Mol Breeding (2010) 25:369–379
DOI 10.1007/s11032-009-9336-1

Genetic and molecular analysis of wheat tan spot resistance effective against *Pyrenophora tritici-repentis* races 2 and 5

P. K. Singh · M. Mergoum · T. B. Adhikari · T. Shah · F. Ghavami · S. F. Kianian

Received: 2 March 2009 / Accepted: 10 September 2009 / Published online: 27 September 2009

Abstract Tan spot, a major foliar disease of wheat (*Triticum aestivum* L.), is caused by an ascomycete *Pyrenophora tritici-repentis*. Both culture filtrates and conidiospore inocula induce disease symptoms in susceptible wheat genotypes. The objectives of this study were to determine and map the genetic control of resistance to spore inocula and culture filtrates of *P. tritici-repentis* races 2 and 5. The F_1 and F_2 generations and an $F_{2:6}$ recombinant inbred lines (RIL) population were developed from a cross between the resistant ND 735 and the susceptible Steele-ND. Disease assessments of the segregating generations were done at the seedling stage using culture filtrates and spore inocula under controlled environmental conditions. Genetic and mapping analyses of the F_1 and F_2 generations and the RIL by both methods indicated that the same single recessive gene, *Tsr1*, located on chromosome 5BL, controlled resistance and insensitivity to necrosis induced by race 2. A second recessive gene, designated *Tsr6*, located on chromosome 2BS, conferred resistance/insensitivity to chlorosis induced by spore inocula or culture filtrates of race 5. Diversity Arrays Technology markers *wPt-3049* (2.9 cM) and *wPt-0289* (4.6 cM) were closely linked to *Tsr1* and *Tsr6*, respectively. The results further indicated that culture filtrates can be used as surrogates for spore inoculation. *Tsr1* and *Tsr6* can be selected by marker-assisted selection in breeding for resistance to tan spot.

Keywords Yellow spot · Necrosis · Chlorosis · Diversity arrays technology (DArT) and simple sequence repeat (SSR) markers · *Triticum aestivum* · Host resistance

P. K. Singh · T. Shah
International Maize and Wheat Improvement Center (CIMMYT), Apdo Postal 6-641, 06600 Mexico, DF, Mexico
e-mail: pk.singh@cgiar.org

M. Mergoum (✉) · F. Ghavami · S. F. Kianian
Department of Plant Sciences, NDSU Dept # 7670, P.O. Box 6050, Fargo, ND 58108, USA
e-mail: Mohamed.Mergoum@ndsu.edu

T. B. Adhikari
Department of Plant Pathology, North Dakota State University, NDSU Dept 7660, P.O. Box 6050, Fargo, ND 58108, USA

Introduction

Tan spot, also known as yellow spot or yellow leaf blotch, is an important leaf spotting disease caused by the fungus *Pyrenophora tritici-repentis* (*PTR*) (Died.) Dreches (anamorph *Drechslera tritici-repentis* (Died.) Shoemaker). Worldwide increases in the incidence and severity of tan spot have been attributed to changes in cultural practices including shifts

from conventional tillage and stubble burning to conservation tillage systems, shorter crop rotations, continuous wheat cultivation, and use of susceptible cultivars (De Wolf et al. 1998; Ciuffetti and Tuori 1999). Foliar diseases like tan spot reduce the photosynthetic area of leaves resulting in reduced grain filling, lower numbers of grains per spike, and lower yields; particularly when the top two leaves, penultimate and flag leaves, are severely infected. Tan spot on average causes yield losses of 5–10%; but these can be higher than 50% under conditions favorable for disease development (De Wolf et al. 1998). This disease also causes significant losses in grain quality by grain shriveling, red smudge, and black point (Fernandez et al. 1998).

Pyrenophora tritici-repentis infects wheat plants at any growth stage. The tan spot disease involves two distinct symptoms: tan necrosis and extensive chlorosis, for which the host responses are independently inherited (Lamari and Bernier 1991; Singh and Hughes 2005). Resistance is expressed as small dark brown lesions that do not increase in size with time or leaf wetness duration whereas susceptibility is expressed as small dark brown spots surrounded by tan necrosis and/or extensive chlorosis that often covers the entire leaf (Lamari and Bernier 1989b). Currently, isolates of *PTR* are classified into eight races based on their ability to induce chlorosis and/or necrosis on a set of wheat differentials (Lamari et al. 2003). Races 1–5 of *PTR* have been reported to occur in North America (Lamari et al. 1998; Ali and Francl 2003).

A range of molecular markers has been used in tagging major genes and quantitative trait loci associated with resistance to tan spot, including random amplified polymorphic DNA markers (RAPD; Stock et al. 1996), restriction fragment length polymorphisms (RFLP; Faris et al. 1996), amplified length polymorphisms (AFLP; Haen et al. 2004), microsatellite or simple sequence repeat (SSR; Singh et al. 2008b), and expressed sequence tags as PCR or RFLP markers (Lu et al. 2006). Recently, a high throughput genome analysis method called Diversity Arrays Technology (DArT) was developed to analyze plant and animal genomes with no prior DNA sequence knowledge of the organism(s) being investigated. DArT generates whole-genome fingerprints by scoring the presence versus absence of thousands of unique DNA fragments in a genomic sample by using a microarray platform (Jaccoud et al. 2001). DArT detects DNA polymorphisms at several hundred genomic loci in a single assay without relying on DNA sequence information in wheat. This novel technology has been used in studies of genetic diversity (White et al. 2008), association analysis (Crossa et al. 2007), and high throughput profiling of the genome (Akbari et al. 2006). However, the utility of the high throughput DArT markers has not been examined in wheat-*PTR* interactions.

Numerous studies have been conducted to reveal the genetics of resistance to various *PTR* races using spore inocula and/or culture filtrates to induce tan spot symptoms. However, the results from these studies vary with the genetic material used, isolate/race tested, environmental conditions, and the disease assessment scale used. By using spore inocula many studies have found that resistance is oligogenic (Lamari and Bernier 1989c, 1991; Orolaza et al. 1995; Gamba et al. 1998; Gamba and Lamari 1998; Anderson et al. 1999; Singh and Hughes 2005, 2006b); however, polygenic control has also been reported (Nagle et al. 1982; Elias et al. 1989; Faris et al. 1997).

The wheat-*PTR* pathosystem follows the toxin model whereby a compatible interaction between the host plant and pathogen leads to susceptibility, the result of interaction of the pathogen-produced toxin and its specific toxin-receptor in the host (Lamari et al. 2003; Singh and Hughes 2005). To date three host-specific toxins, Ptr ToxA, Ptr ToxB, and Ptr ToxC, have been reported and characterized. Toxin Ptr ToxA, a 13.2 kDa protein produced by races 1, 2, 7, and 8, induces necrotic symptoms in sensitive wheat genotypes (Lamari et al. 2003). The gene *Tsn1*, controlling insensitivity to Ptr ToxA, is located on the long arm of chromosome 5B (Faris et al. 1996; Anderson et al. 1999). Toxin Ptr ToxB is a 6.6 kDa protein produced by races 5, 6, 7, and 8 and induces chlorotic symptoms in sensitive wheat genotypes (Lamari et al. 2003). Friesen and Faris (2004) reported that the gene *Tsc2*, controlling insensitivity to Ptr ToxB, is located on the short arm of chromosome 2B. Ptr ToxC is a non-ionic, polar, low molecular weight molecule produced by race 1, and insensitivity to this toxin conferred by gene *Tsc1* mapped on the short arm of chromosome 1A (Effertz et al. 2002).

Genetic studies have established that sensitivities to Ptr ToxA, Ptr ToxB, and Ptr ToxC and

susceptibility to the races that produce them are each controlled by the same gene loci (Lamari and Bernier 1989c; Orolaza et al. 1995; Gamba et al. 1998; Gamba and Lamari 1998; Singh and Hughes 2006a). However, other studies (Riede et al. 1996; Zhang and Jin 1998; Friesen et al. 2003) reported differential responses to toxin infiltration and fungal spore inoculation. Tuori et al. (1995) and Meinhardt et al. (1998) suggested that multiple toxins might be produced by isolates of *PTR* that induce necrotic and/or chlorotic symptoms. Recently, Chu et al. (2008) reported four novel QTLs associated with tan spot resistance; however, none of these QTLs corresponded to known toxin insensitivity loci. Therefore, it is important to assess the relationship between culture filtrate (toxin) and spore inoculation and the loci involved in insensitivity and resistance. The specific objectives of this study were to (1) investigate the inheritance of resistance to tan spot caused by culture filtrate and spore inoculation of *PTR* races 2 and 5, (2) establish the relationship between the host reaction to culture filtrate and spore inoculation and (3) identify DArT and SSR markers linked to the resistance gene(s) to determine the locations of the resistance gene(s) and explore the possibility of marker-assisted selection for tan spot resistance.

Materials and methods

Plant material

Segregating generations of a cross of susceptible cultivar Steele-ND (Singh et al. 2006) and the resistant germplasm ND 735 (Mergoum et al. 2006) were developed for this study. The F_1 and F_2 generations and an $F_{2:6}$ recombinant inbred lines (RIL) population were used. Seedlings of 7–9 F_1 and 236–240 F_2 plants were screened separately with spore inocula of both races and their culture filtrates. A total of 138 RIL, with a minimum of 16 and 12 plants/RIL, were tested for determination of the inheritance of resistance to both spore inoculations and culture filtrates. Within line populations of this size allowed 99 and 90% probabilities, respectively, of correctly distinguishing between segregating and non-segregating families assuming that a single gene controlled resistance (Hanson 1959).

Disease screening

Single-spore cultures of isolates Ptr 86-124 (race 2) and Ptr DW13 (race 5) maintained at the Department of Plant Pathology, NDSU, Fargo, were used to produce spore inocula, which was prepared using a modification of the method of Lamari and Bernier (1989a). Briefly, inoculum was obtained by placing 0.5-cm-diameter dried mycelial plugs of each isolate, previously stored at −20°C, on 10-cm petri plates containing V8-potato dextrose agar (V8-PDA) (150 ml V8-juice, 10 g PDA, 10 g agar, 3 g $CaCO_3$, and 850 ml distilled water). The cultures were incubated in the dark at 20–22°C for 6 days. The plates were then flooded with sterile distilled water and the mycelia were flattened with the base of a sterile test tube. Excess water was decanted from the petri-dishes and the plates were incubated under continuous light at 22–24°C for 1 day followed by 1 day in the dark in an incubator at 16°C to induce conidiophores and conidia production, respectively. Approximately, 25 ml sterile distilled water was added to each plate and the conidia were dislodged with a camel-hair brush. Spore concentrations were measured with a haemocytometer and adjusted to 3,000 conidia/ml before inoculation.

Disease screening was performed using a randomized complete block design. Seeds of each RIL, parents, and checks were planted in a plastic cone 3.8 cm in diameter and 20 cm long (Stuewe and Sons, Inc. Tangent, OR) filled with Sunshine Mix # 1 (Fison Horticulture, Vancouver, BC). Four to five seeds were planted in each cone. Plants at the two-leaf stage were inoculated until runoff with the conidial suspension using a hand sprayer. Following inoculation, seedlings were incubated for 24 h while maintaining continuous leaf wetness in a mist chamber located in a growth room at 22/17°C (day/night) with a 16-h photoperiod and then returned to benches in the same growth room. Wheat cultivars Erik, 6B-365, 6B-662 and Glenlea were included as checks in each test to verify the inoculation and the race used. Eight days after inoculation, the seedlings were rated for disease reaction based on the 1–5 lesion-type rating scale developed by Lamari and Bernier (1989a). Plants with ratings of 1 and 2 were classified as resistant, and those with ratings of 3–5 as susceptible. In the family segregation ratios RIL having all plants with ratings of 1 and 2 were

classified homozygous resistant, those having all plants with ratings of 3–5 homozygous susceptible, and those with both resistant and susceptible plants were classified as segregating.

Culture filtrate screening

Culture filtrates from races 2 and 5 that produce toxins Ptr ToxA and Ptr ToxB, respectively, were collected by the procedures of Orolaza et al. (1995). The same isolates were used for both culture filtrates and spore inocula. Approximately 25 µl of culture filtrate was infiltrated into wheat leaves until water-like soaking of the tissue was observed using a 1-ml syringe without a needle. The infiltrated second leaf was marked by a non-toxic permanent marker. Plant reactions to the culture filtrates were recorded 4 days after infiltration. Check genotypes, Erik and 6B-365 (insensitive to Ptr ToxA and Ptr ToxB), Glenlea (sensitive to Ptr ToxA), and 6B-662 (sensitive to Ptr ToxB), were included in each test to verify the validity of the infiltrations and the toxin(s) in the culture filtrates. Presence or absence of necrosis at the site of infiltration indicated sensitivity or insensitivity, respectively, to the culture filtrate from race 2 (Ptr ToxA). Similarly, presence or absence of chlorosis at the site of infiltration indicated sensitivity or insensitivity, respectively, to the culture filtrate from race 5 (Ptr ToxB).

Molecular marker analysis

DNA extraction was performed on bulks of at least ten plants per RIL following methods described by Guidet et al. (1991) with some modifications. DNA concentrations were measured by a NanoDrop 1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). Samples were diluted to 100 ng/µl, and 20 µl of diluted sample were sent for DArT marker analysis by Triticarte Pty. Ltd. (Canberra, Australia; http://www.triticarte.com.au) as described by Akbari et al. (2006). Of the 138 RILs in this population, 118 were used for mapping the tan spot resistance genes. In total, 2,300 DArT markers, spread across the wheat genome, were screened on the entire population. Microsatellite primers from the *gwm* (Röder et al. 1998) and *wmc* (Somers et al. 2004) series, previously mapped to chromosome 2B and 5B, were used to screen the parental lines for polymorphisms and those markers found to be polymorphic were assayed on the RIL population. Amplification conditions for each marker followed protocols described previously by Röder et al. (1998) and Somers et al. (2004). PCR products were separated on 6% non-denaturing polyacrylamide gels and stained with ethidium bromide as described by Shi et al. (2001). The DArT and SSR marker data were combined with the disease response data to develop the genetic maps.

Statistical analysis

Chi-squared tests for goodness-of-fit to various genetic models was applied to the segregation data from the F_2 and RIL populations to determine the number of genes involved in genetic control of resistance/insensitivity to spore suspensions/culture filtrates of *PTR* races 2 and 5. Linkage analysis and map construction were carried out using Mapmaker 3.0 software (Lander et al. 1987). In linkage analysis, a probability value of 10^{-5} was used as a cut off value to determine linkage between loci.

Results

Phenotypic analyses

Steele-ND and ND 735 gave consistent susceptible and resistant reactions to *PTR* races 2 and 5, respectively, throughout this study. All the F_1 plants of the cross Steele-ND/ND 735 gave susceptible/sensitive reactions when tested with spore inocula/culture filtrates of race 2, indicating that resistance/insensitivity was recessive (Table 1). The F_2 generation of the cross population, when screened with spore inoculum, segregated for 1 resistant: 3 susceptible confirming that resistance was recessive and controlled by a single gene. The RIL segregation pattern again confirmed that a single gene controlled resistance to spore inoculum of *PTR* race 2 in the cross between Steele-ND and ND 735 (Table 2). Further the frequency distribution of the mean disease score of the RILs show a binomial distribution, confirming that a single gene controls resistance to race 2 in the Steele-ND/ND 735 (Fig. 1). Similarly, a single gene control for insensitivity was observed in the F_2 generation and RIL in the above cross when challenged with culture

Table 1 Reaction of F_1 and F_2 plants of cross Steele-ND/ND 735 to *Pyrenophora tritici-repentis* races 2 and 5 and chi-squared tests of F_2 segregation ratios for reaction to spore inoculation culture infiltration with two races

Cross: Steele-ND/ND 735	F_1 plants		F_2 plants		Ratio tested	χ^2 value	Prob. value[b]
	R[a]	S	R	S			
Race 2							
Spore suspension	0	7	63	177	1:3	0.2	0.65
Culture filtrate	0	8	68	172	1:3	1.4	0.24
Race 5							
Spore suspension	0	9	64	172	1:3	0.6	0.45
Culture filtrate	0	8	68	170	1:3	1.6	0.20

[a] For spore suspension inoculated plants were rated on a 1–5 lesion-type rating scale (Lamari and Bernier 1989a). Plants with ratings of 1–2 were classified as resistant (R) and those with ratings of 3–5 were classified as susceptible (S). For culture filtrate infiltrated plants were classified as insensitive (R) and sensitive (S)

[b] Probability of obtaining deviations from the expected ratio by chance alone. A probability value >0.05 indicates that the observed population does not differ significantly from expected hypothesis

Table 2 Family segregation ratios for $F_{2:6}$ RIL from Steele-ND/ND 735 when tested with spore suspensions and culture filtrates of *Pyrenophora tritici-repentis* races 2 and 5

$F_{2:6}$ RILs		Spore suspension			χ^2 value[a]	Prob. value[b]
		Resistant	Segregating	Susceptible		
Race 2						
Culture	Insensitive	66	0	0	2.7	0.26
Filtrate	Segregating	0	4	0		
	Sensitive	0	0	68		
Race 5						
Culture	Insensitive	57	0	0	2.6	0.28
Filtrate	Segregating	0	7	0		
	Sensitive	0	0	74		

[a] Assuming that a single gene controls resistance/insensitivity to spore suspension/culture filtrate the expected family segregation ratio is 15 homozygous resistant/insensitive: 2 segregating: 15 homozygous susceptible/sensitive. Segregating for resistance are those RILs that have both resistant/insensitive and susceptible/sensitive plants

[b] Probability of obtaining deviations from the expected ratio by chance alone. A probability value >0.05 indicates that segregation in the observed population does not differ significantly from the expected ratio

filtrate of race 2. All RIL gave the same reactions to both spore inoculum and culture filtrate indicating toxin Ptr ToxA, present in the culture filtrate, is a pathogenicity factor in the development of tan necrosis caused by *PTR* race 2.

All the F_1 plants tested gave susceptible/sensitive reaction to spore inocula and culture filtrates of race 5 in the cross between Steele-ND and ND 735 (Table 1). The segregation pattern observed in the F_2 generation was an acceptable fit to 1 resistant: 3 susceptible, confirming resistance is recessive to chlorosis induced by spore inoculum of *PTR* race 5 and is likely controlled by a single gene. The single gene control of resistance to race 5 in the cross Steele-ND/ND 735 was confirmed by testing the RILs which segregated for 15 homozygous resistant: 2 segregating: 15 homozygous susceptible lines (Table 2). The frequency distribution of mean RIL disease score for race 5 reactions shows a binomial distribution indicative of single gene control (Fig. 1). Similarly, a single gene controlling insensitivity was observed in the F_2 generation and RIL in the cross Steele-ND/ND 735 when screened with culture filtrate of race 5. All the 138 RIL gave exact reaction to

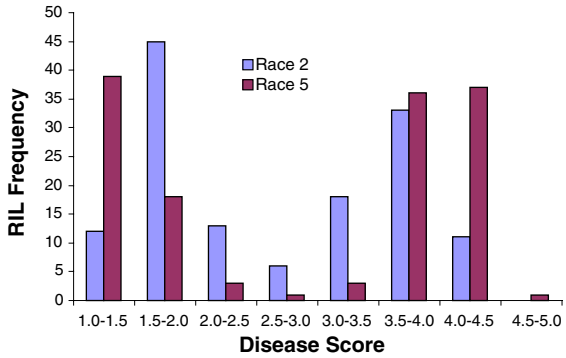

Fig. 1 Frequencies of RIL in disease score groups when tested with *Pyrenophora tritici-repentis* races 2 and 5. Disease evaluations were based on a 1–5 lesion type scale for both the races

spore inoculum and culture filtrate indicating toxin Ptr ToxB, present in the culture filtrate, is a pathogenicity factor in the development of chlorosis caused by *PTR* race 5 in this population. The genes controlling resistance to *PTR* races 2 and 5 are independently inherited (data not shown).

Molecular marker analysis

Among 2,300 DArT markers screened, 429 (18.65%) were polymorphic. The genetic map generated by these 429 markers on 118 RILs was distributed across the entire genome of the wheat as there were 42 linkage groups each accounting for a chromosome arm. The average distance between the markers was about 5 cM, and there were 2–36 markers in each linkage group (data not shown). Additional SSR markers from the *gwm* (Röder et al. 1998) and *wmc* (Somers et al. 2004) series, previously mapped to chromosome 2B and 5B were used to screen the parental lines for DNA polymorphisms. Ten SSR markers were polymorphic on the parents and used on the entire RIL population. As the DArT markers had shown the chromosome regions associated with race 2 and 5 resistances, only a few SSR markers were needed to anchor the map to establish reference points and to allow meaningful comparisons with other genetic maps based on SSR polymorphisms. Figures 2 and 3 show the genetic maps developed using DArT markers and SSR markers help us to locate the genes for resistance to *PTR* races 2 and 5.

Linkage analysis reveals the gene, controlling resistance/insensitivity to race 2, which was previously designated as *Tsr1* was located on the long arm of chromosome 5B. The gene *Tsr1* is flanked by the DArt markers *wPt-8285* and *wPt-3049* at distances of 7.0 and 2.9 cM, respectively (Fig. 2). The gene, controlling resistance/insensitivity to race 5 was located on the short arm of chromosome 2B. This gene, designated *Tsr6,* following recommendations of McIntosh et al. (2008) is flanked by the DArt marker *wPt-0289* and SSR marker *Xwmc25* at distances of 4.6 and 18.7 cM, respectively (Fig. 3).

Discussion

In this study, resistance to tan spot of wheat was observed to be race-specific following the gene-for-gene model involving the recognition of pathogen-produced host-selective toxins by specific genes in the host (Lamari et al. 2003; Singh and Hughes 2005). The identification of multiple major genes for resistance to tan spot and no interaction among them indicates that in order to develop complete resistance to all races of *PTR,* breeders need to incorporate all the resistance genes (Singh et al. 2008a). Since resistance to tan spot is recessive the use of marker assisted selection, utilizing DArT and SSR markers, to hasten the selection process by permitting the identification of heterozygotes may be advantageous.

A single recessive gene, previously designated *Tsr1*, located on long arm of chromosome 5B controlled both insensitivity to culture filtrates and resistance following inoculation with spore suspensions of *PTR* race 2. This finding was in agreement with previous studies (Lamari and Bernier 1989c; Stock et al. 1996; Gamba and Lamari 1998; Gamba et al. 1998; Singh and Hughes 2006a) reporting that the same gene conferred resistance to both spore suspensions and insensitivity to culture filtrates. Based on comparative mapping the gene identified and mapped in this study is the same gene which has been mapped by independent research groups to the long arm of chromosome 5B (Faris et al. 1996; Stock et al. 1996; Anderson et al. 1999), and designated *Tsr1* (McIntosh et al. 2008). However, our findings differ with other studies reporting resistance to be quantitative (Friesen et al. 2003), race non-specific (Faris and Friesen 2005), or not to involve host-selective toxin insensitivity genes (Chu et al. 2008). The different conclusions be attributable to different

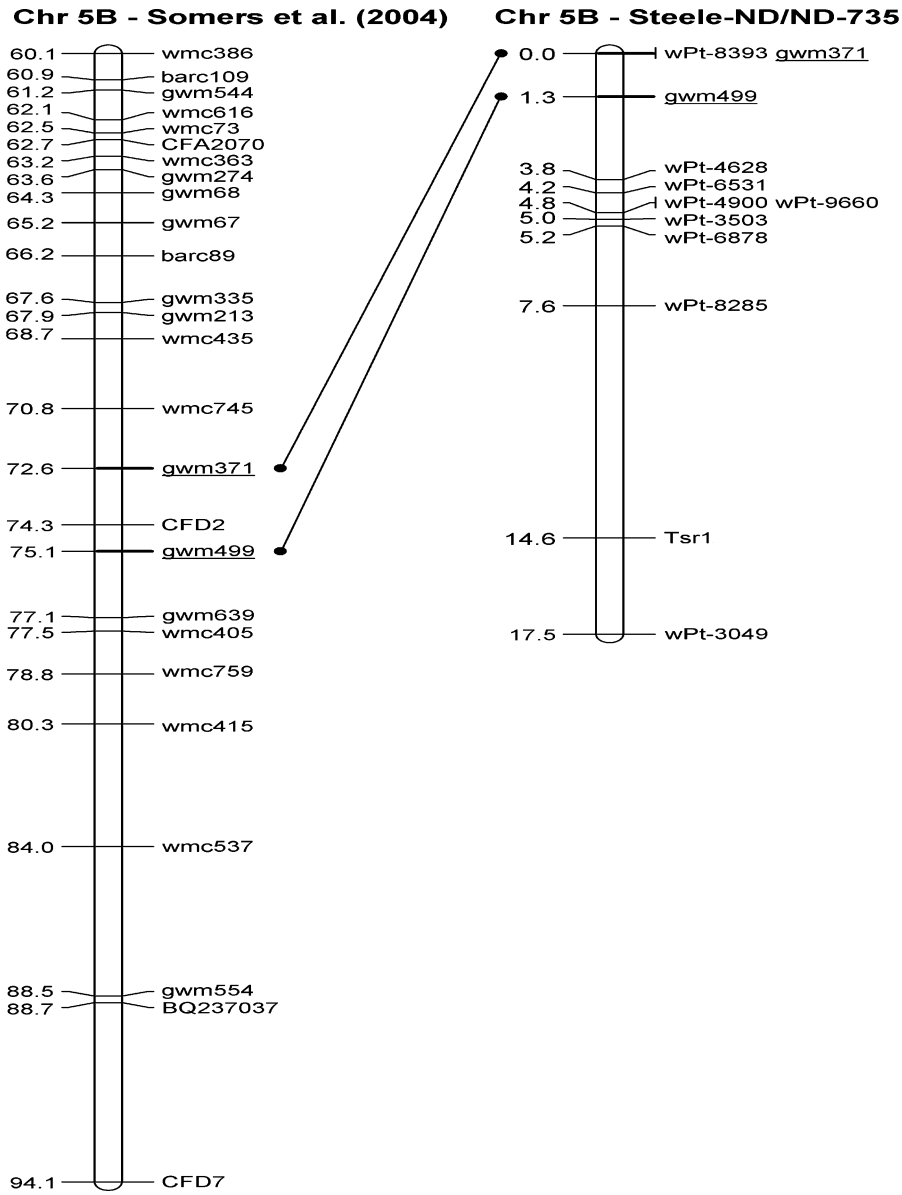

Fig. 2 Genetic map of chromosome 5BL showing the location of gene *Tsr1* for resistance to tan spot and insensitivity to culture filtrate of *Pyrenophora tritici-repentis* race 2. A section of chromosome 5BL from Somers et al. (2004) is presented for comparison

germplasms, races/isolates, disease inducement methodologies, environmental conditions, as well as different disease evaluation analysis methods.

In the present study resistance to *PTR* race 5 segregated as a monogenic recessive gene designated *Tsr6*, was effective against both the spore suspension and culture filtrate of race 5. Similar observations were made by other researchers (Orolaza et al. 1995; Gamba and Lamari 1998; Gamba et al. 1998). However, Singh et al. (2008a) observed that resistance to race 5 was controlled by a single dominant gene whereas Friesen and Faris (2004) reported resistance to be quantitative with the toxin-insensitivity gene, *Tsc2*, mapping to the distal region of chromosome 2BS accounting for 69% of the phenotypic variation. The mode of inheritance of resistance to chlorosis also varied from recessive, through partially dominant to dominant depending upon the parental genotypes (Singh and Hughes 2006b). Moreover, the expression of tan spot chlorotic symptom is continuous and highly influenced by environment (Singh and Hughes 2006b). The germplasm used in this study showed contrasting high levels of resistance and susceptibility and discrete differences in reaction permitted a

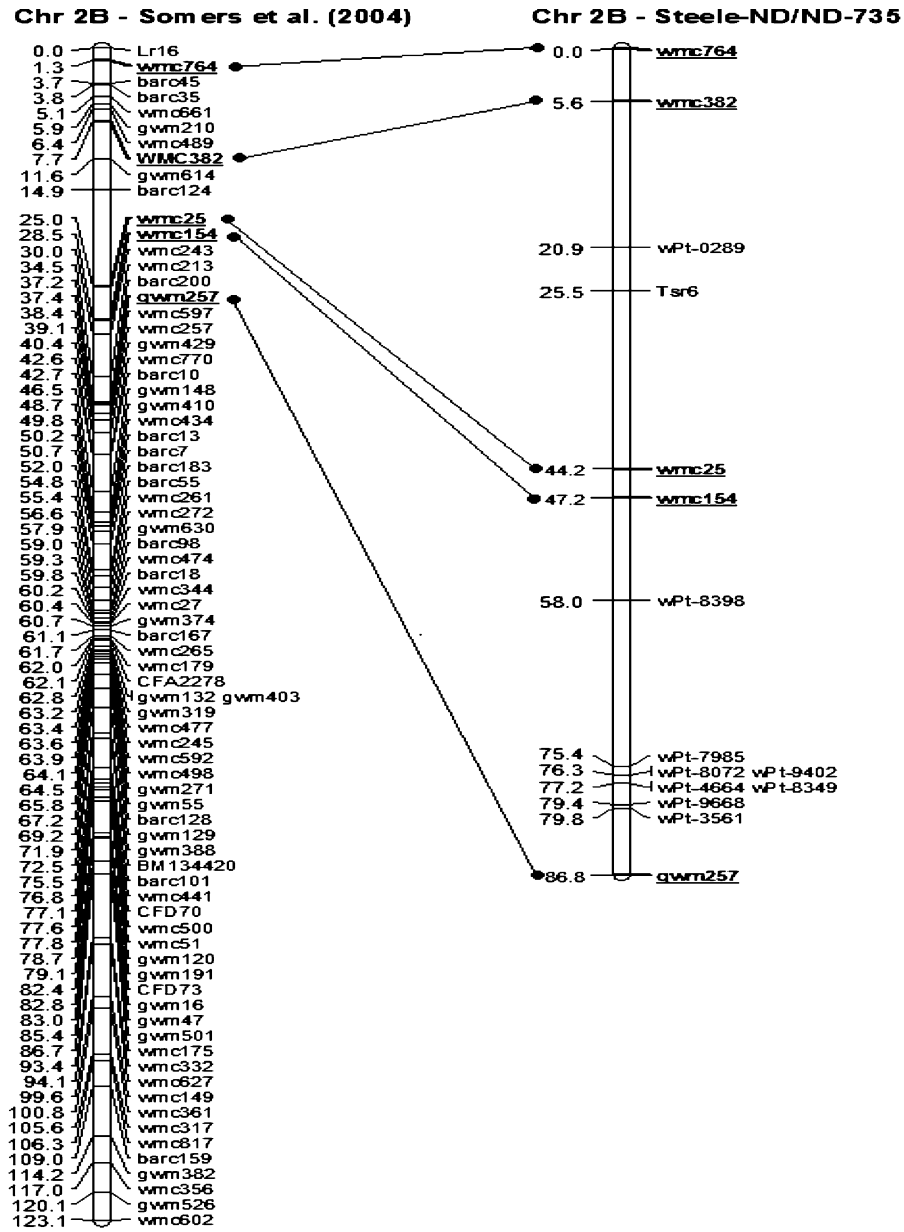

Fig. 3 The genetic map of chromosome 2BS showing the location of *Tsr6* for resistance to tan spot and insensitivity to culture filtrate of *Pyrenophora tritici-repentis* race 5

qualitative interpretation of tan spot response. Parlevliet (1989) reported that it is more likely to find major genes in genetic studies conducted in controlled environmental conditions as strong selection for resistance is achieved in controlled conditions and this tends to select for major genes. Additionally, when studying genetics of highly resistant cultivars to foliar diseases, the mode of inheritance is often found to be controlled by major gene(s) as the population can easily be phenotyped in discrete classes (Parlevliet 1989).

This study clearly demonstrates that culture filtrates of *PTR* races 2 and 5 can be used as surrogates to spore inoculation when screening for tan spot resistance. This is an important finding especially with regard to race 5 as a previous study (Singh et al. 2006) indicated very high frequencies of susceptibility among both durum and common wheat germplasm for *PTR* race 5. Currently race 5 and its combination races (6, 7, and 8) producing Ptr ToxD toxin, are not widely distributed in the USA, Canada, and other major wheat producing areas (Lamari et al. 1998; Ali

and Francl 2003), but the widespread occurrence of high susceptibility levels to race 5 in current cultivars and germplasm indicates considerable genetic vulnerability. Due to quarantine restriction and limited prevalence of *PTR* race 5 most wheat breeding programs do not screen for tan spot resistance for race 5. Since resistance to race 5, is independent to the resistance to race 1, the race generally used in disease screening, breeding programs fail to screen for race 5 resistance in their germplasms. Use of race 5 culture filtrate in screening as a surrogate of spore inoculum of race 5 will be greatly beneficial in screening for complete tan spot resistance. Additional advantages for using culture filtrates from both race 2 and 5 include (1) culture filtrates take half the time (4 days) to produce results compared with spore inoculation (7–8 days), (2) culture filtrates are less costly in terms of labor and space (3) the responses to culture filtrates are very clear and do not require high levels of technical expertise and (4) culture filtrate infiltration is less influenced by environmental conditions than pathogen inoculation and can easily be combined with screening for resistance to other leaf-spotting diseases (Singh and Hughes 2006a).

In this study, DArT markers in combination with SSR markers were used for the first time to map tan spot resistance genes. The genetic maps generated were compared with previously published maps (Somers et al. 2004) and similar orders and distances among SSR markers were observed confirming an accurate phenotypic and genotypic analysis. In addition, the chromosomal locations of several DArT markers were determined further enhancing the utility of these markers. These results suggest that DArT technology can be successfully used for gene discovery and to explore association between molecular markers and tan spot resistance genes. These novel molecular markers can be utilized for the initial gene identification and location and subsequently the identified region can be subjected to high density saturation mapping to identify molecular markers closely linked to genes of interest for marker-assisted selection or the possible map-based cloning of the gene.

Acknowledgments Financial support from State Board of Agricultural Research and Education, ND, and Minnesota Wheat Research and Promotion Council, MN, is gratefully acknowledged. The molecular component of this work was supported in part by the National Research Initiative, USDA Cooperative State Research, Education and Extension Service CAP grant number 2006-55606-16629.

References

Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden MJ, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A (2006) Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theor Appl Genet 113:1409–1420

Ali S, Francl LJ (2003) Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and non-cereal grasses in the great plains. Plant Dis 87:418–422

Anderson JA, Effertz RJ, Faris JD, Francl LJ, Meinhardt SW, Gill BS (1999) Genetic analysis of sensitivity to a *Pyrenophora tritici-repentis* necrosis inducing toxin in durum and common wheat. Phytopathology 89:293–297

Chu CG, Friesen TL, Xu SS, Faris JD (2008) Identification of novel tan spot resistance loci beyond the known host-selective toxin insensitivity genes in wheat. Theor Appl Genet 117:873–881

Ciuffetti LM, Tuori RP (1999) Advances in the characterization of the *Pyrenophora tritici-repentis*-wheat interaction. Phytopathology 89:444–449

Crossa J, Burgueño J, Dreisigacker S, Vargas M, Herrera-Foessel SA, Lillemo M, Singh RP, Trethowan R, Warburton M, Franco J, Reynolds M, Crouch JH, Ortiz R (2007) Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics 177:1889–1913

De Wolf ED, Effertz RJ, Ali S, Francl LJ (1998) Vistas of tan spot research. Can J Plant Pathol 20:349–370

Effertz RJ, Meinhardt SW, Anderson JA, Jordahl JG, Francl LJ (2002) Identification of a chlorosis-inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitivity locus in wheat. Phytopathology 92:527–533

Elias E, Cantrell RG, Horsford RM Jr (1989) Heritability of resistance to tan spot in durum wheat and its association with other agronomic traits. Crop Sci 29:299–304

Faris JD, Friesen TL (2005) Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. Theor Appl Genet 111:386–392

Faris JD, Anderson JA, Francl LJ, Jordahl JG (1996) Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. Phytopathology 86:459–463

Faris JD, Anderson JA, Francl LJ, Jordahl JG (1997) RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis*. Theor Appl Genet 94:98–103

Fernandez MR, DePauw RM, Clarke JM, Fox SL (1998) Discoloration of wheat kernels by *Pyrenophora tritici-repentis*. Can J Plant Pathol 20:380–383

Friesen TL, Faris JD (2004) Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. Theor Appl Genet 109:464–471

Friesen TL, Ali S, Kianian S, Francl LJ, Rasmussen JB (2003) Role of host sensitivity to Ptr ToxA in development of tan spot of wheat. Phytopathology 93:397–401

Gamba FM, Lamari L (1998) Mendelian inheritance of resistance to tan spot (*Pyrenophora tritici-repentis*) in selected genotypes of durum wheat (*Triticum turgidum*). Can J Plant Pathol 20:408–414

Gamba FM, Lamari L, Brûlé-Babel AL (1998) Inheritance of race-specific necrotic and chlorotic reactions induced by *Pyrenophora tritici-repentis* in hexaploid wheats. Can J Plant Pathol 20:401–407

Guidet F, Rogowsky R, Taylor C, Song W, Langridge P (1991) Cloning and characterization of a new rye-specific repeat sequence. Genome 34:81–87

Haen KM, Lu HJ, Friesen TL, Faris JD (2004) Genomic targeting and high-resolution mapping of the *Tsn1* gene in wheat. Crop Sci 44:951–962

Hanson WD (1959) Minimum family sizes for the planning of genetic experiments. Agron J 51:711–715

Jaccoud D, Peng K, Feintein D, Kilian A (2001) Diversity arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res 29:4e25

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

Lamari L, Bernier CC (1989b) Virulence of isolates of *Pyrenophora tritici-repentis* on 11 wheat cultivars cytology of differential host reactions. Can J Plant Pathol 11:284–290

Lamari L, Bernier CC (1989c) Toxin of *Pyrenophora tritici-repentis*: host specificity, significance in disease, and inheritance of host reaction. Phytopathology 79:740–744

Lamari L, Bernier CC (1991) Genetics of tan necrosis and extensive chlorosis in tan spot of wheat caused by *Pyrenophora tritici-repentis*. Phytopathology 81:1092–1095

Lamari L, Gilbert J, Tekauz A (1998) Race differentiation in *Pyrenophora tritici-repentis* and surveys of physiologic variation in western Canada. Can J Plant Pathol 20: 396–400

Lamari L, Strelkov SE, Yahyaoui A, Orabi J, Smith RB (2003) The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. Phytopathology 93:391–396

Lander ES, Green P, Abrahamson J, Barlow A, Day MJ, Lincoln SE, Newberg L (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genetics 121:174–181

Lu HJ, Fellers JP, Friesen TL, Meinhardt SW, Faris JD (2006) Genomic analysis and marker development for the *Tsn1* locus in wheat using bin-mapped ESTs and flanking BAC contigs. Theor Appl Genet 112:1132–1142

McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers J, Morris C, Somers DJ, Appels R, Devos KM (2008) Catalogue of gene symbols for wheat. In: Appels R, Eastwood R, Lagudah E, Langridge P, Mackay M, McIntyre L, Sharp P (eds) Proceedings of the 11th international wheat genetics symposium. Brisbane, Australia

Meinhardt SW, Zhang H, Effertz RJ, Francl LJ (1998) Characterization of additional peaks of necrosis activity from *Pyrenophora tritici-repentis*. Can J Plant Pathol 20: 436–437

Mergoum M, Frohberg RC, Ali S, Singh PK, Rasmussen JB, Miller JD (2006) Registration of spring wheat germplasm ND 735 combining tan spot, leaf, and stem rusts. Crop Sci 46:1004–1005

Nagle BJ, Frohberg RC, Hosford RM Jr (1982) Inheritance of resistance to tan spot of wheat. In: Hosford RM Jr (ed) Tan spot of wheat and related diseases workshop. North Dakota State University, Fargo, pp 40–45

Orolaza NP, Lamari L, Ballance GM (1995) Evidence of a host-specific chlorosis toxin from *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat. Phytopathology 85:1282–1287

Parlevliet JE (1989) Identification and evaluation of quantitative resistance. In: Leonard KJ, Fry WE (eds) Plant disease epidemiology, vol 2: genetics, resistance, and management. MaGraw-Hill, New York, pp 215–248

Riede CR, Francl LJ, Jordahl JG, Meinhardt SW (1996) Additional sources of resistance to tan spot of wheat. Crop Sci 36:771–777

Röder MS, Korzum V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Shi J, Ward R, Wang D, Lewis J (2001) Application of a high throughput, low cost, non-denaturing polyacrylamide gel system for wheat microsatellite mapping. In: Erlanger KY, Canty SM, Lewis J, Silver L, Ward RW (eds) National fusarium head blight forum. Kinko's Publisher, Okemos, pp 25–30

Singh PK, Hughes GR (2005) Genetic control of resistance to tan necrosis induced by *Pyrenophora tritici-repentis*, races 1 and 2, in spring and winter wheat genotypes. Phytopathology 95:172–177

Singh PK, Hughes GR (2006a) Inheritance of insensitivity to culture filtrate of *Pyrenophora tritici-repentis*, race 2, in wheat. Plant Breed 125:206–210

Singh PK, Hughes GR (2006b) Inheritance of resistance to the chlorosis component of tan spot of wheat caused by *Pyrenophora tritici-repentis* races 1 and 3. Euphytica 152:413–420

Singh PK, Mergoum M, Ali S, Adhikari TB, Elias EM, Anderson JA, Glover KD, Berzonsky WA (2006) Evaluation of elite wheat germplasm for resistance to tan spot. Plant Dis 90:1320–1325

Singh PK, Mergoum M, Ali S, Adhikari TB, Hughes GR (2008a) Genetic analysis of resistance to *Pyrenophora tritici-repentis* races 1 and 5 in tetraploid and hexaploid wheat. Phytopathology 98:702–708

Singh PK, Mergoum M, Gonzalez-Hernandez JL, Ali S, Adhikari TB, Kianian SF, Elias EM, Hughes GR (2008b) Genetics and molecular mapping of resistance to necrosis inducing race 5 of *Pyrenophora tritici-repentis* in tetraploid wheat. Mol Breed 21:293–304

Somers DJ, Issac P, Edwards K (2004) A high-density wheat microsatellite consensus map for wheat bread (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Stock WS, Brûlé-Babel AL, Penner GA (1996) A gene for resistance to a necrosis-inducing isolate of *Pyrenophora tritici-repentis* located on 5BL of *Triticum aestivum* cv. Chinese spring. Genome 39:598–604

Tuori RP, Wolpert TJ, Ciuffetti LM (1995) Purification and immunological characterization of toxic components from cultures of *Pyrenophora tritici-repentis*. Mol Plant Micro Inter 8:41–48

White J, Law JR, MacKay I, Chalmers KJ, Smith JSC, Kilian A, Powell W (2008) The genetic diversity of UK, US, and Australian cultivars of *Triticum aestivum* measured by DArT markers and considered by genome. Theor Appl Genet 116:439–453

Zhang X, Jin Y (1998) Sensitivity to Ptr ToxA and tan spot infection responses in *Aegilops/Triticum* complex. Can J Plant Pathol 20:415–418