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REVIEW

Genetics of tan spot resistance in wheat

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Abstract Tan spot is a devastating foliar disease of wheat caused by the necrotrophic fungal pathogen *Pyrenophora tritici-repentis*. Much has been learned during the past two decades about the genetics of wheat–*P. tritici-repentis* interactions. Research has shown that the fungus produces at least three host-selective toxins (HSTs), known as Ptr ToxA, Ptr ToxB, and Ptr ToxC, that interact directly or indirectly with the products of the dominant host genes *Tsn1*, *Tsc2*, and *Tsc1*, respectively. The recent cloning and characterization of *Tsn1* provided strong evidence that the pathogen utilizes HSTs to subvert host resistance mechanisms to cause disease. However, in addition to host–HST interactions, broad-spectrum, race non-specific resistance QTLs and recessively inherited qualitative 'resistance' genes have been identified. Molecular markers suitable for marker-assisted selection against HST sensitivity genes and for race non-specific resistance QTLs have been developed and used to generate adapted germplasm with good levels of tan spot resistance. Future research is needed to identify novel HSTs and corresponding host sensitivity genes, determine if the recessively inherited resistance genes are HST insensitivities, extend the current race classification system to account for new HSTs, and determine the molecular basis of race non-specific resistance QTLs and their relationships with host–HST interactions at the molecular level. Necrotrophic pathogens such as *P. tritici-repentis* are likely to become increasingly significant under a changing global climate making it imperative to further characterize the wheat–*P. tritici-repentis* pathosystem and develop tan spot resistant wheat varieties.

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Introduction

Tan spot, also known as yellow spot, is caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.) Shoem.) and affects all forms of cultivated wheat including both durum (*Triticum turgidum* L., $2n = 4x = 28$, AABB genomes) and common wheat (*T. aestivum* L., $2n = 6x = 42$, AABBDD genomes). Tan spot occurs worldwide and is a significant disease nearly everywhere wheat is cultivated. The fungus overwinters in stubble residue, thus the practice of retaining residue and no-till farming to reduce soil erosion has led to an increase in tan spot incidence. The fungus causes large, tan-colored lesions often surrounded by chlorotic haloes in susceptible genotypes, and in highly susceptible genotypes, the lesions tend to coalesce leading to large areas of dead leaf tissue (Fig. 1). Lesion development results in a decreased capacity for photosynthesis, which translates into plant stress and ultimately yield loss.

Studies on yield losses attributed to tan spot have indicated that the highest yield reductions are observed when tan spot occurs on older plants, such as the boot and flowering stages, as opposed to when tan spot only occurs on juvenile plants (Rees and Platz 1983; Shabeer and Bockus 1988; De Wolf et al. 1998 for review). Tan spot-induced yield reductions are primarily attributed to reduction in kernel weight and the number of grains per head (Shabeer and Bockus 1988), and also through reductions in the number of tillers, dry matter accumulation, leaf area index, and

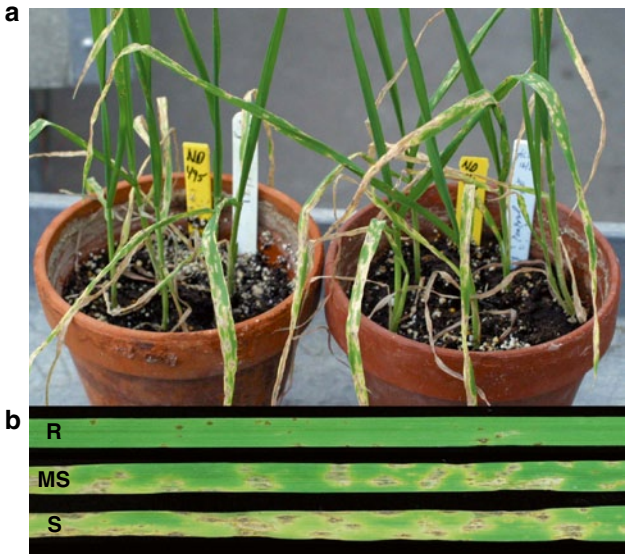

Fig. 1 Tan spot in wheat. **a** Wheat seedlings showing typical tan spot symptoms for a highly susceptible wheat line. **b** Individual wheat leaves showing typical tan spot reactions of resistant (R), moderately susceptible (MS), and susceptible (S)

grain size (Rees and Platz 1983). Rees et al. (1982) indicated that yield losses can reach up to 49 % in susceptible genotypes when conditions favor tan spot development. Tan spot can also affect the quality of the grain by causing pink smudge (Schilder and Bergstrom 1994).

The tan spot fungus was first described in 1823 (Hosford 1982) and was identified in Europe, the USA, and Japan in the early 1900s (Diedicke 1902; Dreschler 1923; Nisikado 1928). At that time, the fungus was considered a saprophyte occasionally causing minor to severe spotting in wheat crops around the world (Hosford 1982). After 1940, reports of more severe tan spot outbreaks came about, and tan spot epidemics began to occur in the 1970s in Canada, the USA, Australia, and the southern cone of Africa (Hosford 1971; Tekauz 1976; Rees and Platz 1992). The timing of these epidemics largely coincided with the adoption of reduced or no-till practices to retain stubble residue for reduction of soil erosion. Strong scientific evidence also indicates that *P. tritici-repentis* acquired a gene known as *ToxA* through horizontal gene transfer from *Stagonospora nodorum* (Friesen et al. 2006), which allowed *P. tritici-repentis* to produce the host-selective toxin (HST) known as Ptr ToxA. This acquisition rendered Ptr ToxA-producing strains virulent on all wheat genotypes harboring the *Tsn1* gene (Faris et al. 1996) (more on this below). Therefore, cultural practices along with the acquisition of the *ToxA* gene were likely the primary causes of tan spot becoming an economically significant disease of wheat.

Tan spot outbreaks can be controlled by using cultural practices such as appropriate crop rotations or tillage treatments (Sutton and Vyn 1990; Bockus and Claasen 1992). The disease can also be managed through the use of fungicides or even biological control methods (De Wolf et al. 1998 for review). However, these methods are not always practical or cost effective. The use of genetically resistant cultivars is the most inexpensive and environmentally sound option for controlling losses due to the tan spot disease.

Host interactions with biotrophic pathogens, such as the wheat–rust systems, tend to have gene-for-gene relationships (Flor 1956) where the recognition of a pathogen-produced effector (avirulence gene product) by a dominant host resistance (R) gene results in an incompatible (resistant) interaction (Fig. 2). In these systems, the lack of pathogen recognition results in a compatible interaction, which leads to susceptibility. It has recently become known that host interactions with necrotrophic pathogens, such as the wheat–*P. tritici-repentis* system, involve the recognition of HSTs, also known as necrotrophic effectors, by dominant host sensitivity genes. Recognition of an HST by a host sensitivity gene results in a compatible interaction, which leads to susceptibility, whereas the lack of HST recognition by the host results in an incompatible interaction and leads to resistance. If the host does not possess the sensitivity gene or if the pathogen does not produce the HST, the result is a resistance response. Therefore, the host–HST

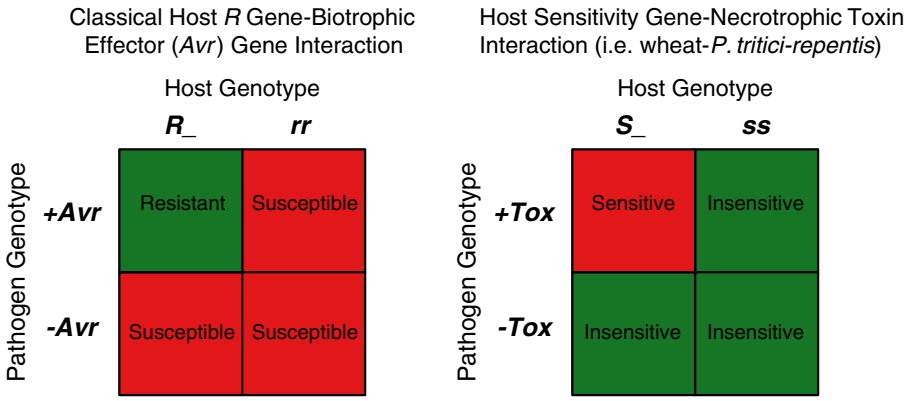

Fig. 2 Comparison of the genetic interactions that occur in classical gene-for-gene relationships involving a host and biotrophic pathogen (*left*), and the inverse gene-for-gene interactions that involve specific host sensitivity genes and corresponding host-selective toxins as observed in the wheat–*Pyrenophora tritici-repentis* system (*right*)

interactions present in host–necrotrophic pathogen pathosystems such as the wheat–*P. tritici-repentis* system are the inverse of the classical host–biotrophic pathogen systems (Wolpert et al. 2002). However, as reviewed below, we now know that the wheat–*P. tritici-repentis* system is more complex than merely an inverse gene-for-gene model based on evidence provided by the identification of broad-spectrum—or race non-specific—resistance quantitative trait loci (QTLs) in some materials (Faris and Friesen 2005; Chu et al. 2008b, 2010; Faris et al. 2012).

In the past few decades, tan spot and other necrotrophic diseases of wheat have become economically significant by causing substantial yield losses and reducing grain quality. Fortunately, significant advances in our understanding of the genetics of tan spot resistance and the development of resistant germplasm have been made. We provide a comprehensive review of our current knowledge and understanding of the genetics of wheat–tan spot interactions, and the tools including resistant sources, genes, QTLs, and molecular markers available to aid in the development of tan spot resistant cultivars.

Pathogen virulence

Prior to the early 1990s, most studies on *P. tritici-repentis* virulence focused on evaluating the quantitative variation of disease caused by the pathogen, describing general parameters such as lesion size (Misra and Singh 1972; Cox and Hosford 1987) or the percent leaf area infected (Nagle et al. 1982; Schilder and Bergstrom 1990). Then, in what could be considered as landmark work, Lamari and Bernier (1989a, 1991) showed that the symptoms of necrosis and chlorosis induced in the host by *P. tritici-repentis* infection were genetically distinct, and they developed a lesion-type rating scale that has since been widely adapted. Furthermore, they showed that chlorosis and necrosis could each be expressed independently depending on the *P. tritici-repentis* isolate and the host genotype (Lamari and Bernier 1989a, b). This knowledge led to the classification of *P. tritici-repentis* isolates into pathotypes, based on their ability to induce chlorosis and/or necrosis on the leaves of specific wheat genotypes (Lamari and Bernier 1989a). In this initial classification, pathotype 1 had the ability to produce necrosis and chlorosis (nec+chl+). Pathotype 2 could produce only necrosis (nec+chl−) and pathotype 3 could only produce chlorosis (nec−chl+). Pathotype 4 was considered avirulent and could not produce either symptom. The first wheat differential hosts used to characterize *P. tritici-repentis* isolates into one of the four pathotypes were the hexaploid wheat genotypes Glenlea and 6B365. Pathotype 1 caused necrosis on Glenlea and chlorosis on 6B365, pathotype 2 caused necrosis only on Glenlea, and pathotype 3 caused chlorosis only on 6B365 (Lamari and Bernier 1989a). Subsequent work by Lamari and Bernier (1991) led to the identification of hexaploid wheat genotypes (Norstar and 6B699) that developed both necrosis and chlorosis symptoms when inoculated with pathotype 1, necrosis with pathotype 2, and chlorosis with pathotype 3. Together, these studies demonstrated that both the pathogen and the host harbored independent genetic loci for governing expression of the necrosis and chlorosis symptoms of tan spot.

This method of characterizing *P. tritici-repentis* isolates was adequate until isolates from Algeria were identified that could produce chlorosis on wheat genotypes that pathotype 3 isolates could not, such as the cultivar Katepwa (Lamari et al. 1995). Also, the Algerian isolates were avirulent on 6B365, for which pathotype 3 isolates were virulent. These results indicated that the pathotype classification system was not sufficient to accommodate the virulence patterns of *P. tritici-repentis* isolates, and therefore a race-based classification system was proposed where isolates were classified according to their virulence pattern on a set of host differentials (Lamari et al. 1995). Pathotypes 1 through 4 were then considered as races one through four using the same differential lines as were previously used to characterize pathotypes (Glenlea and 6B365). The Algerian isolates were considered as race 5 and the line 6B662 was adopted as the differential for this race. The hexaploid wheat landrace Salamouni is used as the universal resistant differential.

Isolates have since been identified that combine the virulences of the initial races described. Just as race 1 isolates combine the virulences of races 2 and 3, race 6 isolates combine the virulences of races 3 and 5, race 7 isolates combine the virulences of races 2 and 5, and race 8 isolates combine virulences of races 2, 3, and 5 (Table 1) (see Strelkov and Lamari 2003; Lamari and Strelkov 2010 for reviews). The virulence factors responsible for host range and race classification are HSTs, also known as necrotrophic effectors, and are described in more detail below. However, Ali et al. (2010) reported the identification of *P. tritici-repentis* isolates from Arkansas that caused necrosis on the race 2-differential Glenlea, but did not produce the HST known as Ptr ToxA, which, by definition, is the virulence factor used to classify race 2 isolates. Therefore, the Arkansas isolates represent a new race that does not conform to the current race classification system, indicating that a modification of the system is needed. It is also possible that additional races exist that have not yet been characterized.

Host-selective toxins produced by the tan spot fungus

To date, three HSTs produced by *P. tritici-repentis* have been described and have been designated Ptr ToxA, Ptr ToxB, and Ptr ToxC according to standardized nomenclature agreed upon by the tan spot community (Ciuffetti et al. 1998). As described above, isolates are classified into races based on their virulences on host differentials, which are determined by the HSTs that are produced. Races 2, 3, and 5 each produce a single known HST, specifically Ptr ToxA, Ptr ToxC, and Ptr ToxB, respectively (Table 1) (see Strelkov and Lamari 2003; Lamari and Strelkov 2010 for reviews). Races 1, 6, and 7 each produce two HSTs with race 1 producing Ptr ToxA and Ptr ToxC, race 6 producing Ptr ToxB and Ptr ToxC, and race 7 producing Ptr ToxA and Ptr ToxB. Race 8 isolates produce all three HSTs.

Of the three HSTs, Ptr ToxA and Ptr ToxB, which cause necrosis and chlorosis, respectively (Fig. 3), have been isolated and substantially characterized. A recent review by Ciuffetti et al. (2010) (and references therein) describes in detail our current understanding of the molecular biology and biochemistry associated with Ptr ToxA and Ptr ToxB, and the responses they evoke in the host. Therefore, descriptions of the nature of these HSTs and their functions for purposes of this review will remain relatively brief.

Ptr ToxA, which induces necrosis in sensitive wheat genotypes (Fig. 3), was the first HST to be identified, isolated, and characterized (see De Wolf et al. 1998; Ciuffetti and Tuori 1999; Ciuffetti et al. 2010 for reviews). It was shown to be a small secreted protein (~13.2 kDa) that is imported into the cells of sensitive hosts and probably located to the chloroplasts where it may be involved in

Table 1 The eight current races of *Pyrenophora tritici-repentis* and the host-selective toxins that they produce

Race	HSTs produced	Symptoms on host differentials			
		Salamouni	Glenlea	6B662	6B365
1	Ptr ToxA PtrToxC	–	Necrosis	–	Chlorosis
2	Ptr ToxA	–	Necrosis	–	–
3	Ptr ToxC	–	–	–	Chlorosis
4	None	–	–	–	–
5	Ptr ToxB	–	–	Chlorosis	–
6	Ptr ToxB Ptr ToxC	–	–	Chlorosis	Chlorosis
7	Ptr ToxA Ptr ToxB	–	Necrosis	Chlorosis	–
8	Ptr ToxA Ptr ToxB Ptr ToxC	–	Necrosis	Chlorosis	Chlorosis

Fig. 3 Reaction of differential wheat lines to Ptr ToxA and Ptr ToxB. *Top panel* shows leaves of Salamouni (insensitive), 6B662 (insensitive) and Glenlea (sensitive) infiltrated with Ptr ToxA. *Bottom panel* shows leaves of Salamouni (insensitive), 6B662 (sensitive) and Glenlea (insensitive) infiltrated with Ptr ToxB

the disruption of photosynthesis (Ciuffetti et al. 2010 for review). Friesen et al. (2006) showed that *P. tritici-repentis* likely acquired the *ToxA* gene from the necrotrophic wheat pathogen *S. nodorum* through a horizontal gene transfer event. Thus, ToxA is a major virulence component of two important wheat pathogens and the corresponding host sensitivity gene, *Tsn1* (see below), is a major susceptibility gene for two diseases (Faris et al. 2010).

Ptr ToxB is also a small secreted protein (~6.5 kDa), but induces chlorosis in sensitive wheat genotypes (Fig. 3) (Ciuffetti et al. 2010 for review). Compared to Ptr ToxA, less is known about the mode of action of Ptr ToxB. However, unlike the *ToxA* gene, which is single copy, the *ToxB* gene is a multicopy gene much more variable in sequence among isolates. Another difference is that, whereas the *ToxA* gene has only been found in isolates that express functional Ptr ToxA, homologs of the *ToxB* gene have been found not only in races known to produce Ptr ToxB, i.e., races 5, 6, 7, and 8, but also in races 3 and 4, which do not produce Ptr ToxB in quantities sufficient to cause chlorosis. Furthermore, *ToxB* homologs have also been identified in other *Pyrenophora* species such as *P. bromi* (Died.) Drechsler (Andrie et al. 2008). Both Ptr ToxA and Ptr ToxB have been shown to activate host responses that are typically observed in resistance responses to biotrophic pathogens (e.g., upregulation of WRKY transcription factors, pathogenesis-related proteins and receptor-like kinases; activation of phenylpropanoid and jasmonic acid pathways; accumulation of reactive oxygen species; photosystem disruption) (Adikari et al. 2009; Pandelova et al. 2009, 2012), thereby providing additional evidence that necrotrophic pathogens such as *P. tritici-repentis* subvert host resistance mechanisms to cause disease.

Unlike Ptr ToxA and Ptr ToxB, Ptr ToxC is not a protein, but rather a non-ionic, polar, low molecular mass molecule (Effertz et al. 2002), but like Ptr ToxB, it induces chlorosis in sensitive wheat genotypes. Ptr ToxC has not been purified or fully characterized, and the gene(s) responsible for its production have not been cloned. However, studies have shown that it is an important virulence factor associated with the development of tan spot disease (Faris et al. 1997, 1999; Effertz et al. 2001, 2002).

It is most likely that *P. tritici-repentis* produces HSTs in addition to the three previously described. Meinhardt et al. (2003) and Ciuffetti et al. (2003) both reported a putative Ptr ToxD with the former eliciting chlorosis and the latter inducing necrosis on specific wheat genotypes, but neither Ptr ToxD has been described in the peer-reviewed literature. Gamba and Lamari (1998) indicated that some race 3 and race 5 isolates produced toxic components that caused necrosis (as opposed to chlorosis) in a specific durum wheat line indicating that they may produce HSTs in addition to Ptr ToxC and PtrToxB, respectively. Thus, it has been proposed to add the durum lines 4B-160 and Coulter to the differential set to account for the necrosis-inducing virulences of some isolates currently classified as race 3 and race 5 (Singh et al. 2008b; 2010b). In addition, recent studies evaluating gene action in specific populations have revealed several recessive tan spot resistance genes (Singh et al. 2006, 2008a; Tadesse et al. 2006a, b, 2008, 2010) (see below). Because HSTs are most often recognized by specific dominant sensitivity genes in the host to cause disease, the observance of recessive resistances is another indication that additional host–HST interactions are operating in this system, and future work will undoubtedly lead to the discovery of new HSTs produced by *P. tritici-repentis*.

Genes that confer HST sensitivity in the wheat–*Pyrenophora tritici-repentis* system

Tsn1

Tomas and Bockus (1987) were the first to show cultivar specificity of a necrosis-inducing HST present in culture filtrates of *P. tritici-repentis* (which was later designated as Ptr ToxA) suggesting that genetic variation for sensitivity to the HST existed among wheat genotypes. In addition, they showed a strong correlation between HST sensitivity and susceptibility to tan spot, suggesting that Ptr ToxA functioned as a pathogenicity or virulence factor (Tomas and Bockus 1987). Soon after, Lamari and Bernier (1989c) validated the findings of Tomas and Bockus (1987) by showing that the toxin was host specific and strongly associated with disease development. They and others further evaluated the inheritance of HST sensitivity in F_2 populations and showed that sensitivity to Ptr ToxA and susceptibility to necrosis-inducing isolates were controlled by the same dominant gene, i.e., insensitivity to the HST and resistance to the fungus were conferred by a recessive gene (Lamari and Bernier 1989c; Faris et al. 1996). However, whereas these early experiments indicated a very strong association between sensitivity to Ptr ToxA and resistance to tan spot, some later studies demonstrated that sensitivity to Ptr ToxA did not always lead to high levels of susceptibility and indicated that Ptr ToxA does not define susceptibility, but influences disease severity depending on the genetic background of the host (Friesen et al. 2003; Cheong et al. 2004; Faris and Friesen 2005; Chu et al. 2008b, 2010; Singh et al. 2008c; Faris et al. 2012).

In 1996, two groups conducted studies to determine the chromosomal location of the wheat gene conferring sensitivity to Ptr ToxA, and both groups proposed the symbols *tsn1* and *Tsn1* to designate the recessive and dominant alleles, respectively (Faris et al. 1996; Stock et al. 1996). Stock et al. (1996) used the Chinese Spring-Kenya Farmer

disomic chromosome substitution lines and Chinese Spring monosomic stocks to infer the location of *Tsn1* on chromosome 5B. Faris et al. (1996) conducted molecular mapping experiments using restriction fragment length polymorphism (RFLP) analysis in F_3 families to show that *Tsn1* was located on the long arm of chromosome 5B.

Numerous sources of resistance to necrosis and insensitivity to Ptr ToxA were reported, and in some cases allelism tests have been conducted to determine if various resistance sources harbored the same or different resistance/insensitivity genes (Sykes and Bernier 1991; Gamba and Lamari 1998; Gamba et al. 1998; Anderson et al. 1999; Singh and Hughes 2005; Singh et al. 2008a). However, Anderson et al. (1999) used the Chinese Spring nullisomic–tetrasomic stocks (Sears 1954) and the Chinese Spring chromosome deletion lines (Endo and Gill 1996) to demonstrate that insensitivity to Ptr ToxA was not governed by a gene product per se, but instead was due to the lack of a gene for sensitivity. Given this finding, the allelism test results involving resistance sources were rendered largely insignificant. It also refuted a previous claim that Chinese Spring plants homozygous for null HST sensitivity alleles are sensitive to Ptr ToxA (Stock et al. 1996).

Faris et al. (2000) developed marker-saturated deletion-based physical maps and showed that *Tsn1* lied within a gene-dense region of the wheat genome that was also expected to have a recombination frequency about 11-fold higher than the genome-wide average. Thus, the locus was considered to be amenable to map-based cloning methods, and Haen et al. (2004) reported the first saturation and high-resolution maps of the region in tetraploid and hexaploid wheat populations. Lu et al. (2006) used a LDN BAC library (Cenci et al. 2003) to assemble BAC contigs of 205 and 228 kb flanking *Tsn1* and developed the PCR-based markers designated *Xfcp1* and *Xfcp2* (Fig. 4), which were suitable for marker-assisted selection (MAS).

In addition, chromosome walking led to the expansion of the BAC contigs (Lu and Faris 2006). Further detailed analysis of colinearity of the predicted genes on this contig with genes in the rice genome revealed that a small region around *Tsn1* was conserved with a region of rice chromosome 9 and two genes including a potassium transporter (PT) and a U2 small nuclear (sn) ribonucleoprotein (RNP) auxiliary factor (U2 snRNP) were introduced as possible candidates (Lu and Faris 2006). Faris et al. (2010) completed the assembly of a BAC contig spanning the *Tsn1* locus and delineated the *Tsn1* gene to six possible candidates including the previously identified PT and U2 snRNP genes (Fig. 5). Association mapping reduced the number of candidates to four, including the PT and U2 snRNP genes along with a putative gene encoding a hypothetical protein and a gene harboring serine/threonine protein kinase (S/TPK), nucleotide binding (NB), and leucine-rich repeat (LRR) domains. Subsequent comparative sequence analysis of these four genes in wild-type wheat genotypes and corresponding ethyl methanesulfonate (EMS)-induced mutants revealed and validated that the S/TPK-NB-LRR-like gene was *Tsn1*.

The largest class of plant genes that confer resistance to biotrophic pathogens, such as rusts, powdery mildews, etc., is the NB-LRR class of R genes (Eitas and Dangl 2010), but some are also of the S/TPK class. Prior to the cloning of *Tsn1*, two other HST sensitivity genes, *Pc* from *Sorghum bicolor* and *LOV1* from *Arabidopsis thaliana*, were cloned and found to possess NB and LRR domains, thus resembling classic plant R genes (Lorang et al. 2007; Nagy and Bennetzen 2008). Therefore, *Tsn1*, *Pc*, and *LOV1* together represent three examples of HST sensitivity genes that resemble classic R genes in structure, but function to confer susceptibility to necrotrophic pathogens providing strength to the notion that necrotrophs have gained the ability to subvert resistance mechanisms acquired by plants to combat biotrophic pathogens. However, it is interesting to note that *Tsn1* is unique among these three genes in that it also harbors the S/TPK domain (Fig. 5), which is essential for function along with the NB and LRR domains (Faris et al. 2010). It was further indicated that *Tsn1* likely originated through a gene-fusion event in the diploid B-genome progenitor (*Aegilops speltoides* or closely related species) of polyploid wheat (Faris et al. 2010).

In addition, functional analysis revealed that *Tsn1* expression was regulated by the circadian clock and light, and that the presence of ToxA led to down-regulation of *Tsn1* (Faris et al. 2010). However, yeast two-hybrid experiments indicated that the Tsn1 and ToxA proteins probably do not interact directly, suggesting that intermediate proteins may be involved in forming a protein complex that results in the recognition of ToxA by Tsn1.

From a more practical standpoint, the physical mapping, sequencing, and cloning of the *Tsn1* locus allowed the development of a functional marker designated *Xfcp623* (Faris et al. 2010) (Figs. 4, 5). However, this marker is typically dominant because ToxA-insensitive genotypes of durum and common wheat are homozygous for null alleles of *Tsn1* (Faris et al. 2010). Co-dominant PCR-based simple sequence repeat (SSR) markers, *Xfcp394* and *Xfcp620*, which delimit the *Tsn1* locus to a 351-kb segment (Figs. 4, 5), have also been developed (Zhang et al. 2009; Faris et al. 2010).

While this 351 kb segment accounts for about 0.09 cM of genetic distance in the LDN × LDN-DIC 5B population, which was the population we used for map-based cloning of *Tsn1*, we hypothesized that recombination frequencies were relatively suppressed due to the fact that the *T. dicoccoides* accession harbored a deletion of more than 300 kb in size that included the *Tsn1* gene

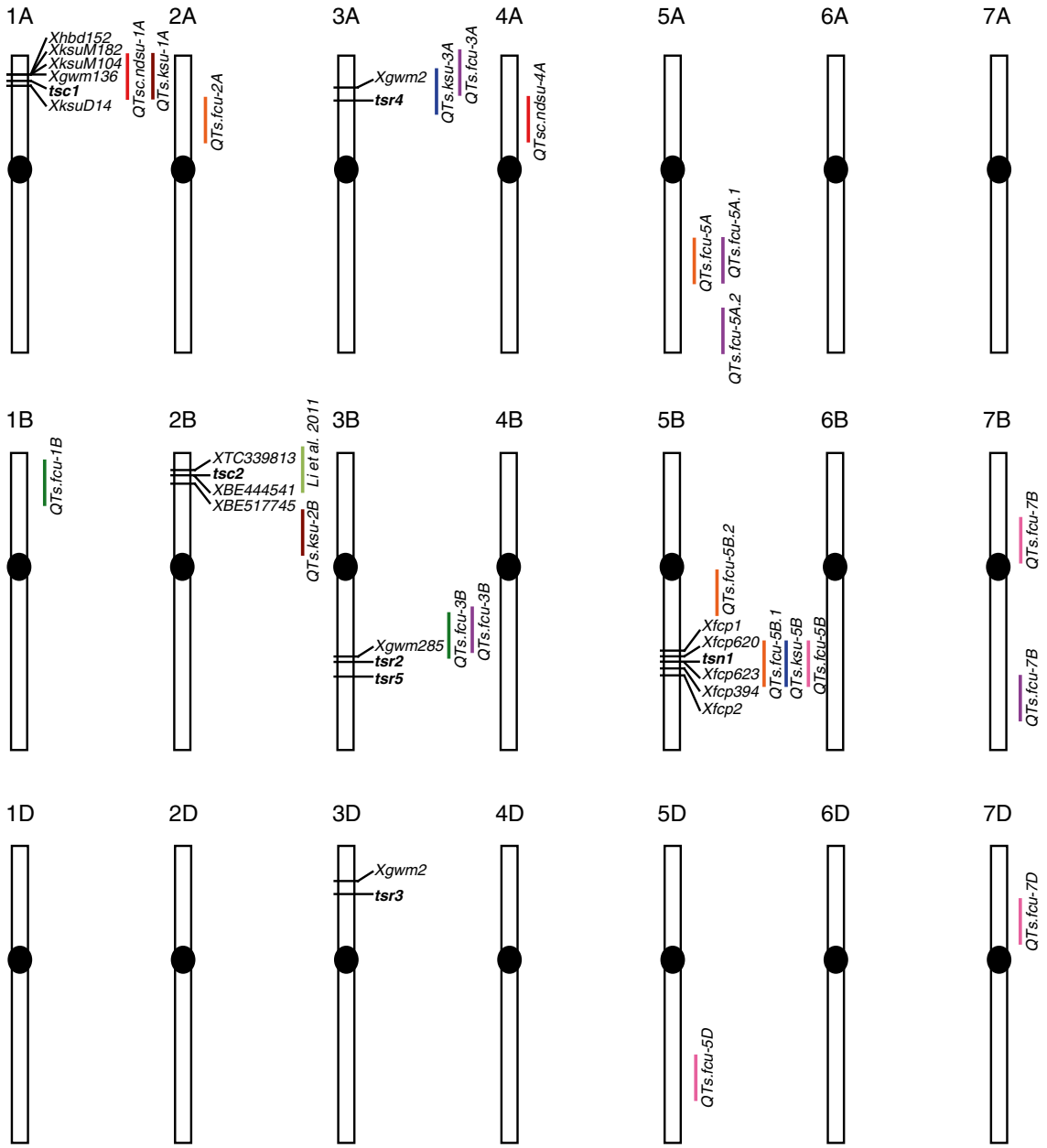

Fig. 4 Genomic positions of tan spot HST insensitivity genes (*tsn1*, *tsc1*, *tsc2*), tan spot resistance genes (*tsr2*, *tsr3*, *tsr4*, *tsr5*), and QTLs associated with tan spot resistance. Molecular markers tightly linked to HST insensitivity and resistance genes are shown as well. *Colored bars next to QTL designations* indicate the approximate locations of the QTLs, and the *different colors* indicate QTLs identified from different studies, i.e., *red* Faris et al. (1997), *green* Faris and Friesen (2005), *orange* Chu et al. (2008b), *blue* Singh et al. (2008c), *purple* Chu et al. (2010), *dark red* Sun et al. (2010), *light green* Li et al. (2011), and *pink* Faris et al. (2012) (color figure online)

(Fig. 5). Indeed, evaluation of recombination frequencies within the same region of a population derived from *T. dicoccoides* accession 36-12, which has *Tsn1*, and the LDN EMS-induced *Tsn1*-dirupted mutant LDNems230 revealed that recombination between *Xfcp394* and *Xfcp620* accounted for 2.3 cM in genetic distance, which is a 26-fold increase in recombination frequency. This result demonstrated two things. First, it showed that the markers flanking *Tsn1* would be very useful for selecting against it because a donor line (ToxA insensitive) would possess the null alleles thereby including the deletion, thus suppressing recombination. Second, it demonstrated the difficulties that even a small deleted segment can impose on a map-based cloning project, i.e., had the phenotype of the insensitive parent not been due to a deletion, chromosome walking would have been much more efficient due to presumably higher recombination frequencies at the *Tsn1* locus.

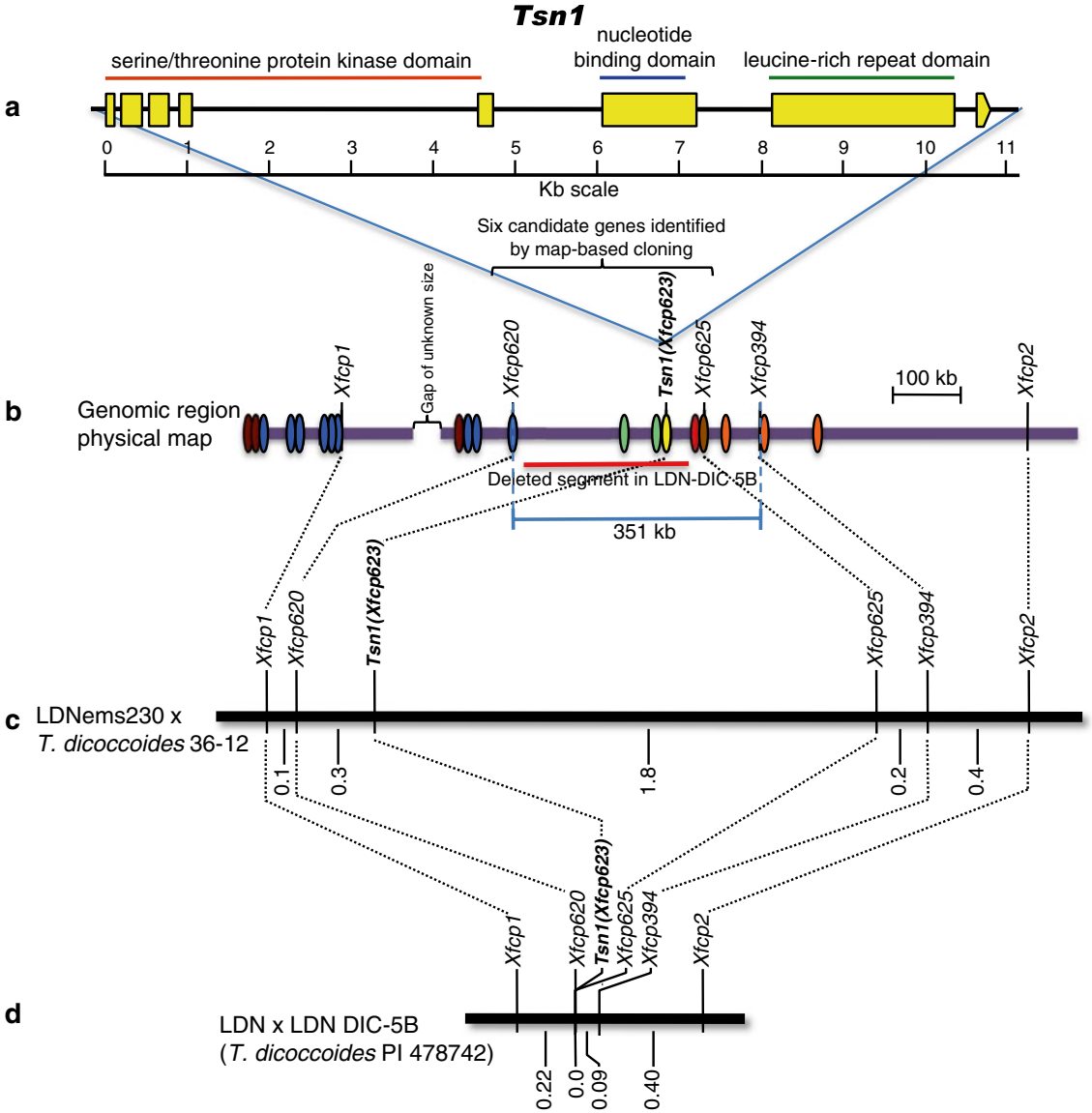

Fig. 5 Physical and genetic linkage maps of *Tsn1* and the *Tsn1* genomic region. **a** The structure of the *Tsn1* gene from Faris et al. (2010). *Yellow boxes* indicate exons. Locations of the serine/threonine protein kinase, nucleotide binding, and leucine-rich repeat domains are indicated by the *colored lines above the illustration*, and a *kilobase scale* is indicated below. **b** The physical map of the *Tsn1* genomic region assembled, sequenced, and annotated through map-based cloning (Lu et al. 2006; Lu and Faris 2006; Faris et al. 2010). The locations and names of markers are indicated in *black along the map*, and genes are indicated by *colored ovals* where *orange* zinc finger-like genes, *brown* potassium transporter, *red* U2 snRNP auxiliary factor, *yellow Tsn1*, *green* F box-like genes, *blue* protein kinase genes, and *dark red* subtilisin protease-like genes. The *horizontal bracket above the map* indicates the region that contained the six candidate genes identified based on recombination in the mapping population of LDN × LDN-DIC 5B. The *red line* represents the segment deleted from LDN-DIC 5B, and the *blue line* represents an approximately 351 kb segment delineated by markers *Xfcp394* and *Xfcp620* used to compare recombination rates between the two mapping populations across the segment involved in the deletion. **c**, **d** Genetic linkage maps of the *Tsn1* region developed in F_2 populations derived from LDNems230 (*Tsn1*-disrupted mutant) × the *Triticum dicoccoides* accession 36-12 (**c**) and from the wild type durum variety Langdon (LDN) crossed with a LDN-*T. dicoccoides* chromosome 5B disomic substitution line (LDN-DIC 5B) (**d**). *Markers* are indicated across the *tops* of the maps and map distances are shown across the *bottoms* (color figure online)

Tsc1

The chlorosis symptom of tan spot was recognized as a factor genetically independent of necrosis (Lamari et al. 1991), and resistance to chlorosis caused by races 1 and 3 (which both produce Ptr ToxC) was reported to be controlled by a dominant gene in some crosses, but was incompletely dominant in others (Lamari and Bernier 1991). However,

follow-up experiments by the same group indicated that resistance to race 3-induced chlorosis was conferred by a single recessive gene (Gamba and Lamari 1998; Gamba et al. 1998). More recently, inheritance studies conducted by Singh and Hughes (2006) suggested that resistance to race 1-induced chlorosis was dominant. These conflicting results are likely due to the fact that the expression of race 1- and 3-induced chlorosis is continuous and influenced by environmental conditions (Strelkov et al. 2002). Indeed, Duguid and Brule-Babel (2001) indicated that inheritance of resistance to chlorosis induced by race 3 isolates could range from recessive to partially dominant to completely dominant depending on the genetic makeup of the wheat lines under evaluation.

Faris et al. (1997) conducted QTL analysis of reaction to a *P. tritici-repentis* race 1 isolate in a population of recombinant inbred (RI) lines derived from the common wheat variety Opata 85 and the synthetic hexaploid wheat W-7984. This population was the subject of an extensive mapping effort by members of the International Triticeae Mapping Initiative (ITMI) and now contains thousands of molecular markers (http://wheat.pw.usda.gov/). Analysis of the ITMI mapping population revealed that it did not segregate for reaction to the necrosis component of the race 1 isolate, but a major QTL located on the short arm of chromosome 1A was found to be associated with chlorosis induction and designated *QTsc.ndsu-1A* (Fig. 4). Effertz et al. (2001) used the ITMI population to show that *QTsc.ndsu-1A* had major effects for resistance to chlorosis in adult plants as well as seedlings, and that it also conferred resistance to chlorosis induced by a race 3 isolate. Furthermore, *QTsc.ndsu-1A* and its effects on resistance to tan spot chlorosis induced by race 1 and race 3 were validated in a different RI population (Effertz et al. 2001).

Effertz et al. (2002) partially purified Ptr ToxC from a race 1 isolate and showed that insensitivity to the HST was governed by a single gene on the short arm of chromosome 1A that coincided with susceptibility to chlorosis produced by conidial inoculations using race 1 and race 3 isolates. Therefore, the Ptr ToxC insensitivity gene, designated *tsc1*, was likely responsible for the effects of *QTsc.ndsu-1A*. The *tsc1* gene mapped 5.7 cM distal to the *XGli1* locus, which was detected by an RFLP (Effertz et al. 2002). This study did not include evaluation of the mode of inheritance of *Tsc1*, and since this study was conducted, additional experiments on the *Tsc1*–Ptr ToxC interaction have not been reported. Therefore, whether or not resistance to chlorosis induction by race 1 and 3 isolates is dominant or recessive is yet a matter of controversy, but mode of inheritance studies for reaction to Ptr ToxC would shed much light on the matter.

The study by Effertz et al. (2002) located the chromosomal position of *Tsc1* relative to RFLP markers, which are not suitable for MAS. Because subsequent research on *Tsc1* has not been done, PCR-based markers suitable for MAS against *Tsc1* have not been reported. We genotyped the ITMI population with several PCR-based markers known to detect loci on the short arm of chromosome 1A and evaluated linkage distances between them and the *Tsc1* locus. The results of the linkage analysis suggest that SSR markers *Xhbd152*, *XksuM182*, *XksuM104*, and *Xgwm136*, which cosegregated with each other 4.7 cM distal to *Tsc1*, and the sequence-tagged site (STS) marker *XksuD14* on the proximal side of *Tsc1* (Fig. 6) might be useful for selecting against a functional *Tsc1* allele.

Tsc2

The discovery of *P. tritici-repentis* isolates that caused chlorosis on the wheat lines Katepwa and 6B662, which were previously shown to be resistant to chlorosis-inducing isolates, led to the adoption of the race classification system (Lamari et al. 1995) and the subsequent identification of Ptr ToxB, the HST responsible for this novel chlorosis induction (Orolaza et al. 1995; Strelkov et al. 1999). Orolaza et al. (1995) showed that sensitivity to partially purified Ptr ToxB cultures was controlled by a single dominant gene in the host. Friesen and Faris (2004) mapped the Ptr ToxB sensitivity gene on the short arm of chromosome 2B using the ITMI population and designated the gene *Tsc2* (Fig. 4). They showed that the *Tsc2*–Ptr ToxB interaction accounted for 69 % of the variation in disease caused by race 5, and identified additional QTLs with minor effects on chromosomes 2A and 4A, the latter of which was the same as the 4A QTL associated with resistance to race 1 reported by Faris et al. (1997, 1999). The parent of the ITMI population sensitive to Ptr ToxB was W7984, which is a synthetic hexaploid wheat derived from the tetraploid durum wheat variety Altar84 and an accession of *Ae. tauschii*. Therefore, Altar84 was the donor of Ptr ToxB sensitivity, indicating that sensitivity to Ptr ToxB in tetraploid wheat was governed by the *Tsc2* locus on the short arm of chromosome 2B.

Abeysekara et al. (2010) conducted saturation mapping of the *Tsc2* genomic region using a population of RI lines derived from the hexaploid wheat lines Salamouni (Ptr ToxB insensitive) and Katepwa (Ptr ToxB sensitive), and developed markers closely linked to *Tsc2* using wheat ESTs and colinearity with the rice and *Brachypodium* genomes. *Tsc2* was physically located to the most distal deletion bin on chromosome arm 2BS and delineated to a 3.3-cM segment by EST-based markers *XTC339813* and *XBE517745* (Fig. 4). In addition, the marker *XBE444541*, which was initially detected by an RFLP, cosegregated with *Tsc2* and was converted to a PCR-based marker. *XBE444541* was demonstrated to be diagnostic for the *Tsc2* Ptr ToxB

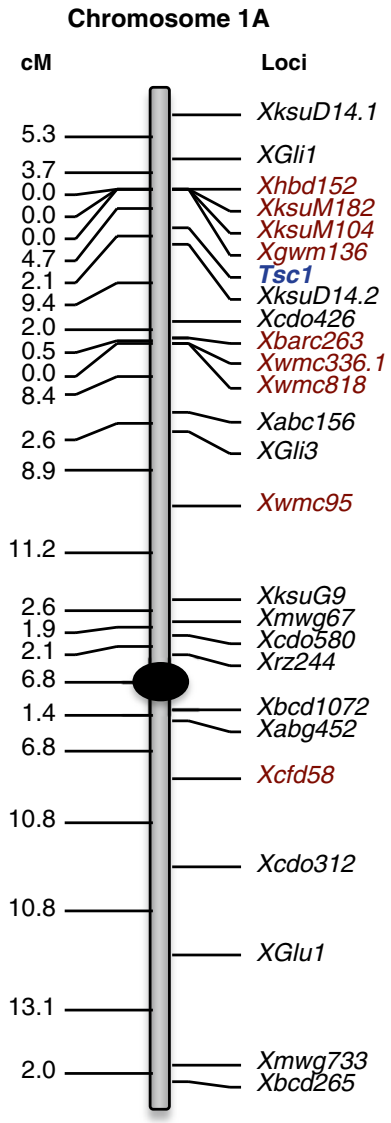

Fig. 6 Genetic linkage map of chromosome 1A in the International Triticeae Mapping Initiative (ITMI) mapping population constructed by Van Deynze et al. (1995) using restriction fragment length polymorphism (RFLP) markers (shown in *black*), and additional simple sequence repeat (SSR) markers (*dark red*) added to the linkage group by J. Faris and Z. Zhang (unpublished). The markers *XksuD14.1* and *XksuD14.2* were mapped as RFLP markers, but can now be assayed using sequence-tagged site (STS) primers as well. The *Tsc1* locus is shown in *blue* and *bold*. The data for Ptr ToxC reactions, which was used for the mapping of *Tsc1*, was kindly provided by Dr. James Anderson, University of Minnesota, and was the same data reported in Effertz et al. (2002). The *black oval* indicates the approximate position of the centromere, with the short arm of 1A toward the *top* and the long arm toward the *bottom* (color figure online)

sensitivity allele and recommended for MAS. Because this work was conducted in a true hexaploid population, as opposed to using a synthetic hexaploid wheat as with the ITMI population (Friesen and Faris 2004), it also showed that the gene governing sensitivity to Ptr ToxB (*Tsc2*) was likely the same for both tetraploid and hexaploid wheat.

Some previous reports regarding the inheritance of resistance to chlorosis caused by race 5 isolates were inconsistent. Singh et al. (2008a) reported that a single dominant gene conferred resistance, whereas Singh et al. (2010a) indicated that resistance was conferred by a single recessive gene. In a population of F_2 plants derived from Salamouni and Katepwa, Abeysekara et al. (2010) demonstrated that resistance was clearly recessive and that a single dominant gene conferred sensitivity to Ptr ToxB. Discrepancies in gene action observations were probably due to that fact that chlorosis symptoms induced by Ptr ToxB-producing isolates are quantitative just as with Ptr ToxC-producing isolates, and the apparent mode of inheritance may be influenced by environmental factors as well as the genetic backgrounds of the parents. For this reason, Abeysekara et al. (2010) argued that, if it is feasible, it is better to investigate the mode of inheritance using HST infiltrations as opposed to conidial inoculations.

Qualitative resistance genes

Nomenclature

The identification of additional genes conferring resistance to tan spot in a qualitative manner (see below) led to the need to revisit wheat gene nomenclature for naming genes associated with tan spot. Previously, qualitative genes were only associated with response to HST-containing fungal culture filtrates, and the '*Tsc*' and '*Tsn*' designations referred to '*tan spot chlorosis*' and '*tan spot necrosis*' HSTs, respectively. The subsequent identification of qualitative genes that conferred resistance to tan spot as observed through conidial inoculations (as opposed to infiltration of culture filtrates or partially purified cultures) led to the naming of several genes as *Tsn2–Tsn5* in the literature (Singh et al. 2006, 2008b; Tadesse et al. 2006a, b), even though these genes were not necessarily associated with necrosis caused by a HST. Therefore, in 2007, the tan spot research community discussed the situation and together decided that qualitative genes identified through only conidial inoculations would be given the designation '*Tsr*' for '*tan spot resistance*,' and genes associated with reaction to HST-containing cultures would continue to be given '*Tsc*' and '*Tsn*' designation depending on the symptom exhibited by the HST (McIntosh et al. 2008). Therefore, genes for response to HSTs are to be designated independently from those that confer resistance to the fungus, and synonyms are noted when it is shown that a HST insensitivity gene also confers resistance to the fungus. For example, the recessive allele of *Tsn1* also confers resistance

to tan spot caused by Ptr ToxA-producing isolates and therefore has the synonym *Tsr1*. *Tsn2–Tsn5* have been redesignated as *Tsr2–Tsr5*, and *Tsc2* is synonymous with *Tsr6*. *Tsc1* has not been given a synonymous *Tsr* designation because no one has evaluated tan spot caused by a Ptr ToxC-producing isolate as a Mendelian trait as of yet.

Tsr2

Some relatively recent genetic experiments have led to the identification of genes, in addition to the HST sensitivity genes, that monogenically control reaction to different *P. tritici-repentis* races and isolates. As mentioned above, Gamba and Lamari (1998) indicated that some race 3 and race 5 isolates, which caused chlorosis in hexaploid wheat due to the *Tsc1* and *Tsc2* genes, respectively, caused necrosis in some tetraploid wheat lines. Singh et al. (2006) evaluated a set of LDN-DIC disomic chromosome substitution lines and indicated that chromosome 3B of *T. dicoccoides* harbored a gene conferring resistance to necrosis induced by the race 3 isolate Ptr 331-9. They subsequently mapped a resistance locus to the long arm of chromosome 3B in a population of RI lines derived from the cross between a resistant *T. turgidum* ssp. *turgidum* accession (PI 352519) and the susceptible durum variety Coulter using molecular markers. It was presumed that the chromosome 3B susceptibility loci in LDN and Coulter were the same, although allelism tests were not conducted. Evaluation of F_2 plants derived from the *T. turgidum* ssp. *turgidum* × Coulter cross indicated that resistance behaved in a recessive manner, which might suggest that Coulter, LDN, and probably other tetraploids harbor a gene on chromosome arm 3BL that confers sensitivity to a yet unidentified necrosis-inducing HST produced by at least some race 3 isolates. The gene conferring susceptibility to necrosis induced by Ptr 331-9 and mapping to 3BL is designated *Tsr2* (Fig. 4).

Tsr3

Tadesse et al. (2006a) used monosomic analysis to identify single genes in three synthetic hexaploid wheat lines that conferred resistance to the *P. tritici-repentis* race 1 isolate ASC1b. Synthetic hexaploids are synthesized by crossing an AB-genome tetraploid such as *T. turgidum* ssp. *durum*, or close relative, with an accession of the diploid D-genome progenitor *Ae. tauschii* followed by embryo rescue and chromosome doubling to obtain a synthetic AABBDD hexaploid. Synthetics are often used in breeding and germplasm enhancement programs to increase genetic variation and as a resource for desirable genes. Tadesse et al. (2006a) showed that the synthetic hexaploids XX41 and XX45 were highly resistant to tan spot caused by ASC1b, and XX110 was moderately resistant. Because the tetraploid parental lines LDN (tetraploid parent for XX41 and XX45) and *T. turgidum* ssp. *dicoccum* A38 (tetraploid parent for XX110) were susceptible to ASC1b and the parental *Ae. tauschii* accessions were resistant, it was presumed that resistance was derived from the diploid *Ae. tauschii* accessions CI 00017 (XX41), RL 5565 (XX45), and CI 33 (XX110). Therefore, the synthetic hexaploid accessions were each crossed to the Chinese Spring D-genome monosomic lines. Chromosome counts were conducted in the F_1 generation and monosomic plants were selfed to produce the F_2 generation, which was then tested for reaction to tan spot caused by ASC1b. Significant deviation from the expected 1:3 ratios were observed in the F_2 populations derived from crosses between the synthetic hexaploids and Chinese Spring monosomic-3D, suggesting that each of the three synthetic hexaploid lines harbored a gene on chromosome 3D that conferred resistance to tan spot caused by ASC1b. It was concluded that XX41 and XX110 harbored single recessive resistance genes and XX45 harbored a single dominant resistance gene, and these genes were designated as *tsn3*, *tsn-syn1*, and *Tsn-syn2*, respectively, as allelism tests were not conducted to determine whether or not they were the same gene.

Some issues stemming from this work may be worth further experimentation. First, it should be noted that LDN, the AB-genome donor of the synthetic hexaploids XX41 and XX45, is sensitive to Ptr ToxA because it carries *Tsn1* (Faris and Friesen 2009; Faris et al. 2010), and ASC1b is a race 1 isolate, which means that it produces Ptr ToxA. However, Tadesse et al. (2006a) reported no indication of a chromosome 5B locus associated with resistance/susceptibility. Even though they reported LDN as being susceptible, they presumed the susceptibility was due to the lack of the 3D gene from *Ae. tauschii*. Follow-up work could be done to address the question as to why XX41 and XX45 were not susceptible to ASC1b given the fact that they should harbor *Tsn1*. It may be that the *Tsn1*–Ptr ToxA interaction is not relevant in these genetic backgrounds, such as was reported by Faris and Friesen (2005), or that their strain for ASC1b did not express the *ToxA* gene, or it was expressed at very low levels. Variability in *ToxA* expression has been reported in the wheat pathogen *S. nodorum* and was strongly correlated with levels of disease (Faris et al. 2011).

As mentioned above, the observance of recessive resistance in the wheat–tan spot system may be considered an indication (although not proof) of an interaction between a HST and dominant host gene for HST sensitivity. In the case of Tadesse et al. (2006a), it is possible that the recessive resistances identified in XX41 and XX110 could indicate the lack of a dominant HST sensitivity locus, which, based on their results, would presumably lie on chromosome 3D of Chinese Spring. However, this would not explain the fact that a few susceptible plants—presumably

nullisomic—were observed in the F_2 populations derived from the crosses between these synthetics and Chinese Spring. If resistance was indeed conferred by the lack of a gene for susceptibility, nullisomics would be resistant and it would therefore be impossible to obtain susceptible progeny in this situation. Also, the identification of a putative dominant resistance gene in XX45 does not lend itself to the host–HST model. Therefore, more research is needed to characterize the genetic nature of resistance/susceptibility in these synthetic hexaploids and Chinese Spring.

In subsequent research, Tadesse et al. (2007) conducted molecular mapping of the genes identified in the synthetic wheat lines XX41, XX45, and XX110 using $F_{2:3}$ populations derived from crosses between the synthetic lines and Chinese Spring. Molecular mapping experiments indicated that all three resistance genes, now designated as *tsr3a*, *Tsr3b*, and *tsr3c*, reside on the short arm of chromosome 3D near loci identified by the marker *Xgwm2* (Tadesse et al. 2007) (Fig. 4). This result along with results of allelism tests reported in this research indicated that the three genes are either alleles of one another or very closely linked. However, it is once again worthy to note that the resistance genes evaluated in the three synthetic wheat lines may not be resistance genes 'per se,' but instead resistance may be due to the lack of the susceptibility gene in Chinese Spring. If this is the case, the results obtained from allelism tests using crosses between resistant lines are not useful.

Tsr4

In another study, Tadesse et al. (2006b) used monosomic analysis again to investigate the genetics of resistance to the tan spot race 1 isolate ASC1a [different from ASC1b in Tadesse et al. (2006a)] in the hexaploid wheat landrace Salamouni. They showed that Salamouni was resistant to ASC1a, whereas Chinese Spring was susceptible, and segregation ratios in an F_2 population derived from Salamouni and euploid Chinese Spring indicated that a single recessive gene conferred resistance. Segregation analysis of F_2 populations derived from Salamouni and 20 of the 21 Chinese Spring monosomic lines revealed that the population derived from Salamouni × Chinese Spring monosomic 3A deviated significantly from the expected 1:3 ratio, thus indicating that the recessive resistance gene in Salamouni was on chromosome 3A. As mentioned above, the identification of recessive resistance might suggest the action of a novel host–HST interaction, but just as they reported in Tadesse et al. (2006a), here they also reported the existence of several susceptible F_2 plants derived from the critical cross and speculated that they were nullisomic for Salamouni 3A. In a host–HST model, such plants would not be susceptible. However, Tadesse et al. (2006b) did recognize the possibility that the recessive resistance in Salamouni could be due to the lack of a susceptibility gene harbored by Chinese Spring, and they tested this by inoculating Chinese Spring lines nullisomic for chromosome 3A. Indeed, the Chinese Spring nullisomic 3A plants were resistant to ASC1a, providing strong evidence that resistance in Salamouni was actually due to the lack of a gene for susceptibility, even though this idea was contradicted by the observance of susceptible plants in the Salamouni × Chinese Spring monosomic 3A population.

Very similar experiments were conducted by Tadesse et al. (2010) to investigate the gene action, inheritance, and chromosomal location of a tan spot resistance gene in the winter wheat cultivar Red Chief. Using the same isolate (ASC1a), they evaluated segregating populations derived from crosses between Red Chief and the 21 monosomic lines of Chinese Spring to show that a single recessive gene on chromosome 3A conferred resistance. Furthermore, they conducted molecular mapping experiments using SSR markers in an F_2 population derived from a cross between Red Chief and euploid Chinese Spring, and showed that the gene was located on the short arm of chromosome 3A approximately 15 cM proximal to the marker *Xgwm2* (Fig. 4). Because this gene was located on chromosome 3A and identified with the *P. tritici-repentis* isolate ASC1a, it was suggested that it was the same gene as identified in the landrace Salamouni by Tadesse et al. (2006b) designated *tsr4*. It was also noted that the marker *Xgwm2* detects loci (which are likely homoeologous) on both chromosomes 3A and 3D. Because *tsr3* mapped just proximal to the *Xgwm2* locus on chromosome 3D (Tadesse et al. 2007), it is conceivable that *tsr3* and *tsr4* are perhaps homoeologous genes. If *tsr3* and *tsr4* are not resistance genes per se, and instead functional susceptibility genes govern the interactions due to host–HST interactions as speculated above, this would mean that Chinese Spring would possess dominant susceptibility genes *Tsr3* and *Tsr4* on chromosome arms 3AS and 3DS, which are likely homoeologous and confer susceptibility to effectors (HSTs) produced by isolates ASC1b and ASC1a, respectively. More experiments are needed to test this hypothesis.

Tsr5

Singh et al. (2008b) conducted molecular mapping and investigated the genetics of necrosis in tetraploid wheat induced by the race 5 isolate DW13 using a population derived from a cross between *T. turgidum* ssp. *turgidum* accession (PI 352519) and the durum variety Coulter, which was the same population of RI lines used for mapping the *Tsr2* gene (Singh et al. 2006). A single recessive gene conferring resistance to necrosis induced by DW13 spore inoculations was identified and mapped to the long arm of durum chromosome 3B. The gene, designated *Tsr5*,

mapped approximately 8.3 cM distal to *Tsr2* (Fig. 4) suggesting that *Tsr2* and *Tsr5* are not the same gene and that the necrosis induced by the race 3 isolate Ptr 331-9 and the race 5 isolate DW13 is due to different virulence factors, which could possibly be HSTs. However, Singh et al. (2008b) also infiltrated the parents of the population, the tan spot differential lines, and the LDN-DIC substitution lines with culture filtrates derived from DW13. The Ptr ToxB differential line 6B662 exhibited chlorosis in response to the infiltrations as expected, but all other lines, including those that were susceptible to DW13-induced necrosis such as Coulter and LDN, were insensitive to the culture filtrate. This would suggest that either: (1) the DW13-induced necrosis is not due to a host–HST interaction or (2) the necrosis is due to a host–HST interaction but the HST was not produced and/or present in a functional form in their culture filtrates. The latter is most probable, and it is possible that manipulations of the culture growth conditions would yield the HST in vitro.

Qualitative resistance in Ethiopian cultivars

Tadesse et al. (2008) reported the identification of three Ethiopian common wheat cultivars with resistance to tan spot caused by isolates ASC1a and DW16. Inheritance studies indicated that all three cultivars contained a single recessive resistance gene. The results of allelism studies conducted by crossing the resistant cultivars with each other led the authors to believe that the resistance harbored by all three cultivars to both isolates was controlled by a common gene. Subsequent monosomic analysis indicated that the resistance gene was located on chromosome 3B, which might suggest that it was the same as *Tsr2* (Singh et al. 2006). However, this study contained some discrepancies and contradictions compared to earlier findings reported by the same group. First, the cultivar Glenlea was reported in this study to be highly susceptible to both ASC1a and DW-16, which are classified as race 1 and race 5 isolates (Tadesse et al. 2006b). Glenlea, by definition, is a differential line that is susceptible to Ptr ToxA-producing races but resistant to all others, including race 5 (Lamari et al. 2003). Therefore, the fact that Glenlea was reported as susceptible to the race 5 isolate DW16 would indicate that either the Glenlea seed source or the DW16 cultures were not correct. Second, when they reported on the characterization of resistance derived from Salamouni to the same isolate (ASC1a), Tadesse et al. (2006b) mentioned that the inoculation of Chinese Spring plants that were nullisomic for chromosome 3A (the chromosome reported to harbor the resistance gene *tsr4* in that study) were resistant, indicating that the lack of the Chinese Spring gene for susceptibility (the *Tsr4* allele) resulted in resistance. If this were the case, then Tadesse et al. (2008) should have observed segregation of resistant and susceptible plants in the cross between Chinese Spring monosome 3A and the Ethiopian lines in the F_1 generation because theoretically half of the plants would have contained the Chinese Spring 3A monosome and the other half would have been null for Chinese Spring 3A, and hence the *Tsr4* allele. Furthermore, F_1 plants monosomic for Red Chief chromosome 3A that were selfed to produce the F_2 generation would yield all resistant plants because the Chinese Spring 3A chromosome would be missing from all plants. However, no such phenotypes resulting from the Chinese Spring 3A monosome crosses were reported. Therefore, more experimentation is needed to resolve these discrepancies.

Quantitative resistance

Tan spot reactions are quantitative because they are affected by genotypic backgrounds, variations in environmental conditions, and experimental error, which is why the quantitative reaction-type rating scale developed by Lamari and Bernier (1989b) is now widely used. Therefore, genetic studies are best conducted using experiments with multiple replicates, appropriate statistical designs, and analysis of QTLs to more accurately account for loci involved in governing tan spot reactions and to estimate their relative effects.

Some studies conducted to estimate the heritability of resistance to tan spot were conducted prior to the discovery by Lamari and Bernier (1991) that the chlorosis and necrosis symptoms were genetically independent. For example, Nagle et al. (1982) studied the inheritance of resistance in multiple tetraploid and hexaploid wheat populations, and Elias et al. (1989) evaluated the heritability of resistance in a tetraploid population. Although both studies indicated good heritability of resistance, they also indicated that resistance was quantitatively controlled.

Faris et al. (1997) were the first to take a QTL mapping approach to identify tan spot resistance loci. They evaluated the ITMI population of RI lines with isolates Pti-2 (race 1), 86-124 (race 2), and D308 (race 3). As mentioned above, the ITMI population did not segregate for reaction to race 2, but Faris et al. (1997) reported a QTL on the short arm of chromosome 1A with major effects for resistance to chlorosis induced by races 1 and 3, and designated the QTL as *QTsc.ndsu-1A* (Fig. 4). In addition to this QTL, a relatively minor QTL was identified on the short arm of chromosome 4A. Subsequent work suggested that genes known to be involved in pathogen defense responses might be responsible for the effects of the 4AS QTL (Faris et al. 1999). Effertz et al. (2001) validated *QTsc.ndsu-1A* in a different population and later demonstrated that the effects of

the QTLs were due to a compatible *Tsc1*–Ptr ToxC interaction (Effertz et al. 2002).

Doubled haploid populations derived from crosses between the Australian varieties Cranbook/Halberd and Krichauff/Brookton were evaluated for resistance to tan spot caused by several local isolates (Cheong et al. 2004). In both populations, a locus on the long arm of chromosome 5B corresponding to the location of the *Tsr1* (*Tsn1*) gene was significantly associated with resistance. The locus explained 39 % of the variation in the Krichauff/Brookton population and up to 60 % of the variation in the Cranbook/Halberd population indicating the importance of a compatible *Tsn1*–Ptr ToxA interaction in these materials.

Faris and Friesen (2005) were the first to identify and characterize the effects of race non-specific tan spot resistance QTLs. They evaluated a hexaploid spring wheat population of RI lines derived from a cross between the Brazilian line BR34 (resistant) and the North Dakota hard red spring wheat (HRSW) variety Grandin (referred to as the BG population) for reaction to isolates Pti-2 (race 1), 86-124 (race 2), OH99 (race 3), and DW5 (race 5). They also screened the population with purified Ptr ToxA to map the *Tsn1* locus and evaluate the effects of a compatible *Tsn1*–Ptr ToxA interaction on the development of disease caused by the Ptr ToxA-producing isolates (Pti-2 and 86-124). Analysis revealed QTLs on the short arm of chromosome 1B and the long arm of 3B, designated *QTs.fcu-1B* and *QTs.fcu-3B*, respectively, that were significantly associated with resistance to all four races (Fig. 4). *QTs.fcu-1B* explained from 13 to 29 % of the variation on its own, and *QTs.fcu-3B* explained from 13 to 41 % of the variation. Additional QTLs that were not race non-specific were reported on other chromosome arms, but their effects were relatively minor. Also, Faris and Friesen (2005) reported no significant association between the *Tsn1* locus and resistance/susceptibility to the four races, including the Ptr ToxA-producing isolates, indicating that the *Tsn1*–Ptr ToxA interaction did not play a role in conferring tan spot susceptibility in the BG population. Faris and Friesen (2005) speculated that the broad-spectrum, race non-specific resistance genes might have precluded the gene-for-gene interactions such as *Tsn1*–Ptr ToxA. The *QTs.fcu-3B* and *QTs.fcu-1B* QTLs have been targeted for the development of tan spot resistant germplasm through MAS (JD Faris and SS Xu, unpublished) (see below).

Chu et al. (2008b) evaluated a doubled haploid population derived from the tan spot resistant synthetic hexaploid wheat line TA4152-60 and the tan spot susceptible HRSW line ND495 for reaction to isolates of races 1, 2, 3, and 5. They reported that the *Tsn1*–Ptr ToxA interaction was significantly associated with disease caused by the Ptr ToxA-producing isolates Pti2 (race 1) and 86-124 (race 2). However, this was the only host–HST interaction found to be associated with reaction to tan spot in this population. Race non-specific QTLs on chromosome arms 2AS and 5BL (proximal to the *Tsn1* locus) (Fig. 4) were associated with resistance to all isolates tested and explained from 14 to 26 % of the variation. A QTL on 5AL was associated with disease caused by races 1, 2, and 5, and a QTL on 4AL was associated with resistance to race 3 only. The identification of these race-specific and race non-specific QTLs increased the complexity of the wheat–tan spot system.

Singh et al. (2008c) used a race 1 isolate collected in Kansas to evaluate a population of RI lines derived from the Indian spring wheat cultivars WH542 (resistant) and HD29 (susceptible). Simple interval mapping (SIM) analysis indicated that genomic regions on chromosomes 1B, 3A, 3B, 5B, and 6B were significantly associated with resistance, but composite interval analysis confirmed only the QTLs on 3A and 5B (Fig. 4). The 5B QTL, which explained 18 % of the phenotypic variation, was detected by markers known to be tightly linked to the *Tsn1* locus indicating that the effects of this QTL were likely due to a compatible *Tsn1*–Ptr ToxA interaction. The 3A QTL was on the short arm and explained 23 % of the variation. It is quite possible that the effects of this QTL were due to the *Tsr4* locus, which was shown by Tadesse et al. (2010) to also map to 3AS.

Evaluation of a tetraploid wheat doubled haploid population derived from the durum wheat variety Lebsock and the *T. turgidum* ssp. *carthlicum* accession PI 94749 by Chu et al. (2010) revealed five QTLs significantly associated with resistance to isolate Pti2 (race 1) and/or isolate 86-124 (race 2). Two of the QTLs were located on chromosome 5A, and chromosomes 3A, 3B, and 7B had one QTL each (Fig. 4). It was speculated that the 3B QTL may have been the same as the race non-specific QTL identified by Faris and Friesen (2005) based on the positions of common markers. The 3A QTL may have been identical to that reported by Singh et al. (2008c), and could also be due to the effects of *Tsr4* (Tadesse et al. 2010). One of the QTLs on 5A appeared to be the same as a 5A QTL reported by Chu et al. (2008b), whereas the second 5A QTL appeared to be novel. No tan spot resistance QTL had previously been reported on chromosome 7B; therefore, this QTL was considered novel as well. It is important to note that the *Tsn1* gene segregated in this population as well, but no QTL was detected at the *Tsn1* locus indicating that, as observed by Faris and Friesen (2005), the *Tsn1*–Ptr ToxA interaction did not play a role in the development of disease in this population.

Sun et al. (2010) used a QTL approach to evaluate resistance to a race 1 isolate (AZ-00) in a population of RI lines derived from a cross between the Chinese landrace Wangshuibai (resistant) and the Chinese breeding line Ning7840 (susceptible). One major QTL on the short arm

of chromosome 1A was identified that explained 39 % of the phenotypic variation (Fig. 4). The position of the QTL coincided with the known position of *Tsc1*, and therefore, the effects of the QTL were attributed to the *Tsc1*–Ptr ToxC interaction. Although isolate AZ-00 produced Ptr ToxA, both parents possessed *Tsn1* and therefore the population did not segregate for reaction to Ptr ToxA. A second QTL was identified on the short arm of chromosome 2B as well, but it explained only 4 % of the variation.

The American soft red winter wheat variety Ernie is immune to tan spot in Australia and was evaluated for QTLs using a doubled haploid population derived from crossing Ernie with the susceptible Australian variety Batavia (Li et al. 2011). Using a method similar to that described by Adee and Pfender (1989), the population was assayed by artificially inoculating two highly susceptible durum wheat varieties with field-infected plants and laying pieces of infected straws from the durum plants on each pot containing seedlings of the doubled haploid population. The authors reported a major QTL on the short arm of chromosome 2B (Fig. 4) with resistance effects derived from Ernie that explained as much as 38 % of the variation in disease, and it was significant across three experiments. They also reported several minor QTLs on chromosomes 1A, 5B, and 6A that were significant in two of the three experiments, and QTLs on 3B, 3D, 7A, and 7D that were significant in only one of the three experiments. Further validation of the 2BS QTL in four additional populations showed that it reduced disease severity by an average of 50 %. The position of the 2BS QTL might coincide with that of the *Tsc2* locus, although the absence of any common markers between the map generated by Li et al. (2011) and those presented by Friesen and Faris (2004) and Abeysekara et al. (2010) precludes any meaningful comparisons. It is not known whether the inoculum used by Li et al. (2011) contained Ptr ToxB, because experiments were not conducted to determine the race of the fungus used for inoculation. Li et al. (2011) presumed that the *P. tritici-repentis* used in their experiments did not produce Ptr ToxB because PCR experiments by Antoni et al. (2010) indicated that, of 119 Australian isolates tested, all possessed the *ToxA* gene, but none possessed the *ToxB* gene. Based on this, Li et al. (2011) speculated that the 2BS QTL derived from Ernie might be a novel QTL. However, this could not be determined for certain without classifying the race used in the inoculation experiments, or infiltrating the population with Ptr ToxB to map the *Tsc2* locus and determining if the gene underlays the peak of the QTL.

In a recent QTL study, Faris et al. (2012) evaluated a population of RI lines derived from a cross between the Canadian spring wheat variety Katepwa (susceptible) and the hexaploid wheat landrace Salamouni for reaction to two race 1 isolates (ASC1 and Pti2), a race 2 isolate (86-124), and the isolate AR LonB2. The latter isolate, as mentioned above, was collected from Arkansas and does not conform to the current race classification system because it caused necrosis on the race 2-differential Glenlea, but it does not produce Ptr ToxA (Ali et al. 2010). Faris et al. (2012) showed that the *Tsn1* locus was significantly associated with susceptibility to the three Ptr ToxA-producing isolates (ASC1, Pti2, and 86-124), but the amount of disease variation explained by the *Tsn1* locus was 5, 22, and 30 %, indicating that the effects of a compatible *Tsn1*–Ptr ToxA interaction varied among these isolates. A QTL on the long arm of chromosome 5D was specific to tan spot caused by ASC1, and a non-specific QTL on 7BS (Fig. 4) was associated with Pti2, 86-124, and AR LonB2, but not ASC1, indicating that genetic differences exist among isolates (i.e., ASC1 and Pti2) classified as the same race. As expected, the *Tsn1* locus was not significantly associated with reaction to AR LonB2, but a QTL specifically associated with disease caused by AR LonB2 was identified on 7DS. It is worthy to note that no resistance locus on chromosome 3A derived from Salamouni was identified in this research as was reported by Tadesse et al. (2006b) providing further support for the notion that Salamouni does not harbor a resistance gene per se on chromosome 3A, but rather Chinese Spring has a susceptibility gene on 3A.

Attempts to use association mapping to identify tan spot resistance loci in wheat have also been made. For example, Gurung et al. (2011) reported the identification of genomic regions associated with resistance to isolates Pti2 (race 1) and DW7 (race 5) using DArT markers. They reported QTLs on chromosomes 1D, 2A, 2B, 2D, 4A, 5B, and 7D associated with resistance to Pti2 that explained from 1.3 to 3.1 % of the variation. They also reported QTLs on chromosomes 2D, 6A, and 7D associated with resistance to DW7 that explained from 2.2 to 5.9 % of the variation. Together, these results and those of the other QTL studies indicate that race non-specific resistance QTLs play important roles in governing reaction to tan spot and that the wheat–tan spot system is much more complex than the current race classification system would suggest.

Sources of tan spot resistance

The studies discussed above provide examples of good sources of tan spot resistance. In addition to these, additional studies have been conducted to evaluate collections of wheat varieties (Rees and Platz 1990; Riede et al. 1996), *Ae. tauschii* accessions (Cox et al. 1992; Siedler et al. 1994), tetraploid wheat relatives (Chu et al. 2008a), wheat–alien species derivatives (Oliver et al. 2008), and synthetic hexaploid wheat lines (Riede et al. 1996; Siedler et al. 1994; Xu et al. 2004; Friesen et al. 2008; Morris et al.

2010). In each case, numerous good sources of resistance were identified and considered to be useful for further discovery of new resistance genes and for incorporation into wheat breeding programs for the introgression of tan spot resistance. The genetics of resistance in sources used for breeding and introgression should be well characterized. It is useful to continue to evaluate and characterize wheat relatives, alien species, and other germplasm sources for broad-spectrum and race non-specific resistance QTLs and also for potentially novel major resistance genes. However, it is worthy to note that new sources that confer insensitivity to the known *P. tritici-repentis* HSTs Ptr ToxA, Ptr ToxB, and Ptr ToxC are not needed because to obtain insensitivity requires the removal of the sensitivity allele, and numerous wheat and durum accessions lacking all three HST sensitivity genes exist, thus making it unnecessary to use wild relatives, alien species, or otherwise unadapted material to do so.

Breeding and marker-assisted selection for tan spot resistance

When developing varieties with resistance to biotrophic pathogens, such as the rusts, wheat breeders need to be concerned about the durability of the resistance genes incorporated. It is well documented that the use of single major R genes that confer gene-for-gene resistance can be overcome by the pathogens in a relatively short period of time due to the ability of rust fungi to rapidly evolve and 'defeat' the R genes. Depending on the degree of selection pressure, a given R gene may be effective for only a few years before being overcome. In a few extreme cases, R genes have been defeated during the variety development stage and before release of the variety to growers. This has caused breeders to implement strategies to pyramid multiple R genes together in a single variety and/or incorporate genes that confer broad-spectrum, or horizontal, resistance.

When breeding for resistance to tan spot, and other necrotrophic pathogens that produce HSTs, breeders need to be less concerned about pathogen evolution to overcome resistance. The first goal for developing tan spot resistant varieties should be to eliminate the HST sensitivity genes from the breeding material. Once this is accomplished, the pathogen cannot evolve to become virulent on a host gene that is not present. Instead, it would have to undergo mutation that would allow it to subvert a different host gene, or it would have to acquire a new HST gene for which the corresponding sensitivity gene was present in the variety. Both cases would require a gain of function to occur, which is much less likely to happen compared to losing function as is the case when new virulent races of rust (or other biotrophs) emerge. However, while these scenarios may be unlikely, they are not impossible, and the horizontal transfer of the *ToxA* gene from *S. nodorum* to *P. tritici-repentis* is a documented example of the latter (Friesen et al. 2006).

Another phenomenon that breeders must be aware of is the possibility that the same gene that confers sensitivity to a necrotroph-produced HST also confers resistance to a biotroph. While there have been no cases of this phenomenon yet reported in any wheat-pathogen system, the oat *Pc-2* gene, which confers resistance to oat crown rust (*Puccinia coronata*), also confers sensitivity to the HST victorin produced by *Cochliobolus victoriae* and susceptibility to Victoria blight caused by the same fungus (Walton 1996). Therefore, it is possible that the incorporation of a R gene for biotroph resistance might also yield susceptibility to a necrotroph, or that the elimination of a necrotroph susceptibility gene might lead to the loss of resistance to a biotroph.

While breeding for resistance to necrotrophic pathogens such as *P. tritici-repentis* presents some unique concerns and challenges, there are documented cases of success. For example in Kansas, tan spot resistant varieties harboring the same genetic resistance have been used for many years without experiencing reduced resistance or overcoming it by *P. tritici-repentis* (Bockus et al. 2011), thereby validating the notion that tan spot resistance is durable once incorporated.

When breeding for tan spot resistance, several factors need to be considered. First, it is important to select a source of resistance that has been well characterized genetically, i.e., its reaction to the known HSTs and to disease caused by various isolates representing all races of *P. tritici-repentis* should be known. Second, the general strategy for developing tan spot resistant germplasm should be to remove any HST sensitivity genes from the recurrent parent and at the same time introgress broad-spectrum race non-specific resistance QTLs from the donor parent. And third, selections will be much more efficient with the use of molecular markers as opposed to conducting phenotypic evaluations. Because HST sensitivity genes are dominant, heterozygotes cannot be distinguished from those plants that are homozygous for the recurrent parent in backcrossing schemes using purified HSTs or culture filtrates. However, co-dominant markers allow one to easily distinguish these two classes and thus make selections when plants are juveniles for subsequent backcrosses. In addition, conidial inoculations for reaction to tan spot are relatively laborious and can be affected by environmental factors, making the selection of genomic regions harboring non-specific resistance QTLs with the use of markers much more efficient as well.

We have followed this recipe for developing tan spot resistant germplasm. We selected the North Dakota HRSW variety Alsen as our recurrent parent. Alsen has extremely good quality and tolerance to Fusarium head blight

because it harbors the gene *Fhb1* (Liu et al. 2006), but it is highly susceptible to tan spot and also *S. nodorum* blotch (SNB), which is also a foliar disease caused by a necrotrophic pathogen that produces numerous HSTs (Friesen and Faris 2010). We selected an RI line derived from the BR34 × Grandin RI population that was insensitive to all *P. tritici-repentis* and *S. nodorum* HSTs, and harbored the tan spot race non-specific resistance QTLs reported by Faris and Friesen (2005) as our donor parent. In this line, all resistance loci were derived from BR34, and approximately 50 % of the genome is derived from Grandin, a North Dakota HRSW with excellent quality. Alsen was insensitive to Ptr ToxB, but sensitive to Ptr ToxA as well as the *S. nodorum* HSTs SnTox2 and SnTox3. Therefore, we used markers to select against the Alsen alleles at *Tsn1* and also *Snn2* and *Snn3*, which confer sensitivity to SnTox2 and SnTox3, respectively. We concomitantly used markers to select for BR34 alleles at marker loci linked to the tan spot resistance QTLs and to select for the Alsen allele of *Fhb1* to retain Fusarium head blight tolerance. After two rounds of backcrossing and MAS, the BC_2F_2 plants carrying homozygous BR34 alleles at the marker loci were selected and advanced to the BC_2F_5 generation. The BC_2F_5 plants were evaluated for reaction to *P. tritici-repentis* races 1, 2, 3 and 5, and a highly virulent *S. nodorum* isolate. Under greenhouse inoculations, lines derived by MAS were resistant to all three *P. tritici-repentis* races and the *S. nodorum* isolate (Fig. 7). These lines will soon be tested under field conditions. This work demonstrates that MAS for the development of adapted tan spot resistant germplasm can be very effective. Seed of the tan spot resistant MAS lines is freely available upon request from the authors.

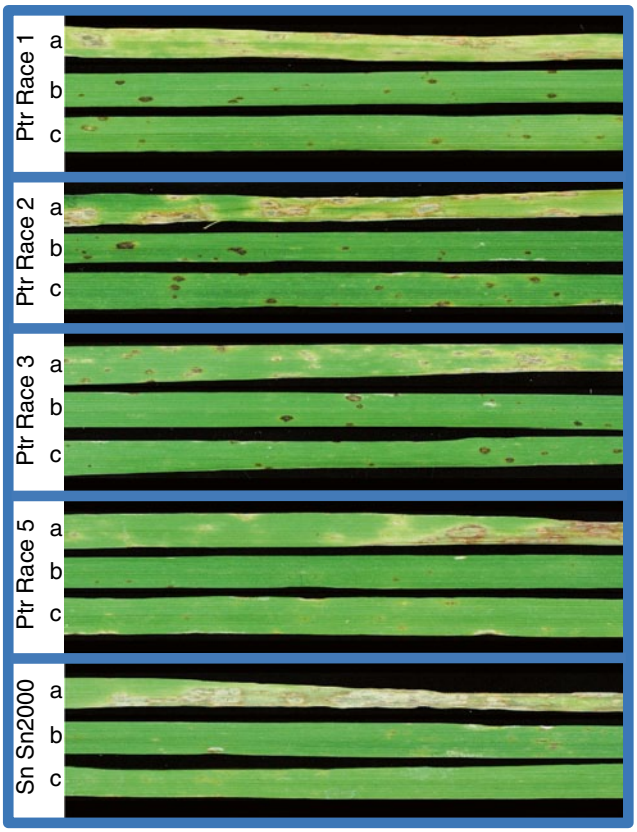

Fig. 7 Reactions of the recurrent parent Alsen (**a**), the resistant donor recombinant inbred line derived from BR34 × Grandin (**b**), and a BC_2F_5 line derived by marker-assisted selection (**c**) to *P. tritici-repentis* (Ptr) races 1, 2, 3, and 5, and to the highly virulent *S. nodorum* isolate Sn2000

Future work

Much progress has been made in describing and understanding the wheat–tan spot system over the past two decades. We know that the fungus produces HSTs that, when recognized by specific host sensitivity genes, induce disease, and that these host–HST relationships follow an inverse gene-for-gene model. However, we also know that race non-specific QTLs that confer broad-spectrum—and potentially durable—resistance exist. In addition, isolates that do not conform to the current race classification system have been described, and novel qualitative "resistance" genes have been reported, which have common attributes of HST insensitivity genes. Therefore, the wheat–tan spot system is much more complex than currently described in the literature, and much additional work is needed to characterize this pathosystem.

On the pathogen side, more research is needed to identify and characterize additional HSTs, because there is strong evidence indicating that HSTs in addition to Ptr ToxA, Ptr ToxB, and Ptr ToxC exist, such as the existence of a putative Ptr ToxD (Meinhardt et al. 2003; Ciuffetti et al. 2003), the existence of recessive resistance genes on chromosomes 3A, 3B, and 3D (Singh et al. 2006, 2008b; Tadesse et al. 2006a, b), the induction of necrosis on wheat lines by isolates that do not produce Ptr ToxA (Ali et al. 2010), and the selective induction of chlorosis on a durum wheat line by race 1 isolates due to factor(s) distinct from Ptr ToxC (Gamba and Lamari 1998).

As more HSTs are identified and characterized, the corresponding host sensitivity genes should also be identified and characterized, and markers suitable for MAS need to be developed so that breeders and geneticists can efficiently remove the sensitivity alleles from germplasm and breeding lines. Interactions between novel HSTs and host genes need to be characterized as well to understand their effective role in governing tan spot susceptibility. More work to identify and characterize broad-spectrum resistance QTLs will also be useful as well. The molecular cloning of more HST sensitivity genes and broad-spectrum resistance

QTLs will allow further characterization of wheat–*P. tritici-repentis* interactions, and shed light on the relationship between host–HST interactions and race non-specific resistance mechanisms at the molecular level. It will also provide the tools necessary for the development of functional markers, which can be used to conduct highly efficient MAS for the pyramiding of HST insensitivity genes together with race non-specific resistance loci leading to the development of wheat and durum varieties with superior tan spot resistance.

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