducted by using reported QTL from 14 previous QTL mapping studies. Three meta-QTL located on chromosomes 2A, 3B, and 5A showed large genetic effects in multiple populations and conferred resistance to multiple races. Integrating those race-nonspecific QTL could provide high and stable tan spot resistance in wheat.

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LITERATURE REVIEW

Wheat

Wheat is one of the staple food crops in the world and provides about 20% of total calories and proteins for humans. Food and Agriculture Organization of the United Nations forecasted a total of 765 million metric tons of wheat produced globally in 2019 (www.fao.org/faostat). Modern cultivated wheat belongs to the genus *Triticum*, Tribe *Triticeae*, Family *Poaceae*. Tetraploid durum wheat (*T. turgidum* ssp. *durum*, 2n = 4x = 28, AABB) and hexaploid bread wheat (*T. aestivum* L. ssp. *aestivum*, 2n = 6x = 42, AABBDD) are cultivated mostly. About 95% of wheat production is hexaploid bread wheat (Shewry 2009). The origin of hexaploid wheat was found in somewhere on Iranian highlands or nearby areas (Beldrok 2000). Bread wheat is primary source of breads, cookies, cakes, noodles, etc. The unique milling and baking properities compared to other diploid and tetraploid were attributed to D sub-genome (Beldrok 2000). About one quarter of bread wheat production in U.S. is spring wheat, around 400 to 600 million bushels per year (https://www.ers.usda.gov/topics/crops/wheat). Durum wheat is a tetraploid species and one of first domesticated crops. Durum wheat is mainly used for pasta production in the European and North American regions and is also used for bread making in Middle East and North Africa (Troccoli et al. 2000). A total of two million hectares is annually planted for durum wheat and around 75 million bushels are produced (https://www.ndwheat.com/buyers/NorthDakotaWheatClasses/Durum). The United States is one of the top six durum wheat-producing countries with an average of 1.8 million Mg. per year. In US, over half of the durum wheat is produced in North Dakota (Elias and Manthey 2016).

Tan spot and its impact on wheat

Tan spot is a major foliar disease in wheat worldwide that is caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoemaker (Ali and Francl 2003). The disease is referred to as yellow spot, yellow blotch, or leaf blight (Hosford 1971). The fungus overwinters in a structure of pseudothecia on crop residues from last growing season, and ascospores are ejected from mature pseudothecia and dispersed by wind in the beginning of the growing season. Also, the asexual spore conidia can be produced by previous wheat stubble and adjacent infected plants, and germinated conidia can cause secondary infection (De Wolf et al. 1998; McMullen and Tika 2009). The spreading of infection may repeat several times in a growing season and causes the increase of disease incidence and severity. Two lesions types, necrosis and chlorosis, can be caused by tan spot on susceptible wheat leaves (Tuori et al. 1995). The infected leaf area by the fungus shows small, oval to diamond-shaped spots at the early stage, and the spots enlarge and are often surrounded by yellow borders (McMullen and Tika 2009). Kernels can be often infected and exhibit reddish discoloration (McMullen and Tika 2009).

The disease can potentially decrease test weight and yield by 50% in North Dakota (McMullen and Tika 2009). Rees et al. (1982) measured yield loss caused by tan spot in the field. In the high-disease treatment, a total of 49% yield loss was observed. Even in the low-disease treatment, grain yield could be reduced by 27% (Rees et al. 1982). Another study found that grain yield, test weight, and thousand grain weight were reduced by 29%, 5%, and 15%, respectively, with high inoculum conditions (Bhathal et al. 2003). In a survey of the Australian wheat industry, tan spot cost an average of 212×10^6 Australian dollars annually and was regarded as the most important disease of wheat in Australia (Murray and Brennan 2009). According to

NDSU Integrated Pest Management survey, regions in North Dakota were reported over 50% of tan spot incidence and/or severity in 2014, 2015, 2016, and 2017 (https://www.ag.ndsu.edu/ndipm).

Necrotrophic effectors produced by *Ptr*

Ptr produces necrotrophic effectors (NEs), also known as host-selective toxins (HSTs), which compromises wheat immune system and results in necrosis and chlorosis. To date, three NEs, Ptr ToxA, Ptr ToxB, and Ptr ToxC, have been identified (De Wolf et al. 1998; Effertz et al. 2002;Ciuffetti et al. 2010). The first isolated NE was Ptr ToxA, which was a size of 13.2 kDa protein (Tuori et al. 1995), and the effector induced necrosis in susceptible wheat genotypes (Ciuffetti et al. 1997). The *ToxA* gene coding Ptr ToxA was cloned and reported as a single copy gene in the isolates investigated (Ciuffetti et al. 1997, 2010). Friesen et al. (2006) postulated the *ToxA* gene was transferred from another wheat pathogen *Parastagonospora nodorum* to *Ptr*, because of low diversity of *Ptr* compared to high diversity of *Parastagonospora nodorum* and time of epidemics of *Ptr* reported.

Ptr ToxB, which induces chlorosis, was purified and characterized from culture filtrate of an Algerian isolate (Strelkov et al. 1999). The effector was also a protein with molecular mass of 6.61 kDa (Strelkov et al. 1999). The toxin showed no effects on the greening of etiolated tissue, which indicated Ptr ToxB degraded chlorophyll and led to chlorosis (Strelkov et al. 1998). Instead of single copy like *ToxA*, multiple copies (two to ten) of *ToxB* were found in race 5, and high copy number conferred high virulence in susceptible genotypes (Ciuffetti et al. 2010).

Ptr ToxC inducing chlorosis hasn't been well characterized (Liu et al. 2017). Ptr ToxC was reported to be a polar, non-ionic, low mass molecule (Faris et al. 2013). Effertz et al. (2002)

partially purified Ptr ToxC from an isolate of race 1 and observed extensive chlorosis by infiltration with the purified products.

Race classification

Based on the production of NEs and pathogenicity on a differential line set, *Ptr* isolates have been categorized into eight races (Lamari and Strelkov 2010; Faris et al. 2013). Races 2, 3 and 5 produce one NE each, Ptr ToxA, Ptr ToxC, and Ptr ToxB, respectively. Race 1 produces Ptr ToxA and Ptr ToxC, and race 4 is considered avirulent. Race 6 was reported with an isolate from Algeria and produces Ptr ToxB and Ptr ToxC (Strelkov et al. 2002). The last two races in the current classification system were identified by screening a collection of isolates from the Fertile Crescent and Caucasus regions (Lamari et al. 2003). Race 7 produces Ptr ToxA and ToxB, and race 8 produces all known NEs. A number of isolates from Arkansas induced symptoms in differential lines by lacking corresponding genes coding toxin, which indicated further amelioration needed for current classification system (Ali et al. 2010).

Host-pathogen interaction

Interaction between the NE and the wheat host was featured with an inverse gene-for-gene model (Wolpert et al. 2002; Strelkov and Lamari 2003; Ciuffetti et al. 2010; Faris et al. 2013; Liu et al. 2017). Contrast to dominant resistance gene in the host, a dominant gene conditioning susceptibility of the host, corresponds to a NE secreted by *Ptr*. The susceptibility gene corresponding to Ptr ToxA is *Tsn1* that was previously mapped to long arm of chromosome 5B (Faris et al. 1996; Lu et al. 2006) and encodes a protein with serine/threonine protein kinase, nucleotide binding, and leucine-rich repeat domains (Faris et al. 2010). Sensitivity to Ptr ToxB was conditioned by *Tsc2* localized in chromosome arm 2BS (Friesen and Faris 2004). *Tsc1* conferring sensitivity to Ptr ToxC, was first identified on chromosome arm 1AS by Faris et al.

(1997). Several qualitative resistance genes were identified and conferred resistance to specific races in certain populations. Singh et al. (2006) identified a resistance gene *tsr2* on the long arm of 3B in a tetraploid population. A gene mapped to 3DS in synthetic hexaploid wheat was named *tsr3*, conferring resistance to ASC1b, an isolate of race 1 (Tadesse et al. 2006a). Another gene, *tsr4* conferring resistance to ASC1a an isolate of race 1, was mapped to 3A using a population derived from a cross between resistant line Salamouni and Chinese Spring (Tadesse et al. 2006b). The two genes, *tsr3* and *tsr4* were mapped proximal to the marker alleles from a single SSR marker *Xgwm2* on 3A and 3D respectively, which suggested that *tsr3* and *tsr4* could be homeologous genes (Faris et al. 2013). Singh et al. (2008) identified and localized *tsr5* conferring resistance to DW13 (an isolate from race 5) on 3BL in tetraploid wheat. A race-nonspecific qualitative gene, *Tsr7*, conferred dominant resistance in wild emmer wheat (Faris et al. 2020), and the gene is likely the same as the QTL identified in BR34 (Faris and Friesen 2005) and Penawawa (Kariyawasam et al. 2016).

Genetic basis of resistance to tan spot in wheat

However, the wheat-*Ptr* interaction system is more sophisticated than the inverse gene-for-gene model. In addition to qualitative genes conferring resistance to tan spot, over 100 QTL have been identified from previous QTL mapping studies in wheat (Friesen and Faris 2004; Faris and Friesen 2005; Chu et al. 2008b; Chu et al. 2010; Sun et al. 2010; Faris et al. 2012; Faris et al. 2013; Kariyawasam et al. 2016; Liu et al. 2017). QTL conditioning resistance to more than one race have been identified. Two race-nonspecific QTL, *QTs.fcu-1BS* and *QTs.fcu-3BL*, were first identified with resistance to *Ptr* races 1, 2, 3, and 5 in BR34 × Grandin population (Faris and Friesen 2005). *QTs.fcu-5AL* localized between 138.4 and 140.1 cM in chromosome 5AL was associated with races 1, 2 and 5 (Chu et al. 2008). Another two QTL on chromosome 5A,

QTs.fcu-5A.1 and *QTs.fcu-5A.2*, were discovered related to races 1 and 2 in different mapping population (Chu et al. 2010). The QTL *QTs.fcu-5A.1* might be identical to *QTs.fcu-5AL* (Chu et al. 2010). The QTL *QTs.fcu-7B* conferring resistance to races 1 and 2, and Arkansas isolate in Salamouni × Katepwa population (Faris et al. 2012). A soft white spring wheat cultivar Penawawa is sensitive to Ptr ToxA but shows resistance to all *Ptr* races (Kariyawasam et al. 2016). A major race-nonspecific QTL conferring resistance to all races was identified on long arm of chromosome 3B (Kariyawasam et al. 2016). Another race-nonspecific QTL on chromosome 5A associated with resistance to races 1, 2, 3, and 5, and Arkansas isolate was identified in a tetraploid wheat population derived from a cross between Ben and PI 41025 (Galagedara 2018).

QTL mapping and meta-QTL analysis

QTL mapping with a population derived from two inbred parents is a common method used for genetic dissection of tan spot resistance in wheat. QTL identified from one single bi-parental mapping population may not play an important role in other populations, limiting inference space of the QTL studies and applications of marker-assisted selection (Holland 2007). In addition, small population size of one single bi-parental mapping population allows QTL with large effect detected only, maps a QTL in a large region, and overestimates effect of the identified QTL (Xu 2003). Using interconnected populations was one approach to overcome those potential drawbacks of QTL mapping with one single bi-parent mapping population. Using interconnected populations sharing one common parent could capture genetic diversity, improve power of QTL detection, and increase mapping resolution (Holland 2007; Yu et al. 2008). The effect of QTL in a specific population and interactions between QTL and population can be delineated by using interconnected populations (Holland 2007). An interconnected population

consisting of ten spring wheat populations was evaluated for stem rust resistance (Bajgain et al. 2016). The joint analysis detected 59 resistance QTLs, and only 14 of these QTLs were identified in single-population QTL mapping. Successful utilization of interconnected population in genetic dissecting of stem rust resistance in wheat (Bajgain et al. 2016), flowering time in maize (Buckler et al. 2009), leaf architecture in maize (Tian et al. 2011), inflorescence architecture in maize (Brown et al. 2011), biomass yield in *Miscanthus* (Dong et al. 2018) has been also reported.

Meta-QTL analysis, utilizing the QTL reported from previous mapping studies, is another method that could enhance genetic dissecting of a complex trait. In meta-QTL analysis, a consensus map is constructed by assembling individual linkage maps. Then, QTL identified in individual studies are projected onto the consensus map and are subject to subsequent statistical analysis (Goffinet and Gerber 2000). Meta-QTL analysis can reduce the interval of a QTL and further facilitate fine mapping and candidate gene analysis (Sosnowski et al. 2012). A meta-QTL analysis on grain weight of tetraploid wheat resulted in a short region (~60 Mb), and candidate gene analysis revealed a wheat ortholog to *OsGRF4* (Avni et al., 2018). Meta-QTL analysis considers genetic background and environmental factors that can test stability of QTL effects, and find co-localization of genetic factors controlling one single trait or multiple traits. In a meta-QTL analysis of FHB resistance in wheat, a total of 209 initial QTL involving four different types of resistance were clustered into 43 meta-QTL on 21 chromosomes (Liu et al. 2009). Meta-QTL analysis can remove redundant QTL and prioritize QTL for marker-assisted selection in breeding program. Soriano and Royo (2015) conducted meta-QTL analysis on leaf rust resistance in wheat and collected 144 resistant QTL identified from 20 bi-parental populations. A total of 71 meta-QTL clustered from 477 initial QTL were identified, which provided valuable

information for marker-assisted selection for phenology, biomass and yield of durum wheat (Soriano et al. 2017).

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PAPER 1: QTL MAPPING OF RESISTANCE TO TAN SPOT INDUCED BY RACE 2 OF *PYRENOPHORA TRITICI-REPENTIS* IN TETRAPLOID WHEAT

Abstract

Durum is a tetraploid species of wheat and a staple food crop in the world. Tan spot, caused by the necrotrophic fungal pathogen *Pyrenophora tritici-repentis* (*Ptr*), is a major foliar disease of both tetraploid durum wheat and hexaploid bread wheat. Understanding the *Ptr*-wheat interaction and identifying major QTL can facilitate the development of resistant cultivars and effectively mitigate the negative effect of this disease. Over 100 QTL have already been discovered in hexaploid bread wheat, wheareas few mapping studies have been conducted in durum wheat. Utilizing resistant resources and identifying novel resistant loci in tetraploid wheat will be beneficial for the development of tan spot resistant durum varieties. In this study, we evaluated four interconnected tetraploid wheat populations for their reactions to the race 2 isolate 86-124, which produces Ptr ToxA. *Tsn1,* the wheat gene that confers sensitivity to Ptr ToxA, was not associated with tan spot severity in any of the four populations. We found a total of 12 tan spot resistance QTL among three mapping populations. The QTL located on chromosomes 3A and 5A were detected in multiple populations and co-localized with race-nonspecific QTL identified in other mapping studies. Together, these QTL can confer high levels of resistance and can be used for the improvement of tan spot resistance in both hexaploid bread and durum wheat breeding. Two QTL on chromosomes 1B and 7A, respectively, were found only in one population when inoculated with a *ToxA* knockout strain 86-124ΔToxA only, indicating that their association with tan spot was induced by other unidentified necrotrophic effectors, but under the absence of Ptr ToxA. Given the complex *Ptr*-wheat interaction, the removal of

dominant susceptibility genes combined with integrating major race-nonspecific resistance loci should be an efficient and effective way to improve tan spot resistance.

Introduction

Durum wheat (*Triticum turgidum* ssp. *durum*, 2*n* =4*x* = 28, AABB) is a tetraploid species of wheat and one of the first domesticated food crops in the world. Durum wheat is mainly used for pasta, couscous, and burghul. In total, about 16.7 million hectares of durum wheat is annually planted in the world and primarily grown in the Mediterranean Basin and Northern America (Shewry 2009). Tan spot is a major foliar disease caused by the fungal pathogen *Pyrenophora tritici-repentis* (*Ptr*) affecting both tetraploid durum wheat and hexaploid bread wheat. *Ptr* can produce necrotrophic effectors (NEs), infect wheat leaves, and kill plant cells leading to reduced photosynthetic leaf area and reductions in grain yield up to 50% (McMullen and Adhikari 2009). Growing resistant cultivars is an effective approach to mitigate the deleterious effects of the disease. Understanding the *Ptr*-wheat interaction and identifying major QTL for resistance to tan spot can facilitate the development of resistant cultivars via molecular breeding approaches.

It was proposed that the *Ptr*-wheat interaction follows an inverse gene-for-gene model (Ciuffetti et al. 2010), where recognition of a particular NE by a host sensitivity gene leads to dominant susceptibility. Three Ptr NEs, also known as host selective toxins (HSTs), have been identified (Ciuffetti et al. 1998), including Ptr ToxA, Ptr ToxB, and Ptr ToxC. Currently, *Ptr* isolates are classified into eight races based on the three known NEs (Strelkov and Lamari 2003). The most prevalent races of *Ptr* in North America are race 1 and race 2 (Aboukhaddour et al. 2013; Ali and Francl 2003), both of which produce Ptr ToxA. Most *Ptr* isolates collected in Australia also produce Ptr ToxA (Antoni et al. 2010). Three dominant host sensitivity genes have been identified in wheat, *Tsc1* (Effertz et al. 2002), *Tsc2* (Orolaza et al. 1995; Friesen and Faris

2004; Abeysekara et al. 2010), and *Tsn1* (Faris et al. 1996; Stock et al. 1996). *Tsn1,* which confers sensitivity to *Ptr* ToxA, is located on chromosome 5B and encodes a protein with serine/threonine protein kinase, nucleotide binding, and leucine-rich repeat domains (Faris et al. 2010).

Previous studies have suggested that the *Ptr*-wheat interaction appears more complex than an inverse gene-for-gene model. First, numerous quantitative trait loci (QTL) have been found to be associated with reaction to tan spot (Faris et al. 2013). Furthermore, while some QTL mapping studies in hexaploid wheat showed that the Ptr ToxA-*Tsn1* interaction was important to the development of tan spot (Chu et al. 2008; Faris et al. 2012; Kariyawasam et al. 2018; Liu et al. 2017; Singh et al. 2008), other studies found no association between *Tsn1* and the development of tan spot induced by *Ptr* isolates producing Ptr ToxA (Faris and Friesen 2005; Galagedara 2018). This discrepancy was validated in genetically diverse wheat breeding populations. See et al. (2018) evaluated 40 Australian spring wheat varieties and found that the importance of the Ptr ToxA-*Tsn1* interaction was dependent on the genetic background. Lastly, although the soft white spring wheat cultivar 'Penawawa' was sensitive to Ptr ToxA, it was highly resistant to all tested *Ptr* races due to a race-nonspecific QTL on chromosome arm 3BL (Kariyawasam et al. 2016), which was also identified in another hexaploid source by Faris and Friesen (2005). It was hypothesized that the race-nonspecific QTL might preclude the development of tan spot by working upstream of and thus downplay the Ptr ToxA-*Tsn1* interaction (Kariyawasam et al. 2016).

Compared to hexaploid bread wheat, fewer mapping studies for tan spot resistance have been conducted in tetraploid wheat. Three tetraploid wheat mapping populations segregating for *Tsn1* were evaluated for their reaction to *Ptr* isolates producing Ptr ToxA (Chu et al. 2010;

Galagedara 2018; Virdi et al. 2016), and none of them detected *Tsn1* as a significant QTL. Most previous studies used mapping populations derived from a resistant parent insensitive to Ptr ToxA. However, the interaction between the Ptr NEs and tetraploid wheat lines with different genetic backgrounds is not well understood. For example, the durum wheat line Rusty is sensitive to Ptr ToxA but shows high levels of resistance to an isolate of race 1 (Pti 2) and an isolate of race 2 (86-124), both of which produce Ptr ToxA; whereas another durum wheat line, Iumillo, is insensitive to Ptr ToxA but shows high levels of susceptibility to both race 1 and race 2 isolates; and the wild emmer wheat accession PI 466979 is insensitive to Ptr ToxA and highly resistant to race 1 and race 2 isolates (Zhaohui Liu, unpublished data).

The findings mentioned above demonstrate the high level of complexity of *Ptr*-wheat interactions and suggest that genes other than *Tsc1, Tsc2* and *Tsn1* with important roles have yet to be identified. Tetraploid wheat accessions with broad genetic diversity likely harbor novel resistance loci, which can be easily transferrable to elite durum wheat. In this study, we employed four interconnected tetraploid wheat populations derived from crosses using Rusty as the common parent and evaluated their reaction to 86-124. Our objectives were to (1) determine if the Ptr ToxA-*Tsn1* interaction was associated with susceptibility to tan spot in these tetraploid wheat mapping populations, and (2) identify novel QTL associated with resistance to 86-124.

Materials and methods

Four tetraploid wheat accessions including PI 387336 (*T. turgidum* ssp. *turgidum*), PI 387696 (*T. turgidum* ssp. *carthlicum*), PI 466979 (*T. turgidum* ssp. *dicoccoides*) and Iumillo (*T. turgidum* ssp. *durum*) were crossed to the durum line Rusty (*T. turgidum* ssp. *durum*). Rusty is susceptible to most isolates of *Puccinia graminis* f. sp. *tritici* (Klindworth et al. 2006), a biotrophic fungal pathogen that causes stem rust in wheat. Approximately 200 recombinant

inbred lines (F_7) from each cross were developed via the single-seed descent method. The four interconnected populations are referred to as RP336, RP696, RP979, and RIum, respectively.

Necrotrophic effector infiltration and fungal inoculation

The parental lines were evaluated for reaction to Ptr ToxA by infiltration as described by Kariyawasam et al. (2016). Approximately 20 µl of Ptr ToxA culture filtrates was infiltrated into the fully expanded secondary leaf of a wheat seedling using a 1 ml syringe with the needle removed. The infiltrated areas were marked using a felt pen, and plants were placed in a growth chamber at 21 °C with a 12-h photoperiod. The reactions were scored 5 days after infiltration as sensitive (necrosis or chlorosis developed in the marked area) or insensitive (no reaction in the marked area).

To evaluate reaction to fungal inoculations, all genotypes including recombinant inbred lines, parents, and checks were planted in cones (Stuewe & Sons, Inc., Corvallis, OR) filled with Sunshine SB100 soil (Sun Grow Horticulture, Bellevue, WA). Four wheat lines, Salamouni (insensitive to all three NEs and resistant to all *Ptr* races), Glenlea (susceptible to Ptr ToxA-producing races), 6B662 (susceptible to Ptr ToxB-producing races), and 6B365 (susceptible to Ptr ToxC-producing races) were used as checks. Three seeds of each genotype were planted in each container. Cones were held by RL98 trays (Stuewe & Sons, Inc., Corvallis, OR). To eliminate edge effects, the highly susceptible hard red winter wheat cultivar Jerry was planted in the cones along the borders of each RL98 tray. The seedlings were grown in a greenhouse. The experimental design was a randomized complete block design with three replications.

The *Ptr* race 2 isolate 86-124 was used to inoculate the seedlings. The two-to-three leaf stage seedlings were subsequently used for fungal inoculation. A *ToxA* knockout strain of 86-124 (86-124ΔToxA) was also evaluated. This knockout strain was obtained from the genetic

modification of 86-124 by replacing the whole *ToxA* coding region with the hygromycin resistance gene (JB Rasmussen et al. unpublished data). Fungal spore suspension was prepared as described by Lamari and Bernier (1989). For disease rating, the second leaf of each seedling was evaluated using a 1-5 scale, in which 1 represents high resistance and 5 represents high susceptibility (Lamari and Bernier 1989). The average score was calculated for each genotype and used for further QTL mapping analysis.

Marker genotyping

The four mapping populations were genotyped using genotyping-by-sequencing (GBS). DNA of each genotype was isolated with Wizard Genomic DNA purification Kit (A1125; Promega, Madison, WI) and quantified with a Quant-iT PicoGreen dsDNA assay kit (P7589; Thermo Fisher Scientific Inc., Berley, MA). GBS libraries were constructed based on the protocol of Poland et al. (2012). Two restriction enzymes, *Pst*I and *Mse*I were used to digest 100 ng DNA of each sample to reduce genome complexity. A unique barcoded adaptor and a common adaptor were ligated with fragments from each sample. The sample volume of the barcoded products was pooled together. Before the pooled sample was amplified by PCR, it was purified with the QIAquick PCR purification kit (28104, Qiagen Inc., Hilden, Germany). For PCR amplification, 50 ng of template DNA with NEB 2X Taq Master Mix and two primers (5 nmol each) in a 200 µl of total volume was amplified on a thermocycler. The whole amplification cycle included 18 cycles with 10 sec of denaturation at 98℃, followed by 30 sec of annealing at 65℃, and finally 30 sec extension at 72℃. The product from PCR was cleaned with the QIAquick PCR purification kit. The library was sequenced using an Illumina (San Diego, CA) HiSeq 2500 at the Genomic Sequencing and Analysis Facility at the University of Texas Southwestern Medical Center at Dallas, TX. Sequences generated were single-end, 100-bp. All

sequences were submitted to the National Center for Biotechnology Information Short Read Archive (SRP216546). The TASSEL-GBS pipeline was used for SNP discovery and genotype calling as described in Glaubitz et al. (2014). The reference dependent approach was performed with the *Triticum aestivum* IWGSC1.0 RefSeq v1.0 as the reference genome (Appels et al. 2018). SNP markers were named with their physical positions mapped to the reference genome.

Construction of genetic linkage maps

For each mapping population, SNP markers polymorphic between the two parents were selected first. For a given marker, heterozygous calls were treated as missing values. Individuals with missing values over 50% were discarded, and SNP markers with missing values more than 50% were also removed. Furthermore, SNP markers with minor allele frequency less than 0.3 were considered as distorted markers and also eliminated. The resulting SNP markers were grouped for each mapping population using MSTmap (Wu et al. 2008) implemented in R language package "ASMap" (Taylor and Butler 2015). For each linkage group, markers were ordered using JoinMap 4.0 (Van Ooijen 2006) with regression algorithm and distances between markers were calculated using the Kosambi mapping function (Kosambi 1943).

QTL mapping in the four interconnected populations

Multiple QTL mapping analysis was performed using the R language package "R/qtl" (Arends et al. 2010; Broman et al. 2003). An interval mapping with Haley-Knott regression method was first used to identify initial QTL using the function *scanone*. A test with 1000 permutations was performed to generate the LOD significance threshold, which was set at $\alpha < 0.01$. After identifying initial QTL, multiple QTL mapping analysis was performed and the best model was selected using the function *stepwiseqtl*. The QTL position was further refined using the function *refineqtl*. For each identified QTL, 1.5 LOD confidence interval, the explained

phenotypic variation, QTL effect, etc. were calculated. The corresponding interval on the reference genome was reported according to physical positions of the flanking markers.

Results

Ptr ToxA infiltration and fungal inoculation

Based on the infiltration, Rusty, PI 387336, and PI 387696 were sensitive to Ptr ToxA, indicating their possession of the dominant allele *Tsn1*, whereas PI 466979 and Iumillo were insensitive to Ptr ToxA and therefore lacked the *Tsn1* allele (Table 1). Therefore, RP979 and RIum segregated for *Tsn1*. The five parents and four mapping populations were also evaluated for reaction to the race 2 isolate 86-124, which is known to produce Ptr ToxA. Rusty and PI 466979 were highly resistant to 86-124 while the other three parents showed reaction types that ranged from moderately susceptible to highly susceptible (Table 1). The average score of lesion types was calculated for each genotype. Quantitative variation was found for all four mapping populations, indicating polygenic inheritance of reaction to 86-124 (Table 2 and Figure 1). Out of the four populations, RP979 showed the lowest average score and range (Table 2).

The RP336 population was also evaluated for reaction to a *ToxA* knockout strain 86-124ΔToxA. The average score was significantly lower with a mean difference of 0.94 compared to the inoculation of 86-124 (Table 2). Nevertheless, the observed quantitative variation indicated that 86-124ΔToxA might produce other unknown NEs inducing necrosis. Furthermore, the correlation between the reactions to 86-124 and to 86-124ΔToxA was 0.62, suggesting that some loci might be associated with reaction to both strains.

Table 1. Reactions of the five parents to infiltration of Ptr ToxA and inoculation of isolate 86-124.

Genotype (Species)	Ptr ToxA	86-124
PI 387336 (*T. turgidum* ssp. *turgidum*)	Sensitive	4.0
PI 387696 (*T. turgidum* ssp. *carthlicum*)	Sensitive	5.0
PI 466979 (*T. turgidum* ssp. *dicoccoides*)	Insensitive	1.5
Iumillo (*T. turgidum* ssp. *durum*)	Insensitive	3.5
Rusty (*T. turgidum* ssp. *durum*)	Sensitive	1.0

Table 2. Average lesion-type reaction to isolate 86-124 and 86-124ΔToxA of the four mapping populations.

Population	Isolate	Population average	Population range
RP336	86-124	3.31	1.83-4.33
	86-124ΔToxA	2.40	1.00-4.25
RP696	86-124	2.94	1.00-4.67
RP979	86-124	1.69	1.00-3.33
RIum	86-124	2.64	1.00-4.17

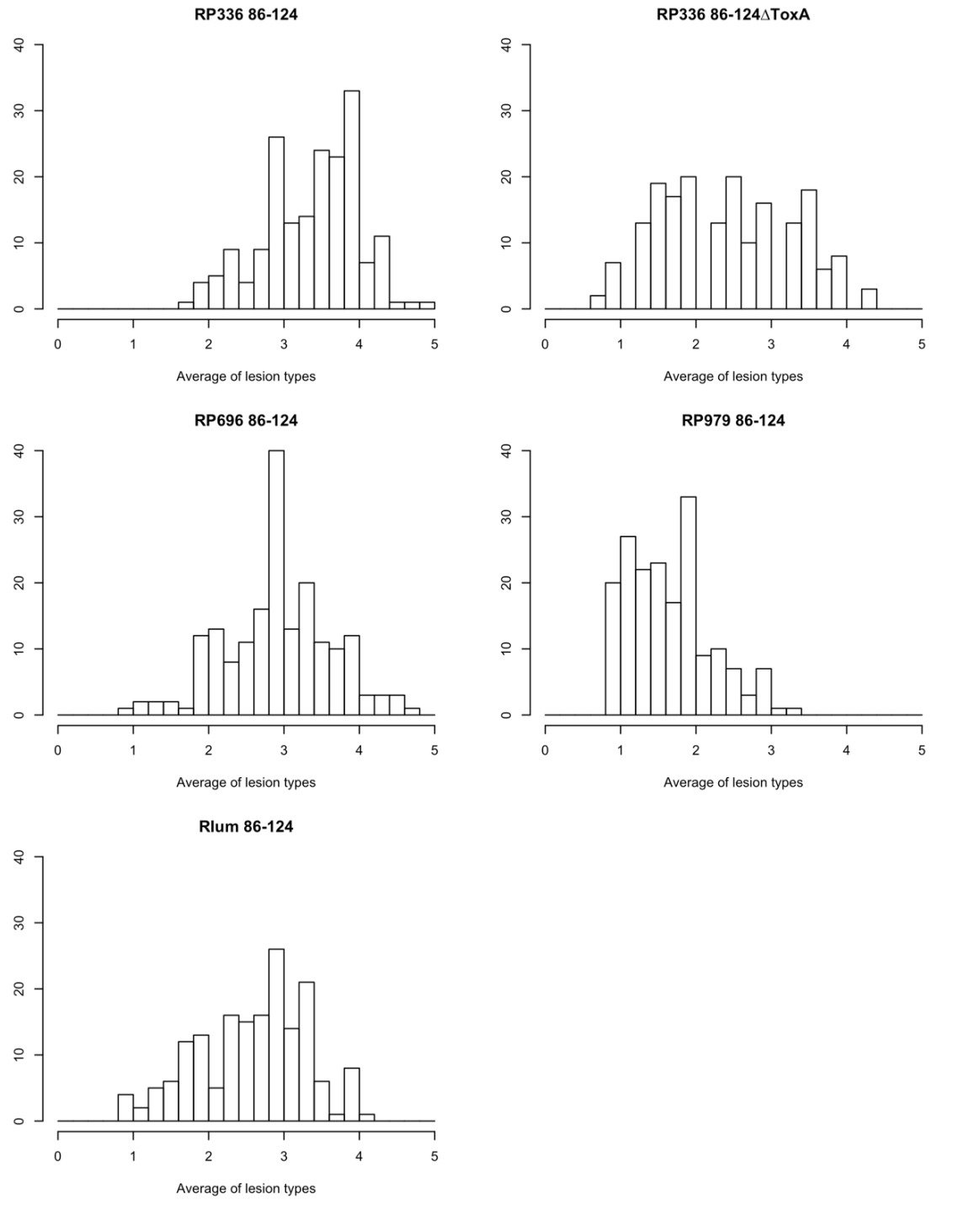

Figure 1. Histograms of average lesion-type reactions to isolates 86-124 and 86-124ΔToxA of the four tetraploid wheat mapping populations. The RP336, RP696, RP979, and RIum populations were evaluated for reaction to isolate 86-124. The population RP336 was also evaluated for a *PtrToxA*-knockout strain 86-124ΔToxA. The *x* axis is the average lesion-type scale and *y* axis is the number of genotypes.

Genetic linkage maps

A total of 257 millions reads were obtained for RP336, 263 millions reads for RP696, 349 millions reads for RP979, and 263 million reads for RIum. After filtering, 7,212 SNP markers were retained for RP336, 4,746 SNP markers for RP696, 8,146 SNP markers for RP979, and 8,815 SNP markers for RIum. Co-segregating markers were excluded by using an *in-house* script. The resulting SNP markers were used for linkage map construction. The four populations had 14 linkage groups each with 2,059 to 3,692 SNP markers mapped and total lengths of 2724.1 cM to 2,968.1 cM (Table 3). A total of 9,071 SNP markers were uniquely mapped on one of the four linkage maps, 1,495 SNP markers commonly mapped on two linkage maps, 435 commonly mapped on three linkage maps, and 40 SNP markers shared by all four linkage maps.

QTL mapping

No QTL was significantly associated with resistance to 86-124 in the RP979 population. In total, 12 QTL were identified in the other three mapping populations. None of the 12 QTL were located on chromosome 5B where *Tsn1* is known to reside. The favorable alleles of the 12 identified QTL were all derived from Rusty (Table 4).

For the mapping population RP336, three QTL were identified, one located on chromosome 2B and the other two on 5A (Table 4). One of the two QTL on chromosome 5A, RP336_86-124_5A.2 showed the highest effect and explained 13.8% of the total phenotypic variation (Table 4). When inoculated with strain 86-124ΔToxA, four QTL were identified and located on chromosomes 1B, 3A, 5A, and 7A, respectively (Table 4). The most significant QTL, RP336_86-124Δ*ToxA*_5A, explained 36.4% of total phenotypic variation. The genetic intervals of the two most significant QTL (RP336_86-124_5A.2 and RP336_86-124Δ*ToxA*_5A)

overlapped with each other (Table 4). It is likely that there is one a gene in the region conferring resistance to both 86-124 and 86-124Δ*ToxA*.

For the mapping population RP696, four QTL were identified, one located on chromosome 2B, one on chromosome 3A, and two on chromosome 5A (Table 4). RP696_86-124_3A was the most significant QTL and explained 13.6% of the phenotypic variation. For the population RIum, only one QTL, RIum_86-124_3A was identified and located on chromosome 3A and explained 9.8% of total phenotypic variation (Table 4).

Discussion

The Ptr ToxA-*Tsn1* interaction was not associated with the development of tan spot in durum wheat

Ptr isolates that produce Ptr ToxA are prevalent in major wheat growing areas of the world. Both bread and durum wheat genotypes with the dominant *Tsn1* allele are sensitive to Ptr ToxA. However, the importance of the Ptr ToxA-*Tsn1* interaction to the development of tan spot caused by *Ptr* isolates that produce Ptr ToxA was found to be dependent on the genetic background in hexaploid bread wheat (Faris and Friesen 2005; Chu et al. 2008; Faris et al. 2012; Kariyawasam et al. 2016; Kariyawasam et al. 2018; Liu et al. 2017). A few mapping studies were conducted in tetraploid wheat (Chu et al. 2010; Virdi et al. 2016; Galagedara 2018), none of which found significant association between *Tsn1* and susceptibility to tan spot induced by either race 1 or race 2 isolates, both of which produce Ptr ToxA. Similarly, a genome-wide association study using 371 durum wheat landraces and breeding lines found no association between *Tsn1* and susceptibility to tan spot (Galagedara 2018). In this study, we evaluated four interconnected tetraploid wheat populations for their reactions to the race 2 isolate 86-124.

Table 3. Distribution of SNP markers mapped on 14 linkage groups in each mapping population.

Population	RP336		RP696		RP979		RIum	
Chr	No. Markers	Length (cM)	No. Markers	Length (cM)	No. Markers	Length (cM)	No. Markers	Length (cM)
1A	214	192.4	158	144.6	272	193.5	166	196.2
1B	191	194.1	166	161.3	183	148.6	257	159.9
2A	165	244.4	126	234.6	285	230.4	214	423.4
2B	251	231.0	156	177	326	235.7	217	176.3
3A	235	249.7	140	236.1	284	227.0	226	246.6
3B	224	228.5	164	208	327	220.0	224	245.3
4A	190	160.8	124	159.6	267	177.5	96	184.5
4B	146	86.2	120	159.1	155	149.3	98	125.2
5A	187	257.9	126	218.8	242	232.7	165	258.8
5B	222	256.7	161	234.9	273	213.8	212	224.5
6A	190	202.8	137	144.6	236	174.4	198	178.8
6B	225	213.8	118	236.6	302	162.3	288	157.5
7A	212	203.1	185	253.3	296	240.8	277	249.8
7B	242	136.7	178	155.7	244	170.2	273	141.4
Overall	2,894	2,858.4	2,059	2,724.1	3,692	2,776.3	2,911	2,968.1

Table 4. QTL identified for resistance to tan spot from the three mapping populations

Population	QTL	Chr[a]	Genetic Position (cM)[b]	Genetic Interval (cM)[c]	Physical Interval (Mbp)[d]	LOD[e]	R^2 (%)[f]	Effects[g]
RP336	RP336_86-124_2B	2B	14.2	0.0-34.2	2.8-30.5	4.3	6.9	-0.39
	RP336_86-124_5A.1	5A	86.0	68.0-92.0	46.6-475.6	4.9	7.9	-0.33
	RP336_86-124_5A.2	5A	238.4	234.8-244.0	665.9-679.6	8.2	13.8	-0.58
RP336	RP336_86-124ΔToxA_1B	1B	90.5	74.0-106.7	445.6-598.7	4.2	5.2	-0.47
	RP336_86-124ΔToxA_3A	3A	13.6	8.0-26.0	7.6-20.2	6.5	8.3	-0.40
	RP336_86-124ΔToxA_5A	5A	236.0	234.8-238.0	665.9-681.5	23.1	36.4	-1.08
	RP336_86-124ΔToxA_7A	7A	3.5	0.1-12.0	1.9-12.2	3.6	4.4	-0.29
RP696	RP696_86-124_2B	2B	10.0	5.0-20.0	58.1-118.6	4.0	7.1	-0.36
	RP696_86-124_3A	3A	7.9	2.0-16.0	7.4-17.4	7.3	13.6	-0.36
	RP696_86-124_5A.1	5A	53.1	32.0-59.9	45.0-452.9	3.9	7.0	-0.33
	RP696_86-124_5A.2	5A	193.6	188.0-202.0	663.4-688.3	5.2	9.6	-0.34
RIum	RIum_86-124_3A	3A	21.1	17.3-32.9	10.8-26.2	4.2	9.8	-0.49

[a] Chromosome

[b] Genetic position of the QTL on the linkage map

[c] 1.5 LOD confidence interval of the QTL on the linkage map

[d] Physical interval of the QTL on the wheat reference genome

[e] Logarithm of the odds (LOD) score of the QTL

[f] The percentage of total variation explained by the QTL

[g] The estimated additive effect of the QTL favorable allele

Consistent with previous mapping studies in tetraploid wheat, the *Tsn1* locus was not associated with tan spot susceptibility in any of the four mapping populations, including RP979 and RIum which are segregated for sensitivity to Ptr ToxA. Therefore, there continues to be no evidence that the Ptr ToxA-*Tsn1* interaction plays a significant role in the development of tan spot in tetraploid wheat. The lack of significance is possibly due to low expression levels of the *ToxA* gene in 86-124 when inoculated on durum wheat (Virdi et al. 2016), but the cause for the lack of expression is yet unknown.

QTL identified in the tetraploid mapping populations

In addition to removing dominant susceptibility genes like *Tsn1*, integrating other resistance loci will offer broad-spectrum protection against tan spot. Many QTL for tan spot resistance have been identified from hexaploid bread wheat, the introduction of which to durum wheat can be, however, impeded by ploidy level differences (Padmanaban et al. 2017). The resistant sources and loci directly identified from tetraploid wheat are desirable for durum wheat. In this study, a total of 12 QTL associated with reaction to 86-124 were identified from three mapping populations, out of which 10 QTL on chromosomes 2B, 3A, and 5A were identified in two or three mapping populations. No QTL was identified from the population RP979, in which both the average and the range of disease reaction scale were quite low, likely due to both parents being resistant, where QTL with very minor effects might be segregated but went undetected.

To investigate potential co-localized QTL, QTL reported from the current study and previous mapping studies were positioned on the reference genome *Triticum aestivum* IWGSC1.0 RefSeq v1.0 (Appels et al. 2018).

RP336_86-124_2B and RP696_86-124_2B were identified on chromosome 2B from two different populations. No markers on the reference genome between 0.0-50.0 Mbp were mapped on the RP696 linkage map. It is possible that the two QTL are co-localized although they did not overlap according to their physical positions (Table 4). *Tsc2,* a dominant gene conditioning sensitivity to Ptr ToxB, was mapped to chromosome 2B (Abeysekara et al. 2010; Virdi et al. 2016). The marker *XBE444541* closely linked to *Tsc2* is located at ~24.3 Mbp, which falls into the physical interval of RP336_86-124_2B. However, the fact that isolate 86-124 does not produce Ptr ToxB suggests RP336_86-124_2B comprises a gene different from *Tsc2*. Li et al. (2011) identified a major QTL on chromosome 2B in hexaploid bread wheat, which was detected in three independent trials explaining over 30% of total phenotypic variation. The flanking markers of this QTL were positioned to 1.7-7.7 Mbp on chromosome 2B and co-localized with RP336_86-124_2B. Therefore, it is possible that RP336_86-124_2B is the same as the one reported by Li et al. (2011).

The three QTL identified on chromosome 3A from three mapping populations in this study overlapped and were located at 7.4-26.2 Mbp (Table 4). Chu et al. (2010) identified a race-nonspecific QTL, *QTs.fcu-3A* conferring resistance to race 1 and race 2 in a tetraploid wheat population derived from Lebsock and PI 94749. The QTL was flanked by microsatellite markers *Xbarc321* to *Xwmc11*, which mapped to positions ~11.7 Mbp and ~16.2 Mbp, respectively. Therefore, *QTs.fcu-3A* is co-localized with the three QTL on chromosome 3A identified in this study. In hexaploid wheat, a recessive resistance gene designated *tsr4* was mapped to chromosome 3A (Tadesse et al. 2006, 2007, 2010), and a closely linked marker, *Xgwm2*, mapped at ~ 60.2 Mbp on chromosome 3A. Also, Singh et al. (2008) identified a QTL, *QTs.ksu.3AS,* that conferred resistance to race 1 in hexaploid bread wheat, and its two linked markers *Xbarc45* and

Xbarc86 both aligned to ~54.5 Mbp on chromosome 3A. Although the two QTL found in hexaploid wheat are not very distant from the 3A QTL found in tetraploid wheat in this study and that of Chu et al. (2010), further study or meta-QTL analysis is needed to determine if all of them are co-localized.

A total of five QTL were found on chromosome 5A from three mapping populations in this study. RP696_86-124_5A.1 and RP336_86-126_5A.1 overlapped with each other and were located at the interval of 46.6-475.6 Mbp on chromosome 5A (Table 4). The other three QTL, RP696_86-124_5A.2, RP336_86-124_5A.2, and RP336_86-124Δ*ToxA*_5A overlapped and located at the interval of 665.9-681.5 Mbp (Table 4). Chu et al. (2010) also identified two QTL, *QTs.fcu-5A.1* and *QTs.fcu-5A.2,* in a tetraploid wheat population derived from Lebsock and PI 94749. Both QTL showed resistance to *Ptr* race 1 and race 2. The two most closely flanking markers for *QTs.fcu-5A.1* were *Xbarc360* and *Xgwm6.1*, which mapped to 458.5 Mbp and 496.5 Mbp, respectively. Therefore, *QTs.fcu-5A.1* is co-localized with RP336_86-124_5A.1 and RP696_86-124_5A.1. The two flanking markers for *QTs.fcu-5A.2*, *Xwmc110* and *Xwmc727*, were mapped to 644.9 Mbp and 687.8 Mbp on chromosome 5A, respectively. Therefore, *QTs.fcu-5A.2* co-localized with RP336_86-124_5A.2, RP696_86-124_5A.2, and RP336_86-124Δ*ToxA*_5A. Like the QTL on chromosome 3A, the five QTL identified on chromosome 5A in this study are also co-localized with the two QTL found by Chu et al. (2010) in the tetraploid mapping population. In addition, Chu et al. (2008) mapped a race-nonspecific QTL designated *QTs.fcu-5AL* on chromosome 5A in a hexaploid wheat population. The closely linked flanking markers, *Xbarc1061* and *Xwmc96*, mapped to 559.5 Mbp and 638.3 Mbp, respectively. Another race-nonspecific QTL, *QTs.zhl-5A,* was identified in hexaploid wheat (Kariyawasam et al. 2016), and the two flanking SSR markers, *Xwmc327* and *Xbarc319*, were positioned to 547.4 Mbp and

625.7 Mbp, respectively. The two race-nonspecific QTL identified in hexploid bread wheat are not distant from the 5A QTL identified in this study and by Chu et al. (2010) in tetraploid wheat. Therefore, given the different significance threshold and mapping methods used in previous studies, it is possible that they might all co-localize.

Interestingly, the QTL on chromosomes 1B and 7A were found only in the RP336 population, and detected as significant QTL when inoculated with Ptr ToxA knockout strain 86-124ΔToxA only, but not the isolate 86-124. It had been reported that some isolates do not produce Ptr ToxA but can induce necrosis on wheat (Ali et al. 2010 and Guo et al. 2018). It is likely that when the *ToxA* gene is disrupted, other NEs may be upregulated in the pathogen to increase disease, which would then lead to the identification of other host susceptibility loci involved. Therefore, there should be epistasis between host and NEs as well, which has been evident in a similar pathogen, *Parastagonospora nodorum* (Faris et al. 2011; Haugrud et al. 2019; Phan et al. 2016).

Faris and Friesen (2005) identified a race-nonspecific QTL, *QTsfcu-1BS*, in hexaploid wheat population derived from a cross between BR34 and Grandin. BLAST analysis of the two flanking markers *Xgdm33* and *Xgdm125* failed to detect significant hits on chromosome 1B of the reference sequence. Therefore, it is not clear whether RP336_86-124ΔToxA_1B is co-localized with *QTsfcu-1BS*. Five QTL associated with tan spot resistance were found on chromosome 7A from previous studies (Kalia et al. 2018; Li et al. 2011; Stadlmeier et al. 2019). However, BLAST analysis of their closely linked markers found that they were located over 100 Mbp from the QTL RP336_86-124ΔToxA_7A. Therefore, RP336_86-124ΔToxA_7A is likely a novel QTL.

Over 100 QTL have been identified for tan spot resistance through QTL mapping in hexaploid bread wheat and tetraploid wheat. Recently, several genome-wide association studies have also revealed numerous regions associated with tan spot resistance (Dinglasan et al. 2019; Juliana et al. 2018; Kollers et al. 2014; Liu et al. 2015; Perez-Lara et al. 2017; Singh et al. 2016). Some QTL were commonly identified in multiple populations even across tetraploid and hexaploid wheat, which can provide resistance in a broad genetic background. Some QTL were found to confer resistance to multiple races (Chu et al. 2008; Faris and Friesen 2005; Kariyawasam et al. 2016). To develop a cultivar with high and stable resistance to tan spot, pyramiding multiple major resistance loci is a promising approach. The QTL identified in this research provide useful targets for the improvement of tan spot resistance in tetraploid wheat and possibly hexaploid wheat as well.

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PAPER 2: META-QTL ANALYSIS OF TAN SPOT RESISTANCE IN WHEAT

Abstract

Tan spot, caused by the fungal pathogen *Pyrenophora tritici-repentis* (*Ptr*), is a major foliar disease worldwide in both bread wheat and durum wheat and can reduce grain yield due to reduction in photosynthetic area of leaves. Developing and growing resistant cultivars is a cost-effective and environmentally friendly approach to mitigate negative effects of the disease. Understanding the genetic basis of tan spot resistance can enhance the development of resistant cultivars. With that goal, over 100 QTL associated with resistance to tan spot induced by a variety of *Ptr* races and isolates have been identified from previous QTL mapping studies. Meta-QTL analysis can identify redundant QTL amongst various studies and reveal major QTL for targeting in marker-assisted selection applications. In this study, we performed a meta-QTL analysis of tan spot resistance using the reported QTL from 15 previous QTL mapping studies. An integrated linkage map with a total length of 4,080.5 cM containing 47,309 markers was assembled from 21 individual linkage maps and three previously published consensus maps. Nineteen meta-QTL were clustered from 104 initial QTL projected on the integrated map. Three of the 19 meta-QTL located on chromosomes 2A, 3B, and 5A show large genetic effects and confer resistance to multiple races in multiple bread wheat and durum wheat mapping populations. The integration of those race-nonspecific QTL is a promising strategy to provide high and stable resistance to tan spot in wheat.

Introduction

Tan spot, also known as yellow spot, is a major foliar disease worldwide on both bread wheat (*Triticum aestivum* L., 2n=6x=42, AABBDD) and durum wheat (*T. turgidum* L. 2n=4x=28, AABB). Tan spot is caused by a fungal pathogen, *Pyrenophora tritici-repentis* (*Ptr*),

and characterized by large lesions with tan-color surrounded by chlorotic haloes on leaves of susceptible wheat lines. Tan spot caused grain yield loss ranging from 5% to 31% due to reduction in photosynthetic area of leaves (Shabeer and Bockus 1988; Bhathal et al. 2003). In Australia, annual loss due to tan spot varied from AUD212 M to AUD676 M (Dinglasan et al. 2019). Crop rotation and fungicide applications are viable practices to reduce the effect of tan spot. However, developing resistant cultivars is more cost-effective and environmentally friendly. Understanding the genetic basis of tan spot resistance can enhance the development of resistant cultivars.

Ptr produces necrotrophic effectors (NEs), also known as host-selective toxin (HSTs), which compromise wheat immune system and results in necrotic and/or chlorotic symptoms. Three Ptr NEs including Ptr ToxA, Ptr ToxB, and Ptr ToxC have been identified. Ptr ToxA is a small protein and induces necrosis (Manning 2005). Ptr ToxB is also a protein but induces chlorosis (Ciuffetti et al. 2010b). Ptr ToxC is an unknown low weight molecule and induces chlorosis (Effertz et al. 2002). Existence of a putative Ptr ToxD was also reported (Meinhardt et al. 2003; Ciuffetti et al. 2003). Some isolates do not produce Ptr ToxA but can induce necrosis, also suggesting that other uncharacterized NEs are present (Ali et al. 2010; Guo et al. 2018). Currently, *Ptr* isolates are classified into eight races based on the three known NEs they produce (Strelkov and Lamari 2003). Race 1 produces Ptr ToxA and Ptr ToxC; Race 2 produces Ptr ToxA; Race 3 produces Ptr ToxC; Race 4 produces none; Race 5 produces Ptr ToxB; Race 6 produces Ptr ToxB and Ptr ToxC; Race 7 produces Ptr ToxA and Ptr ToxB; and Race 8 produce all three known NEs.

Previous studies revealed that the interaction between wheat and the three known NEs follows an inverse gene-for-gene model (Ciuffetti et al. 2010), i.e. the recognition of a NE by a

host gene leads to dominant susceptibility. Three dominant host sensitivity genes have been identified and named as *Tsc1*, *Tsc2*, and *Tsn1* (Singh et al. 2010; Faris et al. 2013). *Tsn1* confers sensitivity to Ptr ToxA is located on chromosome 5B and the first dominant sensitive gene cloned in wheat (Faris et al. 2010). *Tsn1* harbors serine/threonine protein kinase (S/TPK), nucleotide binding (NB), and leucine-rich repeat (LRR) domains. *Tsc1* is located on chromosome 1A and confers sensitivity to Ptr ToxC (Effertz et al. 2002). *Tsc2* is located on chromosome 2B and confers sensitivity to Ptr ToxB (Abeysekara et al. 2010; Friesen and Faris 2004; Orolaza et al. 1995). Dominant sensitivity loci were also found on chromosomes 3A, 3B, and 3D (Singh et al. 2006; Singh et al. 2008; Tadesse et al. 2006; Tadesse et al. 2008), which suggests the presence of additional unknown NEs. Cloning other dominant sensitive genes will facilitate the better understanding of the interactions between Ptr NEs and wheat.

In addition to the host dominant sensitivity genes, over 100 QTL related to resistance to tan spot induced by varied races and isolates have been identified from previously published mapping studies, suggesting that *Ptr*-wheat interaction is more complex than a reverse gene-for-gene model (Faris et al. 2013). Some QTL were race-nonspecific, conferring resistance to multiple races in common wheat (Chu et al. 2008; Faris et al. 2012; Faris and Friesen 2005; Kariyawasam et al. 2016) and durum wheat (Chu et al. 2010). In addition to removal of the dominant susceptible genes, integrating other quantitative resistance loci should provide high and stable resistance to tan spot. Given a large number of the identified QTL, it is practical to remove redundancies and target a few major QTL in breeding programs.

Meta-QTL analysis, combining data from various mapping studies, can be used for removal of redundancies and for candidate gene discovery as well as marker-assisted selection (MAS) (Goffinet and Gerber 2000). In meta-QTL analysis, a dense consensus linkage map is

constructed based on various individual linkage maps, and QTL identified from each study are projected onto the consensus map. It can further narrow down a QTL to a smaller region to facilitate the discovery of causal gene and enable the selection of closer linked markers for effective MAS as well. Meta-QTL analysis can also estimate global effect of a QTL region commonly identified from multiple populations and/or environments and prioritize the identified QTL for MAS (Yu et al. 2014; Maccaferri et al., 2016). Meta-QTL analysis has been conducted for Fusarium head blight resistance (Liu et al. 2009; Loffer et al. 2009; Venske et al. 2019), grain weight (Avni et al. 2018), leaf rust resistance (Soriano and Royo 2015), root-related traits (Soriano and Alvaro 2019), and stem rust resistance (Yu et al. 2014) in wheat. In this study, a meta-QTL analysis of tan spot resistance was performed using results from previous mapping studies. We expect to obtain a deeper and wider understanding of the genetic basis of tan spot resistance in wheat and prioritize meta-QTL for potential MAS in breeding.

Materials and methods

Collection of tan spot resistance loci identified in previous studies

Tan spot resistance loci were collected from 14 previous mapping studies comprising 18 mapping populations. Out of the 18 mapping populations, eight populations were evaluated with isolates of race 1; twelve populations were evaluated with race 2 isolates; five populations were evaluated with race 3 isolates; six populations were evaluated with race 5 isolates; five populations were evaluated with locally collected isolates; and eight populations were evaluated for multiple *Ptr* races. Except for one population RP979 (Liu et al. 2019) without QTL identified, there were 135 QTL in total with LOD over 3.0 identified from the other 17 populations (Appendix Table 1) and used for further meta-QTL analysis. To better organize the QTL identified from different studies, QTL were renamed according to their population name,

Ptr isolate associated, and chromosomal location. Multiple QTL mapped on the same linkage group from the same population and associated with the same isolate(s) were distinguished by a number following the chromosome name, e.g. BG_OH99_3B.1 and BG_OH99_3B.2, whereas QTL mapped on the same linkage group from the same population but detected at different environments or trials were distinguished by a unique letter following the chromosome name, e.g. CW_2A.a and CW_2A.b. QTL associated with infection caused by uncharacterized local isolates were renamed based on population name and chromosomal location. There were 29 QTL associated with resistance to race 1 isolates, 34 QTL associated with race 2, 12 QTL associated with race 3, and 13 QTL associated with race 5. There were 65 QTL mapped on the A sub-genome, 59 QTL mapped on the B sub-genome, and 11 QTL mapped on the D sub-genome. Chromosome 5A possessed the most QTL with 22, and chromosome 5D harbored one QTL only.

Construction of a consensus linkage map

The R package "LP merge" (Endelman and Plomion 2014) was used to assemble an integrated map with three consensus linkage maps (Somers et al. 2004; Marone et al. 2012; Maccaferri et al. 2014) and 21 individual linkage maps. The 21 individual linkage maps were derived from 21 mapping populations, including the 18 populations with QTL identified as introduced above, 10 tetraploid and 11 hexaploid. The 21 populations are Attlia ×CDC Go (Zou et al. 2017), Altar × Langdon (Virdi et al. 2016), BR34 × Grandin (Faris and Friesen 2005), Ben × PI41025 (Galagedara 2018), Calingiri × Wyalkatchem (Shankar et al. 2017), Divide × PI272527 (DP527, Faris and Xu, unpublished), Ernie × Betavia (Li et al. 2011), IGW2547 × Annuello (Shankar et al. 2017), Joppa × 10Ae564 (Joppa10Ae, Zhao et al. 2018),LMPG-6 × PI626573 (Liu et al. 2017), Lousie × Penawawa (Kariyawasam et al. 2016), Lebsock × PI94749 (Chu et al. 2010), MAGIC population (Stadlmeier et al. 2019), Rusty × Iumillo (RIum, Liu et al.

2019), Rusty × PI387336 (RP336, Liu et al. 2019), Rusty × PI387696 (RP696, Liu et al. 2019), Rusty × PI466979 (RP979, Liu et al. 2019), Rusty × PI183883 (RP883, Liu et al. 2019; Sharma et al. 2019), Salamouni × Katepwa (Faris et al. 2012), TA4152-60 × ND495 (Chu et al. 2008), and TA161-L1 × TAM105 (Kalia et al. 2018). The brief information of the 21 linkage maps and mapping populations are listed in Appendix Table 2. The majority of QTL markers were DArT, SSR, and SNP.

Out of the 21 linkage maps, five were constructed with SNP markers using the Illumina iSelect 9K or 90K array and four using genotype-by-sequencing (GBS). To better bridge SNP markers genotyped by different platforms across linkage maps, four populations, BP, DP527, Joppa10Ae, and RP883 originally genotyped with Illumina iSelect 9K or 90K SNP array were genotyped again using GBS as described by Poland et al. (2012). TASSEL-GBS pipeline was used for SNP discovery and genotype calling as described in Glaubitz et al. (2014). The *Triticum aestivum* IWGSC1.0 RefSeq v1.0 was used as reference genome (Appels et al. 2018). The resulting GBS SNP markers were named based on their physical positions mapped to the reference genome. For each population, individuals with missing values over 50% were discarded. The SNP markers with missing values more than 50% were also removed. Furthermore, the SNP markers with segregation ratios exceeding 0.7:0.3 were considered as distorted markers thus also eliminated. We re-constructed the linkage maps using both Illumina iSelect array SNP markers from original studies and GBS SNP markers from the four re-genotyped mapping populations. The resulted SNP markers were grouped for each mapping population using MSTmap (Wu et al. 2008) implemented in R language package ASMap (Taylor and Butler 2015). For each linkage group, markers were ordered using JoinMap 4.0 (Van Ooijen

2006) and distances between markers were calculated using the Kosambi mapping function (Kosambi 1943).

Three previously published consensus maps were built with SSR and/or DArT markers and were included to increase connectivity among individual maps with SSR and/or DArT markers (Appendix Table 2). The three previously published consensus maps were the 2004 Common Wheat SSR Integrated map (Somers et al. 2004), the 2012 Durum Wheat SSR/DArT Integrated map (Marone et al. 2012), and the 2014 Durum Wheat SSR/DArT Integrated map (Maccaferri et al. 2014). They were assembled from six, six, and twelve individual linkage maps, respectively, none of which were the same as any of the 21 individual mapping populations used in this study.

To assess the quality of the resulting integrated map, we compared it to two other wheat consensus maps previously constructed (Wang et al. 2014; Maccaferri et al. 2015), where correlation of the shared markers' orders between the maps was calculated for each linkage group.

Meta-QTL analysis

Meta-QTL analysis was carried out using BioMercator V4.2 (Sosnowski et al. 2012). Individual QTL were first projected onto the resulted integrated map. Given N QTL projected on one linkage group, BioMercator tests five models assuming presence of 1-, 2-, 3-, 4-, or N-unique QTL, respectively. Akaike Information Criterion (AIC), an estimator of model fitting, was calculated, and the model with the lowest AIC value was selected as the best model.

Identification of co-localized QTL

In addition to the 14 mapping studies, some other previously published QTL mapping studies were not used for the meta-QTL analysis due to lack of detailed information of the

identified QTL or lack of sufficient common markers between the individual linkage map and our integrated linkage map. Besides, there were QTL identified from a number of genome-wide association mapping studies (GWAS) for tan spot resistance in wheat. To identify QTL not included in meta-QTL analysis that's potentially co-localized with the identified meta-QTL, the closely linked markers to the QTL in question were aligned to the same reference genome *Triticum aestivum* IWGSC1.0 RefSeq v1.0 (Appels et al. 2018).

Results

Construction of an integrated linkage map

An integrated linkage map was assembled from 21 individual linkage maps and three previously published consensus maps. The resulting linkage map contained 47,309 markers, including 2,998 DArT, 38,231 SNP, 3,034 SSR, and 3,046 other types of markers like AFLP, STS, and TRAP (Appendix Table 2). The total length is 4,080.5 cM, with a range of 104.0 cM to 409.5 cM across the 21 linkage groups (Table 5). For the linkage groups from sub-genome A and B, marker densities ranged from 8.4 to 15.0 markers per cM (Table 5). The densities for the seven linkage groups from sub-genome D were much lower, ranging from 0.7 to 1.7 markers per cM (Table 5), because all 11 hexaploid wheat individual linkage maps were constructed with mainly SSR or DArT markers but lack of SNP markers.

Table 5. Information of the consensus map and its correlations of common marker orders with previously published consensus maps.

Consensus Map	Markers (no.)	Length (cM)	Marker density[†]	Order correlation[‡]	Order correlation[§]
1A	3083	281.2	11.0	0.97	0.99
1B	3344	252.8	13.2	0.98	0.99
2A	3237	259.6	12.5	0.94	0.98
2B	4052	312.5	13.0	0.98	0.98
3A	2822	271.0	10.4	0.99	0.97
3B	3834	255.7	15.0	0.95	0.96
4A	2770	285.2	9.7	0.96	0.96
4B	1768	211.4	8.4	0.97	0.98
5A	2595	201.0	12.9	0.96	0.98
5B	3639	317.8	11.4	0.99	0.99
6A	2820	233.9	12.1	0.96	0.95
6B	4041	310.8	13.0	0.97	0.99
7A	3718	409.5	9.1	0.95	0.96
7B	3563	370.7	9.6	0.99	0.99
1D	328	212.0	1.5	-	-
2D	432	249.4	1.7	-	-
3D	245	347.9	0.7	-	-
4D	128	104.0	1.2	-	-
5D	281	256.5	1.1	-	-
6D	298	206.8	1.4	-	-
7D	311	290.7	1.1	-	-

[†]Number of markers per cM

[‡]Order correlation with map in (Wang et al. 2014)

[§]Order correlation with map in (Maccaferri et al. 2015)

To assess the quality of the integrated map, common markers mapped on the same linkage group were used to calculate the correlations of the marker orders between the integrated map and two previously published consensus maps, one in tetraploid wheat (Wang et al. 2014) and the other in hexaploid wheat (Wang et al. 2014). The correlations ranged from 0.92 to 0.99 across the 14 linkage groups of the A and B sub-genome (Table 5), indicating high synteny between our integrated map and previously published consensus maps. Because the 11 hexaploid individual linkage maps used to construct our integrated map lacked SNP markers while the hexaploid consensus map from Wang et al. (2014) was constructed solely with SNP markers (genotyped by Illumina iSelect 90K array), few markers on the D sub-genome were shared between our integrated map and the consensus map from Wang et al. (2014). For that reason no correlations were estimated for the D sub-genome linkage groups.

Meta-QTL analysis

Out of the 135 QTL identified from 17 mapping populations, 129 were successfully projected onto the integrated linkage map (Appendix Table 1). Out of the 129 QTL projected to the integrated linkage map, 23 remained as single QTL, and the other 106 QTL were grouped into 20 meta-QTL. One meta-QTL on chromosome 6A consisted of two initial QTL identified from the population EB at two independent greenhouse trials (Table 6; Li et al. 2011), which was determined not as a meta-QTL for further analysis. The remaining 19 meta-QTL were located on chromosomes 1A, 1B, 2A, 2B, 2D, 3A, 3B, 4A, 5A, 5B, 7A, and 7B (Figure 2 and Table 6). The number of clustered QTL per meta-QTL ranged from 2 to 13 (Table 6). Out of the 19 meta-QTL, 14 meta-QTL were clustered from initial QTL identified in multiple populations (Table 6); five meta-QTL were clustered from initial QTL identified in only one population but showed resistance to multiple races (Table 6).

Table 6. Summary of the meta-QTL conferring resistance to tan spot

	Genetic position (cM)	Genetic interval (cM)	Physical interval† (Mbp)	Average LOD	Average R^2	Initial QTL
MQTL-1A.1	24.0	23.5-24.4	3.4-9.4	10.8	18.2	LP_Pti2_1A LP_ARB10_1A LP_3319_1A TT_AZ00_1A IA_1A.a IA_1A.b IA_WAC11137_1A
MQTL-1A.2	43.1	41.8-44.5	7.3-12.1	9.3	19.3	LP573_Asc1_1A LP573_Pti2_1A LP573_3319_1A EB_1A.a EB_1A.b BMW_1A
MQTL-1B	20.3	16.2-24.3	3.6-6.3	7.0	20.8	BG_Pti2_1B BG_86-124_1B BG_OH99_1B BG_DW5_1B
MQTL-2A.1	70.7	69.1-72.2	45.1-58.7	7.5	20.1	CW_2A.a BMW_2A

Table 6. Summary of the meta-QTL conferring resistance to tan spot (continued).

	Genetic position (cM)	Genetic interval (cM)	Physical interval† (Mbp)	Average LOD	Average R^2	Initial QTL
MQTL-2A.2	84.1	82.3-84.9	65.7-262.1	6.0	17.4	TN_Pti2_2A
						TN_86-124_2A
						TN_OH99_2A
						TN_DW5_2A
						LP573_Asc1_2A
						LP573_DW5_2A
						CW_2A.b
						CW_2A.c
						CW_2A.d
						CW_2A.e
						CW_WAC11137_2A.a
						CW_WAC11137_2A.b
						CW_WAC11137_2A.c
MQTL-2B	58.3	54.6-62.1	1.2-27.2	7.2	27.4	EB_2B.a
						EB_2B.b
						EB_2B.c
						RP336_86-124_2B
						AL_DW5_2B
MQTL-2D	156.0	155.6-156.4	312.3-383.0	6.3	6.0	LP_Pti2_2D
						LP_86-124_2D
						LP_ARB10_2D
						LP_3319_2D

Table 6. Summary of the meta-QTL conferring resistance to tan spot (continued).

	Genetic position (cM)	Genetic interval (cM)	Physical interval† (Mbp)	Average LOD	Average R^2	Initial QTL
MQTL-3A	35.2	33.0-37.5	20.0-21.8	5.1	10.1	RP336_86-124KO_3A
						RP696_86-124_3A
						RIumillo_86-124_3A
						LP749_Pti2_3A
						LP749_86-124_3A
MQTL-3B.1	45.5	42.6-48.5	23.9-68.8	5.2	19.2	BG_OH99_3B.1
						EB_3B
MQTL-3B.2	62.8	59.4-66.1	465.2-583.4	17.0	27.0	LP_86-124_3B
						LP_ARB10_3B
						LP_DW5_3B
						LP_Pti2_3B
						LP_3319_3B
						LP749_Pti2_3B
						LP749_86-124_3B
						BG_Pti2_3B
						BG_86-124_3B
						BG_OH99_3B.2
						BG_DW5_3B
MQTL-5A.1	88.5	81.1-95.9	437.4-482.1	4.5	13.0	RP696_86-124_5A.1
						RP336_86-124_5A.1
						LP749_Pti2_5A.1
						LP749_86-124_5A.1

Table 6. Summary of the meta-QTL conferring resistance to tan spot (continued).

	Genetic position (cM)	Genetic interval (cM)	Physical interval† (Mbp)	Average LOD	Average R^2	Initial QTL
MQTL-5A.2	139.9	138.9-141.8	592.1-614.4	7.3	10.8	BP_DW5_5A
						BP_86-124_5A
						BP_Pti2_5A
						BP_ARB10_5A
						BP_331-9_5A
						LP_86-124_5A
						LP_Pti2_5A
						LP_DW5_5A
						LP_ARB10_5A
						LP_331-9_5A
						TN_Pti2_5A
						TN_86-124_5A
						TN_DW5_5A
MQTL-5A.3	174.3	172.9-175.8	671.3-676	8.8	16.2	RP696_86-124_5A.2
						RP336_86-124_5A.2
						RP336_86-124ΔToxA_5A
						LP749_Pti2_5A.2
						LP749_86-124_5A.2
MQTL-5B.1	80.0	74.5-85.5	58.8-448	7.4	21.0	TN_Pti2_5B.1
						TN_86-124_5B.1
						TN_OH99_5B
						TN_DW5_5B

Table 6. Summary of the meta-QTL conferring resistance to tan spot (continued).

	Genetic position (cM)	Genetic interval (cM)	Physical interval† (Mbp)	Average LOD	Average R^2	Initial QTL
MQTL-5B.2	123.8	120.4-125.6	541.7-549.9	10.8	21.5	TN_Pti2_5B.2
						TN_86-124_5B.2
						SK_Pti2_5B
						SK_86-124_5B
						LP573_Asc1_5B
						LP573_Pti2_5B
						LP573_86-124_5B
MQTL-5B.3	167.5	164.7-170.7	663.1-679.4	3.9	12.9	EB_5B.1
						EB_5B.2
						LP573_DW5_5B
MQTL-6A	100.6	92.4-108.8	0.8-22.3	3.1	14.8	EB_6A.a
						EB_6A.b
MQTL-7A	113.6	111.7-115.6	116.1-133.6	5.5	11.3	TT_AZ00_7A.1
						BMW_7A
MQTL-7B.1	80.4	78.4-82.4	21.0-34.0	3.9	6.3	SK_Pti2_7B
						SK_86-124_7B
						SK_ARLonB2_7B
MQTL-7B.2	171.8	170.1-172.1	614.2-622.8	4.2	10.6	LP749_Pti2_7B
						LP749_86-124_7B
						IA_7B
						CW_7B

†Physical position was estimated by adjacent GBS SNP or blastn result of marker primer.

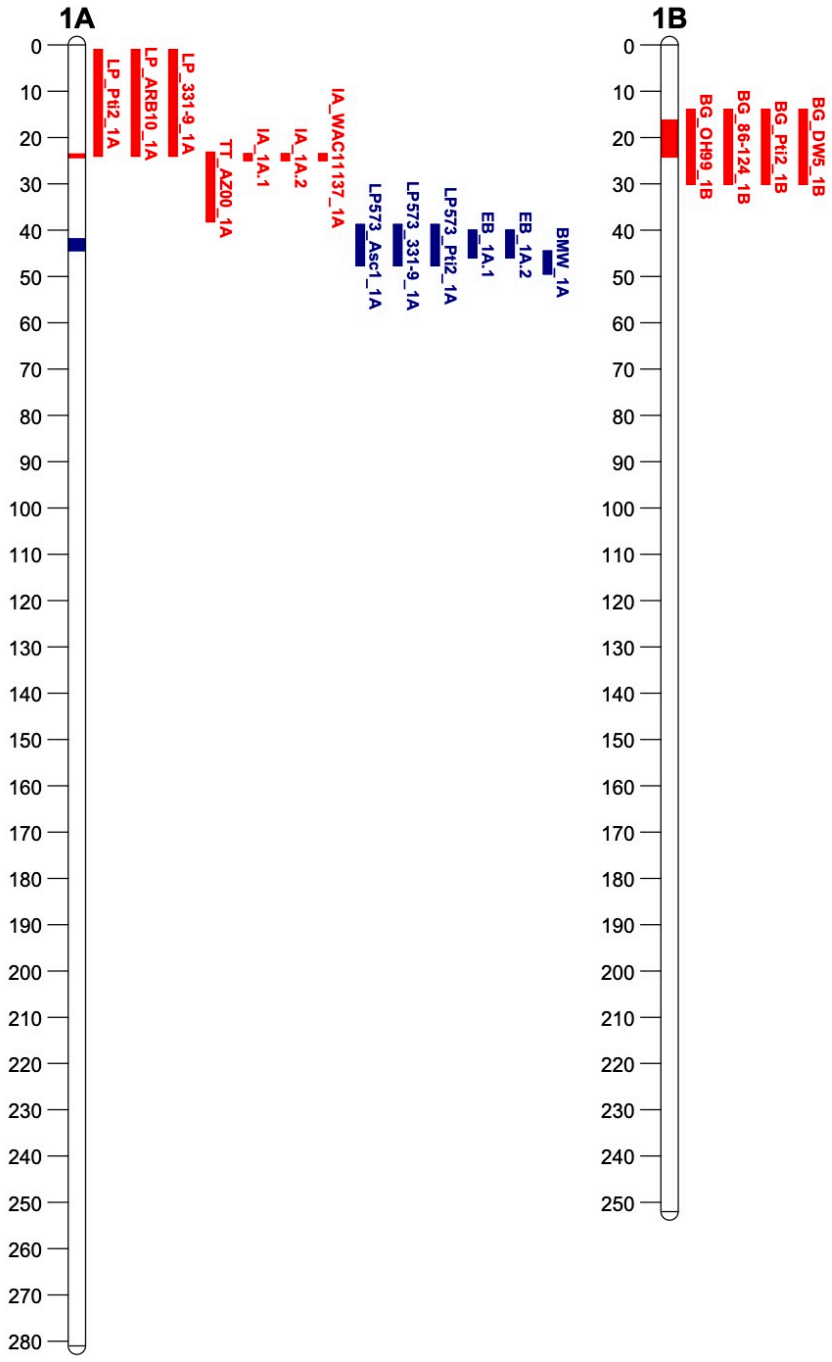

Figure 2. Distribution of meta-QTL on chromosomes.

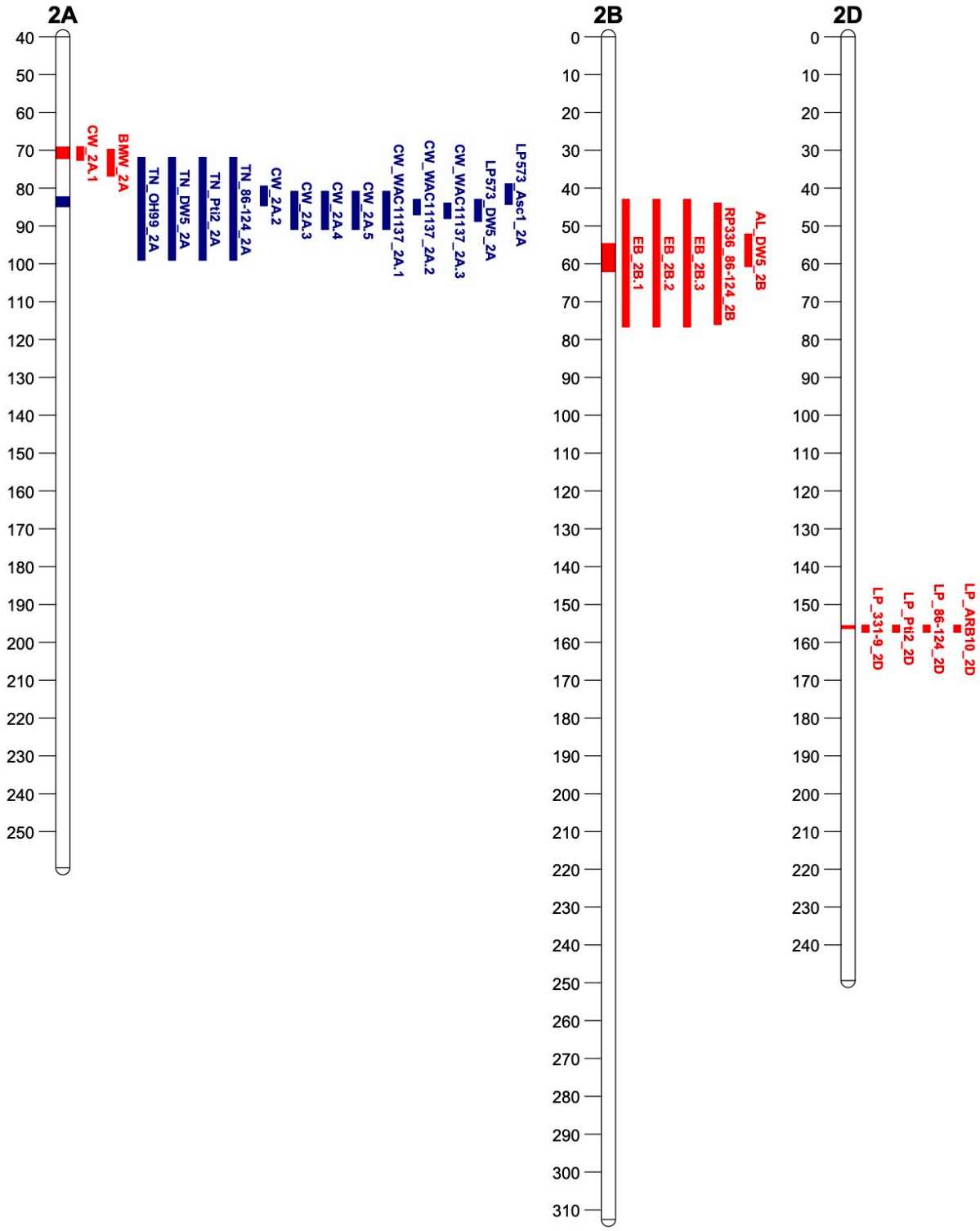

Figure 2. Distribution of meta-QTL on chromosomes (continued).

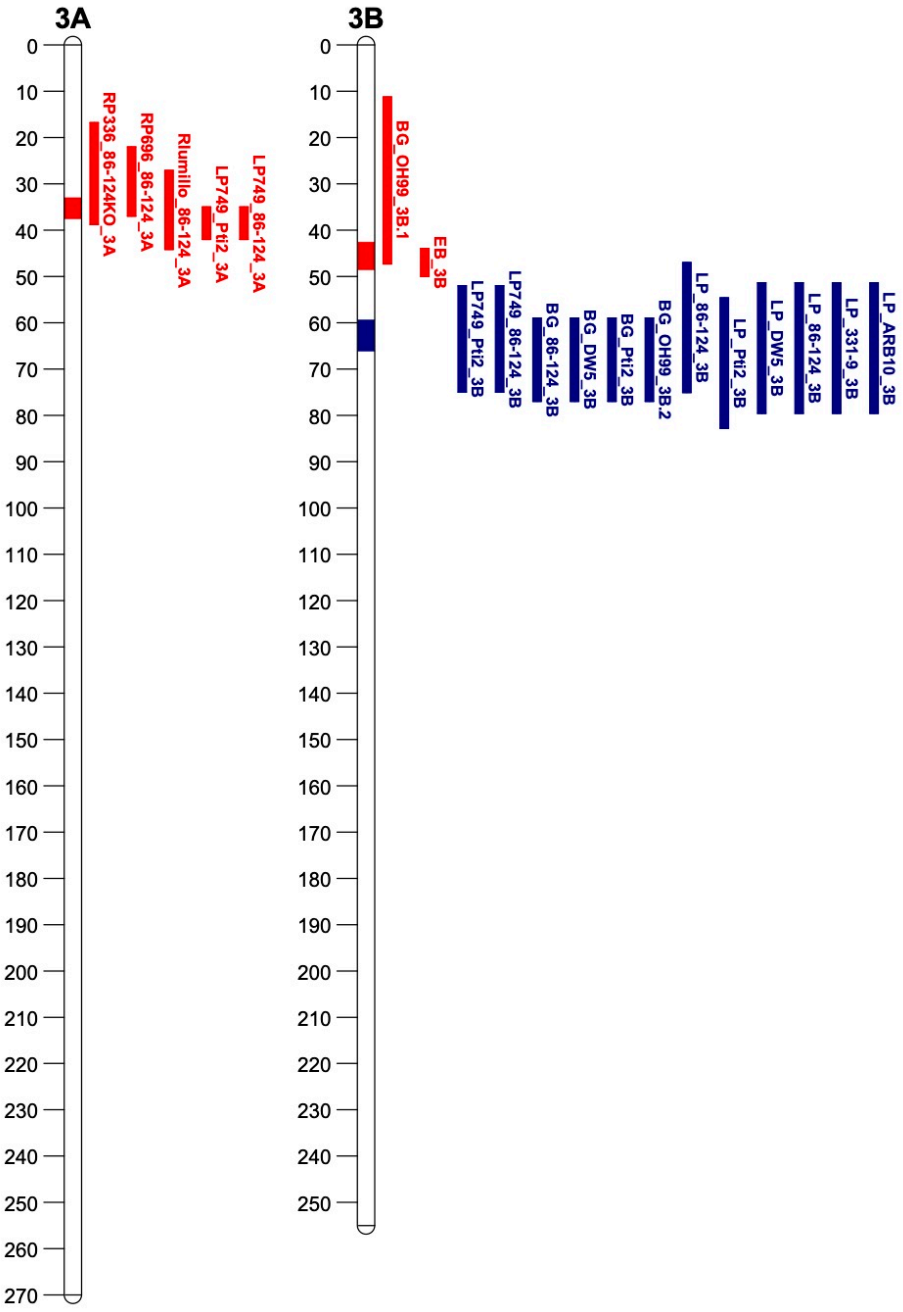

Figure 2. Distribution of meta-QTL on chromosomes (continued).

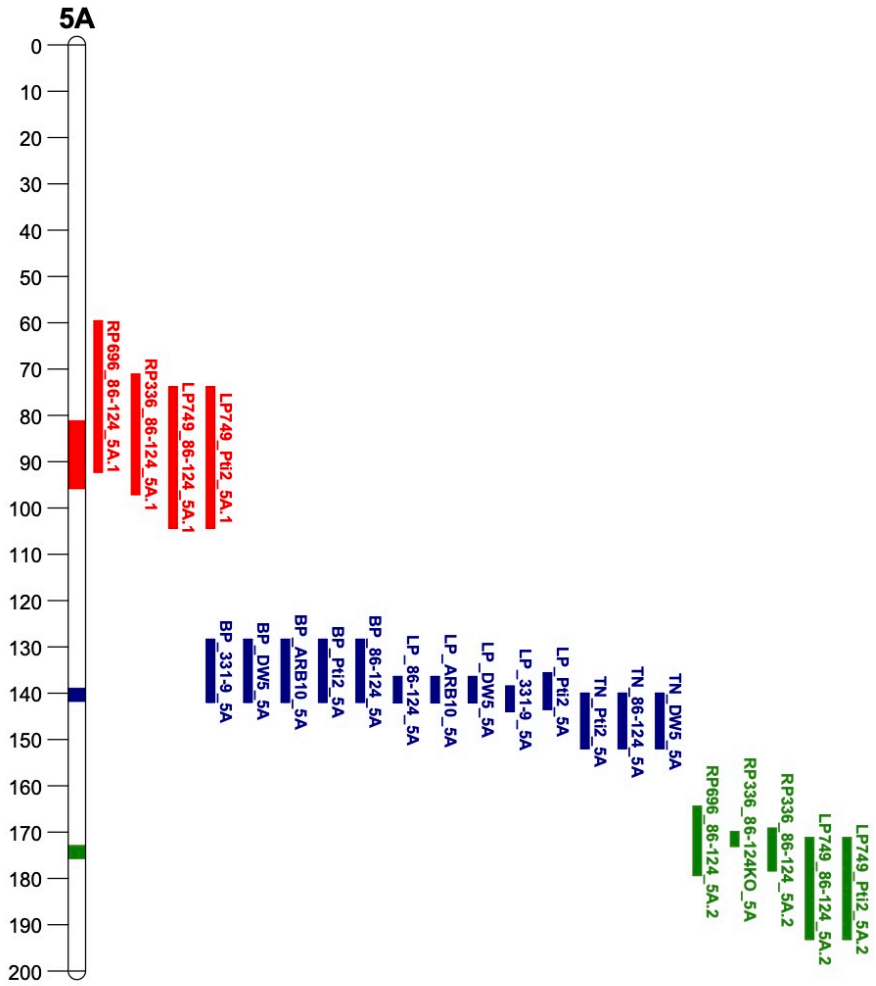

Figure 2. Distribution of meta-QTL on chromosomes (continued).

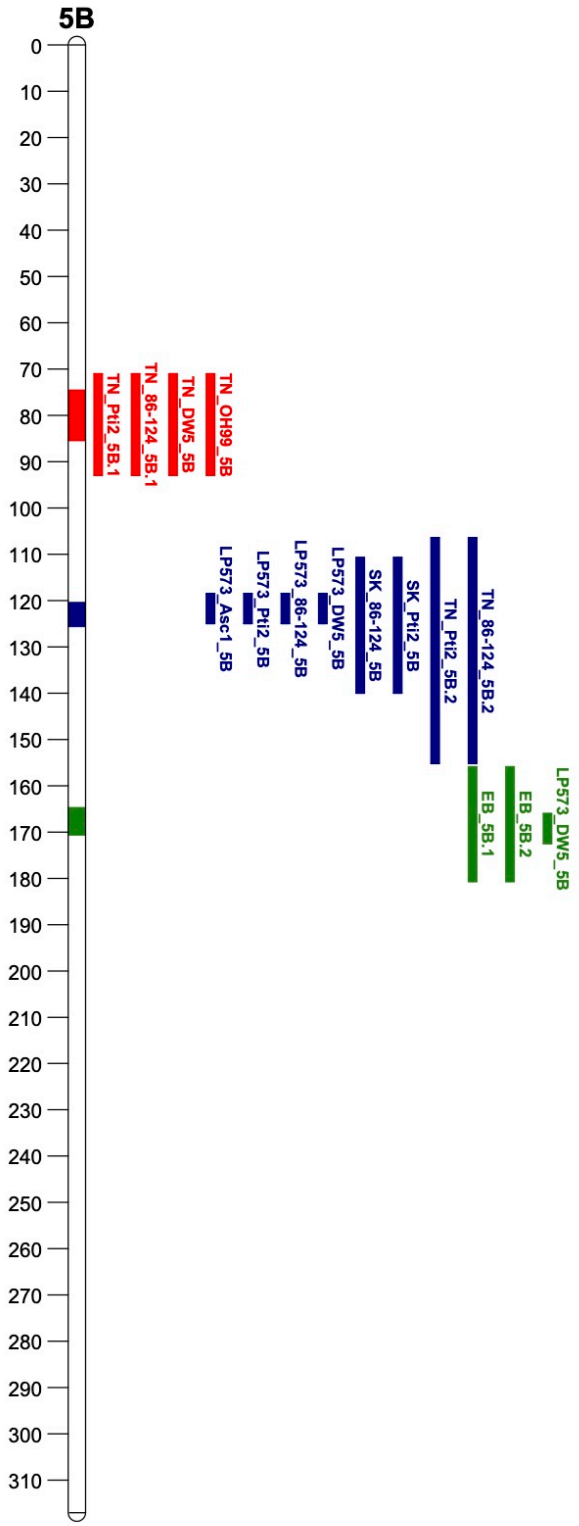

Figure 2. Distribution of meta-QTL on chromosomes (continued).

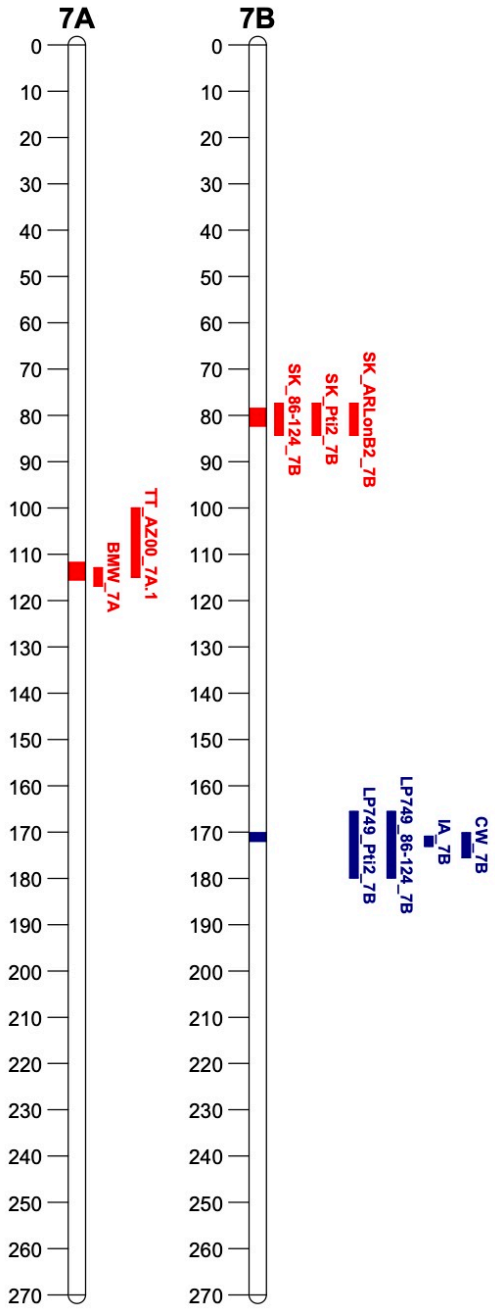

Figure 2. Distribution of meta-QTL on chromosomes (continued).

There were two meta-QTL detected on chromosome 1A. MQTL-1A.1 located at ~24.0 cM with an interval of 23.5-24.4 cM on the integrated linkage map and 3.4-9.4 Mbp on the reference genome (Figure 2 and Table 6). The MQTL-1A.1 comprised seven initial QTL identified from the three hexaploid bread wheat mapping populations IA, LP, and TT, conferring resistance to isolates of races 1 and 3, AR CrossB10, and Australia local isolates (Table 6 and Appendix Table 1). AR CrossB10 is an unclassified isolate, which does not produce Ptr ToxA but induces necrosis (Ali et al. 2010). AR CrossB10 likely produces Ptr ToxC because it can cause extensive chlorosis on Ptr ToxC susceptible check 6B365 (Kariyawasam et al. 2016). The initial QTL identified in LP were believed to be *Tsc1*, which confers susceptibility to Ptr ToxC, because it was close to *Tsc1* detected in previous studies (Faris 1997; Effertz et al. 2001) and significant associations were detected for the tested Ptr ToxC-producing isolates but not races 2 and 5 (Kariyawasam et al. 2016). Sun et al. (2010) identified a locus associated with resistance to race 1 isolate AZ-00, from which the closely linked marker *Xcfa2153* was aligned to ~7.2 Mbp on chromosome 1A and fell in the region of the MQTL-1A.1. Association mapping using durum wheat collections found that markers mapped near ~2.9-6.4 Mbp were significantly associated with resistance to Ptr ToxC-producing isolates (Galagedara 2018). Another association mapping study in hexaploid bread wheat collections also found that the markers near ~1.6-2.2 Mbp were significantly associated with resistance to a race 1 isolate WAC13611 from Australia and a race 1 isolate P4 from Russia (Dinglasan et al. 2019).

MQTL-1A.2 located at ~43.1 cM with an interval of 41.8-44.5 cM (7.3-12.1 Mbp) and comprised six initial QTL identified from three hexaploid bread wheat mapping populations including LP573, EB and BMW, conferring resistance to isolates of races 1 and 3 and uncharacterized local isolates collected from German. The initial QTL identified in LP573 was

proposed to be *Tsc1* because of the association with tested Ptr ToxC-producing isolates only (Liu et al. 2017). Patel et al. (2013) identified a QTL associated with resistance to AR CrossB10 using 535 spring wheat lines, where the closest linked marker *wPt-671823* was mapped to ~10 Mbp and fell in the region of MQTL-1A.2. Association mapping of a durum wheat breeding population found markers significantly associated with resistance to a race 1 isolate ND12 within the MQTL-1A.2 (Li et al. unpublished data).

There might be a structural variant or multiple copies of *Tsc1* on chromosome arm 1AS and both MQTL-1A.1 and MQTL-1A.2 contain the gene *Tsc1*. Differences on population size, marker density, significant thresholds used in individual QTL mapping studies resulted in varied confidence intervals, which can further affect the meta-QTL result. In addition, most association mapping studies in wheat were lack of high-density markers and could not finely map the locus. Therefore, it is also possible that MQTL-1A.1 and MQTL-1A.2 are one locus but were mapped as two meta-QTL because of experimental errors.

On chromosome 1B, four initial QTL were clustered to MQTL-1B, which located at 20.3 cM with interval of 16.2-24.3 cM (3.6-6.3 Mbp) (Figure 2 and Table 6). The four initial QTL conferred resistance to races 1, 2, 3 and 5, but were identified from one hexaploid bread wheat mapping population BG only (Faris and Friesen 2005). The four initial QTL were overlapped and originally named as *QTs.fcu-1BS*, which was one of the first reported race-nonspecific QTL (Faris and Friesen 2005). The desirable resistant allele was from BR-34, a Brazilian hard red spring wheat line (Faris and Friesen 2005). These evidences indicated that MQTL-1B might provide resistance in diverse genetic background in both common and durum wheat populations although it was detected in only one bi-parental mapping population. Singh et al. (2016) identified a locus associated with resistance to a race 1 isolate from Mexico using 170 elite bread

wheat lines from CYMMIT, from which the closest linked marker *wPt-8949* was mapped near ~3.9 Mbp. GWAS in durum wheat collections found markers around 4.8-6.1 Mbp significantly associated with resistance to race 1 isolate Pti2, however, this region was not significantly associated with resistance to other races tested (Galagedara 2018). Therefore, there might be another gene related to resistance to race 1.

MQTL-2A.1 located between 69.1 to 72.2 cM (45.1 to 58.7 Mbp) and comprised two initial QTL identified from two hexaploid wheat mapping populations, conferring resistance to local uncharacterized isolates from Australia and German, respectively (Figure 2 and Table 6). MQTL-2A.2 located between 82.3 to 84.9 cM (65.7 to 262.1 Mbp) and is another race-nonspecific QTL (Figure 2 and Table 6). MQTL_2A.2 contained 13 initial QTL identified from three hexaploid bread wheat mapping populations CW, LP573, and TN, conferring resistance to races 1, 2, 3, 5, and uncharacterized local isolates from Australia (Figure 2, Table 6, and Appendix Table 1). Out of the 13 initial QTL, seven QTL identified in CW population confer resistance to Australia local isolates under varied environments and growth stages (Appendix Table 1; Shankar et al. 2017); four initial QTL identified in TN population showed resistance to races 1, 2, 3, and 5 (Chu et al. 2008); two initial QTL found in population LP573 conferred resistance to races 1 and 3 (Liu et al. 2017). The LP573 population was also evaluated for resistance to isolates of races 2 and 5, but no significant resistance was identified in this region (Liu et al. 2017). The lack of resistance to races 2 and 5 in LP573 could be due to the interaction between MQTL-2A.2 and population and/or epistatic interaction of MQTL-2A.2 with other loci in LP573. MQTL-2A.1 and MQTL-2A.2 could represent the same locus because their initial QTL overlapped (Figure 2 and Table 6). The resulting separation of two meta-QTL could be due to the relative narrow intervals determined for the QTL identified from the CW population.

MQTL-2B spanned from 54.6 to 62.1 cM (1.2 to 27.2 Mbp) and comprised five initial QTL identified from three populations AL, EB, and RP336 (Figure 2 and Table 6). Reaction to Ptr ToxB infiltration suggested that the initial QTL identified from the AL population was the gene *Tsc2*, which conditions sensitivity to Ptr ToxB (Virdi et al. 2016). Abeysekara et al. (2010) finely mapped *Tsc2* and developed a diagnostic marker *XBE444541*. The marker *XBE444541* was mapped near ~24.1 Mbp on chromosome 2B. GWAS in durum wheat collections found that the most significant marker associated with reaction to infiltration of Ptr ToxB mapped near ~24.1 Mbp and the most significant marker associated with resistance to race 5 isolate DW5 was mapped on ~24.3 Mbp of chromosome 2B (Galagedara 2018). These evidences indicated that MQTL-2B is co-localized with *Tsc2*. However, the initial QTL identified in the RP336 population confers resistance to race 2 isolate 86-124, which does not produce Ptr ToxB (Liu et al. 2019). In addition, the EB population was tested using infected straws with Australia local isolates (Li et al. 2011), from which Ptr ToxB was rarely produced. GWAS using 295 bread wheat lines found a marker around 7.8 Mbp significantly associated with Australia local isolates too (Dinglasan et al. 2019). Therefore, it is possible that MQTL-2B is composed of another gene conferring resistance/susceptibility to NEs other than Ptr ToxB (Liu et al. 2019).

MQTL-2D was located at interval 155.6-156.4 cM (312.3-383.0 Mbp) and comprised four initial QTL identified from one hexaploid bread wheat mapping population LP (Table 6). MQTL-2D is race-nonspecific because the four individual QTL conferred resistance to isolates of races 1, 2, 3, and AR CrossB10 (Table 6).

MQTL-3A located at interval 33.0-37.5 cM (20.0-21.8 Mbp) and consisted of five initial QTL identified from the tetraploid wheat mapping populations LP749, RIum, RP336, and RP696 (Figure 2 and Table 6). The five individual QTL were related to resistance to races 1 and 2. The

four populations were not screened for resistance to other Ptr races (Chu et al. 2010; Liu et al. 2019), therefore, it is unknown if this meta-QTL provides race-nonspecific resistance.

MQTL-3B.1 was located at interval 42.6-48.5 cM (23.9-68.8 Mbp) and comprised two initial QTL identified from the two hexaploid wheat populations BMW and CW (Figure 2 and Table 6). The two initial QTL conferred resistance to race 1 isolate OH99 and Autstralia local isolate (Table 6). MQTL-3B.2 was comprised of 11 initial QTL identified from the hexaploid bread wheat mapping populations BG and LP and the tetraploid wheat mapping population LP749, which located between 59.4 to 66.1 cM (465.2 to 583.4 Mbp) on chromosome 3B (Figure 2 and Table 6). The four initial QTL identified from the BG population confer resistance to races 1, 2, and 3 (Faris and Friesen 2005). In the LP population, five initial QTL confered resistance to races 1, 2, 3, and 5 and unclassified isolate AR CrossB10 (Kariyawasam et al. 2016). The two initial QTL identified from the tetraploid wheat population LP749 confered resistance to races 1 and 2 (Table 6), from which no other races were evaluated (Chu et al. 2010). MQTL-3B.2 had the highest average LOD score across all meta-QTL and explained about 27% of the total variation on average (Table 6). Association mapping using a durum wheat collection also found markers in this region (around 466.6-474.3 Mbp) significantly associated with resistance to races 1, 2, 3, and 5 (Galagedara 2018). GWAS with 295 hexaploid bread wheat has revealed a marker near ~479.5 Mbp significantly associated with resistance to tan spot induced by Australia local isolates (Dinglasan et al. 2019).

Three meta-QTL were identified on chromosome 5A. MQTL-5A.1 was located at interval 81.1-95.9 cM (437.4-482.1 Mbp) and consisted of four initial QTL identified from the three tetraploid wheat mapping populations LP749, RP336, and RP696 (Figure 2 and Table 6). MQTL-5A.3 was located at interval 172.9-175.8 cM (671.3-676.0 Mbp) and consisted of four

initial QTL identified from the same three tetraploid wheat mapping populations (Figure 2 and Table 6). Both meta-QTL conferred resistance to races 1 and 2 (Table 6). Same as the MQTL-3A, no other races were evaluated for those tetraploid mapping populations (Chu et al. 2010; Liu et al. 2019). Therefore, it is unknown if the two meta-QTL are race-nonspecific. MQTL-5A.2 was located at interval 138.9-141.8 cM (592.1-614.4 Mbp) and is another race-nonspecific QTL identified in both bread wheat and durum wheat (Figure 2 and Table 6). MQTL-5A.2 is composed of 13 initial QTL identified from the hexaploid wheat mapping populations LP and TN and the tetraploid wheat mapping population BP (Table 6). Five initial QTL identified from the LP population confer resistance to races 1, 2, 3, 5, and unclassified isolate AR CrossB10 (Table 6 and Appendix Table 1). In the TN population, three initial QTL provided resistance to races 1, 2, and 5 (Table 6). In the tetraploid wheat mapping population BP, five initial QTL conferred resistance to races 1, 2, 3, and 5 and unclassified isolate AR CrossB10 (Table 6).

Three meta-QTL were also found on chromosome 5B. MQTL-5B.1 was located at interval 74.5-85.5 cM (58.8-448.0 Mbp). MQTL-5B.1 comprised four initial QTL identified from one hexaploid wheat mapping population TN, and conferred resistance to races 1, 2, 3, and 5 (Figure 2 and Table 6). This meta-QTL explained 21.0% of total variation on average which could be an over-estimate due to the small population size of TN. MQTL-5B.2 was located at interval 120.4-125.6 cM (541.7-549.9 Mbp) and consisted of eight initial QTL identified from three hexaploid wheat mapping populations including LP573, SK, and TN, conferring resistance to races 1 and 2, both producing Ptr ToxA (Table 6). LP573 was evaluated for races 1, 2, 3, and 5, but this region was detected only for Ptr ToxA-producing isolates (Liu et al. 2017). Infiltration of purified Ptr ToxA was carried out for the LP573 population and results indicated that the identified QTL co-localized with *Tsn1* (Liu et al. 2017). GWAS of reaction to Ptr ToxA

infiltration found most significant markers near ~546.7 Mbp in both bread wheat and durum wheat collections (Galagedara 2018; Liu et al. 2015; Perez et al. 2017). The diagnostic marker *Xfcp623* of *Tsn1* was mapped at ~546.6 Mb on chromosome 5B. Thus, the causal gene of MQTL-5B.2 is *Tsn1*. MQTL-5B.3 was located at interval 164.7-170.7 cM (663.1-679.4 Mbp) and consisted of three initial QTL identified in the hexaploid wheat mapping populations EB and LP573. The two initial QTL in EB conferred resistance to Australia local isolates (Table 6). GWAS using 295 hexaploid bread wheat has found a marker near ~697.0 Mbp significantly associated with resistance to Australia local isolates (Dinglasan et al. 2019). One initial QTL identified in LP573 conferred resistance to race 5 isolate DW5, producing Ptr ToxB (Liu et al. 2017). Interestingly, GWAS of sensitivity to Ptr ToxB using 83 Canadian spring wheat cultivars found significant markers near ~656.7-658.5 Mbp (Perez et al. 2017). Given the fact that Australia local isolates rarely produced Ptr ToxB, MQTL-5B.3 may cover more than one gene and confer resistance or susceptibility to Ptr ToxB and/or other unknown NEs.

MQTL-7A was located at interval 111.7-115.6 cM (116.1-133.6 Mbp) and consisted of two initial QTL identified from the hexaploid wheat mapping populations TT and BMW. The initial QTL identified in the TT population conferred resistance to the race 1 isolate AZ00 (Kalia et al. 2018). The initial QTL identified in the BMW population conferred resistance to isolates collected from German (Stadlmeier et al. 2019).

MQTL-7B.1 was located at interval 78.4-82.4 cM (21.0-34.0 Mbp) and comprised three initial QTL identified from the SK population, conferring resistance to races 1 and 2 and isolate AR LonB2 (Figure 2 and Table 6). Similar to AR CrossB10, AR LonB2 is an unclassified isolate, which does not produce Ptr ToxA but induces necrosis (Ali et al. 2010). The initial QTL

was named as *QTs.fcu-7B* in the original study and conferred resistance to race 1 isolate Pti2 but not to race 1 isolate Asc1 (Faris et al. 2012).

MQTL-7B.2 was located at interval 170.1-172.1 cM (614.2-622.8 Mbp) and contained two initial QTL from the tetraploid wheat mapping population LP749, one initial QTL from the hexaploid wheat mapping population IA and one individual QTL from the hexaploid wheat mapping population CW (Figure 2 and Table 6). The four initial QTL confer resistance to races 1 and 2 and Australia local isolates.

Discussion

Genetic architecture of tan spot resistance revealed by meta-QTL analysis

To gain a better understanding of the genetic architecture of tan spot resistance, we performed a meta-QTL analysis using reported QTL from previous mapping studies. In total, 123 QTL identified from 17 mapping populations in 14 previous QTL mapping studies were projected onto the integrated linkage map. Out of the 123 projected QTL, 104 QTL were grouped into 19 meta-QTL, suggesting a majority of previously mapped tan spot resistance loci were shared by multiple populations and/or showed resistance to multiple races/isolates.

Three known Ptr NEs-wheat interactions including Ptr ToxA-*Tsn1*, Ptr ToxB-*Tsc2*, and Ptr ToxC-*Tsc1* have been characterized in wheat. MQTL-1A.1 and MQTL-1A.2 co-localized with *Tsc1*; MQTL-2B co-localized with *Tsc2*; MQTL-5B.2 co-localized with *Tsn1*. Per their initial QTL studies, the three genes showed varied effects on the development of disease across mapping populations (Table 6, Chu et al. 2008; Faris et al. 2012; Liu et al. 2017). Evaluation of Australian wheat varieties suggested that the importance of the Ptr ToxA-*Tsn1* interaction in tan spot development depended on the wheat host genetic background (See et al. 2018). Seven tetraploid mapping populations were evaluated with races 1 and/or 2 in one of our previous

studies (Liu et al. 2019), but none of them found *Tsn1* as a significant QTL even for the five populations segregated for *Tsn1*. It was proposed that some non-race specific QTL might work upstream of the Ptr ToxA-*Tsn1* interaction precluding the development of tan spot and therefore played a more important role in some populations (Kariyawasam et al., 2016). Expression levels of *Tsn1* were varied across isolates and pathogen races, which could also explain the discrepancy of the importance.

Six meta-QTL including MQTL-1B, MQTL-2A.2, MQTL-2D, MQTL-3B.2, MQTL-5A.2, and MQTL-5B.1, comprising of 50 individual QTL, conferred resistance to multiple races and are likely race non-specific. However, only 19 out of 371 lines have the desirable resistant allele. The rare frequency of the desirable allele in breeding population magnifies its importance on improvement of tan spot resistance. We have developed diagnostic markers for the three meta-QTL and will investigate their frequencies of the desirable alleles in elite wheat breeding populations.

MQTL-3A, MQTL-5A.1, and MQTL-5A.3 comprised of 14 individual QTL identified from four tetraploid wheat mapping populations that were only evaluated for resistance to races 1 and 2. The 11 hexaploid wheat mapping populations used for meta-QTL analysis were evaluated with multiple races and none of them found QTL in these three meta-QTL regions. It is possible that those loci are tetraploid wheat specific. However, the resistance alleles of the individual QTL were from the parents Rusty and Lebsock both of which are sensitive to Ptr ToxA but highly resistant to races 1 and 2. Therefore, it will be meaningful to test if those meta-QTL provide race-nonspecific resistance by screening other races for those tetraploid wheat populations.

KASP markers linked to the major meta-QTL were developed for marker-assisted selection. Given a large number of QTL identified in wheat, selecting proper QTL and their linked markers is critical to efficiently improve tan spot resistance via MAS. Those QTL offering resistance to multiple races with large additive effects in diverse genetic background should be prioritized. In addition to known dominant genes conferring susceptibility to Ptr ToxA, Ptr ToxB, and Ptr ToxC, we found two major race-nonspecific QTL, MQTL-3B and MQTL-5A.2, both of which provide resistance to all tested races and showed large additive effects in multiple mapping populations. We have developed and will test six KASP markers, three markers for each meta-QTL, in a panel of elite hard red spring wheat and a panel of elite durum wheat breeding lines.

Implication of marker-assisted selection for breeding tan spot resistance in wheat

MAS is an effective and efficient approach to improve a quantitative trait like tan spot resistance by selecting major QTL or genes. Meta-QTL analysis could remove redundancies and prioritize QTL for MAS. Meta-QTL with a large effect, a small confidence interval, and high number of initial QTL identified from diverse populations is preferred (Loffler et al. 2009). Removal of the three known dominant susceptible genes in breeding populations is highly recommended although they play a major role only in some populations and some genotypes. In addition to three known dominant susceptible genes, our meta-QTL analysis found that three race-nonspecific meta-QTL including MQTL_2A.2, MQTL_3B, and MQTL_5A.2 also meet the criteria. Integrating desirable alleles of those race-nonspecific meta-QTL could provide high and stable tan spot resistance induced by varied races and isolates. Of them, MQTL-2A.2, MQTL-3B, and MQTL-5A.2 were identified in multiple mapping populations and showed large additive effects. Those meta-QTL provide broad spectrum resistance in diverse background and are

importance of tan spot resistance improvement in breeding. MQTL-2A.2 conferred resistance to tan spot not only at the seedling stage under controlled environmental condition but also at tilling and adult stages under field trials. It will be interesting to know if other race-nonspecific QTL could also provide resistance in adult plants. MQTL-3B showed the largest effect among all 19 meta-QTL. GWAS in durum wheat collections including 171 landrace and 200 cultivated breeding lines found the MQTL-3B region was significant associated with resistance to races 1, 2, 3, and 5 (Galagedara 2018). Most previous studies were conducted at the seedling stage under controlled environments. It will be meaningful to test if those race-nonspecific meta-QTL provide resistance at other growth stages under field conditions. Further validation of those loci in an active breeding population under field condition is essential for implementing those meta-QTL for marker-assisted selection in a breeding program. It is also crucial to map putative novel loci under field conditions.

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APPENDIX

Table A1. List of population and disease evaluation information of tan spot resistance QTL identified in 17 mapping populations for meta-QTL analysis.

QTL	Population	Cross	Isolate	Race	Environment	Growth stage
ACDC_2B	ACDC	Attlia/CDC Go	Canada local isolates	NA	Field	Milk stage
ACDC_2D	ACDC	Attlia/CDC Go	Canada local isolates	NA	Field	Milk stage
ACDC_6B	ACDC	Attlia/CDC Go	Canada local isolates	NA	Field	Milk stage
AL_86124_6B	AL	Altar/Langdon	86-124	race 2	Greehouse	Seedling stage
AL_DW5_2B	AL	Altar/Langdon	DW5	race 5	Greehouse	Seedling stage
AL_DW5_4B	AL	Altar/Langdon	DW5	race 5	Greehouse	Seedling stage
AL_L13-35_4B	AL	Altar/Langdon	L13-35	race 2	Greehouse	Seedling stage
AL_L13-35_5B	AL	Altar/Langdon	L13-35	race 2	Greehouse	Seedling stage
BG_86124_1B	BG	BR34/Grandin	86-124	race 2	Greehouse	Seedling stage
BG_86124_3B	BG	BR34/Grandin	86-124	race 2	Greehouse	Seedling stage
BG_DW5_1B	BG	BR34/Grandin	DW5	race 5	Greehouse	Seedling stage
BG_DW5_3B	BG	BR34/Grandin	DW5	race 5	Greehouse	Seedling stage
BG_OH99_1B	BG	BR34/Grandin	OH99	race 3	Greehouse	Seedling stage
BG_OH99_3B.1	BG	BR34/Grandin	OH99	race 3	Greehouse	Seedling stage
BG_OH99_3B.2	BG	BR34/Grandin	OH99	race 3	Greehouse	Seedling stage
BG_Pti2_1B	BG	BR34/Grandin	Pti2	race 1	Greehouse	Seedling stage
BG_Pti2_3B	BG	BR34/Grandin	Pti2	race 1	Greehouse	Seedling stage
BMW_2A	BMW	MAGIC population*	German local isolates	NA	Field	Seedling stage
BMW_3D	BMW	MAGIC population*	German local isolates	NA	Field	Seedling stage
BMW_7A	BMW	MAGIC population*	German local isolates	NA	Field	Seedling stage
BMW_1A	BMW	MAGIC population*	German local isolates	NA	Field	Seedling stage
BP_331-9_5A	BP	Ben/PI 41025	331-9	race 3	Greehouse	Seedling stage
BP_86-124_3A	BP	Ben/PI 41025	86-124	race 2	Greehouse	Seedling stage
BP_86-124_5A	BP	Ben/PI 41025	86-124	race 2	Greehouse	Seedling stage
BP_AR CrossB10_5A	BP	Ben/PI 41025	AR CrossB10	NA	Greehouse	Seedling stage
BP_DW5_5A	BP	Ben/PI 41025	DW5	race 5	Greehouse	Seedling stage
BP_DW5_5B	BP	Ben/PI 41025	DW5	race 5	Greehouse	Seedling stage
BP_Pti2_5A	BP	Ben/PI 41025	Pti2	race 1	Greehouse	Seedling stage

Table A1. List of population and disease evaluation information of tan spot resistance QTL identified in 17 mapping populations for meta-QTL analysis (continued).

QTL	Population	Cross	Isolate	Race	Environment	Growth stage
CW_2A.a	CW	Calingiri/Wyalkatchem	Australia local isolates	NA	Greenhouse	Seedling stage
CW_2A.b	CW	Calingiri/Wyalkatchem	Australia local isolates	NA	Field	Booting stage
CW_2A.c	CW	Calingiri/Wyalkatchem	Australia local isolates	NA	Greehouse	Seedling stage
CW_2A.d	CW	Calingiri/Wyalkatchem	Australia local isolates	NA	Field	Tillering stage
CW_2A.e	CW	Calingiri/Wyalkatchem	Australia local isolates	NA	Field	Adult stage
CW_6B	CW	Calingiri/Wyalkatchem	Australia local isolates	NA	Field	Tillering stage
CW_7B	CW	Calingiri/Wyalkatchem	Australia local isolates	NA	Field	Booting stage
CW_WAC11137_2A.a	CW	Calingiri/Wyalkatchem	Australia local isolates	NA	Field	Tillering stage
CW_WAC11137_2A.b	CW	Calingiri/Wyalkatchem	Australia local isolates	NA	Field	Adult stage
CW_WAC11137_2A.c	CW	Calingiri/Wyalkatchem	Australia local isolates	NA	Field	Adult stage
EB_1A.a	EB	Ernie/Betavia	Australia local isolates	NA	Greehouse	Seedling stage
EB_1A.b	EB	Ernie/Betavia	Australia local isolates	NA	Greehouse	Seedling stage
EB_2B.a	EB	Ernie/Betavia	Australia local isolates	NA	Greehouse	Seedling stage
EB_2B.b	EB	Ernie/Betavia	Australia local isolates	NA	Greehouse	Seedling stage
EB_2B.c	EB	Ernie/Betavia	Australia local isolates	NA	Greehouse	Seedling stage
EB_3B	EB	Ernie/Betavia	Australia local isolates	NA	Greehouse	Seedling stage
EB_3D	EB	Ernie/Betavia	Australia local isolates	NA	Greehouse	Seedling stage
EB_5B.a	EB	Ernie/Betavia	Australia local isolates	NA	Greehouse	Seedling stage
EB_5B.b	EB	Ernie/Betavia	Australia local isolates	NA	Greehouse	Seedling stage
EB_6A.a	EB	Ernie/Betavia	Australia local isolates	NA	Greehouse	Seedling stage
EB_6A.b	EB	Ernie/Betavia	Australia local isolates	NA	Greehouse	Seedling stage
EB_7A	EB	Ernie/Betavia	Australia local isolates	NA	Greehouse	Seedling stage
EB_7D	EB	Ernie/Betavia	Australia local isolates	NA	Greehouse	Seedling stage
IA_1A.a	IA	IGW2547/Annuello	Australia local isolates	NA	Field	Tillering stage
IA_1A.b	IA	IGW2547/Annuello	Australia local isolates	NA	Greenhouse	Adult stage
IA_1A.c	IA	IGW2547/Annuello	Australia local isolates	NA	Field	Tillering stage
IA_2B	IA	IGW2547/Annuello	Australia local isolates	NA	Field	Tillering stage
IA_2D	IA	IGW2547/Annuello	Australia local isolates	NA	Field	Tillering stage
IA_4B	IA	IGW2547/Annuello	Australia local isolates	NA	Field	Tillering stage

Table A1. List of population and disease evaluation information of tan spot resistance QTL identified in 17 mapping populations for meta-QTL analysis (continued).

QTL	Population	Cross	Isolate	Race	Environment	Growth stage
IA_7B	IA	IGW2547/Annuello	Australia local isolates	NA	Field	Tillering stage
IA_WAC11137_1A	IA	IGW2547/Annuello	Australia local isolates	NA	Greenhouse	Adult stage
IA_WAC11137_4A	IA	IGW2547/Annuello	Australia local isolates	NA	Field	Adult stage
IA_WAC11137_5B	IA	IGW2547/Annuello	Australia local isolates	NA	Field	Adult stage
LP_331-9_1A	LP	Louise/Penawawa	331-9	race 3	Greehouse	Seedling stage
LP_331-9_2D	LP	Louise/Penawawa	331-9	race 3	Greehouse	Seedling stage
LP_331-9_3B	LP	Louise/Penawawa	331-9	race 3	Greehouse	Seedling stage
LP_331-9_5A	LP	Louise/Penawawa	331-9	race 3	Greehouse	Seedling stage
LP_86-124_2D	LP	Louise/Penawawa	86-124	race 2	Greehouse	Seedling stage
LP_86-124_3B	LP	Louise/Penawawa	86-124	race 2	Greehouse	Seedling stage
LP_86-124_5A	LP	Louise/Penawawa	86-124	race 2	Greehouse	Seedling stage
LP_AR CrossB10_1A	LP	Louise/Penawawa	AR CrossB10	NA	Greehouse	Seedling stage
LP_AR CrossB10_2D	LP	Louise/Penawawa	AR CrossB10	NA	Greehouse	Seedling stage
LP_AR CrossB10_3B	LP	Louise/Penawawa	AR CrossB10	NA	Greehouse	Seedling stage
LP_AR CrossB10_5A	LP	Louise/Penawawa	AR CrossB10	NA	Greehouse	Seedling stage
LP_DW5_3B	LP	Louise/Penawawa	DW5	race 5	Greehouse	Seedling stage
LP_DW5_5A	LP	Louise/Penawawa	DW5	race 5	Greehouse	Seedling stage
LP_Pti2_1A	LP	Louise/Penawawa	Pti2	race 1	Greehouse	Seedling stage
LP_Pti2_2D	LP	Louise/Penawawa	Pti2	race 1	Greehouse	Seedling stage
LP_Pti2_3B	LP	Louise/Penawawa	Pti2	race 1	Greehouse	Seedling stage
LP_Pti2_5A	LP	Louise/Penawawa	Pti2	race 1	Greehouse	Seedling stage
LP573_331-9_1A	LP573	LMPG-6/PI 626573	331-9	race 3	Greehouse	Seedling stage
LP573_86-124_5B	LP573	LMPG-6/PI 626573	86-124	race 2	Greehouse	Seedling stage
LP573_Asc1_1A	LP573	LMPG-6/PI 626573	Asc1	race 1	Greehouse	Seedling stage
LP573_Asc1_2A	LP573	LMPG-6/PI 626573	Asc1	race 1	Greehouse	Seedling stage
LP573_Asc1_2B	LP573	LMPG-6/PI 626573	Asc1	race 1	Greehouse	Seedling stage
LP573_Asc1_5B	LP573	LMPG-6/PI 626573	Asc1	race 1	Greehouse	Seedling stage
LP573_DW5_2A	LP573	LMPG-6/PI 626573	DW5	race 5	Greehouse	Seedling stage
LP573_DW5_5B	LP573	LMPG-6/PI 626573	DW5	race 5	Greehouse	Seedling stage

Table A1. List of population and disease evaluation information of tan spot resistance QTL identified in 17 mapping populations for meta-QTL analysis (continued).

QTL	Population	Cross	Isolate	Race	Environment	Growth stage
LP573_Pti2_1A	LP573	LMPG-6/PI 626573	Pti2	race 1	Greehouse	Seedling stage
LP573_Pti2_5B	LP573	LMPG-6/PI 626573	Pti2	race 1	Greehouse	Seedling stage
LP749_86-124_3A	LP749	Lebsock/PI 94749	86-124	race 2	Greehouse	Seedling stage
LP749_86-124_3B	LP749	Lebsock/PI 94749	86-124	race 2	Greehouse	Seedling stage
LP749_86-124_5A.1	LP749	Lebsock/PI 94749	86-124	race 2	Greehouse	Seedling stage
LP749_86-124_5A.2	LP749	Lebsock/PI 94749	86-124	race 2	Greehouse	Seedling stage
LP749_86-124_7B	LP749	Lebsock/PI 94749	86-124	race 2	Greehouse	Seedling stage
LP749_Pti2_3A	LP749	Lebsock/PI 94749	Pti2	race 1	Greehouse	Seedling stage
LP749_Pti2_3B	LP749	Lebsock/PI 94749	Pti2	race 1	Greehouse	Seedling stage
LP749_Pti2_5A.1	LP749	Lebsock/PI 94749	Pti2	race 1	Greehouse	Seedling stage
LP749_Pti2_5A.2	LP749	Lebsock/PI 94749	Pti2	race 1	Greehouse	Seedling stage
LP749_Pti2_7B	LP749	Lebsock/PI 94749	Pti2	race 1	Greehouse	Seedling stage
RIumillo_86-124_3A	RIum	Rusty/Iumillo	86-124	race 2	Greehouse	Seedling stage
RP336_86-124_2B	RP336	Rusty/PI 387336	86-124	race 2	Greehouse	Seedling stage
RP336_86-124_5A.1	RP336	Rusty/PI 387336	86-124	race 2	Greehouse	Seedling stage
RP336_86-124_5A.2	RP336	Rusty/PI 387336	86-124	race 2	Greehouse	Seedling stage
RP336_86-124ΔToxA_1B	RP336	Rusty/PI 387336	86-124ΔToxA	race 2	Greehouse	Seedling stage
RP336_86-124ΔToxA_3A	RP336	Rusty/PI 387336	86-124ΔToxA	race 2	Greehouse	Seedling stage
RP336_86-124ΔToxA_5A	RP336	Rusty/PI 387336	86-124ΔToxA	race 2	Greehouse	Seedling stage
RP336_86-124ΔToxA_7A	RP336	Rusty/PI 387336	86-124ΔToxA	race 2	Greehouse	Seedling stage
RP696_86-124_2B	RP696	Rusty/PI 387696	86-124	race 2	Greehouse	Seedling stage
RP696_86-124_3A	RP696	Rusty/PI 387696	86-124	race 2	Greehouse	Seedling stage
RP696_86-124_5A.1	RP696	Rusty/PI 387696	86-124	race 2	Greehouse	Seedling stage
RP696_86-124_5A.2	RP696	Rusty/PI 387696	86-124	race 2	Greehouse	Seedling stage
SK_86-124_5B	SK	Salamouni/Katepwa	86-124	race 2	Greehouse	Seedling stage

Table A1. List of population and disease evaluation information of tan spot resistance QTL identified in 17 mapping populations for meta-QTL analysis (continued).

QTL	Population	Cross	Isolate	Race	Environment	Growth stage
SK_86-124_7B	SK	Salamouni/Katepwa	86-124	race 2	Greehouse	Seedling stage
SK_AR LonB2_7B	SK	Salamouni/Katepwa	AR LonB2		Greehouse	Seedling stage
SK_AR LonB2_7D	SK	Salamouni/Katepwa	AR LonB2		Greehouse	Seedling stage
SK_Asc1_5D	SK	Salamouni/Katepwa	Asc1	race 1	Greehouse	Seedling stage
SK_Pti2_5B	SK	Salamouni/Katepwa	Pti2	race 1	Greehouse	Seedling stage
SK_Pti2_7B	SK	Salamouni/Katepwa	Pti2	race 1	Greehouse	Seedling stage
TN_86-124_2A	TN	TA4152-60/ND495	86-124	race 2	Greehouse	Seedling stage
TN_86-124_5A	TN	TA4152-60/ND495	86-124	race 2	Greehouse	Seedling stage
TN_86-124_5B.1	TN	TA4152-60/ND495	86-124	race 2	Greehouse	Seedling stage
TN_86-124_5B.2	TN	TA4152-60/ND495	86-124	race 2	Greehouse	Seedling stage
TN_DW5_2A	TN	TA4152-60/ND495	DW5	race 5	Greehouse	Seedling stage
TN_DW5_5A	TN	TA4152-60/ND495	DW5	race 5	Greehouse	Seedling stage
TN_DW5_5B	TN	TA4152-60/ND495	DW5	race 5	Greehouse	Seedling stage
TN_OH99_2A	TN	TA4152-60/ND495	OH99	race 3	Greehouse	Seedling stage
TN_OH99_4A	TN	TA4152-60/ND495	OH99	race 3	Greehouse	Seedling stage
TN_OH99_5B	TN	TA4152-60/ND495	OH99	race 3	Greehouse	Seedling stage
TN_Pti2_2A	TN	TA4152-60/ND495	Pti2	race 1	Greehouse	Seedling stage
TN_Pti2_5A	TN	TA4152-60/ND495	Pti2	race 1	Greehouse	Seedling stage
TN_Pti2_5B.1	TN	TA4152-60/ND495	Pti2	race 1	Greehouse	Seedling stage
TN_Pti2_5B.2	TN	TA4152-60/ND495	Pti2	race 1	Greehouse	Seedling stage
TT_AZ00_1A	TT	TA161-L1/TAM105	AZ00	race 1	Greehouse	Seedling stage
TT_AZ00_6A	TT	TA161-L1/TAM105	AZ00	race 1	Greehouse	Seedling stage
TT_AZ00_7A.1	TT	TA161-L1/TAM105	AZ00	race 1	Greehouse	Seedling stage
TT_AZ00_7A.2	TT	TA161-L1/TAM105	AZ00	race 1	Greehouse	Seedling stage

Table A2. List of genetic mapping results of tan spot resistance QTL identified in 17 mapping populations for meta-QTL analysis.

QTL	Interval for the QTL on the integrated map	LOD	Variation explained (%)	Original name of the QTL	Chr	Projected
ACDC_2B	NA	3.0	7.0	*QTs.dms-2B*	2B	No
ACDC_2D	36.1~52.7	3.6	10.0	*QTs.dms-2D*	2D	Yes
ACDC_6B	46.9~54.2	3.2	6.9	*QTs.dms-6B*	6B	Yes
AL_86124_6B	149.8~158.3	6.9	22.0	*QTs.fcu-6B*	6B	Yes
AL_DW5_2B	52.2~60.7	12.0	26.0	*QTs.fcu-2B*	2B	Yes
AL_DW5_4B	NA	8.6	12.0	*QTs.fcu-4B*	4B	No
AL_L13-35_4B	NA	4.0	11.0	*QTs.fcu-4B*	4B	No
AL_L13-35_5B	42~44.9	4.2	12.0	*QTs.fcu-5B*	5B	Yes
BG_86124_1B	13.9~30.1	5.5	14.0	*QTs.fcu-1BS*	1B	Yes
BG_86124_3B	59~77	7.2	24.0	*QTs.fcu-3BL*	3B	Yes
BG_DW5_1B	13.9~30.1	5.9	13.0	*QTs.fcu-1BS*	1B	Yes
BG_DW5_3B	59~77	13.5	41.0	*QTs.fcu-3BL*	3B	Yes
BG_OH99_1B	13.9~30.1	8.6	29.0	*QTs.fcu-1BS*	1B	Yes
BG_OH99_3B.1	44~50	5.0	13.0	*QTs.fcu-3BS*	3B	Yes
BG_OH99_3B.2	59~77	4.6	12.0	*QTs.fcu-3BL*	3B	Yes
BG_Pti2_1B	13.9~30.1	8.1	27.0	*QTs.fcu-1BS*	1B	Yes
BG_Pti2_3B	59~77	6.8	17.0	*QTs.fcu-3BL*	3B	Yes
BMW_2A	69.8~76.8	4.7	6.6	*QTs.lfl-2A*	2A	Yes
BMW_3D	81~88	4.4	7.1	*QTs.lfl-3D*	3D	Yes
BMW_7A	112.9~116.9	3.5	10.6	*QTs.lfl-7A.1*	7A	Yes
BMW_1A	44.5~49.5	5.1	6.8	*QTs.lfl-1A*	1A	Yes
BP_331-9_5A	128.4~142	7.1	15.0	-	5A	Yes
BP_86-124_3A	NA	4.8	10.0	-	3A	No
BP_86-124_5A	128.4~142	6.1	13.0	-	5A	Yes
BP_AR CrossB10_5A	128.4~142	4.2	9.0	-	5A	Yes
BP_DW5_5A	128.4~142	4.4	10.0	-	5A	Yes

Table A2. List of genetic mapping results of tan spot resistance QTL identified in 17 mapping populations for meta-QTL analysis (continued).

QTL	Interval for the QTL on the integrated map	LOD	Variation explained (%)	Original name of the QTL	Chr	Projected
BP_DW5_5B	129~131	4.8	10.0	-	5B	Yes
BP_Pti2_5A	128.4~142	6.5	14.0	-	5A	Yes
CW_2A.a	69.1~72.6	10.3	33.5	-	2A	Yes
CW_2A.b	79.5~84.6	6.2	19.3	-	2A	Yes
CW_2A.c	83.9~86.9	8.6	27.4	-	2A	Yes
CW_2A.d	83.9~86.9	8.7	25.6	-	2A	Yes
CW_2A.e	83.9~86.9	5.2	14.8	-	2A	Yes
CW_6B	NA	3.8	10.0	-	6B	No
CW_7B	170.2~175.5	3.5	9.9	-	7B	Yes
CW_WAC11137_2A.a	83.9~86.9	4.5	16.2	-	2A	Yes
CW_WAC11137_2A.b	83~86	4.1	15.1	-	2A	Yes
CW_WAC11137_2A.c	84~88	11.4	27.4	-	2A	Yes
EB_1A.a	40~46	3.0	18.4	-	1A	Yes
EB_1A.b	40~46	2.7	15.8	-	1A	Yes
EB_2B.a	43~76.6	7.4	38.2	-	2B	Yes
EB_2B.b	43~76.6	6.1	29.8	-	2B	Yes
EB_2B.c	43~76.6	6.4	36.2	-	2B	Yes
EB_3B	11.2~47.3	5.4	25.3	-	3B	Yes
EB_3D	0~25.6	3.7	23.2	-	3D	Yes
EB_5B.a	155.9~180.7	2.9	11.9	-	5B	Yes
EB_5B.b	155.9~180.7	3.9	24.4	-	5B	Yes
EB_6A.a	91.5~114.8	2.7	13.2	-	6A	Yes
EB_6A.b	91.5~114.8	3.4	16.3	-	6A	Yes
EB_7A	18.4~40.1	3.6	16.9	-	7A	Yes
EB_7D	19~53.7	3.8	21.1	-	7D	Yes
IA_1A.a	23.5~25	8.9	25.4	-	1A	Yes
IA_1A.b	23.5~25	3.6	28.1	-	1A	Yes

Table A2. List of genetic mapping results of tan spot resistance QTL identified in 17 mapping populations for meta-QTL analysis (continued).

QTL	Interval for the QTL on the integrated map	LOD	Variation explained (%)	Original name of the QTL	Chr	Projected
IA_1A.c	29~34	4.7	16.6	-	1A	Yes
IA_2B	152.9~156.1	3.5	9.6	-	2B	Yes
IA_2D	124.7~155.1	7.3	19.7	-	2D	Yes
IA_4B	73.8~86.6	3.0	8.0	-	4B	Yes
IA_7B	170.9~173.1	7.2	18.6	-	7B	Yes
IA_WAC11137_1A	23.5~25	3.1	12.8	-	1A	Yes
IA_WAC11137_4A	1.1~14.8	4.1	12.9	-	4A	Yes
IA_WAC11137_5B	136.9~144.5	6.5	21.0	-	5B	Yes
LP_331-9_1A	1~24	23.1	22.0	*QTs.zhl-1A*	1A	Yes
LP_331-9_2D	155.5~157.3	4.7	3.0	*QTs.zhl-2D*	2D	Yes
LP_331-9_3B	51.4~79.6	34.3	41.0	*QTs.zhl-3B*	3B	Yes
LP_331-9_5A	138.5~144	5.2	6.0	*QTs.zhl-5A*	5A	Yes
LP_86-124_2D	155.5~157.3	6.4	7.0	*QTs.zhl-2D*	2D	Yes
LP_86-124_3B	51.4~79.6	44.0	53.0	*QTs.zhl-3B*	3B	Yes
LP_86-124_5A	136.4~142.1	13.9	13.0	*QTs.zhl-5A*	5A	Yes
LP_AR CrossB10_1A	1~24	14.4	3.5	*QTs.zhl-1A*	1A	Yes
LP_AR CrossB10_2D	155.5~157.3	5.5	5.0	*QTs.zhl-2D*	2D	Yes
LP_AR CrossB10_3B	51.4~79.6	13.6	22.0	*QTs.zhl-3B*	3B	Yes
LP_AR CrossB10_5A	136.4~142.1	7.9	14.0	*QTs.zhl-5A*	5A	Yes
LP_DW5_3B	51.4~79.6	36.3	22.0	*QTs.zhl-3B*	3B	Yes
LP_DW5_5A	136.4~142.1	18.2	8.0	*QTs.zhl-5A*	5A	Yes
LP_Pti2_1A	1~24	11.2	9.0	*QTs.zhl-1A*	1A	Yes
LP_Pti2_2D	155.5~157.3	8.6	9.0	*QTs.zhl-2D*	2D	Yes
LP_Pti2_3B	54.6~82.8	18.4	30.0	*QTs.zhl-3B*	3B	Yes
LP_Pti2_5A	135.6~143.5	8.4	13.0	*QTs.zhl-5A*	5A	Yes
LP573_331-9_1A	38.8~47.7	14.0	23.0	*QTs.zhl-1A*	1A	Yes
LP573_86-124_5B	118.5~125	23.0	36.0	*QTs.zhl-5B*	5B	Yes

Table A2. List of genetic mapping results of tan spot resistance QTL identified in 17 mapping populations for meta-QTL analysis (continued).

QTL	Interval for the QTL on the integrated map	LOD	Variation explained (%)	Original name of the QTL	Chr	Projected
LP573_Asc1_1A	38.8~47.7	15.0	25.0	*QTs.zhl-1A*	1A	Yes
LP573_Asc1_2A	78.9~84.3	4.7	9.0	*QTs.zhl-2A*	2A	Yes
LP573_Asc1_2B	164.8~190.7	3.8	7.0	*QTs.zhl-2B*	2B	Yes
LP573_Asc1_5B	118.5~125	8.9	16.0	*QTs.zhl-5B*	5B	Yes
LP573_DW5_2A	83~88.8	5.5	10.0	*QTs.zhl-2A*	2A	Yes
LP573_DW5_5B	166~172.5	4.8	9.0	*QTs.zhl-5B*	5B	Yes
LP573_Pti2_1A	38.8~47.7	16.0	27.0	*QTs.zhl-1A*	1A	Yes
LP573_Pti2_5B	118.5~125	14.0	23.0	*QTs.zhl-5B*	5B	Yes
LP749_86-124_3A	35~42	3.1	8.0	*QTs.fcu-3A*	3A	Yes
LP749_86-124_3B	52~75	2.4	5.0	*QTs.fcu-3B*	3B	Yes
LP749_86-124_5A.1	73.8~104.4	4.1	15.0	*QTs.fcu-5A.1*	5A	Yes
LP749_86-124_5A.2	171.2~193.2	4.1	13.0	*QTs.fcu-5A.2*	5A	Yes
LP749_86-124_7B	165.6~179.9	3.0	6.0	*QTs.fcu-7B*	7B	Yes
LP749_Pti2_3A	35~42	4.6	11.0	*QTs.fcu-3A*	3A	Yes
LP749_Pti2_3B	52~75	3.9	8.0	*QTs.fcu-3B*	3B	Yes
LP749_Pti2_5A.1	73.8~104.4	5.1	22.0	*QTs.fcu-5A.1*	5A	Yes
LP749_Pti2_5A.2	171.2~193.2	3.3	8.0	*QTs.fcu-5A.2*	5A	Yes
LP749_Pti2_7B	165.6~179.9	3.1	8.0	*QTs.fcu-7B*	7B	Yes
RIumillo_86-124_3A	27.1~44.2	4.2	9.8	-	3A	Yes
RP336_86-124_2B	44~76	4.3	6.9	-	2B	Yes
RP336_86-124_5A.1	71.1~97.1	4.9	7.9	-	5A	Yes
RP336_86-124_5A.2	169.2~178.4	8.2	13.8	-	5A	Yes
RP336_86-124ΔToxA_1B	58.4~87.4	4.2	5.2	-	1B	Yes
RP336_86-124ΔToxA_3A	16.8~38.8	6.5	8.3	-	3A	Yes

Table A2. List of genetic mapping results of tan spot resistance QTL identified in 17 mapping populations for meta-QTL analysis (continued).

QTL	Interval for the QTL on the integrated map	LOD	Variation explained (%)	Original name of the QTL	Chr	Projected
RP336_86-124ΔToxA_5A	169.9~173.1	23.1	36.4	-	5A	Yes
RP336_86-124ΔToxA_7A	2~13.9	3.6	4.4	-	7A	Yes
RP696_86-124_2B	94.5~110.7	4.0	7.1	-	2B	Yes
RP696_86-124_3A	22~37	7.3	13.6	-	3A	Yes
RP696_86-124_5A.1	59.6~92.3	3.9	7.0	-	5A	Yes
RP696_86-124_5A.2	164.4~179.4	5.2	9.6	-	5A	Yes
SK_86-124_5B	110.7~140	14.5	32.0	*QTs.fcu-5B*	5B	Yes
SK_86-124_7B	77.4~84.3	3.2	5.0	*QTs.fcu-7B*	7B	Yes
SK_AR LonB2_7B	77.4~84.3	3.9	6.0	*QTs.fcu-7B*	7B	Yes
SK_AR LonB2_7D	99.4~108.4	4.5	7.0	*QTs.fcu-7D*	7D	Yes
SK_Asc1_5D	121.7~149.2	4.7	13.0	*QTs.fcu-5D*	5D	Yes
SK_Pti2_5B	110.7~140	10.0	25.0	*QTs.fcu-5B*	5B	Yes
SK_Pti2_7B	77.4~84.3	4.7	8.0	*QTs.fcu-7B*	7B	Yes
TN_86-124_2A	71.9~99	8.6	22.0	*QTs.fcu-2AS*	2A	Yes
TN_86-124_5A	140~152	3.5	9.0	*QTs.fcu-5AL*	5A	Yes
TN_86-124_5B.1	71~93	8.4	22.0	*QTs.fcu-5BL.1*	5B	Yes
TN_86-124_5B.2	106.4~155.2	5.5	14.0	*QTs.fcu-5BL.2*	5B	Yes
TN_DW5_2A	71.9~99	5.0	19.0	*QTs.fcu-2AS*	2A	Yes
TN_DW5_5A	140~152	4.8	14.0	*QTs.fcu-5AL*	5A	Yes
TN_DW5_5B	71~93	4.9	14.0	*QTs.fcu-5BL.1*	5B	Yes
TN_OH99_2A	71.9~99	5.3	14.0	*QTs.fcu-2AS*	2A	Yes
TN_OH99_4A	143.5~160.5	3.6	10.0	*QTs.fcu-4AL*	4A	Yes
TN_OH99_5B	71~93	9.0	26.0	*QTs.fcu-5BL.1*	5B	Yes
TN_Pti2_2A	71.9~99	5.1	14.0	*QTs.fcu-2AS*	2A	Yes
TN_Pti2_5A	140~152	3.5	10.0	*QTs.fcu-5AL*	5A	Yes

Table A2. List of genetic mapping results of tan spot resistance QTL identified in 17 mapping populations for meta-QTL analysis (continued).

QTL	Interval for the QTL on the integrated map	LOD	Variation explained (%)	Original name of the QTL	Chr	Projected
TN_Pti2_5B.1	71~93	7.4	22.0	*QTs.fcu-5BL.1*	5B	Yes
TN_Pti2_5B.2	106.4~155.2	5.8	17.0	*QTs.fcu-5BL.2*	5B	Yes
TT_AZ00_1A	23.2~38.2	11.2	22.0	-	1A	Yes
TT_AZ00_6A	NA	10.7	20.0	-	6A	No
TT_AZ00_7A.1	100~115	7.4	12.0	-	7A	Yes
TT_AZ00_7A.2	167.4~184.6	6.4	14.0	-	7A	Yes

Table A3. Populations used for consensus map construction and meta-QTL analysis.

ID	Mapping population	Cross	Polyploidy level	Populaiton type	Markers mapped
1	ACDC	Attlia/CDC Go	Hexaploid	167 RILs	5,667 90K-SNPs
2	BG	BR34/Grandin	Hexaploid	118 RILs	743 TRAP/SSR
3	BMW	MAGIC population*	Hexaploid	394 RILs	5,435 SNP
4	CW	Calingiri/Wyalkatchem	Hexaploid	247 DHs	413 DArT/SSR/SNP
5	EB	Ernie/Betavia	Hexaploid	153 DHs	899 DArT/SSR
6	IA	IGW2547/Annuello	Hexaploid	97 DHs	426 DArT/SSR/SNPs
7	LP	Louise/Penawawa	Hexaploid	188 RILs	596 SNPs and SSRs
8	LP573	LMPG-6/PI 626573	Hexaploid	240 RILs	848 9K SNPs+19 SSRs
9	SK	Salamouni/Katepwa	Hexaploid	121 RILs	323 SSRs
10	TN	TA4152-60/ND495	Hexaploid	120 DHs	449 SSR/TRAP/others
11	TT	TA161-L1/TAM105	Hexaploid	140 $F_{2:3}$	550 DArTs
12	AL	Altar/Langdon	Tetraploid	127 RILs	100 SSRs+ 2 CAPs+ 741 SNPs
13	BP	Ben/PI 41025	Tetraploid	200 RILs	96 SSRs + 878 9K + 3,909 GBS-SNPs
14	LP749	Lebsock/PI 94749	Tetraploid	146 DHs	281 SSRs
15	RIum	Rusty/Iumillo	Tetraploid	190 RILs	2,911 GBS-SNPs
16	RP336	Rusty/PI 387336	Tetraploid	190 RILs	2,894 GBS-SNPs
17	RP696	Rusty/PI 387696	Tetraploid	190 RILs	2,059 GBS-SNPs
18	RP979	Rusty/PI 466979	Tetraploid	190 RILs	3,692 GBS-SNPs
19	DP527	Divide/PI 272527	Tetraploid	219 RILs	5,145 GBS + 3,005 90K-SNPs
20	Joppa10Ae	Joppa/10Ae564	Tetraploid	205 RILs	3,089 9K + 3,013 GBS-SNPs
21	RP883	Rusty/PI 193883	Tetraploid	190 RILs	29 SSRs + 4,229 GBS + 3,041 90K-SNPs
22	Consensus2004	-	Hexaploid	436 RIL/DHs	1,791 SSRs
23	Consensus2012	-	Tetraploid	765 RILs	1,898 DArT/SSR/Other
24	Consensus2014	-	Tetraploid	1031 RILs	598 SSR/DArT/AFLP/SNP/STS

Table A3. Populations used for consensus map construction and meta-QTL analysis (continued).

ID	*Ptr* race tested	Segregated for Tsn1	Reference
1	Canadian local isolates	Unknown	(Zou et al. 2017)
2	Pti2 (race 1), 86-124 (race 2), OH99 (race 3), and DW5 (race 5)	Yes	(Faris and Friesen 2005)
3	German local isolates	Unknown	(Stadlmeier et al. 2019)
4	Australia local isolates	No	(Shankar et al. 2017)
5	Australia local isolates	Unknown	(Li et al. 2011)
6	Australia local isolates	No	(Shankar et al. 2017)
7	Pti2 (race 1), 86-124 (race 2), 331-9 (race 3), DW5 (race 5), and AR CrossB10	No	(Kariyawasam et al. 2016)
8	Asc1 (race 1), Pti2 (race 1), 86-124 (race 2), 331-9 (race 3), and DW5 (race 5)	Yes	(Liu et al. 2017)
9	Pti-2 (race 1), Asc1 (race 1), 86-124 (race 2) and AR LonB2	Yes	(Faris et al. 2012)
10	Pti2 (race 1), 86-124 (race 2), OH99 (race 3), and DW5 (race 5)	Yes	(Chu et al.2008)
11	AZ-00 (race 1)	Unknown	(Kalia et al. 2018)
12	L13-35 (race 1), 86-124 (race 2), and DW5 (race 5)	Yes	(Virdi et al. 2016)
13	Pti2 (race 1), 86-124 (race 2), 331-9 (race 3), and DW5 (race 5), and AR CrossB10	yes	(Galagedara 2018)
14	Pti2 (race 1) and 86-124 (race 2)	Yes	(Chu et al. 2010)
15	86-124 (race 2)	Yes	(Liu et al. 2019)
16	86-124 (race 2) and 86-124ΔToxA (race 2)	No	(Liu et al. 2019)
17	86-124 (race 2)	No	(Liu et al. 2019)
18	86-124 (race 2)	Yes	(Liu et al. 2019)
19	-	-	(Faris and Xu, unpublished)
20	-	-	(Zhao et al. 2018)
21	-	-	(Sharma et al. 2019)
22	-	-	(Somers et al. 2004)
23	-	-	(Marone et al. 2012)
24	-	-	(Maccaferri et al. 2014)

* Eight founders are Event, BAYP4535, Ambition, Firl3565, Format, Potenzial, Bussard, Julius

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