ROLES OF THE TSN1 AND TSC2 GENES IN CONFERRING SUSCEPTIBILITY OF DURUM WHEAT TO TAN SPOT AND SEPTORIA NODORUM BLOTCH

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Sciences

November 2015

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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ABSTRACT

Tan spot is an important disease caused by the necrotrophic fungus *Pyrenophora tritici-repentis*. Two common necrotrophic effectors produced by this fungus are Ptr ToxA and Ptr ToxB, which recognize host sensitivity genes *Tsn1* and *Tsc2*, respectively. In this research, a tetraploid recombinant inbred line population was evaluated for reaction to the Ptr ToxA and Ptr ToxB-producing isolates 86-124 (race 2) and DW5 (race 5). The results indicated that a compatible *Tsc2*-Ptr ToxB interaction accounted for 26% of the disease variation, which states that this interaction plays a significant role in the development of tan spot. On the contrary, the *Tsn1*-Ptr ToxA interaction was not associated with tan spot caused by 86-124. However, evaluation of a ToxA-producing isolate of *Parastagonospora nodorum*, indicated that the *Tsn1*-ToxA interaction accounted for 38% of the variation. Therefore, the *Tsn1*-ToxA interaction played a significant role in the development of septoria nodorum blotch, but not tan spot.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisor, Dr. Justin D. Faris, for his guidance throughout my graduate studies at North Dakota State University, and providing me with an excellent opportunity to start my research career. His motivation and knowledge has helped me in all the time of research and writing of this thesis, I could not have imagined having a better advisor than Dr. Justin D. Faris, who showed me a better path towards my future career, corrected my writing and financially supported my research. I am very grateful and would have never been able to finish my research without the encouragement of my current and previous lab-mates who always created a friendly environment in the lab.

Besides my advisor, I would like to thank the rest of my thesis committee members, Dr. Phillip E. McClean, Dr. Zhaohui Liu and Dr. Steven Xu, who helped in statistical analysis and reading the disease. I would also like to thank all the lab members of Dr. Liu's lab who were always very co-operative.

In addition, I am very grateful to all the faculty members, staff, and graduate students in the Department of Plant Sciences for their support and guidance.

Most importantly, I would like to thank my parents and brothers for all the support they have provided me over the years. Also, I need to thank my grandfather, who taught me the value of hard work and an education. Without him, I may never have gotten to where I am today.

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GENERAL INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important food grains in the world. Leaf spotting diseases affect the yield and production of wheat in all the major wheat-production areas such as Australia and the Great Plains region of the United States and Canada. Tan spot, also known as yellow spot, which is one of the most common wheat leaf diseases, occurs worldwide wherever common wheat and durum wheat are grown. In 1992, tan spot was ranked as the most economically important wheat disease in North Dakota (McMullen and Nelson, 1992). On average, it causes 5-10% yield loss but under conditions conducive to disease development yield losses have reached 50%. Disease develops in the summer and spring on upper and lower parts of leaves. Symptoms of tan spot include necrosis, chlorosis or both. The tan-brown flecks and tan lesions with yellow borders are the initial necrosis symptoms. The chlorosis symptom consists of yellow areas surrounding lesions on the leaf blades.

Both necrotic and chlorotic lesions result in decreased capacity for photosynthesis which leads to plant stress and ultimately yield loss. Wheat spikes and the kernels can also be affected by this fungus which then can cause red smudge, a disease of the seeds. By using crop rotation, reduced tillage practices or application of fungicides, tan spot outbreaks can be controlled. However, fungicides can leave residues and stubble burning and reduced tillage can increase the risk of soil erosion. Therefore, the use of resistant varieties is the most effective way to control tan spot.

Identification of new resistance sources and building up more resistance genes in a cultivar are very important for better genetic control. But selection of genotypes by using classical genetics and breeding methods is very time consuming. Over the past years, development of molecular markers that are closely associated with resistance genes is considered

to be an effective way to select for desirable traits (Gupta et al., 1999; Huang et al., 2000). Some types of molecular markers developed to date that have been used for genetic mapping in wheat include restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and microsatellites also known as simple sequence repeats (SSRs).

For the development of genetic linkage maps, the use of RFLPs (Chao et al., 1989; Kam-Morgan et al., 1989) and RAPDs (Devos and Gale, 1992), is very limited because of their low level of polymorphism. On the other hand, the markers that have been used broadly for wheat gene mapping are AFLPs and SSRs (Huang et al., 2000; Hartl et al., 1999; Singrün et al., 2004; Schmolke et al., 2005; Mohler et al., 2005). But, SSR markers are used more frequently than other markers because of their higher level of polymorphism, repeatability and PCR-based amplification (Röder et al., 1998; Gupta et al., 1999; Huang et al., 2004). During recent years, single nucleotide polymorphism (SNP) markers have gained attention in molecular genetics and plant breeding because of their abundance in genomes (more than 1 per 1000 bases), ease of use and amenability to high-throughput automation.

The wheat-*P. tritici-repentis* pathosystem relies on pathogen produced necrotrophic effectors (NEs) that are recognized in an inverse gene-for-gene manner to cause disease. The wheat genes *Tsn1* and *Tsc2* confer sensitivity to the NEs Ptr ToxA and Ptr ToxB, respectively, and both the *Tsn1*-Ptr ToxA and *Tsc2*-Ptr ToxB interactions are known to play significant roles in the development of tan spot (Lamari and Bernier, 1989; Faris et al., 1996; Friesen and Faris, 2004). Most studies have been conducted in hexaploid wheat and, while tan spot is known to cause significant losses in durum wheat as well, less is known about how host-NE interactions

affect disease development. The major objective of this study is to determine the roles of the *Tsn1* and *Tsc2* genes in conferring susceptibility of durum wheat to tan spot.

LITERATURE REVIEW

The disease

Pyrenophora tritici-repentis (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem, is a necrotrophic pathogen and the causal agent of tan spot of wheat. It falls under the phylum: Ascomyta, Class: Dothideomycetes, Family: Pleosporaceae, Genus: *Pyrenophora*- anamorph is *Drechslera*, causing leaf spots in cereals and grasses. During the fall and winter the fungus develops one-loculed, black, raised fruiting bodies (pseudothecia) on wheat residue. Sac-like asci (double walled) are formed inside a pseudothecium containing sexual spores (ascospores) with eight ascospores per ascus. Ascospores are the primary inoculum for infection, and infection by ascospores can be seen as lesions found on lower leaves in late winter or early spring wheat residue. Conidia represent the secondary or repeating inoculum for tan spot in a wheat field, and are formed on conidiophores in the lesion on a leaf. Conidiophores are olive-black with a swollen base, and conidia are subhyaline and cylindrically shaped with 4-6 septa (Ellis and Waller, 1976).

Pathogen virulence

Lamari and Bernier (1989) established a *P. tritici-repentis* pathotype classification system. According to that classification system, pathotype 1 produced necrosis and chlorosis, pathotype 2 produced only necrosis, pathotype 3 produced only chlorosis and pathotype 4 was avirulent. The first wheat differential hosts used to characterize these pathotypes were Glenlea and 6B365. Pathotype 1 causes necrosis on Glenlea and chlorosis on 6B365, pathotype 2 causes only necrosis on Glenlea and pathotype 3 causes only chlorosis on 6B365.

It was later found that Norstar and 6B699 (hexaploid wheat genotypes), when inoculated with pathotype 1, developed both necrosis and chlorosis symptoms, necrosis with pathotype 2,

and chlorosis with pathotype 3, which further demonstrated that both host and pathogen harbored loci independently controlling necrosis and chlorosis (Lamari and Bernier, 1989). To fully characterize *P. tritici-repentis* isolates, the pathotype classification system was not sufficient because of the identification of an isolate from Algeria that could cause chlorosis on genotypes that pathotype 3 could not (Lamari et al., 1995).

This led to race-based classification system (Lamari et al., 1995) where Algerian isolates were considered as race 5 and 6B662 was the differential line adopted for this race. Now, pathotypes 1 to 4 were considered as races one to four and the differential lines remained the same, 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 the virulences of races 2, 3 and 5. It has also been reported by Ali et al. (2010) that *P. tritici-repentis* isolates from Arkansas caused necrosis on the race 2 differential Glenlea, but did not produce the necrotrophic effector (NE) known as Ptr ToxA. Therefore, the current classification system needs to be transformed because the Arkansas isolates does not conform to the current race classification system.

Necrotrophic effector production

P. tritici-repentis produces three NEs, i.e. Ptr ToxA, Ptr ToxB and Ptr ToxC, and the following table (Table 1) shows the eight current races of *P. tritici-repentis,* the NEs they produce and their symptoms on host differentials.

Table 1.The eight current races of *Pyrenophora tritici-repentis* and the NEs that they produce.

Race	NEs produced	Host Differentials			
		Salamouni	Glenlea	6B662	6B365
1	Ptr ToxA Ptr ToxC	_[a]	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

The “-[a]” indicates that the host differentials develop resistant symptoms in response to *P. tritici-repentis*.

As shown in Table 1, races 3 and 5 are known to produce Ptr ToxC and Ptr ToxB which cause chlorosis only on the hexaploid wheat differential lines 6B662 and 6B365, but Gamba and Lamari (1998) showed that races 3 and 5 also produced necrosis (as opposed to chlorosis) on the durum wheat lines 4B-160 and Coulter suggesting that some race 3 and race 5 isolates may produce NEs in addition to Ptr ToxC and Ptr ToxB. Therefore, to make the current classification

system more accurate, it has been suggested to add durum lines 4B-160 and Coulter to the set of differentials (Singh et al., 2008, 2010)

Host gene-NE interactions in the wheat-*Pyrenophora tritici-repentis* system

The inverse gene-for-gene system

Plants have developed an innate immune system to defend themselves from invading pathogens. Interaction of a host with a biotrophic pathogen leads to a gene-for-gene relationship (Flor 1956) in which a pathogen produced effector (avirulence gene product) is recognized by a dominant resistance gene resulting in an incompatible (resistant) interaction. Compatible interactions lead to susceptibility because the plant lacks the ability to recognize the effector. Necrotrophic specialist pathogen systems, such as the wheat–*P. tritici-repentis* system, involve the recognition of NEs by dominant host sensitivity genes. In this inverse gene-for-gene system, when an NE is recognized by a host sensitivity gene it leads to a compatible interaction and hence susceptibility, whereas the lack of NE recognition by the host results in an incompatible interaction and leads to resistance. In the absence of the sensitivity gene in the host or if the pathogen does not produce the NE, the result is a resistance response. Therefore, the wheat-*P. tritici-repentis* system is the inverse of classical host-biotrophic pathogen systems (Wolpert et al., 2002)

Tsn1-Ptr ToxA

Ptr ToxA was the first *P. tritici-repentis* NE to be discovered, and it induces necrosis in wheat genotypes (Ballance et al., 1989; Ciuffetti et al., 1997; Tomas et al., 1990; Tuori et al., 1995). It is a small secreted protein (~13.2 kDa) and the product of a single copy gene which encodes a pre-pro protein (Ballance et al., 1996; Ciuffetti et al., 1997). The pre region contains the signal peptide (residues 1 to 22) which targets the protein to the secretory pathway. The pro-

region (N-domain residues; residues 23 to 60) cleaves before the secretion of the mature ToxA (C-domain; residues 61 to 178) (Tuori et al., 1995; Ciuffetti et al., 1997). Tuori et al. (2000) showed that the N-domain is necessary for the proper folding for the formation of disulfide in the C-domain which stabilizes the active conformation of ToxA. The ToxA protein has a beta-barrel fold, a single alpha helix and the solvent exposed amino acid 137-146 with the arginyl-glycyl-aspartic acid (RGD) motif (Sarma et al., 2005). ToxA also contains a stretch of 10 amino acids (aa 137 to 146) which shares similarity with the mammalia protein vitronectin (Manning et al., 2004; Suzuki et al., 1985) and this vitronectin-like sequence of ToxA includes an RGD cell-attachment motif which is required for NE-induced cell death (Manning et al., 2004; Meinhardt et al., 2002).

It has been shown that mutagenesis of the RGD motif to arginyl-glycyl-glutamate (RGE) completely stops the protein internalization and toxic activity. Therefore, the RGD motif is important for receptor binding and internalization into sensitive wheat cells (Manning et al., 2008). ToxA binds to receptors on the plasma membrane via RGD-containing loop, and after it enters into the plasma membrane it internalize into endosomes. After releasing from the endosomes it enters the cytosol and targets the chloroplast. After entering into the chloroplast it interacts with ToxA binding protein 1 (ToxABP1) (Manning et al., 2007). Interaction of ToxA with ToxABP1 could prevent regeneration of PS components, disrupt the electron transport chain and, reactive oxygen species accumulation (Manning and Ciuffetti, 2005; Manning et al., 2009) but, ToxABP1 is not responsible for the specificity of Ptr ToxA-induced necrosis, but involved in downstream events leading to cell death. Homologs of ToxABP1 have been found in the chloroplast of *Arabidopsis thaliana* (Thf1) and in the cyanobacteria *Synechocystis* (Psb29).

Thf1 and Psb29 plays important role in thylakoid formation and PSII biogenesis (Keren et al., 2005).

Tai et al. (2007) conducted Y2H analyses and showed that two domains are required for proper ToxA function. The RGD motif for target recognition and amino acids E145 to D149 for ToxA-ToxA oligomerization and plastocyanin association with ToxA. Plastocyanin is a component of the electron transport chain of photosynthesis. With the help of cDNA library screening, plastocyanin was identified as a host target protein of ToxA. The ToxA-plastocyanin interaction could affect photosystem and blocking of electron flow might lead to formation of ROS which leads to cell death (Manning and Ciuffetti, 2005; Van Breusegem and Dat, 2006).

Lu et al. (2014) conducted Y2H and in-vitro co-immunoprecipitation assays to confirm the existence of a physical interaction between PR-1-5 and ToxA. It has been shown that interaction is governed by two surface-exposed asparagine residues: the N102 residue in ToxA and the N141 residue in PR-1-5. The native PR-1-5 protein is expressed differentially in response to ToxA treatment between ToxA sensitive and insensitive wheat lines. When the ToxA-N102A mutant and PR-1-5-N141A mutant were infiltrated into ToxA insensitive lines, results showed that ToxA-N102A mutant fails to induce necrosis and PR-1-5-N141A mutant showed reduce necrosis activity. The above results indicate that PR-1-5 is a target of ToxA. It was hypothesized that because of certain stress conditions, the PR-1-5 proteins can be induced in sensitive wheat lines. The stress-induced PR-1-5 proteins can then be targeted by ToxA to initiate an initial interaction which could activate the *Tsn1*-controlled cell death pathway.

Tsn1, which is a single locus present on wheat chromosome arm 5BL, is sensitive to Ptr ToxA (Faris et al., 1996). Faris et al. (2010) cloned *Tsn1* and showed that it was a member of the NB-LRR class of R genes which usually confer resistance to biotrophic pathogens (Eitas and

Dangl 2010). The NE sensitivity gene *LOV1* from *Arabidopsis thaliana* confers sensitivity to the effector called victorin produced by the oat pathogen *Cochliobolus victoriae* and the *Pc* from *Sorghum bicolor* confers sensitivity to the effector known as Pc-toxin produced by *Periconia circinata*. Both *LOV1* and *Pc* possess NB-LRR domains as well (Lorang et al., 2007; Nagy and Bennetzen 2008). However *Tsn1* is unique from these because it also contains an S/TPK domain (serine/threonine protein kinase) which is important for function along with the NB-LRR domain. The *Rpg5* stem rust R gene from barley also possesses S/TPK and NBS-LRR domains but *Tsn1* and *Rpg5* do not share ancestory (Faris et al., 2010). *Tsn1* expression is regulated by the circadian clock and light as putting plants under the dark show low *Tsn1* expression (Faris et al., 2010).

Lamari and Bernier (1989) concluded that resistance to the fungus and insensitivity to Ptr ToxA were conferred by a recessive gene. In other words, sensitivity to Ptr ToxA and susceptibility to the fungus were controlled by the same dominant gene. Faris et al. (1996) conducted molecular mapping experiments using RFLP analysis in F_3 families, and the families segregated in the ratio of 15:29:14 homozygous insensitive/segregating/homozygous sensitive, suggesting that a single gene was responsible for insensitivity. The RFLPs flanked the insensitive locus at distances of 5.7 and 16.5 cM on chromosome 5B. The above analyses indicated that gene resides on the long arm of chromosome 5B and designated as *tsn1*.

In the above paragraphs, it has been shown that *Tsn1* interacts with ToxA and causes susceptibility. But this is not always the case, sometimes the *Tsn1*-ToxA interaction is significant in disease susceptibility and sometimes it is not. Depending on the host background *Tsn1* can play a significant role (Cheong et al., 2004; Singh et al., 2008), a minor role (Friesen et al., 2003; Chu et al., 2008), or no role at all (Faris and Friesen 2005). To evaluate the significance of the

Tsn1-ToxA interaction, Faris et al. (2012) used a hexaploid wheat population derived from a cross between the landrace Salamouni and the Canadian bread wheat Katepwa (SK population) consisting of 121 RILs, and evaluated the population for reaction to two isolates of race 1 (Pti2 and Asc1) and one isolate of race 2 (86–124), all of which produce the necrotrophic effector Ptr ToxA. A total of four QTLs were identified and designated *QTs.fcu-5B*, *QTs.fcu-5D*, *QTs.fcu-7B*, and *QTs.fcu-7D*. Among the four QTL identified, *QTs.fcu-5B* was associated with Pti-2 and 86–124 and had the largest effects due to the *Tsn1*–Ptr ToxA interaction. Therefore, *QTs.fcu-5B* is considered a susceptibility QTL due to Ptr ToxA sensitivity. On the contrary, Faris and Friesen (2005), evaluated an RIL population from a cross between the common wheat varieties Grandin and BR34 (BG population) and showed that the *Tsn1*-Ptr ToxA interaction was not a significant factor in the development of tan spot. It could be due to a decrease in NE activity by the race-nonspecific resistance mechanism contained by BR34.

***Tsc2*-Ptr ToxB**

Ptr ToxB is also a small secreted protein (6.5 kDa), and it induces chlorosis in sensitive wheat genotypes (Ciuffetti et al., 2010). It was firstly described that culture filtrates of race five isolates produce chlorosis-inducing NE called Ptr ToxB (Strelkov et al., 1999). Strelkov et al. (2002) and Lamari et al. (2003) identified additional races (races 6, 7 and 8) which also produce Ptr ToxB in combination with other NEs. Races 3 and 4 also contain *ToxB* homologs (Strelkov and Lamari 2003; Martinez et al., 2004) but do not produce any ToxB-related symptoms (Lamari et al., 1995). Homologs of *ToxB* in races 3 and 4 are different because of sequence variation and single copy gene presence.

It has been shown that the *ToxB* open reading frame (ORF) is 261 bp in length present in isolates of races 5 and 6 which translates into 87-aa pre-protein with a 23-aa signal peptide

(Martinez et al., 2001; Strelkov and Lamari, 2003). Martinez et al. (2004) showed that *ToxB* homologs of race 4 had 86% similarity to race 5 *ToxB* and is transcribed at low levels (Amaike et al., 2008). The lack of chlorosis-inducing activity by race 3 Ptr ToxB as compared to race 5 Ptr ToxB is due to differences in the flanking upstream sequence and changes in the start codon (Strelkov et al., 2006). The purified form of Ptr ToxB is hydrophilic in nature and stable when exposed to heat. It has also been hypothesized that Ptr ToxB might act in the apoplast and have some common characteristics shared with apoplastic effectors (Fiqueroa et al., 2015).

To induce symptoms it has been shown that multiple copies of *ToxB* genes are required. (Lamari et al., 2003; Martinez et al., 2004). Race 5 isolates, DW7 from North Dakota (Ali et al., 1999) and Alg3-24 from Eastern Algeria (Strelkov and Lamari, 2003), have been used to clone *ToxB* loci. When six *ToxB* loci from DW7 were aligned, they showed high level of conservation, including identical *ToxB* ORFs (Martinez et al., 2004) and these regions were flanked by retrotransposon-like sequences. Some loci also contained inversions and inverted repeats. Amplication of *ToxB*-containing loci is the result of unequal crossing over with similar sequences in the genome. Strelkov et al. (2002, 2006) and Martinez et al. (2004) showed that more copies led to increased virulence, hence copy number variation of *ToxB* is linked to virulence. So, increased production of *ToxB* can be responsible for an increase in virulence (Strelkov et al., 2006; Amaike et al., 2008).

It has also been shown that the higher levels of *ToxB* transcription correlate with more rapid development of appressoria (Amaike et al., 2008). Andrie et al. (2008) identified homologs of *ToxB* in *Cochliobolus*, *Alternaria*, and other members of the genus *Pyrenophora*. Homologs of *ToxB* (*PbToxB*) are present in single or multiple copies depending on the isolate, in *Pyrenophora bromi*, the causal agent of brown leaf spot of smooth bromegrass (*Bromus*

inermis). The Pb ToxB protein shares 80% similarity with *ToxB* but the genes are expressed at lower levels (R. M. Andrie and L. M. Ciuffetti, unpublished data). There is no symptom development in leaves of bromegrass treated with heterologously expressed Pb ToxB proteins, but these proteins induce chlorosis on ToxB-sensitive wheat cultivars. This suggests that an increase in copy number and gene expression could increase the pathogenicity of *P. bromi* on wheat. Silencing of the *ToxB* gene in a wild-type race 5 isolate of *P. tritici-repentis* showed that Ptr ToxB is not only a pathogenicity factor essential for tan spot development, but also a virulence factor contributing quantitatively to disease severity (Aboukhaddour et al., 2012).

Sensitivity to Ptr ToxB was shown to be controlled by single dominant gene in the host (Orolazo et al., 1995). Gamba et al. (1998) also showed that sensitivity to Ptr ToxB was governed by single dominant gene, by using F_1 and F_2 progeny of a cross between the line Katepwa (race 5 susceptible) and race 5 resistant line Erik. Singh et al. (2008) showed that resistance was controlled by single dominant gene but other research showed that resistance was conferred by a single recessive gene (Singh et al., 2010). Abeysekara et al. (2010) showed that sensitivity to Ptr ToxB was dominant, which agreed with (Singh et al., 2008). Disagreement between the studies could be due to the environmental factors that could influence the mode of action or the different genetic backgrounds of the parents.

Friesen and Faris (2004) mapped *Tsc2* to the distal end of the short arm of chromosome 2B by using the ITMI mapping population, which was derived from the synthetic hexaploid wheat W-7984 and the hexaploid wheat variety Opata 85. The *Tsc2* locus defined a major QTL linked with susceptibility to race 5, and it explained 69% of the phenotypic variation in disease development. Saturation mapping of the *Tsc2* genomic region was done by Abeysekara et al., (2010) using lines derived from the hexaploid wheat lines Salamouni (Ptr ToxB insensitive) and

Katepwa (Ptr ToxB sensitive) (SK population). Rice and *Brachypodium* genomes were used as a reference genomes for development of markers. *Brachypodium distachyon* chromosome 5 and rice chromosome 4 were perfectly colinear with the wheat *Tsc2* region between markers XTC339813 and XBF202540.

Abeysekara et al. (2010) showed that susceptibility to chlorosis in the SK population was due to the *Tsc2*–Ptr ToxB interaction, which accounted for 54 % of the variation in disease caused by the race 5 isolate DW5. Therefore, a compatible *Tsc2*-Ptr ToxB interaction played a major role in conferring susceptibility to race 5 isolates of *P. tritici-repentis*.

***Tsc1*-Ptr ToxC**

Ptr ToxC is a non-ionic, polar, low molecular mass molecule which induces chlorosis in sensitive wheat genotypes. Ptr ToxC is produced by races 1 and 3, and according to Lamari et al. (1991), resistance to chlorosis caused by races 1 and 3 was controlled by a dominant gene in some specific crosses, but further experiments (Gamba and Lamari 1998; Gamba et al., 1998) showed that resistance to race 3 was controlled by a single recessive gene. Singh and Hughes (2006) showed that resistance to race 1-induced chlorosis was dominant. These contradictory results indicated that chlorosis induction by races 1 and 3 is continuous and impacted by environmental conditions (Strelkov et al., 2002).

There is not much evidence regarding the interaction of *Tsc1*-Ptr ToxC but Faris et al. (1997) carried out QTL analysis using the ITMI population derived from the common wheat variety Opata 85 and synthetic hexaploid wheat W-7984 and identified a major QTL located on short arm of chromosome 1A associated with chlorosis induction and labeled it as *QTsc.ndsu-1A*. This QTL had major effects for resistance to chlorosis in adult plants as well as seedlings. Effertz et al. (2002) concluded that the Ptr ToxC insensitivity gene, *tsc1*, was 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.

Qualitative resistance genes

Tsr2

Singh et al. (2006) mapped the *Tsr2* resistance locus to the long arm of chromosome 3B from a population of RI lines obtained from a cross between a resistant *T. turgidum* ssp. *turgidum* accession (PI 352519) and the susceptible durum variety Coulter using molecular markers. It was assumed that the chromosome 3B susceptibility loci in LDN and Coulter were the same. By evaluating F_2 plants obtained from *T. turgidum* ssp. *turgidum* x PI 352519 Coulter cross, it has been suggested that Coulter, LDN and other tetraploids harbor a gene on chromosome 3BL that confers susceptibility by race 3 isolate *Ptr* 331-9.

Tsr3

The synthetic hexaploids XX41, XX54 and XX110 derived by crossing an AB-genome tetraploid, *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, were resistant to tan spot caused by ASC1b (Tadesse et al., 2006). Further it was concluded that XX41 and XX110 contained single recessive genes and XX45 harbored a single dominant resistance gene. Tadesse et al. (2007) conducted molecular mapping experiments and showed that the resistance genes, designated as *tsr3a, Tsr3b, tsr3c* were located on the short arm of chromosome 3D.

However, Faris et al. (2013) raised some concerns over their work because 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 produces Ptr ToxA. No indication of a chromosome 5B locus associated with resistance/susceptibility was reported by Tadesse et al. (2006) even though they mentioned that LDN was susceptible. It must have been assumed by them that the susceptibility was due to the lack of the 3D gene from *Ae*. *tauschii*. So, questions have arisen over why XX41 and XX45 were not susceptible to ASC1b given the fact that they should harbor *Tsn1*. There could be several reasons why the *Tsn1*–Ptr ToxA interaction is not relevant in these genetic backgrounds or their strain for ASC1b did not express the ToxA gene, or maybe it was expressed at very low levels. Tadesse et al. (2007) conducted molecular mapping indicated that all three resistance genes reside on the short arm of chromosome 3D near loci identified by the marker *Xgwm2* for the wheat lines XX41, XX45, and XX110.

Tsr4

Tadesse et al. (2006) showed that Salamouni was resistant to ASC1a, whereas Chinese Spring was susceptible, and segregation ratios in an F_2 population indicated a single recessive gene conferring resistance. Tadesse et al., (2006) concluded that the recessive resistance in Salamouni could be due to the lack of a susceptibility gene harbored by Chinese Spring, and an inoculation test of Chinese Spring and Chinese Spring aneuploids showed that Chinese Spring nullisomic 3A plants were resistant to ASC1a, which provides strong evidence that resistance in Salamouni was actually due to lack of gene for susceptibility. Tadesse et al. (2010) 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

approximately 15 cM proximal to the marker *Xgwm2* on the short arm of chromosome 3A and designated as *tsr4*. It was also noted that the marker *Xgwm2* detects loci on both chromosomes 3A and 3D. Because *tsr3* also mapped just proximal to the *Xgwm2* locus on chromosome 3D (Tadesse et al., 2007), it is possible that *tsr3* and *tsr4* are may be homoeologous genes.

Tsr5

With the help of DW13 spore inoculation, a single recessive gene conferring resistance to necrosis was identified and then mapped to the long arm of durum chromosome 3B (Singh et al., 2006, 2008). *Tsr5* was the name designated to the gene and it mapped approximately 8.3 cM distal to *Tsr2* which suggests that *Tsr2* and *Tsr5* are not the same gene and necrosis produced by the race 3 isolate Ptr 331-9 and the race 5 isolate DW13 is due to different virulence factors.

Quantitative resistance

The first tan spot QTL mapping experiment was performed by Faris et al. (1997) to identify tan spot resistance loci and the first QTL with major effects for resistance to chlorosis by races 1 and 3 was reported on the short arm of chromosome 1A, designated as *QTsc.ndsu-1A* and a minor QTL was reported on the short arm chromosome of 4A. Faris and Friesen (2005) were the first to identify race non-specific tan spot resistance QTLs, which were identified on the short arm of chromosome 1B and the long arm of chromosome 3B. 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). The QTL *QTs.fcu-1B* explained 13 to 29% of the variation and *QTs.fcu-3B* explained 13 to 41% of the variation.

Singh et al. (2008) evaluated a population of RI lines obtained from a cross between WH542 (resistant) and HD29 (susceptible) by using a race 1 isolate. It was revealed that a 5B QTL explained 18% of the phenotypic variation and was tightly linked to *Tsn1* locus indicating that the effects of this QTL were due to a compatible *Tsn1*–Ptr ToxA interaction.

Chu et al. (2008) 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. It was reported that the *Tsn1*-Ptr ToxA interaction was associated with disease caused by Ptr ToxA-producing isolates Pti2 (race 1) and 86-124 (race 2). Race non-specific QTLs on chromosome arms 2AS and 5BL were linked with resistance to all isolates tested and explained about 14 to 26% of the variation. Chu et al. (2010) also indicated five QTLs associated with resistance to isolate Pti2 (race 1) and isolate 86-124 (race 2) after the evaluation of tetraploid wheat doubled haploid population derived from the durum wheat variety Lebsock and the *T. turgidum* ssp. *carthlicum* accession PI 94749. Two of the QTLs were located on chromosomes 5A and 3A and chromosomes 3B and 7B had one QTL each.

Sun et al. (2010) evaluate resistance to a race 1 isolate (AZ-00) in a population of RI lines obtained from a cross between the Chinese landrace Wangshuibai (resistant) and the Chinese breeding line Ning7840 (susceptible) by using a QTL approach. One major QTL on the short arm of chromosome 1A was identified that explained 39% of the phenotypic variation. 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.

Septoria nodorum blotch (SNB)

The disease

Parastagonospora nodorum is a necrotrophic fungal pathogen that causes Septoria nodorum blotch (SNB), a foliar and glume disease of both common wheat and durum wheat. SNB is an economically important disease in many wheat growing areas throughout the world because of its potential to cause significant yield losses and negatively impact grain quality. It has been suggested that resistance to SNB is governed by multiple genes and also influenced by environmental factors (Nelson and Gates, 1982; Wilkinson et al., 1990; Bostwick et al., 1993; Wicki et al., 1999). In recent years, significant progress has been made through a series of studies, which revealed that NEs are major determinants of SNB disease specificity in the *P. nodorum*-wheat pathosystem.

Parastagonospora nodorum-wheat interactions

The wheat-*P. nodorum* pathosystem and wheat-*P. tritici-repentis* pathosystem both follow the inverse gene-for-gene relationship. In both systems sensitivity is conferred by single dominant gene. Recent studies suggest that the *P. nodorum*-wheat pathosystem is controlled by at least eight NEs (SnToxA, SnTox1, SnTox2, SnTox3, SnTox4, SnTox5, SnTox6 and SnTox7) that interact directly or indirectly with dominant host genes (*Tsn1*, *Snn1*, *Snn2*, *Snn3, Snn4, Snn5, Snn6, Snn7*) to induce disease (Friesen et al., 2006, 2007, 2008; Liu et al., 2004, 2006; Abeysekara et al., 2009; Friesen et al., 2012; Gao et al., 2015; Shi et al., 2015).

The first *P. nodorum* NE to be identified was designated as SnTox1 and considered to be proteinaceous (Liu et al., 2004). The corresponding dominant sensitivity gene *Snn1* in wheat was mapped at the distal end of the short arm of chromosome 1B using the ITMI population (Liu et al., 2004). QTL analysis indicated that the *Snn1* locus explained 58% of the phenotypic variation

in SNB susceptibility. Therefore, a compatible *Snn1*-SnTox1 interaction played an important role in causing disease in that population.

The same isolate was used by (Liu et al., 2004) to inoculate the BG population (Friesen et al., 2006; Liu et al., 2006). The results showed a major QTL on chromosome arm 5BL that explained 62% of the phenotypic variation in this population. This QTL also coincided with the *Tsn1* locus (Friesen et al., 2006; Liu et al., 2006) which confers sensitivity to the *P. tritici-repentis* NE Ptr ToxA (Faris et al., 1996; Haen et al., 2004; Lu et al., 2006). Liu et al. (2006) showed that *Tsn1* conferred sensitivity to both Ptr ToxA and the new NE produced by *P. nodorum* by using *Tsn1*-disrupted mutants. With the *P. nodorum* genomic sequence available, a homologous gene sharing 99.7% similarity to the *Ptr ToxA* gene was identified in the *P. nodorum* genome and designated *SnToxA*. Subsequent studies showed that *P. tritici-repentis* acquired the *ToxA* gene from *P. nodorum* through a horizontal transfer event that led to the emergence of a major wheat disease, tan spot (Friesen et al., 2006)

Molecular markers in breeding

Molecular markers are used to generate linkage maps, which are used effectively to map genes of interest. Molecular markers detect known locations within a genome that harbor mutations or alterations in the genome. DNA markers are classified into: low-throughput, hybridization-based markers for example, RFLPs, medium-throughput, PCR-based markers such as RAPDs, AFLPs and SSRs and high-throughput sequence-based markers such as SNPs or genotype-by-sequencing (GBS) markers.

RFLPs were first used for linkage mapping in humans (Botstein et al., 1980). For plant genome mapping RFLPs were considered to be the first generation DNA markers. They are based on Southern blotting. RFLP markers are co-dominant, locus specific and highly

reproducible. The *Tsn1* gene was mapped on chromosome 5B by Faris et al. (1996) using RFLPs. However RFLPs are expensive, time consuming and laborious. Devos and Gale (1992) were the first to use RAPDs for wheat genetic linkage mapping. These are dominant markers, detect high levels of polymorphism, and do not require hybridization and blotting steps like RFLPs. However, their reproducibility level is very low due to non-specific binding of random primers. They cannot detect allelic differences in heterozygotes. AFLP markers are highly reproducible, dominant in nature, and not require prior sequence information. However, content for bi-allelic marker is low and not amenable to automation.

The advent of SSR markers overcame many of the limitations of the above-mentioned DNA marker technologies. They are highly polymorphic, highly reproducible, amenable to automation, locus specific, co-dominant in nature and require small amounts of DNA (Röder et al., 1998; Gupta et al., 1999; Huang et al., 2004). They have been abundantly used for QTL mapping and genetic linkage mapping in wheat. SSRs consist of tandem repeats of short nucleotide motifs, di-, tri- and tetra-nucleotide repeats, e.g. (GT)n, (AAT)n and (GATA)n. These repeats are extensively distributed throughout the genomes of plants and animals. The copy number of these repeats is a source of polymorphism in plants. Despite having so many advantages, these markers still show some limitations in that they are time-consuming and laborious.

During recent years, SNP markers have gained attention in molecular genetics and plant breeding because of their abundance in genomes (more than 1 per 1000 bases), no gel based assays are required and they are suitable to high-throughput automation. SNPs are biallelic in nature and less polymorphic than SSRs, but these drawbacks are compensated by the above

mentioned advantages of SNPs. These markers are powerful tool for MAS (marker assisted selection) of disease resistant wheat lines (Jafar et al., 2012).

Materials and methods

Plant materials

A segregating population of 200 recombinant inbred lines (RILs) was developed from a cross between the durum cultivars 'Altar 84' and 'Langdon'. The RILs were developed by advancing the plants to the F_7 generation by single seed descent (SSD). Plants were grown in cones containing SB100 (Sun Gro Sunshine; Sun Gro Horticulture, Vancouver, BC) soil mix with 10 to 20 granules of Osmocote fertilizer (Scotts Company LLC, Marysville, OH) added to each cone. For disease evaluations and NE infiltrations all plants were grown in the greenhouse at an average temperature of 21 °C with a 16 h photoperiod. Preliminary experiments indicated that Altar 84 was sensitive to Ptr ToxB and insensitive to Ptr ToxA and Langdon was sensitive to Ptr ToxA and insensitive to Ptr ToxB.

Disease evaluations

For *P. tritici-repentis* disease evaluation, a subset of AL population consisting of 127 RILs plus both parents, were screened with race 2 isolates 86-124, L513-119 and L 13-35, which are known to produce Ptr ToxA, and the race 5 isolate DW5 which is known to produce Ptr ToxB. Isolates were grown on V8-potato dextrose agar (Difco PDA; Becton, Dickinson and Company, Sparks, MD) plates for 5 to 7 d in the dark, and inoculum was prepared as described in Lamari and Bernier (1989) and Ali et al. (2010). Parents and the RIL population were planted in a completely randomized design (CRD) consisting of three replicates for conidial inoculations. Each replicate consisted of a single cone per line with three plants per cone placed in racks of 98 cones. The tan spot-susceptible wheat variety 'Jerry' was planted in the borders of each rack to

reduce any edge effects. Plants were inoculated until runoff at the two-to three-leaf stage with 3000 spores per mL and 2 drops of Tween20 (polyoxyethylene sorbitan monolaurate; J.T. Baker Chemical Co., Phillipsburg, NJ) per 100 ml of inoculum. Inoculated plants were placed in a mist chamber with 100% relative humidity at 21 °C for 24 hours and then were subjected to 6 d of incubation in the growth chamber at 21 °C under a 12 h photoperiod. Inoculated plants were rated using a 1 to 5 lesion type scale (Lamari et al., 1989) at 7 d post-inoculation, where 1 is resistant with small, dark brown to black spots without any surrounding chlorosis or necrosis, and 5 is susceptible with dark brown or black centers which may or may not be distinguishable (most lesions consist of coalescing chlorotic or tan necrotic zones).

For *P. nodorum* disease evaluations, the AL population was screened with *P. nodorum* isolate Sn2k which is known to produce SnToxA. The 127 RILs were then inoculated with conidia for disease evaluation. Inoculum production and inoculation methods were described in Liu et al. (2004). After inoculation, plants were placed in a mist chamber with 100% relative humidity at 21 ºC for 24 h, and then moved to a growth chamber at 21 ºC with a 12 h photoperiod. Disease evaluation was carried out at 7 days after inoculation, by scoring lesions on the second leaf using the 0-5 scale described by Liu et al. (2004).

Necrotrophic effector (NE) production and infiltration assays

For NE production, one colony from a streak plate of a yeast strain *Pichia pastoris* X33 was taken and then mixed in 10 ml of yeast-extract peptone dextrose (YPD) media. One yeast colony was placed in a 50 ml test tube containing 10 ml of YPD plus and placed on a shaker at 200 rpm at 30 °C overnight. The cultures were then centrifuged at 3000 rpm for 2 min and the supernatant extracted and used for infiltrations. Infiltrations were conducted using a 1-ml needleless syringe, by holding the secondary leaf between the thumb and forefinger of one hand

and with the other hand pressing the syringe against the leaf tissue until 2 to 3 cm of leaf surface was infiltrated. The boundaries of the infiltrated region were marked using a non-toxic permanent marker. The infiltrated plants were then placed in the growth chamber at 21 °C with a 12-h photoperiod. Plants were evaluated 4 d after infiltration and scored as sensitive or insensitive based on the presence or absence of chlorosis for Ptr ToxB or necrosis for Ptr ToxA

Genotyping

Simple sequence repeat marker identification

Deoxyribonucleic acid (DNA) was isolated from the plant tissues of the 127 RILs of the AL population and of the parental lines as described by Faris et al. (2000). Previously published genetic and physical maps of A and B genome chromosomes were surveyed to identify SSR markers. SSR primers were selected from the following sets: MAG (Xue et al., 2008), GWM (Röder et al., 1998), WMC (Somers et al., 2004), HBG (Torada et al., 2006), CFD (Sourdille et al., 2004) and BARC (Song et al., 2005). SSR primer sets were used to amplify the parental DNA using polymerase chain reaction (PCR) conditions as outlined in Lu and Faris (2006). PCR amplifications were performed in 10 ul reactions consisting of 100 ng of DNA template, 1.5 mM $MgCl_2$, 0.125 mM dNTPs, 4 pmol of primers and 1 unit of Taq DNA polymerase. The PCR conditions were 94°C for 4 mins, followed by 35 cycles of 94°C for 30s, the appropriate annealing temperature for 30 s, and 72 °C for 1 min. The annealing temperature of each primer was obtained from Graingenes website (http://wheat.pw.usda.gov/GG2/index.shtm). Fragments were electrophoresed on 6% polyacrylamide gels which were made using 46 ul of H_2o, 6 ul 10X TBE (Tris-borate-EDTA), 9 ul acrylamide/bis-acrylamide, 40 ul tetramethylethylenediamine (TEMED) and 350 ul of 10% ammonium persulfate (APS). Gels were stained with GelRed (Biotium, inc.) and visualized with a Typhoon 9410 variable mode imager (GE Healthcare,

Waukesha, WI). Markers that revealed polymorphisms between the parents were then used to genotype the 127 lines of the AL population.

Single nucleotide polymorphism (SNP) detection and analysis

To perform the genotyping of the AL population, 138 RILs were selected. Genotyping was performed using the wheat 9K SNP array (Cavanagh et al., 2013) on an Illumina iScan instrument (Illumina, San Diego, CA). DNA was first quantified using a Nanodrop spectrophotometer ND-1000 (Thermo Fisher Scientific, Wilmington, DE) and then amplified by using the Infinium HD assay ultra-reagents: MA1 (Multi-Sample Amplification 1 mix), MA2 (Multi-Sample Amplification 2 mix), MSM (Multi-Sample Amplification Master Mix), and 0.1 N NaOH in MSA3 plates. First 20 ul of MA1 was dispensed in MSA3 plates. Next, 4 ul of DNA and 0.1 NAOH (4 ul) were added to the MSA3 plate. The plates were then sealed with a 96-well cap mat and vortexed for 1 minute at 1600 rpm. After vortexing, plates were centrifuged at 280×g for 1 minute and MSA3 plates were incubated at room temperature for 10 minutes. Next, 34 ul of MA2 and 38 ul of MSM were also added to the MSA3 plates and again the plates were vortexed and centrifuged as described above. After that, MSA3 plates were kept at 37˚C overnight in an Illumina hybridization oven for whole genome amplification.

On the second day, the amplified DNA was enzymatically fragmented by using the end point fragmentation method according to the ‘Infinium HD assay ultra-protocol’ to avoid the over fragmentation of the DNA sample. Plates containing the whole genomic amplified DNA were initially centrifuged at 50×g for 1 minute. In the next step, 25 ul of FMS (Infinium HD assay ultra-reagent) was added. The plates were again vortexed and centrifuged using similar conditions and the plates were kept at 37 ˚C for 1 hr. To precipitate the fragmented DNA, 50 ul of PM1 (Precipitation Solution) was added to the MSA3 plates and the plates were then vortexed

(16000 rpm for 1 minute) and incubated for 5 minutes at 37 ˚C and centrifuged at 50×g for 1 minute. Then, 155 ul of 100% 2-propanol was added to each well of MSA3 plates, which were then sealed with the dry cap mat and were inverted 10 times to mix the reagents. Plates were then incubated for 30 minutes at 40 ˚C. Following incubation, plates were centrifuged at 3,000×g for 20 minutes at 4 ˚C. The liquid was removed and the plates were left inverted at room temperature for 1 hour to allow the pellets to dry.

To resuspended the precipitated DNA, 23 ul of RA1 (resuspension, hybridization and wash solution) was added to each well of the MSA3 plate which were then sealed and placed for incubation at 48 ˚C in the Illumina hybridization oven. After that, the plates were vortexed at 1800 rpm for 1 minute and pulse centrifuged at 280×g. The resuspended DNA was loaded onto the bead chip for hybridization to the bead probes for 16-24 hr in the Illumina hybridization oven. On the third day, beadchips were washed with PB1 (reagent used to prepare BeadChips for hybridization) before staining to remove hybridized DNA fragments. The 'single base extension method' was used to identify SNPs across the hybridized DNA segment. In this method, only one nucleotide base was incorporated during the primer extension step where 2, 4-Dinitrophenol-labeled ddNTPs (ddCTP, ddGTP) and biotin labeled ddNTPs (ddATP, ddTTP) were used. Anti-streptavidin biotin, anti-Ab-dNTP, streptavidin-green, and anti-DNP-red were used for staining the chips. The green and red color peaks were recorded based on the incorporated ddNTPs.

After scanning of bead chip, the data was imported into genome software (Genome Studio Genotyping module V1.0) for analysis. An input file with '.dmap' extension, which contains the files that were used during scanning, was loaded to define the bead locations and raw data was generated. For processing of the raw data, a project file (*.bsc) was created in the genome studio software by using the output file (.idat) and manifest file (contains information

regarding SNP ID and annotation). The output file contains the signal intensities for the 127 samples (including both the parents) at each SNP locus. The samples were clustered into different groups based on their intensities, and the clusters were then analyzed using Genome Studio Genotyping module V1.0. In the next step, genotypes were assigned into significant clusters.

Linkage and quantitative trait loci (QTL) analysis

Linkage analysis was conducted using the computer program MapDisto 1.8.1 (Lorieux 2012) to generate linkage maps. Frist, marker grouping was done by using the command 'find groups' with a logarithm of odds (LOD) > 3.0 and an Rmax value = 30.0, this command divides the initial sequences into different groups. Next, the 'order sequence', 'check inversions', 'ripple order', and 'drop locus' commands were used to determine the best order for each group. The 'order sequence' command provides the best marker order based on the sum of adjacent frequencies (SARF). The 'ripple order' command compares the alternative orders along the linkage group by using a five locus sliding window (Lorieux 2102). The 'check inversion' command leads to fewer errors caused by large gaps within the linkage group (Lorieux 2012). The Kosambi mapping function (Kosambi 1944) was used to calculate the linkage distances.

The linkage data was used for further trait analysis by utilizing computer program QGene v.4.0 (Joehanes and Nelson, 2008). The phenotypic data was regressed on phenotypic data using composite interval mapping. An LOD threshold of 3.6 was determined by performing a permutation test with 1000 iterations. The homogeneity of variances among the three replicates were determined by Bartlett's χ^2 test using SAS program (SAS Institute Inc., Cary, NC). Mean separation of the genotypic means were determined by Fisher's protected LSD at an α level of 0.01.

Results

Marker analysis and linkage map construction

The parental lines of the AL population, Altar 84 and Langdon, were screened with 250 SSR primer pairs. Of these, 119 (47.6%) revealed polymorphisms between the parents and were used to genotype the AL population.

Use of the 9K SNP array yielded a total 833 polymorphic SNP markers. One CAPS marker (*HpyCh4*) that was developed based on the *Snn1* gene sequence on chromosome arm 1BS (Shi et al., unpublished) was added to the marker set along with the two phenotypic markers *Tsn1* and *Tsc2* (see below). Therefore, the initial marker dataset consisted of a total of 955 markers. After initial linkage analysis, a total of 111 markers including 19 SSRs and 92 SNPs were eliminated from the dataset because they were unlinked leaving a total of 844 markers in the dataset including 100 SSRs, 741 SNPs, one CAPS and two phenotypic markers.

These markers were assembled into 14 linkage groups that corresponded to the 14 durum wheat chromosomes (Figure 1) and spanned a total genetic distance of 2,207.15 cM with an average marker density of 2.6 cM/marker (Table 2). The A-genome chromosomes had 414 markers and spanned 1,128.22 cM with an average density of 2.7 cM/marker, whereas the B-genome chromosomes had a total of 430 markers spanning 1,078.93 cM with an average marker density of 2.5 cM/marker (Table 2).

Chromosome 7A was the longest linkage group (262.41 cM) and chromosome 4B was the shortest (107.95 cM). The number of markers per chromosome ranged from 17 (4B) to 104 (5B) (Table 2). Chromosome 6B had the highest marker density at 1.2 cM/marker, whereas chromosome 4B had the lowest with one marker every 6.3 cM (Table 2). Of the 844 markers, 208 (24.6%), had segregation ratios that deviated significantly ($P < 0.05$) from the expected 1:1

ratio (Figure 1, Appendix I). These segregating distorted markers were located on 10 chromosomes (1A, 2A, 2B, 3B, 4B, 5A, 5B, 6A, 6B and 7B) with chromosomes 5B and 6B having 71 and 52 distorted markers, respectively (Figure 1, Table 2, Appendix I).

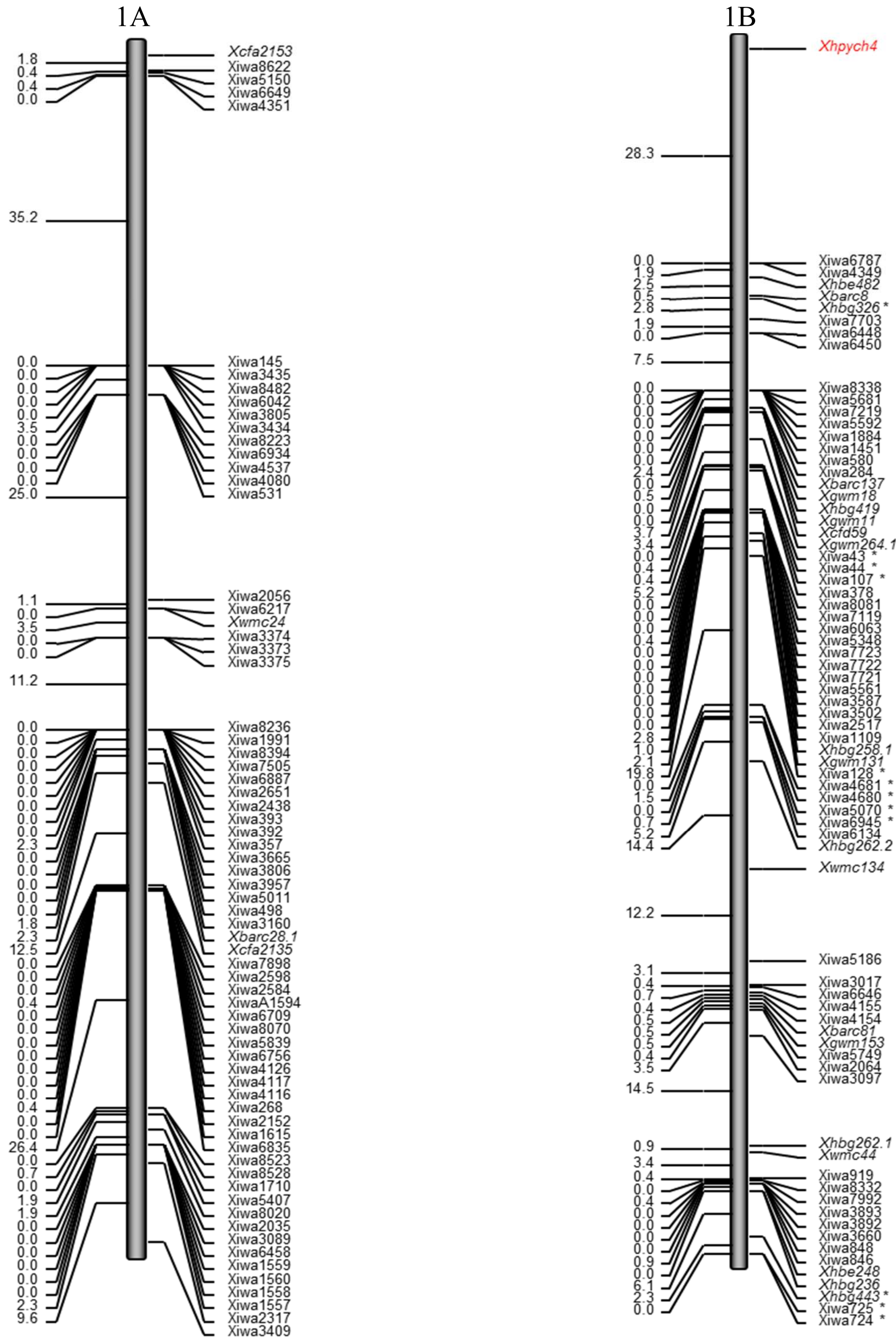
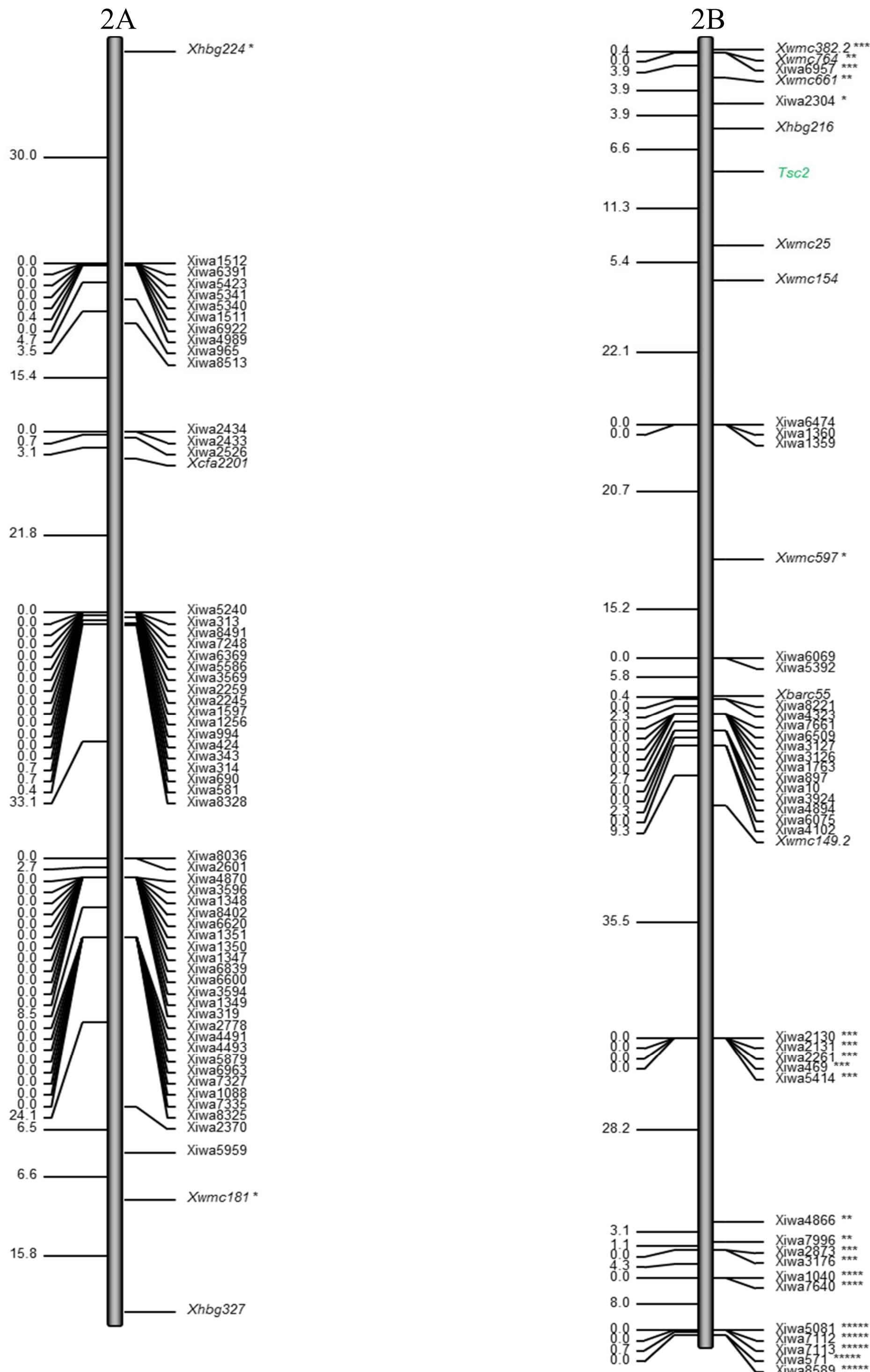


Figure 1. Genetic linkage maps of chromosomes 1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B, 5A, 5B, 6A, 6B, 7A and 7B in the Altar 84×Langdon population developed using simple sequence repeat (SSR) markers, single nucleotide polymorphism (SNP) markers and cleaved amplified polymorphic sequence (CAPS) (shown in red). * indicates markers with distorted segregation ratios. Markers are indicated to the right of the genetic maps.



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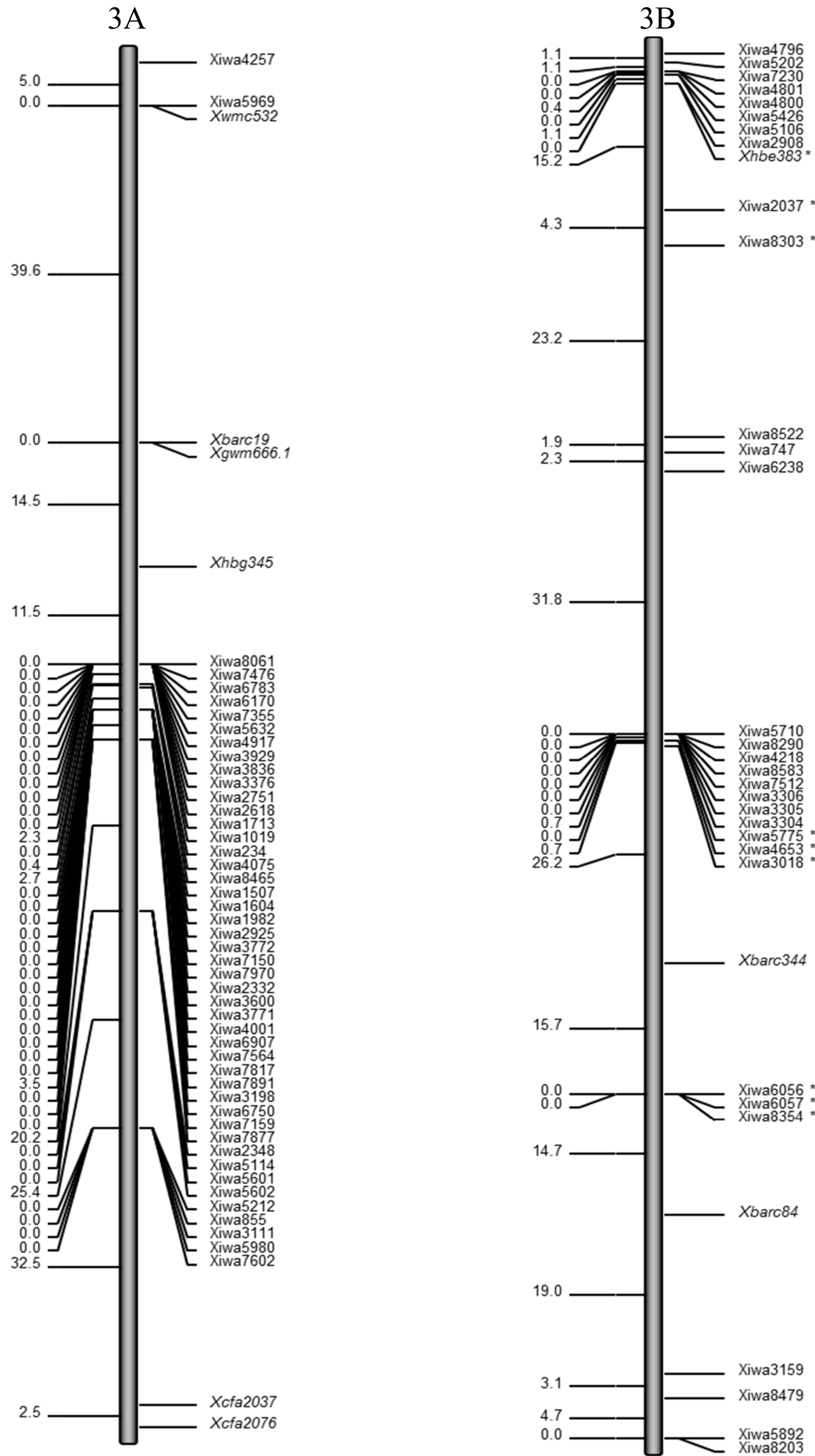

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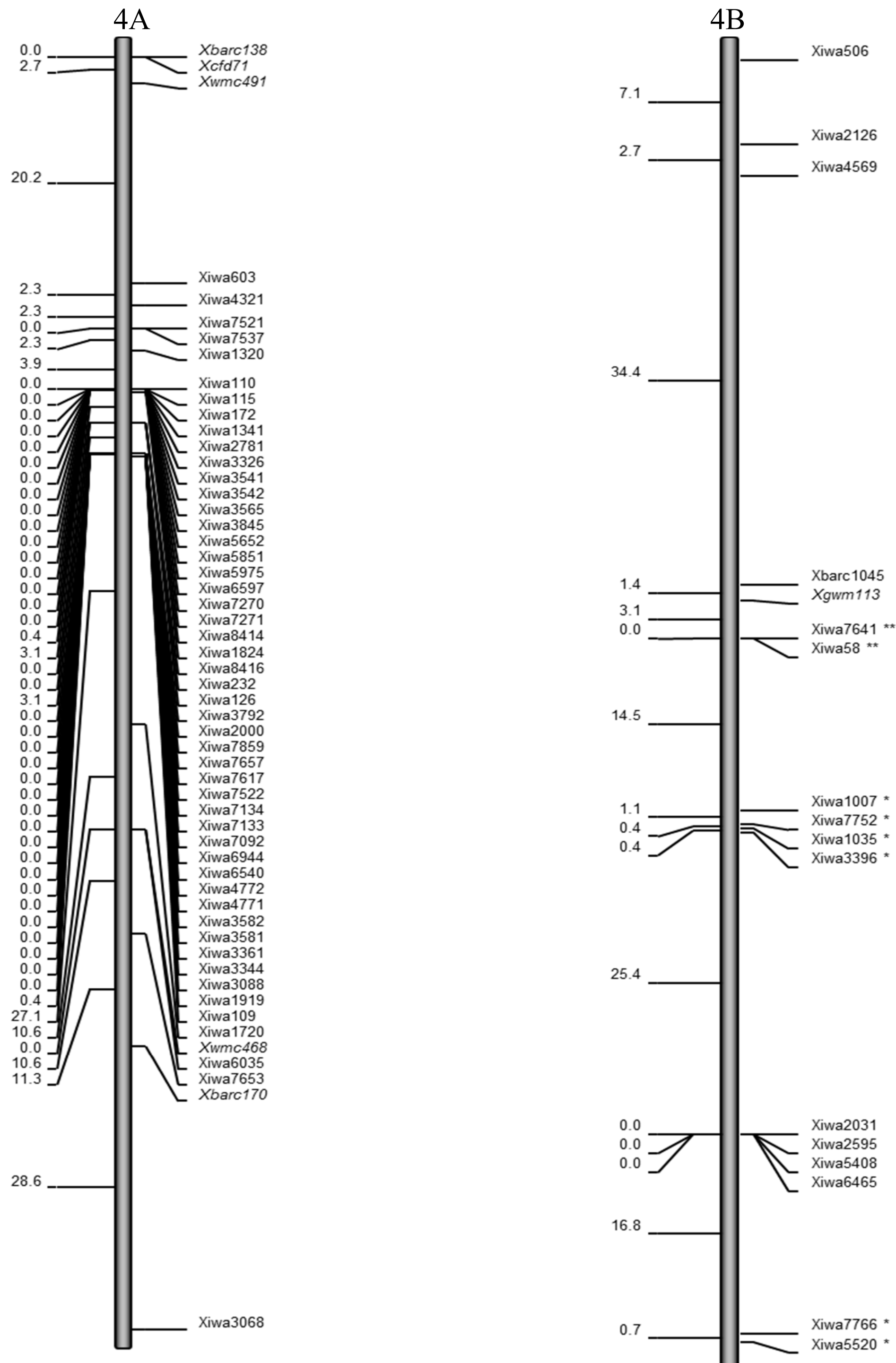


Figure 1. Genetic linkage maps of chromosomes 1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B, 5A, 5B, 6A, 6B, 7A and 7B in the Altar 84×Langdon population developed using simple sequence repeat (SSR) markers, single nucleotide polymorphism (SNP) markers and cleaved amplified polymorphic sequence (CAPS) (shown in red) (continued). * indicates markers with distorted segregation ratios. Markers are indicated to the right of the genetic maps.

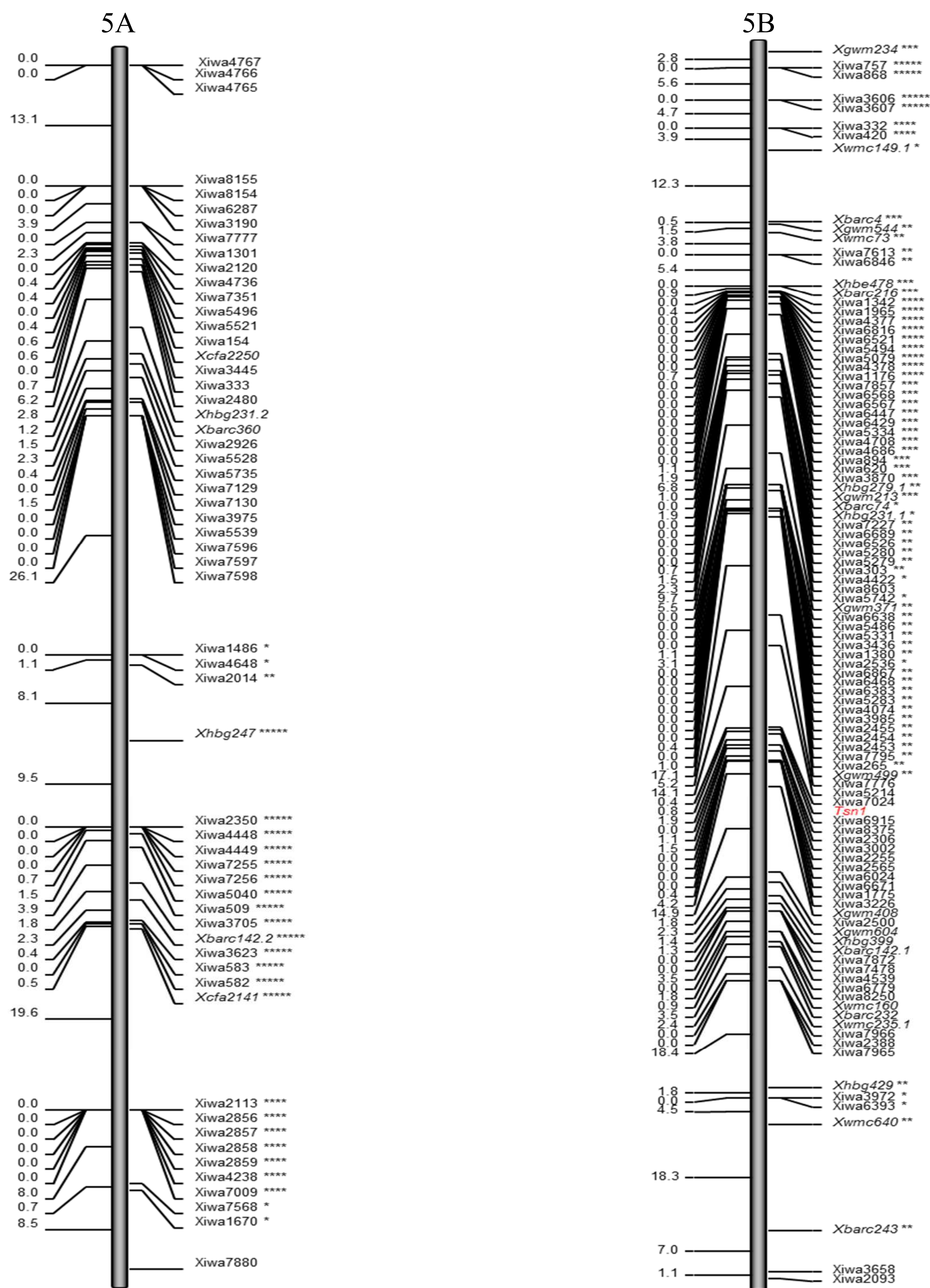
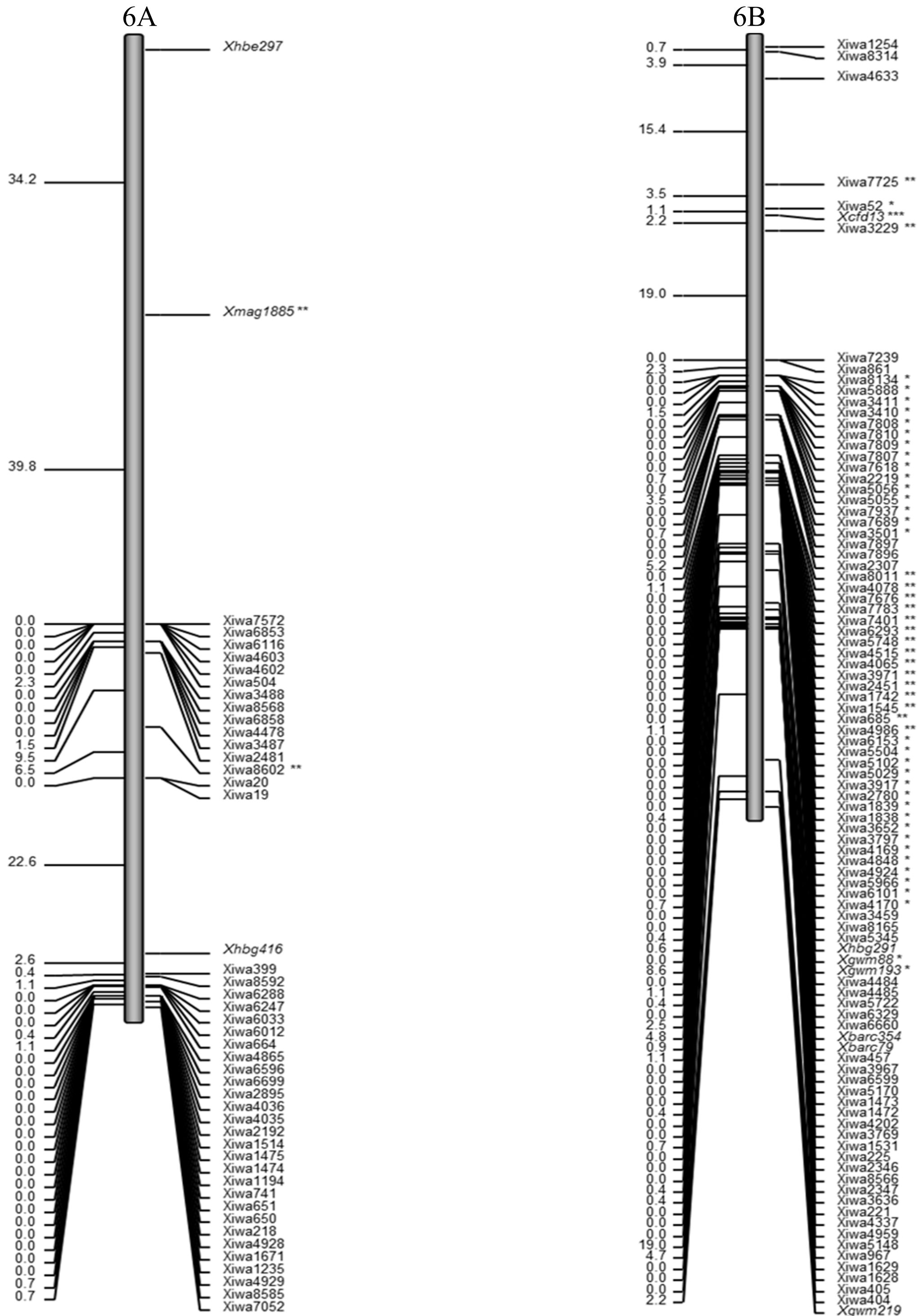


Figure 1. Genetic linkage maps of chromosomes 1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B, 5A, 5B, 6A, 6B, 7A and 7B in the Altar 84×Langdon population developed using simple sequence repeat (SSR) markers, single nucleotide polymorphism (SNP) markers and cleaved amplified polymorphic sequence (CAPS) (shown in red) (continued). * indicates markers with distorted segregation ratios. Markers are indicated to the right of the genetic maps.



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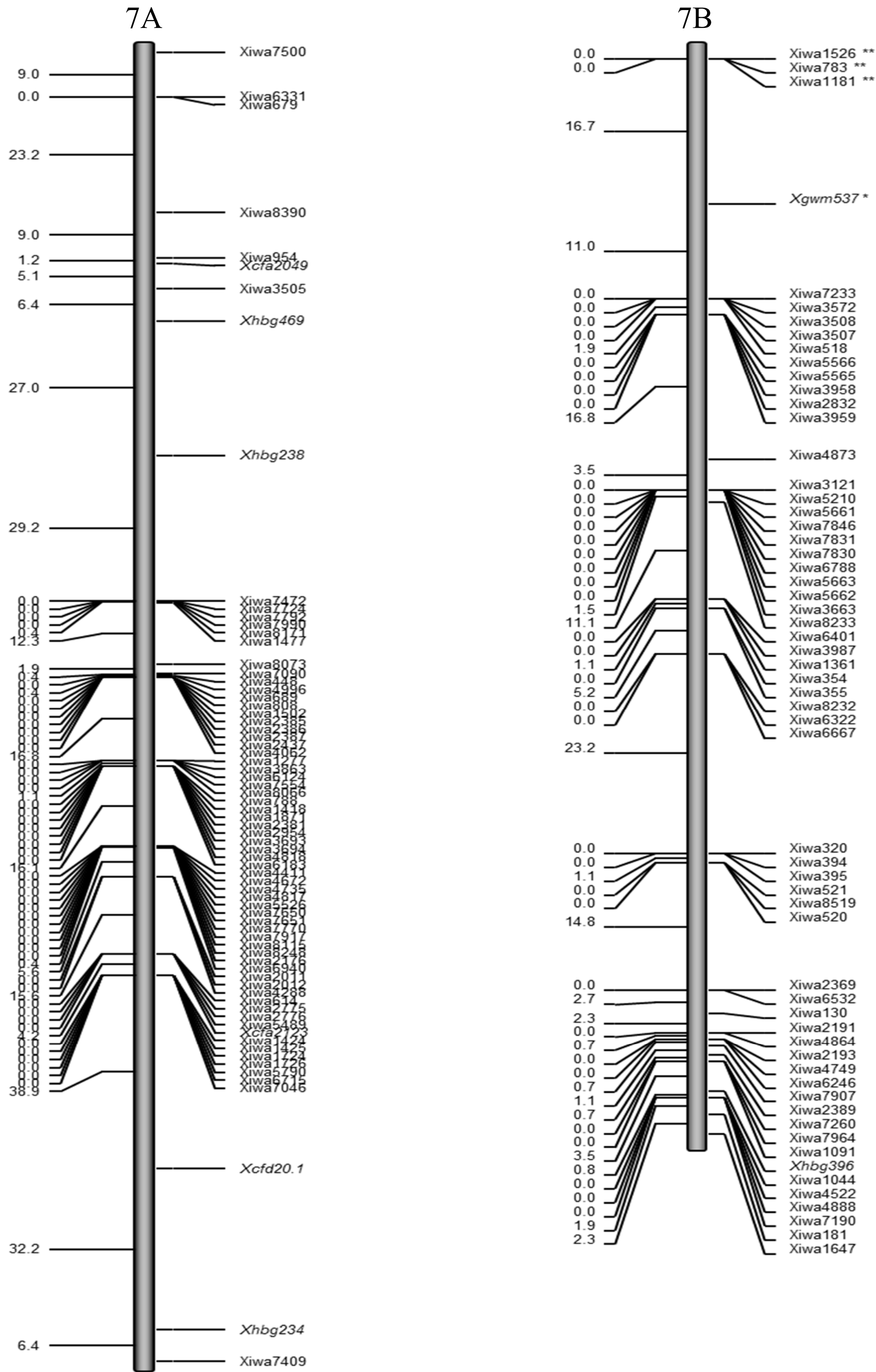

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Table 2. Summary of markers mapped in each chromosome/genome in the Altar 84 × Langdon population.

Chromosome	Markers[y]				Total	Length (cM)[z]	Marker density (cM/marker)	No. of markers with distorted ratios
	SSR	CAPS	SNP	Phenotype				
1A	4	–	65	–	69	144.90	2.1	0
1B	20	1	53	–	74	159.84	2.2	12
2A	4	–	57	–	61	178.68	2.9	2
2B	9	–	36	1	46	196.73	4.2	22
3A	6	–	47	–	53	159.97	3.0	0
3B	3	–	31	–	34	167.19	4.9	9
4A	5	–	50	–	55	128.57	2.3	0
4B	2	–	15	–	17	107.95	6.3	8
5A	6	–	52	–	58	130.76	2.3	26
5B	23	–	80	1	104	211.72	2.0	71
6A	3	–	43	–	46	123.34	2.7	2
6B	7	–	88	–	95	111.10	1.2	52
7A	6	–	66	–	72	262.41	3.6	0
7B	2	–	58	–	60	124.40	2.1	4
A genome	34	–	380	–	414	1,128.22	2.7	30
B genome	66	1	361	2	430	1,078.93	2.5	178
Total	100	1	741	2	844	2,207.15	2.6	208

[y] SSR= simple sequence repeat, CAPS= cleaved amplified polymorphic sequence, SNP= single nucleotide polymorphism

[z] Length in centiMorgans (cM)

Genetic analysis of Ptr ToxA and Ptr ToxB sensitivity

Altar 84 was insensitive to Ptr ToxA and Langdon was sensitive (Figure 2). The AL population, which consisted of 127 RILs, segregated in a ratio of 59 insensitive:68 sensitive for reaction to Ptr ToxA cultures and fit the expected 1:1 ratio for a single host gene conferring sensitivity to Ptr ToxA ($\chi^2_{df=1} = 0.65$, $P = 0.42$). Conversion of the Ptr ToxA reaction scores to genotypic scores allowed me to map the *Tsn1* locus, which mapped to the long arm chromosome 5B as expected flanked by SNP markers *XXiwa7024* and *XXiwa6915* at distances of 0.4 and 0.8 cM, respectively (Figure 1, Appendix I).

Altar 84 was sensitivie to Ptr ToxB and Langdon was insensitive (Figure 2). The AL population segregated in a ratio of 72 insensitive:55 sensitive for reaction to Ptr ToxB and fit the expected 1:1 ratio for a single host gene conferring sensitivity to Ptr ToxB ($\chi^2_{df=1} = 3.28$, $P = 0.07$). The reactions to Ptr ToxB were converted to genotypic scores and analyzed along with the molecular marker data. Linkage analysis showed that the *Tsc2* locus mapped to the short arm of chromosome 2B flanked by SSR markers *Xhbg216* and *Xwmc25* at distances of 6.6 and 11.3 cM, respectively (Figure 1, Appendix I).

Figure 2. Reaction of Langdon (A) and Altar 84 (B) infiltrated with Ptr ToxA. Reaction of Langdon (C) and Altar 84 (D) infiltrated with Ptr ToxB.

AL population reaction to *P. tritici-repentis*

Altar 84, LDN and the AL population were screened with the Ptr ToxA-producing race 2 isolate 86-124. Altar 84 and LDN were resistant and moderately susceptible to 86-124 with average disease reaction types of 1.17 and 3.50, respectively (Figure 3, Table 3). The average disease reaction type for the AL population was 2.31 and reaction types ranged from 1.00 to 4.16 (Figure 3, Table 3). The mean reaction types of Ptr ToxA-insensitive and -sensitive AL lines were 2.21 and 2.31, which were not significantly different at the 0.01 level of probability (LSD = 0.19) (Table 3) indicating that the *Tsn1*-Ptr ToxA interaction was not significant in in the development of tan spot caused by 86-124.

For reaction to the race 5 Ptr ToxB-producing isolate DW5, Altar 84 and LDN were both moderately susceptible with average disease reactions of 3.16 and 3.50, respectively (Figure 3, Table 3). The average disease reaction type for the AL population was 3.02, and reaction types ranged from 1.25 to 4.50 (Figure 3, Table 3) indicating strong transgressive segregation and suggesting that more than one gene was involved in conditioning resistance. The mean reaction

types of Ptr ToxB-insensitive and -sensitive AL lines to DW5 were 2.29 and 1.33, respectively, which were significantly different at the 0.01 level of probability ($LSD_{0.01} = 0.16$) (Table 3). This result indicates that the *Tsc2*-Ptr ToxB interaction played a significant role in the development of tan spot caused by DW5.

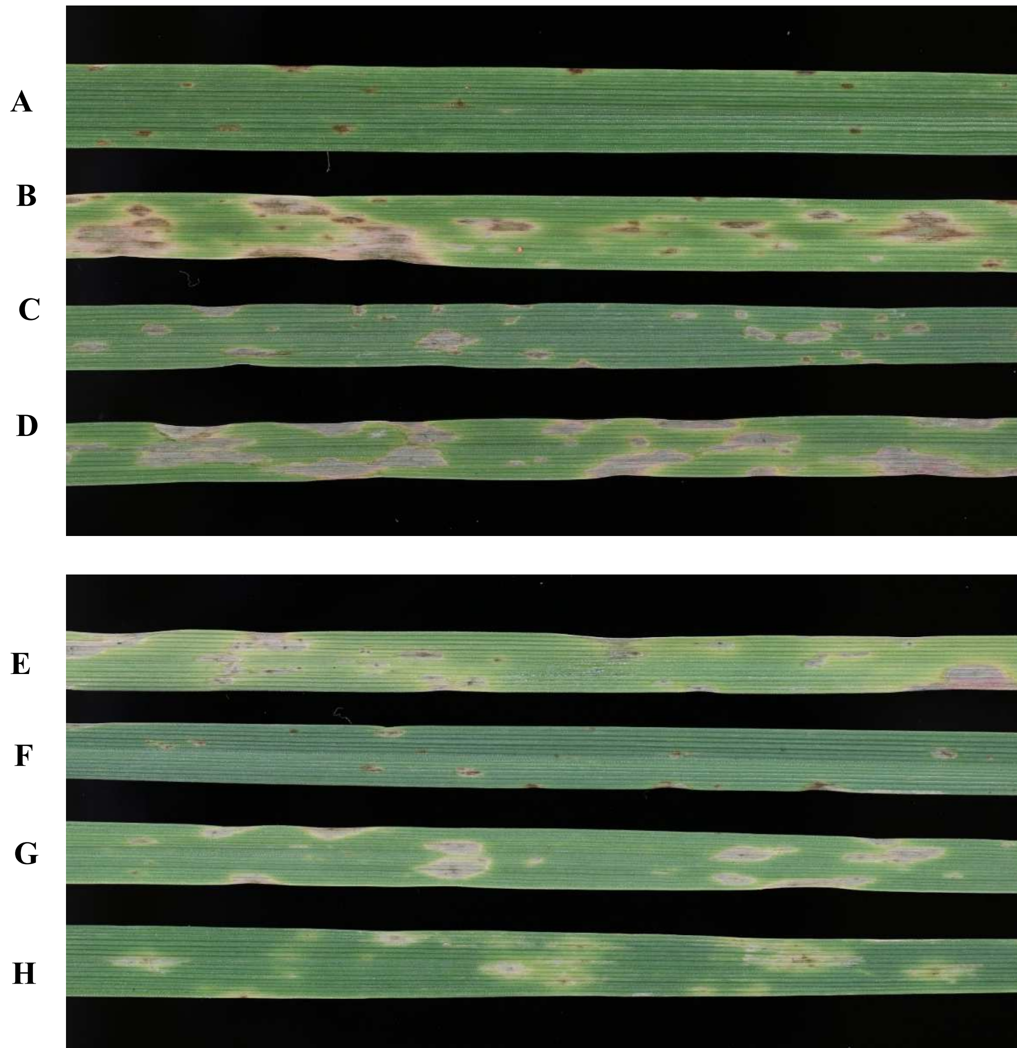

Figure 3. Inoculation of secondary leaves with conidia of 86-124 and DW5. Reaction of Altar 84 (A) (average disease reaction type 1.17) and Langdon (B) (average disease reaction type 3.50) to 86-124, a race 2 isolate. The secondary leaves of recombinant inbred line numbers. AL113 (C) and AL130 (D) showed moderately and highly susceptible reactions to race 2 isolate 86-124. Reaction of Altar 84 (H) (average disease reaction type 3.16) and Langdon (G) (average disease reaction type 3.50) to DW5, race 5 isolate. The secondary leaves of recombinant inbred line numbers AL123 (E) and AL34 (F) showed highly susceptible and highly resistant reactions to DW5 isolate.

AL population reaction to *P. nodorum*

The parents and AL population were screened for reaction to SNB caused by spore inoculations using the *P. nodorum* isolate Sn2K. Altar 84 was resistant to Sn2K and Langdon was highly susceptible with average disease reaction types of 1.33 and 4.83, respectively (Figure 4, Table 3). The average disease reaction type for the AL population was 3.18, and reaction types ranged from 1.16 to 4.80 (Figure 4, Table 3). The mean reaction types of ToxA-insensitive and -sensitive AL lines were 2.00 and 3.00, respectively (Table 3). These means were significantly different at the 0.01 level of probability (LSD = 0.06) (Table 3), indicating that the *Tsn1*-SnToxA interaction played significant role in the development of SNB caused by Sn2K.

Figure 4. Reaction of secondary leaves of Langdon (A) (average disease reaction type 4.83) and Altar 84 (B) (average disease reaction type 1.33) to Sn2K isolate.

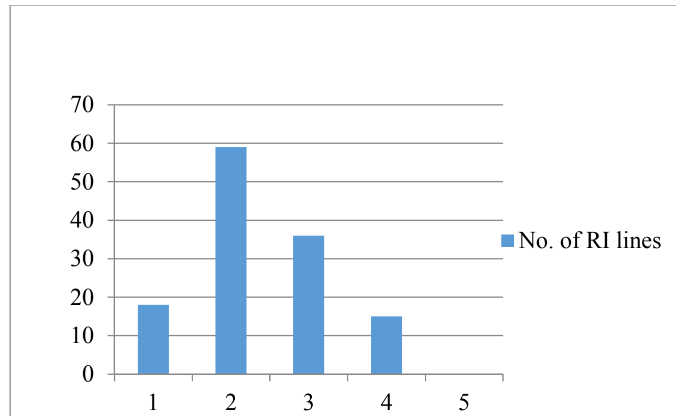

Figure 5. Histograms of lesion type means in the AL population after inoculation with *Pyrenophora tritici repentis* (Ptr) race 2 isolate 86-124. X-axis represent race lesion types and Y-axis represents number of recombinant inbred lines.

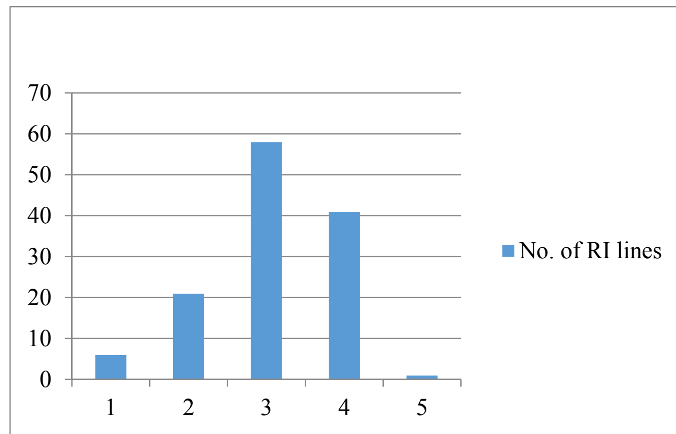

Figure 6. Histograms of lesion type means in the AL population after inoculation with *Pyrenophora tritici repentis* (Ptr) race 5 isolate DW5. X-axis represent race lesion types and Y-axis represents number of recombinant inbred lines.

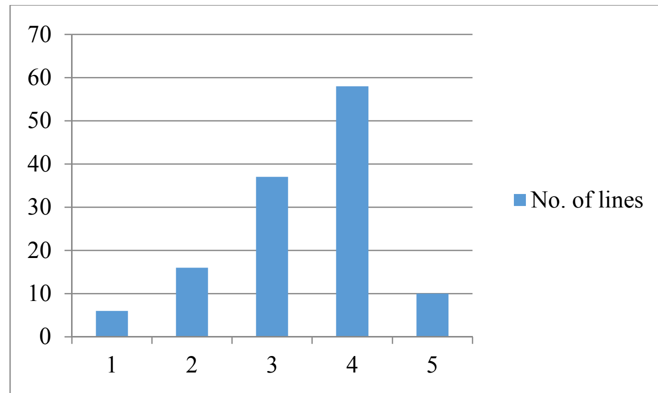

Figure 7. Histograms of lesion type means in the AL population after inoculation with *Parastagonospora nodorum* isolate Sn2K. X-axis represent race lesion types and Y-axis represents number of recombinant inbred lines

Table 3. Average reaction types of Altar, Langdon, and the Altar 84 × Langdon population of recombinant inbred lines to tan spot and Septoria nodorum blotch.

Isolate	Altar 84	Langdon	AL population range	AL population average	Ptr ToxA sensitive lines	Ptr ToxA insensitive lines	Ptr ToxB sensitive lines	Ptr ToxB insensitive lines	Average disease Reaction type difference between sensitive and insensitive lines
86-124 (race 2)	1.17	3.50	1.00-4.16	2.31	2.39	2.21	–	–	0.18
DW5 (race 5)	3.16	3.50	1.25-4.50	3.02	–	–	2.29	1.33	0.96*
Sn2K	1.33	4.83	1.16-4.80	3.18	3.00	2.00	–	–	1.00*

* Indicates that there is significant difference at the 0.01 level of probability

Identification of QTLs associated with tan spot and SNB in the AL population

A Bartlett's Chi-squared test was conducted on the three replicates of data collected for each of the three isolates to determine if replicates were homogeneous. The results indicated that the three replicates for each isolate were homogeneous (86-124: $\chi^2_{df=2} = 2.08$, $P = 0.35$; DW5: $\chi^2_{df=2} = 6.01$, $P = 0.05$; Sn2K: $\chi^2_{df=2} = 0.78$, $P = 0.70$). Therefore, the mean disease reaction types for each isolate were used for QTL analysis.

For the race 2 isolate 86-124, only one QTL was significantly associated with resistance (Table 4). This QTL was on the long arm of 6B between markers *XXiwa5148* and *XXiwa967*, which were at positions 84.4 and 103.4 cM, respectively (Figure 6). This QTL, designated *QTs.fcu-6B*, had an LOD of 6.9 and explained 22% of the phenotypic variation (Table 4). The resistance effects of *QTs.fcu-6B* were contributed by Altar 84 (Table 4). The *Tsn1* locus on 5BL was not significantly associated with resistance to 86-124 (Figure 6), which produces Ptr ToxA. In single factor regression, *Tsn1* had an LOD of only 0.35.

For the race 5 isolate DW5, two significant QTLs were identified. The QTLs were located on chromosomes arms 2BS and 4BL, and were designated *QTs.fcu-2B* and *QTs.fcu-4B,* respectively (Figure 5, Table 4). The peak of *QTs.fcu-2B* was defined by the *Tsc2* locus on chromosome 2BS. It had an LOD of 12.0 and explained 26% of the disease variation (Figure 5, Table 4). Because this QTL represented the *Tsc2*-Ptr ToxB interaction for which Altar 84 contributed the Ptr ToxB-sensitive allele, resistance to this QTL was contributed by Langdon (Table 4).

As second QTL associated with reaction to tan spot caused by DW5 was identified on 4BL and designated as *QTs.fcu-4B* (Figure 5, Table 4). *QTs.fcu-4B* had an LOD of 8.6 and explained 12% of the variation with resistance effects contributed by Altar 84 (Table 4). The

QTL peaked between markers *XXiwa2126* to *Xgwm113* which were located at positions 7.1 and 38.9 cM, respectively (Figure 5, Table 4).

A single QTL designated *QSnb.fcu-5B* associated with SNB caused by the ToxA-producing *P. nodorum* isolate Sn2k was identified on the long arm of 5B (Figure 6). The *Tsn1* locus, with an LOD of 13.0, defined the peak of *QSnb.fcu-5B* and explained 38% of the phenotypic variation (Figure 6, Table 4). Because this QTL is due to the effects of the *Tsn1*-SnToxA interaction, it indicates that this interaction played a significant role in the development of SNB caused by isolate Sn2K.

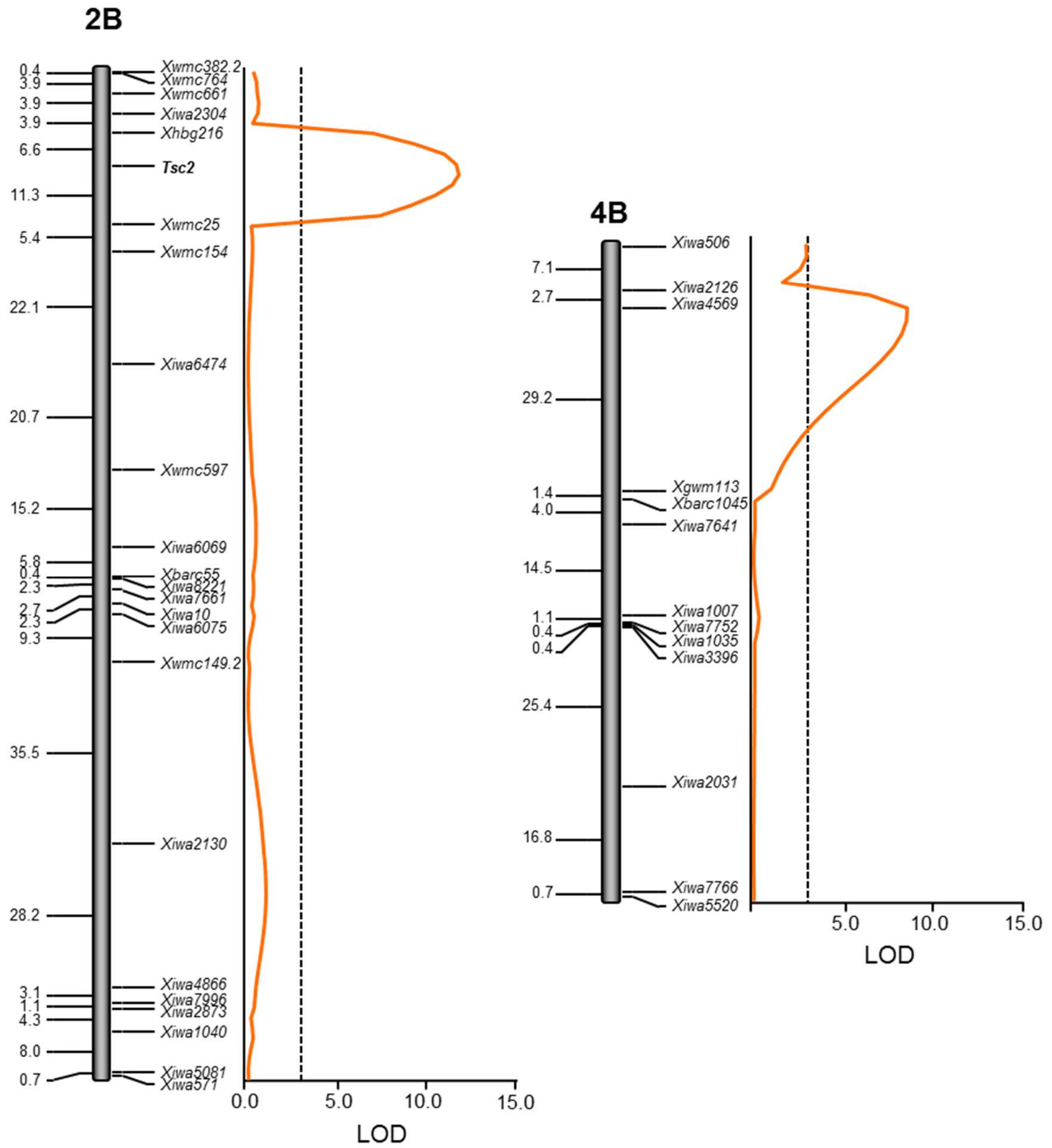

Figure 8. The composite interval regression maps of QTL associated with race 5 isolate DW5. Markers are indicated to the *right side* of the genetic map and their position in centiMorgans (cM) is shown along *left side.* The critical LOD threshold is indicated by the *dotted lines* and the LOD scale is indicated at the bottom.

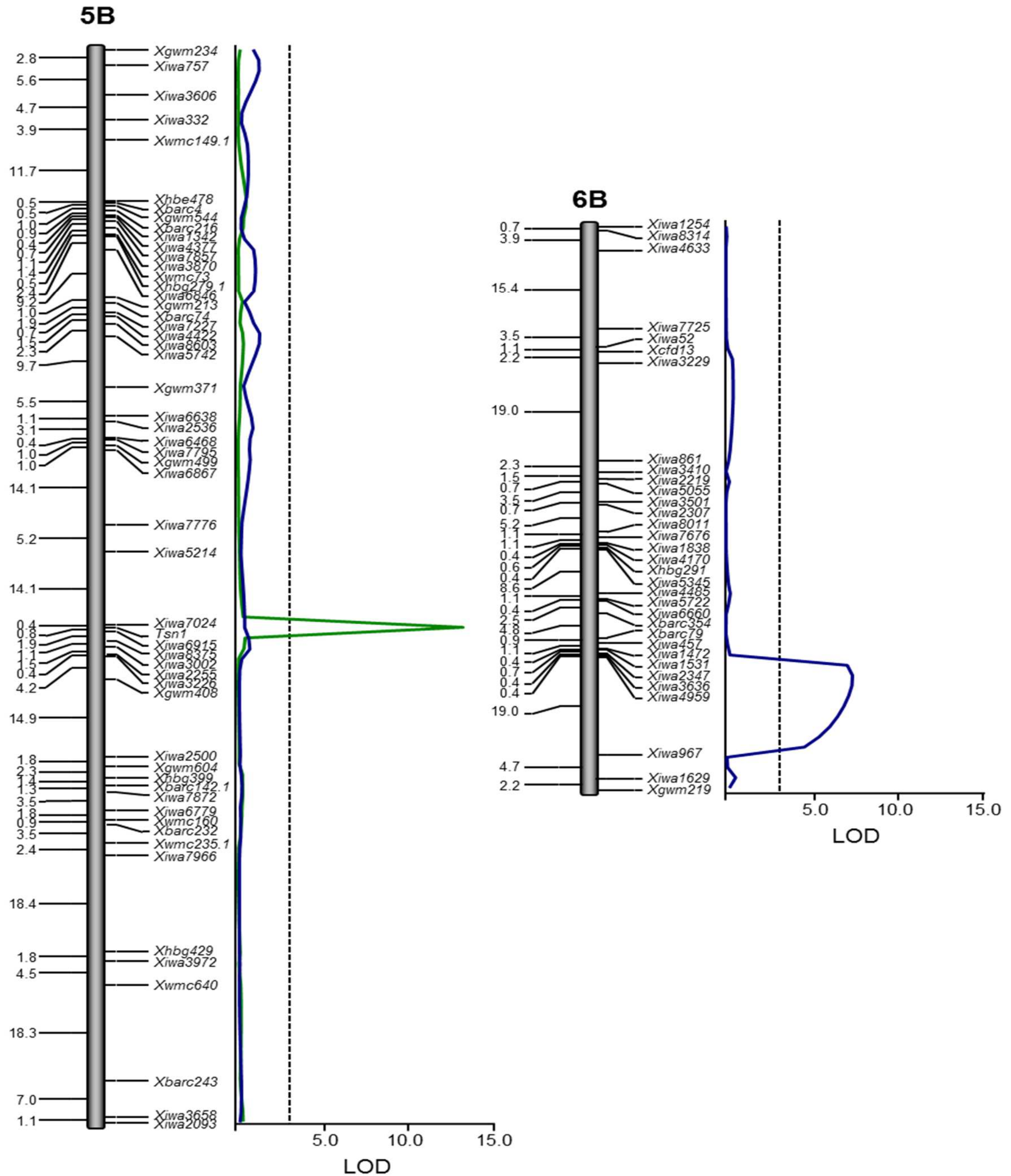

Figure 9. The composite interval regression maps of QTL associated with tan spot and septoria nodorum blotch by race 2 isolate 86-124 (shown in blue) and Sn2K (shown in green), respectively. Markers are indicated to the *right side* of the genetic map and their position in centiMorgans (cM) are shown along *left side.* The critical LOD threshold is indicated by the *dotted lines* and the LOD scale is indicated at the bottom.

Table 4. Composite interval mapping analysis of QTLs associated with resistance to tan spot caused by Ptr races 2 and 5 and resistance to Septoria nodorum blotch caused by Sn2K in the Altar 84 × Langdon population.

QTL	Chromosome	Marker interval	Marker position	DW5 (race 5)			86-124 (race 2)			Sn2K		
				LOD	R^2	Add[b]	LOD	R^2	Add	LOD	R^2	Add
QTs.fcu-2B	2BS	*Tsc2*	18.5	12.0	0.26	0.41	–	–	–	–	–	–
QTs.fcu-4B	4B	IWA2126–*Xgwm113*	7.1 – 38.9	8.60	0.12	−0.56	–	–	–	–	–	–
Qsnb.fcu-5B	5BL	*Tsn1*	110.2	–	–	–	–	–	–	13.0	0.38	−0.59
QTs.fcu-6B	6B	IWA5148–IWA967	84.4 – 103.4	–	–	–	6.90	0.22	−0.51	–	–	–

[b] Add.: the additive effects of the QTL. A negative value indicates resistance effects derived from Langdon

A dash indicates that the marker was not significantly associated with resistance.

Discussion

The construction of linkage maps is necessary to conduct QTL analysis. The 9K SNP array, which was developed to be used for hexaploid wheat, worked well for the development of linkage maps representing the A and B genome chromosomes in the tetraploid wheat population used here. The objective of this research was to evaluate the roles of *Tsn1*-ToxA and *Tsc2*-ToxB interactions, but a whole-genome scan was also desired to determine if loci other than *Tsn1* and *Tsc2* might be associated with disease caused by the Ptr isolates under investigation. For this, the marker density and the large amount of D genome markers offered by the 90K SNP array were not necessary. The goal was to generate linkage maps sufficient to conduct a whole-genome QTL scan, for which the 9K array provided adequate marker coverage without the redundancy and excess data that the 90K array would provide. The SSR markers employed in this research helped me to assign the linkage groups to specific chromosomes with confidence.

The results of this study revealed that the *Tsc2*-ToxB interaction played a significant role in the development of disease by explaining 25% of the disease variation. Friesen and Faris (2004) were the first to map the Ptr ToxB sensitivity gene *Tsc2* on chromosome 2BS, and did so by using the ITMI population. The ITMI population was derived from a cross between the synthetic hexaploid wheat W-7984 and the hard red spring wheat variety Opata 85 (PI591776). W-7984 was derived from durum wheat variety Altar 84 and the *Aegilops tauschii* accession CI 18 (WPI 219). Because Altar 84 donated the B-genome chromosome to W-7984, it also donated the *Tsc2* locus thus rendering W-7984 sensitive to Ptr ToxB. Opata 85 was insensitive to Ptr ToxB, but interestingly both parents were moderately susceptible to conidia produced by the race 5 isolate DW5. When the whole population was inoculated with DW5, Friesen and Faris (2004) observed that all the Ptr ToxB-sensitive lines developed chlorotic symptoms. QTL analysis

revealed a major QTL, which explained 69% of the variation on chromosome 2BS and corresponded to the *Tsc2* gene, indicating that the *Tsc2*-ToxB interaction was significantly associated with development of the disease in the ITMI population. A number of relatively minor QTLs were also identified, which were likely responsible for the susceptibility of Opata 85 to DW5 even though Opata 85 was insensitive to Ptr ToxB.

Abeyesekara et al. (2010) conducted comparative and fine mapping of the *Tsc2* gene in a hexaploid wheat RIL population derived from a cross between the landrace Salamouni and the Canadian hard red spring wheat variety Katepwa (SK population) and showed that the *Tsc2*-Ptr ToxB interaction in this population played a major role in conferring susceptibility to race 5 isolates of *P. tritici-repentis* by explaining 54% of the disease variation. They also evaluated a population of 114 F_2 plants derived from a Salamouni × Katepwa, and showed that sensitivity to Ptr ToxB segregated in a ratio of 91:23 sensitive:insensitive, which fit the expected 3:1 ratio for a single gene. This indicated that Ptr ToxB sensitivity was governed by a dominant allele and suggested that resistance to Ptr ToxB-producing isolates was controlled by the recessive *tsc2* allele.

Singh et al. (2010) conducted an experiment to explore the inheritance of resistance caused by spore inoculations and culture filtrate infiltrations of the race 5 isolate DW13. For their experiment, they used also used a hexaploid wheat population. RIL, F_1 and F_2 plants derived from a cross between the hard red spring wheat variety Steele-ND and the North Dakota hard red spring wheat breeding line ND735 were screened separately for spore inoculations and culture filtrates. They found that resistance to the chlorosis component of DW13 spore inoculations and insensitivity to DW13 culture filtrates was controlled by the same recessive gene. Genetic linkage mapping using a DArT-based linkage map showed that the gene was on

the short arm of chromosome 2B near the known location of *Tsc2* (Friesen and Faris 2004; Abeysekara et al. 2010), indicating it was likely the same gene.

The three studies mentioned above showed that the *Tsc2* gene is a major susceptibility factor in hexaploid wheat. Although the durum variety Altar 84 contributed the dominant *Tsc2* allele for Ptr ToxB sensitivity to the synthetic wheat W-7984, the evaluation of the effects of the *Tsc2*-Ptr ToxB interaction were conducted in a hexaploid wheat background (Friesen and Faris 2004). Therefore in this study, I chose to evaluate a tetraploid population derived from Altar 84 and LDN to determine the effects of the *Tsc2*-Ptr ToxB interaction in a true tetraploid wheat background. The results indicated that the *Tsc2* explained up to 26% of the disease variation. This is the first study to demonstrate that the *Tsc2*-Ptr ToxB interaction plays a significant role in conferring susceptibility in tetraploid wheat, just as it does in hexaploid wheat.

The second objective of this study was to evaluate the role of the *Tsn1*-Ptr ToxA interaction in a tetraploid wheat population. Most of the previous studies pertaining to the *Tsn1*-Ptr ToxA interaction have been conducted in hexaploid wheat (Faris et al. 2013). Some of these studies have demonstrated that the *Tsn1*-Ptr ToxA interaction plays a major role in the development of tan spot caused by Ptr ToxA-producing isolates. For example, early studies by Tomas and Bockus (1987) and Lamari and Bernier (1989) showed that sensitivity to Ptr ToxA and susceptibility to necrosis-inducing isolates was strongly correlated suggesting that the same gene(s) governing sensitivity to Ptr ToxA were also responsible for conditioning susceptibility to the fungus.

Subsequent studies employed genetic mapping and/or QTL analysis to identify loci associated with the effects of necrosis-inducing isolates of *Ptr*. Cheong et al. (2004) evaluated two different hexaploid wheat populations and identified a major QTL associated with disease at

the *Tsn1* locus on chromosome arm 5BL in both populations explaining 39 and 60% of the variation, respectively. Singh et al. (2010) reported that the same gene, *Tsr1* (syn *Tsn1*), controlled resistance to necrosis produced by a race 2 isolate and insensitivity to race 2 culture filtrates, which presumably contained Ptr ToxA.

Other studies have indicated that the *Tsn1*-Ptr ToxA interaction was less significant. For example, Friesen et al. (2003) evaluated a RIL population derived from a cross between the hard red spring wheat cultivars Erik and Kulm and showed that the *Tsn1*-Ptr ToxA interaction accounted for only 24% of the variation. They also showed that *Tsn1*-disrupted mutants were insensitive to Ptr ToxA, but still susceptible to the disease.

More recently, Faris et al. (2012) conducted QTL analysis for reaction to the race 1 isolates ASC1 and Pti2 and the race 2 isolate 86-124, all of which produce Ptr ToxA. They reported that the *Tsn1*-Ptr ToxA interaction accounted for 5 to 30% of the disease variation depending on the isolate. Other QTLs significantly associated with disease caused by these isolates were reported as well, demonstrating that host genetic factors other than *Tsn1* are involved. Similarly, Singh and Bockus (2008) identified a QTL on 5BL corresponding to the location of *Tsn1* that explained 27% of the variation in disease caused by a race 1 isolate. A second QTL explaining 23% of the variation on 3AS was also identified.

In another QTL study, Chu et al. (2008) evaluated a population of doubled haploid lines derived from a cross between the synthetic hexaploid wheat TA4152-60 and the North Dakota hard red spring wheat line ND495 (NC60 population) for reaction to the race 1 isolate Pti2 and the race 2 isolate 86-124. They found that the *Tsn1*-Ptr ToxA interaction accounted for only 17 and 15% of the variation, respectively.

Still, another study in hexaploid wheat showed that the *Tsn1*-Ptr ToxA interaction has no significant role in the development of disease. Faris and Friesen (2005) used a population of RILs derived from the wheat lines BR34 and Grandin (BG population) to identify QTLs associated with resistance to isolates representing races 1, 2, 3, and 5. Although the BG population segregated for reaction to Ptr ToxA, no significant QTL was identified at the *Tsn1* locus indicating that the *Tsn1*-Ptr ToxA interaction did not play a role in the development of disease caused by Ptr ToxA-producing isolates. Instead, the authors reported QTLs on 1BS and 3BL that were associated with resistance to *P. tritici-repentis* races 1, 2, 3, and 5, and were thus considered broad spectrum race-nonspecific resistance QTLs.

All of the above-mentioned studies on the effects of the *Tsn1*-Ptr ToxA interaction were conducted in hexaploid wheat populations and indicate that the interaction can play a major role, a minor role, or have no effect depending on the genetic background. However, only one study prior to the current one involved the evaluation of the *Tsn1*-Ptr ToxA interaction in tetraploid wheat. In that study, Chu et al. (2010a) evaluated a tetraploid wheat doubled haploid population derived from a cross between the durum variety Lebsock and accession PI 94749 of *T. turgidum* ssp. *carthlicum* (LP population) for a reaction to the *P. tritici-repentis* race 2 isolate 86-124 and the race 1 isolate Pti2, which both produce Ptr ToxA. Although the population segregated for reaction to Ptr ToxA infiltrations, the insensitive and sensitive DH lines had average reaction types of 3.0 and 2.9 for reaction to Pti2, and 3.0 and 3.0 for reaction to 86-124, respectively, indicating that sensitivity to Ptr ToxA had no effect on disease. Further QTL analysis showed no significance for the *Tsn1* locus on 5BL for reaction to either 86-124 or Pti2. Instead, race nonspecific resistance QTLs were identified on chromosomes 3A, 3B, 5A, and 7B.

My results agree with those of Chu et al. (2010a) in that the *Tsn1*-Ptr ToxA interaction was not a factor in the development of disease caused by isolate 86-124 in tetraploid wheat. To verify that this result was not specific to 86-124, I conducted single replication inoculations with the race 1 isolates ASC1 and Pti2, and with two additional race 2 isolates, all of which produce Ptr ToxA, and found that none indicated a significant association with the *Tsn1* locus (data not shown). This is the second study to evaluate the effects of the *Tsn1*-Ptr ToxA interaction in tetraploid wheat, and both showed that the interaction played no significant role in the development of disease. To my knowledge, no other study has implicated *Tsn1* in the susceptibility of tetraploid wheat to tan spot.

The reasons for varying levels of significance of the *Tsn1*-Ptr ToxA interaction in different wheat genetic backgrounds are unknown. It is unlikely that the differences are due to structural variation in the *ToxA* gene among isolates because first, Friesen et al. (2006) evaluated Ptr ToxA haplotypes of 54 *Ptr* isolates, and found no sequence variation in the *ToxA* gene, and second, the same race 2 isolate (86-124) has been used in many of the above mentioned studies and has been associated with results indicating a strong role for the *Tsn1*-Ptr ToxA interaction (Lamari and Bernier 1989), a minor role for the interaction (Friesen et al. 2003; Chu et al. 2010; Faris et al. 2012), and no role for the interaction (Faris and Friesen 2005). Faris et al. (2011) showed that differential expression of the *ToxA* gene in different isolates of *P. nodorum* correlated with the level of disease. This might explain differences in the significance of the *Tsn1*-Ptr ToxA interaction among different isolates. For example, Faris et al. (2012) showed that isolates ASC1, Pti2, and 86-124 explained 5, 22, and 30% of the variation, respectively, even though they all contain the same *ToxA* haplotype (Friesen et al. 2006). The same explanation for differences in *Tsn1*-Ptr ToxA relevance among studies that employed the same isolate, 86-124,

may not be expected. However, it is possible that some wheat genotypes possess factors that may suppress expression of the *ToxA* gene or inhibit the recognition of Ptr ToxA by *Tsn1* in plants inoculated with fungal spores, but not in plants infiltrated with Ptr ToxA. In any case, it is most likely that differences in the relevance of the *Tsn1*-Ptr ToxA interaction in causing disease on different wheat genotypes are influenced by host genetic factors other than *Tsn1*.

Although my research indicated that the *Tsn1*-Ptr ToxA interaction played no significant role in the development of tan spot caused by Ptr ToxA-producing isolates of *P. tritici-repentis*, it was highly significant in the development of SNB caused by *P. nodorum* explaining 38% of the variation. Friesen et al. (2006) showed that the *ToxA* gene was horizontally transferred from *P. nodorum* to *P. tritici-repentis* sometime prior to 1940. Liu et al. (2006) evaluated the hexaploid BG population and showed that sensitivity to Ptr ToxA co-segregated with the sensitivity to SnToxA at the *Tsn1* locus. Also, *Tsn1*-disrupted mutants were insensitive to both SnToxA and Ptr ToxA indicating that both NEs were similar in function. They also showed that the *Tsn1*-SnToxA interaction explained 62% of the disease variation for resistance to *P. nodorum* isolate Sn2K. As mentioned above, Faris and Friesen (2005) found that the *Tsn1*-Ptr ToxA interaction was not associated with disease caused by Ptr ToxA-producing isolates. Therefore, *Tsn1* played a major role in conferring susceptibility to SNB, but not to tan spot, which agrees with my findings in the AL population.

Similar results were also obtained in tetraploid wheat with the LP population (Chu et al. 2010; Friesen et al. 2012). Whereas Chu et al. (2010) showed that there was no significant association between the *Tsn1*-Ptr ToxA interaction and the development of tan spot, Friesen et al. (2012) found that the *Tsn1* locus accounted for 31% of the variation in the development of SNB.

The *Tsn1*-ToxA interaction and its role in conferring susceptibility to both tan spot and SNB has also been evaluated in a synthetic hexaploid-derived population. Using the NC60 population, Chu et al. (2008) showed that *Tsn1* accounted for up to 17% of the variation in tan spot resistance, and Chu et al. (2010) reported that the locus accounted for 28% of the variation in SNB.

Friesen and Faris (2009) showed that *Tsn1* was a major susceptibility factor in a different tetraploid wheat population. They evaluated a population of 85 recombinant inbred chromosome lines (RICLs) derived from a cross between LDN and LDN-*T. turgidum* ssp. *dicoccoides* chromosome 5B substitution (LDN-DIC 5B) for a reaction to the *P. nodorum* isolate Sn2K and showed that the *Tsn1* locus accounted for 95% of variation in conferring susceptibility to SNB. They also suggested that *Tsn1* was the only factor in conferring disease in that population because chemically-induced *Tsn1*-disrupted mutants were highly resistant to isolate Sn2K.

The data presented so far indicate that the *Tsn1*-ToxA interaction is sometimes important in conferring susceptibility to tan spot in hexaploid wheat, never associated with susceptibility to tan spot in tetraploid wheat, and always important in conferring susceptibility to SNB in both hexaploid and tetraploid wheat. Possible reasons for differences among reaction to Ptr ToxA-expressing *P. tritici-repentis* isolates are likely due to different host genetic factors as discussed above. The reasons for the varying levels of significance of the *Tsn1*-ToxA interaction in causing tan spot compared to SNB are most likely different. Including my research, there have now been three wheat populations (BG, LP, and AL) that have been evaluated for reaction to ToxA-producing isolates of both *P. tritici-repentis* and *P. nodorum*, and all three have shown that the *Tsn1*-ToxA interaction played no role in the development of tan spot, but a major role in the development of SNB. Because each of these studies evaluated disease produced by ToxA-

producing isolates of both *Ptr* and *P. nodorum* on the same population, the differences observed in the effects of the *Tsn1*-ToxA interaction must be due to differences in the pathogens. As mentioned above, the *ToxA* gene has existed in *P. nodorum* for a very long time and was only recently transferred to *Ptr* (Friesen et al., 2006). It is possible that *ToxA* functions more efficiently in *P. nodorum* compared to *P. tritici-repentis*, or that *P. nodorum* potentially expressed *ToxA* at higher levels than *Ptr*. It is also possible that *P. nodorum* is able to evade recognition by more general host resistance mechanisms that may allow host recognition of *Ptr* leading to circumvention of Ptr ToxA recognition or inhibition of PCD. These are merely speculative explanations and more research is needed to determine the underlying reasons for the vastly different roles of the *Tsn1*-ToxA interaction in conferring tan spot versus SNB.

Instead of finding significance of the *Tsn1* locus on 5BL associated with 86-124, I identified a QTL on the long arm of chromosome 6B that explained 22% of the disease variation with resistance contributed by Altar 84. Singh et al. (2015) conducted association mapping for resistance to tan spot by evaluating 170 historical bread wheat germplasm lines for reaction to a race 1 isolate of *Ptr*. They reported the identification of markers on chromosome 6BS that explained up to 40% of the phenotypic variation. However, the fact that the QTL identified by Singh et al. (2015) was on the short arm of 6B and the QTL I identified in the AL population was on the long arm would suggest they are not the same. It is possible that the QTL we observed on 6BL is due to a yet unidentified host sensitivity gene-NE interaction in the wheat-*Ptr* pathosystem, but more research is needed to address this notion.

The QTL identified on chromosome 4B associated with reaction to tan spot caused by the race 5 isolate DW5 is likely novel, because to my knowledge no tan spot resistance QTL has previously been reported on chromosome 4B. Further validation of this and the QTL on 6B in

different genetic backgrounds is needed to determine the robustness and potential utility of these QTL.

In conclusion, the objectives of this study were to determine the roles of the *Tsn1*-Ptr ToxA and *Tsc2*-Ptr ToxB interactions in tetraploid wheat. First, my results showed that the *Tsc2*-Ptr ToxB interaction played a significant role in the development of tan spot caused by the Ptr ToxB-producing race 5 isolate DW5. This was the first study to demonstrate this for tetraploid wheat. Therefore, durum wheat breeders should determine whether or not their material possesses the *Tsc2* gene and strive to remove it from their lines using marker-assisted selection. Diagnostic markers for *Tsc2* have been developed and proven to be useful for such purposes (Abeysekara et al., 2010). Second, my research showed that the *Tsn1*-ToxA interaction was not associated with the development of tan spot in the AL population, however it played a significant role in the development of SNB. This is the second study to show this result in tetraploid wheat, and validates the work by Chu et al. (2010a) and Friesen et al. (2012) who collectively obtained the same results in a different tetraploid wheat population. Although *Tsn1* may not be relevant for susceptibility to tan spot in these two durum wheat populations, this result needs to be confirmed in other populations as well. Regardless of whether or not *Tsn1* is important for tan spot susceptibility in any durum wheat genotype, breeders should still strive to remove *Tsn1* from their materials in an effort to eliminate SNB susceptibility loci and render their lines more resistant. The *Tsn1* gene has been cloned, and numerous diagnostic markers are available for this purpose (Zhang et al., 2009; Faris et al., 2010). More research is needed to determine why the relevance of the *Tsn1*-Ptr ToxA interaction in the development of tan spot is affected by different host genetic backgrounds, and why the interaction is more significant in the development of SNB than tan spot.

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APPENDIX. MOLECULAR MARKERS MAPPED IN ALTAR 84×LANGDON POPULATION

Marker	Chromosome	Position	Segregation distortion
Xcfa2153	1A	0	ns
Xiwa8622	1A	1.786214556	ns
Xiwa5150	1A	2.153848067	ns
Xiwa6649	1A	2.521481578	ns
Xiwa4351	1A	2.521481578	ns
Xiwa145	1A	37.73368323	ns
Xiwa3435	1A	37.73368323	ns
Xiwa8482	1A	37.73368323	ns
Xiwa6042	1A	37.73368323	ns
Xiwa3805	1A	37.73368323	ns
Xiwa3434	1A	37.73368323	ns
Xiwa8223	1A	41.2549	ns
Xiwa6934	1A	41.2549	ns
Xiwa4537	1A	41.2549	ns
Xiwa4080	1A	41.2549	ns
Xiwa531	1A	41.2549	ns
Xiwa2056	1A	66.26722306	ns
Xiwa6217	1A	67.37845522	ns
Xwmc24	1A	67.37845522	ns
Xiwa3374	1A	70.8720186	ns
Xiwa3373	1A	70.8720186	ns
Xiwa3375	1A	70.8720186	ns
Xiwa8236	1A	82.11901313	ns
Xiwa1991	1A	82.11901313	ns
Xiwa8394	1A	82.11901313	ns
Xiwa7505	1A	82.11901313	ns
Xiwa6887	1A	82.11901313	ns
Xiwa2651	1A	82.11901313	ns
Xiwa2438	1A	82.11901313	ns
Xiwa393	1A	82.11901313	ns
Xiwa392	1A	82.11901313	ns
Xiwa357	1A	82.11901313	ns
Xiwa3665	1A	84.39317487	ns
Xiwa3806	1A	84.39317487	ns
Xiwa3957	1A	84.39317487	ns

Marker	Chromosome	Position	Segregation distortion
Xcfa2135	1A	88.47467771	ns
Xiwa7898	1A	101.0075044	ns
Xiwa2598	1A	101.0075044	ns
Xiwa2584	1A	101.0075044	ns
Xiwa1594	1A	101.0075044	ns
Xiwa6709	1A	101.3724546	ns
Xiwa8070	1A	101.3724546	ns
Xiwa5839	1A	101.3724546	ns
Xiwa6756	1A	101.3724546	ns
Xiwa4126	1A	101.3724546	ns
Xiwa4117	1A	101.3724546	ns
Xiwa4116	1A	101.3724546	ns
Xiwa268	1A	101.3724546	ns
Xiwa2152	1A	101.7374049	ns
Xiwa1615	1A	101.7374049	ns
Xiwa6835	1A	101.7374049	ns
Xiwa8523	1A	128.1349896	ns
Xiwa8528	1A	128.1349896	ns
Xiwa1710	1A	128.8702964	ns
Xiwa5407	1A	128.8702964	ns
Xiwa8020	1A	130.7507739	ns
Xiwa2035	1A	132.6172065	ns
Xiwa3089	1A	132.6172065	ns
Xiwa6458	1A	132.6172065	ns
Xiwa1559	1A	132.6172065	ns
Xiwa1560	1A	132.6172065	ns
Xiwa1558	1A	132.6172065	ns
Xiwa1557	1A	132.6172065	ns
Xiwa2317	1A	134.8913683	ns
Xiwa3409	1A	144.489597	ns
Xhpych4	1B	0	ns
Xiwa6787	1B	28.32159414	ns
Xiwa4349	1B	28.32159414	ns
Xhbe482	1B	30.20911157	ns
Xbarc8	1B	32.66181712	ns

Marker	Chromosome	Position	Segregation distortion
Xhbg326	1B	33.14255585	*
Xiwa7703	1B	35.89739767	ns
Xiwa6448	1B	37.76383027	ns
Xiwa6450	1B	37.76383027	ns
Xiwa8338	1B	45.25692568	ns
Xiwa5681	1B	45.25692568	ns
Xiwa7219	1B	45.25692568	ns
Xiwa5592	1B	45.25692568	ns
Xiwa1884	1B	45.25692568	ns
Xiwa1451	1B	45.25692568	ns
Xiwa580	1B	45.25692568	ns
Xiwa284	1B	45.25692568	ns
Xbarc137	1B	47.66239937	ns
Xgwm18	1B	47.66239937	ns
Xhbg419	1B	48.11282478	ns
Xgwm11	1B	48.11282478	ns
Xcfd59	1B	48.11282478	ns
Xgwm264.1	1B	51.77735101	ns
Xiwa43	1B	55.19128688	*
Xiwa44	1B	55.19128688	*
Xiwa107	1B	55.55359276	*
Xiwa378	1B	55.91589864	ns
Xiwa8081	1B	61.09271779	ns
Xiwa7119	1B	61.09271779	ns
Xiwa6063	1B	61.09271779	ns
Xiwa5348	1B	61.09271779	ns
Xiwa7723	1B	61.45502367	ns
Xiwa7722	1B	61.45502367	ns
Xiwa7721	1B	61.45502367	ns
Xiwa5561	1B	61.45502367	ns
Xiwa3587	1B	61.45502367	ns
Xiwa3502	1B	61.45502367	ns
Xiwa2517	1B	61.45502367	ns
Xiwa1109	1B	61.45502367	ns
Xhbg258.1	1B	64.20986549	ns

Marker	Chromosome	Position	Segregation distortion
Xgwm131	1B	65.24082754	ns
Xiwa128	1B	67.30362902	*
Xiwa4681	1B	87.08491784	*
Xiwa4680	1B	87.08491784	*
Xiwa5070	1B	88.56675051	*
Xiwa6945	1B	88.56675051	*
Xiwa6134	1B	89.2966899	ns
Xhbg262.2	1B	94.45463837	ns
Xwmc134	1B	108.8855535	ns
Xiwa5186	1B	121.0766022	ns
Xiwa3017	1B	124.1336603	ns
Xiwa6646	1B	124.4959662	ns
Xiwa4155	1B	125.2259056	ns
Xiwa4154	1B	125.5882115	ns
Xbarc81	1B	126.0511472	ns
Xgwm153	1B	126.5774229	ns
Xiwa5749	1B	127.0928491	ns
Xiwa2064	1B	127.4577994	ns
Xiwa3097	1B	130.951634	ns
Xhbg262.1	1B	145.4613103	ns
Xwmc44	1B	146.4047329	ns
Xiwa919	1B	149.8077092	ns
Xiwa8332	1B	150.1700151	ns
Xiwa7992	1B	150.1700151	ns
Xiwa3893	1B	150.5323209	ns
Xiwa3892	1B	150.5323209	ns
Xiwa3660	1B	150.5323209	ns
Xiwa848	1B	150.5323209	ns
Xiwa846	1B	150.5323209	ns
Xhbe248	1B	151.4757435	ns
Xhbg236	1B	151.4757435	ns
Xhbg443	1B	157.5656551	*
Xiwa725	1B	159.8397579	*
Xiwa724	1B	159.8397579	*
Xhbg224	2A	0	*

Marker	Chromosome	Position	Segregation distortion
Xiwa1512	2A	30.02560572	ns
Xiwa6391	2A	30.02560572	ns
Xiwa5423	2A	30.02560572	ns
Xiwa5341	2A	30.02560572	ns
Xiwa5340	2A	30.02560572	ns
Xiwa1511	2A	30.02560572	ns
Xiwa6922	2A	30.3879116	ns
Xiwa4989	2A	30.3879116	ns
Xiwa965	2A	35.12615645	ns
Xiwa8513	2A	38.59303213	ns
Xiwa2434	2A	54.01621607	ns
Xiwa2433	2A	54.01621607	ns
Xiwa2526	2A	54.74615547	ns
Xcfa2201	2A	57.83602027	ns
Xiwa5240	2A	79.59988833	ns
Xiwa313	2A	79.59988833	ns
Xiwa8491	2A	79.59988833	ns
Xiwa7248	2A	79.59988833	ns
Xiwa6369	2A	79.59988833	ns
Xiwa5586	2A	79.59988833	ns
Xiwa3569	2A	79.59988833	ns
Xiwa2259	2A	79.59988833	ns
Xiwa2245	2A	79.59988833	ns
Xiwa1597	2A	79.59988833	ns
Xiwa1256	2A	79.59988833	ns
Xiwa994	2A	79.59988833	ns
Xiwa424	2A	79.59988833	ns
Xiwa343	2A	79.59988833	ns
Xiwa314	2A	79.59988833	ns
Xiwa690	2A	80.32982773	ns
Xiwa581	2A	81.05976712	ns
Xiwa8328	2A	81.422073	ns
Xiwa8036	2A	114.5052093	ns
Xiwa2601	2A	114.5052093	ns
Xiwa4870	2A	117.1590593	ns

Marker	Chromosome	Position	Segregation distortion
Xiwa3596	2A	117.1590593	ns
Xiwa1348	2A	117.1590593	ns
Xiwa8402	2A	117.1590593	ns
Xiwa6620	2A	117.1590593	ns
Xiwa1351	2A	117.1590593	ns
Xiwa1350	2A	117.1590593	ns
Xiwa1347	2A	117.1590593	ns
Xiwa6839	2A	117.1590593	ns
Xiwa6600	2A	117.1590593	ns
Xiwa3594	2A	117.1590593	ns
Xiwa1349	2A	117.1590593	ns
Xiwa319	2A	117.1590593	ns
Xiwa2778	2A	125.6422903	ns
Xiwa4491	2A	125.6422903	ns
Xiwa4493	2A	125.6422903	ns
Xiwa5879	2A	125.6422903	ns
Xiwa6963	2A	125.6422903	ns
Xiwa7327	2A	125.6422903	ns
Xiwa1088	2A	125.6422903	ns
Xiwa7335	2A	125.6422903	ns
Xiwa8325	2A	125.6422903	ns
Xiwa2370	2A	149.7437365	ns
Xiwa5959	2A	156.2844195	ns
Xwmc181	2A	162.8968431	*
Xhbg327	2A	178.6786375	ns
Xwmc382.2	2B	0	***
Xwmc764	2B	0.403207894	**
Xiwa6957	2B	0.403207894	***
Xwmc661	2B	4.256687357	**
Xiwa2304	2B	8.110166819	*
Xhbg216	2B	11.96364628	ns
tsc2	2B	18.54283021	ns
Xwmc25	2B	29.8409827	ns
Xwmc154	2B	35.20071032	ns
Xiwa6474	2B	57.32342006	ns

Marker	Chromosome	Position	Segregation distortion
Xiwa1360	2B	57.32342006	ns
Xiwa1359	2B	57.32342006	ns
Xwmc597	2B	78.0515922	*
Xiwa6069	2B	93.20270579	ns
Xiwa5392	2B	93.20270579	ns
Xbarc55	2B	98.9800341	ns
Xiwa8221	2B	99.38001661	ns
Xiwa4323	2B	99.38001661	ns
Xiwa7661	2B	101.6370581	ns
Xiwa6509	2B	101.6370581	ns
Xiwa3127	2B	101.6370581	ns
Xiwa3126	2B	101.6370581	ns
Xiwa1763	2B	101.6370581	ns
Xiwa897	2B	101.6370581	ns
Xiwa10	2B	104.290908	ns
Xiwa3924	2B	104.290908	ns
Xiwa4894	2B	104.290908	ns
Xiwa6075	2B	106.5479495	ns
Xiwa4102	2B	106.5479495	ns
Xwmc149.2	2B	115.8360282	ns
Xiwa2130	2B	151.360997	***
Xiwa2131	2B	151.360997	***
Xiwa2261	2B	151.360997	***
Xiwa469	2B	151.360997	***
Xiwa5414	2B	151.360997	***
Xiwa4866	2B	179.5521094	**
Xiwa7996	2B	182.6091675	**
Xiwa2873	2B	183.712227	***
Xiwa3176	2B	183.712227	***
Xiwa1040	2B	188.0194586	****
Xiwa7640	2B	188.0194586	****
Xiwa5081	2B	196.0027206	*****
Xiwa7112	2B	196.0027206	*****
Xiwa7113	2B	196.0027206	*****
Xiwa571	2B	196.73266	*****

Marker	Chromosome	Position	Segregation distortion
Xiwa8589	2B	196.73266	*****
Xiwa4257	3A	0	ns
Xiwa5969	3A	5.016410738	ns
Xwmc532	3A	5.016410738	ns
Xbarc19	3A	44.59183671	ns
Xgwm666.1	3A	44.59183671	ns
Xhbg345	3A	59.11572931	ns
Xiwa8061	3A	70.58621199	ns
Xiwa7476	3A	70.58621199	ns
Xiwa6783	3A	70.58621199	ns
Xiwa6170	3A	70.58621199	ns
Xiwa7355	3A	70.58621199	ns
Xiwa5632	3A	70.58621199	ns
Xiwa4917	3A	70.58621199	ns
Xiwa3929	3A	70.58621199	ns
Xiwa3836	3A	70.58621199	ns
Xiwa3376	3A	70.58621199	ns
Xiwa2751	3A	70.58621199	ns
Xiwa2618	3A	70.58621199	ns
Xiwa1713	3A	70.58621199	ns
Xiwa1019	3A	70.58621199	ns
Xiwa234	3A	72.86037374	ns
Xiwa4075	3A	72.86037374	ns
Xiwa8465	3A	73.22532399	ns
Xiwa1507	3A	75.89946844	ns
Xiwa1604	3A	75.89946844	ns
Xiwa1982	3A	75.89946844	ns
Xiwa2925	3A	75.89946844	ns
Xiwa3772	3A	75.89946844	ns
Xiwa7150	3A	75.89946844	ns
Xiwa7970	3A	75.89946844	ns
Xiwa2332	3A	75.89946844	ns
Xiwa3600	3A	75.89946844	ns
Xiwa3771	3A	75.89946844	ns
Xiwa4001	3A	75.89946844	ns

Marker	Chromosome	Position	Segregation distortion
Xiwa6907	3A	75.89946844	ns
Xiwa7564	3A	75.89946844	ns
Xiwa7817	3A	75.89946844	ns
Xiwa7891	3A	75.89946844	ns
Xiwa3198	3A	79.39330304	ns
Xiwa6750	3A	79.39330304	ns
Xiwa7159	3A	79.39330304	ns
Xiwa7877	3A	79.39330304	ns
Xiwa2348	3A	99.61863601	ns
Xiwa5114	3A	99.61863601	ns
Xiwa5601	3A	99.61863601	ns
Xiwa5602	3A	99.61863601	ns
Xiwa5212	3A	124.9959379	ns
Xiwa855	3A	124.9959379	ns
Xiwa3111	3A	124.9959379	ns
Xiwa5980	3A	124.9959379	ns
Xiwa7602	3A	124.9959379	ns
Xcfa2037	3A	157.4706682	ns
Xcfa2076	3A	159.9724989	ns
Xiwa4796	3B	0	ns
Xiwa5202	3B	1.103059575	ns
Xiwa7230	3B	2.20611915	ns
Xiwa4801	3B	2.20611915	ns
Xiwa4800	3B	2.20611915	ns
Xiwa5426	3B	2.568425028	ns
Xiwa5106	3B	2.568425028	ns
Xiwa2908	3B	3.671484603	ns
Xhbe383	3B	3.671484603	*
Xiwa2037	3B	18.85695154	*
Xiwa8303	3B	23.16418311	*
Xiwa8522	3B	46.33981072	ns
Xiwa747	3B	48.20624333	ns
Xiwa6238	3B	50.46328477	ns
Xiwa5710	3B	82.23079215	ns
Xiwa8290	3B	82.23079215	ns

Marker	Chromosome	Position	Segregation distortion
Xiwa4218	3B	82.23079215	ns
Xiwa8583	3B	82.23079215	ns
Xiwa7512	3B	82.23079215	ns
Xiwa3306	3B	82.23079215	ns
Xiwa3305	3B	82.23079215	ns
Xiwa3304	3B	82.23079215	ns
Xiwa5775	3B	82.96609892	*
Xiwa4653	3B	82.96609892	*
Xiwa3018	3B	83.7014057	*
Xbarc344	3B	109.9420623	ns
Xiwa6056	3B	125.6552536	*
Xiwa6057	3B	125.6552536	*
Xiwa8354	3B	125.6552536	*
Xbarc84	3B	140.346653	ns
Xiwa3159	3B	159.3951953	ns
Xiwa8479	3B	162.4522534	ns
Xiwa5892	3B	167.1904983	ns
Xiwa8203	3B	167.1904983	ns
Xbarc138	4A	0	ns
Xcfd71	4A	0	ns
Xwmc491	4A	2.705115598	ns
Xiwa603	4A	22.92279114	ns
Xiwa4321	4A	25.17983259	ns
Xiwa7521	4A	27.45399433	ns
Xiwa7537	4A	27.45399433	ns
Xiwa1320	4A	29.71103578	ns
Xiwa110	4A	33.59455878	ns
Xiwa115	4A	33.59455878	ns
Xiwa172	4A	33.59455878	ns
Xiwa1341	4A	33.59455878	ns
Xiwa2781	4A	33.59455878	ns
Xiwa3326	4A	33.59455878	ns
Xiwa3541	4A	33.59455878	ns
Xiwa3542	4A	33.59455878	ns
Xiwa3565	4A	33.59455878	ns

Marker	Chromosome	Position	Segregation distortion
Xiwa3845	4A	33.59455878	ns
Xiwa5652	4A	33.59455878	ns
Xiwa5851	4A	33.59455878	ns
Xiwa5975	4A	33.59455878	ns
Xiwa6597	4A	33.59455878	ns
Xiwa7270	4A	33.59455878	ns
Xiwa7271	4A	33.59455878	ns
Xiwa8414	4A	33.59455878	ns
Xiwa1824	4A	33.95686466	ns
Xiwa8416	4A	37.01392275	ns
Xiwa232	4A	37.01392275	ns
Xiwa126	4A	37.01392275	ns
Xiwa3792	4A	40.07098084	ns
Xiwa2000	4A	40.07098084	ns
Xiwa7859	4A	40.07098084	ns
Xiwa7657	4A	40.07098084	ns
Xiwa7617	4A	40.07098084	ns
Xiwa7522	4A	40.07098084	ns
Xiwa7134	4A	40.07098084	ns
Xiwa7133	4A	40.07098084	ns
Xiwa7092	4A	40.07098084	ns
Xiwa6944	4A	40.07098084	ns
Xiwa6540	4A	40.07098084	ns
Xiwa4772	4A	40.07098084	ns
Xiwa4771	4A	40.07098084	ns
Xiwa3582	4A	40.07098084	ns
Xiwa3581	4A	40.07098084	ns
Xiwa3361	4A	40.07098084	ns
Xiwa3344	4A	40.07098084	ns
Xiwa3088	4A	40.07098084	ns
Xiwa1919	4A	40.07098084	ns
Xiwa109	4A	40.43328671	ns
Xiwa1720	4A	67.53757683	ns
Xwmc468	4A	78.10683797	ns
Xiwa6035	4A	78.10683797	ns

Marker	Chromosome	Position	Segregation distortion
Xiwa7653	4A	88.69635916	ns
Xbarc170	4A	99.98644617	ns
Xiwa3068	4A	128.5697385	ns
Xiwa506	4B	0	ns
Xiwa2126	4B	7.071071995	ns
Xiwa4569	4B	9.724921947	ns
Xbarc1045	4B	8	ns
Xgwm113	4B	45.52337899	ns
Xiwa7641	4B	48.65220294	**
Xiwa58	4B	48.65220294	**
Xiwa1007	4B	63.19884629	*
Xiwa7752	4B	64.31837304	*
Xiwa1035	4B	64.68332329	*
Xiwa3396	4B	65.04827355	*
Xiwa2031	4B	90.42557539	ns
Xiwa2595	4B	90.42557539	ns
Xiwa5408	4B	90.42557539	ns
Xiwa6465	4B	90.42557539	ns
Xiwa7766	4B	107.2187546	*
Xiwa5520	4B	107.948694	*
Xiwa4767	5A	0	ns
Xiwa4766	5A	0	ns
Xiwa4765	5A	0	ns
Xiwa8155	5A	13.13711532	ns
Xiwa8154	5A	13.13711532	ns
Xiwa6287	5A	13.13711532	ns
Xiwa3190	5A	13.13711532	ns
Xiwa7777	5A	17.08204056	ns
Xiwa1301	5A	17.08204056	ns
Xiwa2120	5A	19.33908201	ns
Xiwa4736	5A	19.33908201	ns
Xiwa7351	5A	19.70138789	ns
Xiwa5496	5A	20.06369377	ns
Xiwa5521	5A	20.06369377	ns
Xiwa154	5A	20.42599964	ns

Marker	Chromosome	Position	Segregation distortion
Xcfa2250	5A	21.05093204	ns
Xiwa3445	5A	21.67586443	ns
Xiwa333	5A	21.67586443	ns
Xiwa2480	5A	22.40580383	ns
Xhbg231.2	5A	28.55846996	ns
Xbarc360	5A	31.37778061	ns
Xiwa2926	5A	32.54061997	ns
Xiwa5528	5A	34.02245264	ns
Xiwa5735	5A	36.27949409	ns
Xiwa7129	5A	36.64179996	ns
Xiwa7130	5A	36.64179996	ns
Xiwa3975	5A	38.12363263	ns
Xiwa5539	5A	38.12363263	ns
Xiwa7596	5A	38.12363263	ns
Xiwa7597	5A	38.12363263	ns
Xiwa7598	5A	38.12363263	ns
Xiwa1486	5A	64.18638331	*
Xiwa4648	5A	64.18638331	*
Xiwa2014	5A	65.28944289	**
Xhbg247	5A	73.42402465	*****
Xiwa2350	5A	82.87448313	*****
Xiwa4448	5A	82.87448313	*****
Xiwa4449	5A	82.87448313	*****
Xiwa7255	5A	82.87448313	*****
Xiwa7256	5A	82.87448313	*****
Xiwa5040	5A	83.60442253	*****
Xiwa509	5A	85.0862552	*****
Xiwa3705	5A	88.9697782	*****
Xbarc142.2	5A	90.77221144	*****
Xiwa3623	5A	93.04631421	*****
Xiwa583	5A	93.41126446	*****
Xiwa582	5A	93.41126446	*****
Xcfa2141	5A	93.96066929	*****
Xiwa2113	5A	113.5586677	****
Xiwa2856	5A	113.5586677	****

Marker	Chromosome	Position	Segregation distortion
Xiwa2857	5A	113.5586677	****
Xiwa2858	5A	113.5586677	****
Xiwa2859	5A	113.5586677	****
Xiwa4238	5A	113.5586677	****
Xiwa7009	5A	113.5586677	****
Xiwa7568	5A	121.5419297	*
Xiwa1670	5A	122.2718691	*
Xiwa7880	5A	130.7551001	ns
Xgwm234	5B	0	***
Xiwa757	5B	2.832959601	*****
Xiwa868	5B	2.832959601	*****
Xiwa3606	5B	8.456184066	*****
Xiwa3607	5B	8.456184066	*****
Xiwa332	5B	13.19442892	****
Xiwa420	5B	13.19442892	****
Xwmc149.1	5B	17.04783122	*
Xbarc4	5B	29.35801058	***
Xgwm544	5B	29.83874931	**
Xwmc73	5B	31.29533192	**
Xiwa7613	5B	35.07576193	**
Xiwa6846	5B	35.07576193	**
Xhbe478	5B	40.48843492	***
Xbarc216	5B	40.48843492	***
Xiwa1342	5B	41.39754948	****
Xiwa1965	5B	41.39754948	****
Xiwa4377	5B	41.75985536	****
Xiwa6816	5B	41.75985536	****
Xiwa6521	5B	41.75985536	****
Xiwa5494	5B	41.75985536	****
Xiwa5079	5B	41.75985536	****
Xiwa4378	5B	41.75985536	****
Xiwa1176	5B	41.75985536	****
Xiwa7857	5B	42.48979476	***
Xiwa6568	5B	42.48979476	***
Xiwa6567	5B	42.48979476	***

Marker	Chromosome	Position	Segregation distortion
Xiwa6447	5B	42.48979476	***
Xiwa6429	5B	42.48979476	***
Xiwa5334	5B	42.48979476	***
Xiwa4708	5B	42.48979476	***
Xiwa4686	5B	42.48979476	***
Xiwa894	5B	42.48979476	***
Xiwa620	5B	42.48979476	***
Xiwa3870	5B	43.59285433	***
Xhbg279.1	5B	45.4983621	**
Xgwm213	5B	52.28035281	***
Xbarc74	5B	53.29048605	*
Xhbg231.1	5B	53.29048605	*
Xiwa7227	5B	55.19599382	**
Xiwa6689	5B	55.19599382	**
Xiwa6526	5B	55.19599382	**
Xiwa5280	5B	55.19599382	**
Xiwa5279	5B	55.19599382	**
Xiwa303	5B	55.19599382	**
Xiwa4422	5B	55.92593322	*
Xiwa8603	5B	57.40776588	ns
Xiwa5742	5B	59.66480733	*
Xgwm371	5B	69.35779647	**
Xiwa6638	5B	74.82447541	**
Xiwa5486	5B	74.82447541	**
Xiwa5331	5B	74.82447541	**
Xiwa3436	5B	74.82447541	**
Xiwa1380	5B	74.82447541	**
Xiwa2536	5B	75.93570756	*
Xiwa6867	5B	79.01633797	**
Xiwa6468	5B	79.01633797	**
Xiwa6383	5B	79.01633797	**
Xiwa5283	5B	79.01633797	**
Xiwa4074	5B	79.01633797	**
Xiwa3985	5B	79.01633797	**
Xiwa2455	5B	79.01633797	**

Marker	Chromosome	Position	Segregation distortion
Xiwa2454	5B	79.01633797	**
Xiwa2453	5B	79.01633797	**
Xiwa7795	5B	79.37864384	**
Xiwa265	5B	79.37864384	**
Xgwm499	5B	80.38877709	**
Xiwa7776	5B	97.4678705	ns
Xiwa5214	5B	102.6446896	ns
Xiwa7024	5B	116.7690652	ns
tsn1	5B	117.1690477	ns
Xiwa6915	5B	117.9755159	ns
Xiwa8375	5B	119.8419485	ns
Xiwa2306	5B	119.8419485	ns
Xiwa3002	5B	120.9450081	ns
Xiwa2255	5B	122.4268408	ns
Xiwa2565	5B	122.4268408	ns
Xiwa6024	5B	122.4268408	ns
Xiwa6671	5B	122.4268408	ns
Xiwa1775	5B	122.4268408	ns
Xiwa3226	5B	122.7891466	ns
Xgwm408	5B	127.0043375	ns
Xiwa2500	5B	141.8700256	ns
Xgwm604	5B	143.6249945	ns
Xhbg399	5B	145.9199862	ns
Xbarc142.1	5B	147.2838459	ns
Xiwa7872	5B	148.5998361	ns
Xiwa7478	5B	148.5998361	ns
Xiwa4539	5B	148.5998361	ns
Xiwa6779	5B	152.0667118	ns
Xiwa8250	5B	152.0667118	ns
Xwmc160	5B	153.8372218	ns
Xbarc232	5B	154.7463364	ns
Xwmc235.1	5B	158.2872294	ns
Xiwa7966	5B	160.6472571	ns
Xiwa2388	5B	160.6472571	ns
Xiwa7965	5B	160.6472571	ns

Marker	Chromosome	Position	Segregation distortion
Xhbg429	5B	179.0843738	**
Xiwa3972	5B	180.8868071	*
Xiwa6393	5B	180.8868071	*
Xwmc640	5B	185.3536527	**
Xbarc243	5B	203.622645	**
Xiwa3658	5B	210.5975386	ns
Xiwa2093	5B	211.7170654	ns
Xhbe297	6A	0	ns
Xmag1885	6A	34.16035589	**
Xiwa7572	6A	73.94458422	ns
Xiwa6853	6A	73.94458422	ns
Xiwa6116	6A	73.94458422	ns
Xiwa4603	6A	73.94458422	ns
Xiwa4602	6A	73.94458422	ns
Xiwa504	6A	73.94458422	ns
Xiwa3488	6A	76.20162567	ns
Xiwa8568	6A	76.20162567	ns
Xiwa6858	6A	76.20162567	ns
Xiwa4478	6A	76.20162567	ns
Xiwa3487	6A	76.20162567	ns
Xiwa2481	6A	77.68345834	ns
Xiwa8602	6A	87.19766007	**
Xiwa20	6A	93.73834303	ns
Xiwa19	6A	93.73834303	ns
Xhbg416	6A	116.3339421	ns
Xiwa399	6A	118.9448074	ns
Xiwa8592	6A	119.3071133	ns
Xiwa6288	6A	120.4101729	ns
Xiwa6247	6A	120.4101729	ns
Xiwa6033	6A	120.4101729	ns
Xiwa6012	6A	120.4101729	ns
Xiwa664	6A	120.7724787	ns
Xiwa4865	6A	121.8755383	ns
Xiwa6596	6A	121.8755383	ns
Xiwa6699	6A	121.8755383	ns

Marker	Chromosome	Position	Segregation distortion
Xiwa2895	6A	121.8755383	ns
Xiwa4036	6A	121.8755383	ns
Xiwa4035	6A	121.8755383	ns
Xiwa2192	6A	121.8755383	ns
Xiwa1514	6A	121.8755383	ns
Xiwa1475	6A	121.8755383	ns
Xiwa1474	6A	121.8755383	ns
Xiwa1194	6A	121.8755383	ns
Xiwa741	6A	121.8755383	ns
Xiwa651	6A	121.8755383	ns
Xiwa650	6A	121.8755383	ns
Xiwa218	6A	121.8755383	ns
Xiwa4928	6A	121.8755383	ns
Xiwa1671	6A	121.8755383	ns
Xiwa1235	6A	121.8755383	ns
Xiwa4929	6A	121.8755383	ns
Xiwa8585	6A	122.6108451	ns
Xiwa7052	6A	123.3407845	ns
Xiwa1254	6B	0	ns
Xiwa8314	6B	0.729939395	ns
Xiwa4633	6B	4.613462395	ns
Xiwa7725	6B	20.03664634	**
Xiwa52	6B	23.50352201	*
Xcfd13	6B	24.57882954	***
Xiwa3229	6B	26.77777701	**
Xiwa7239	6B	45.77789623	ns
Xiwa861	6B	45.77789623	ns
Xiwa8134	6B	48.03493767	*
Xiwa5888	6B	48.03493767	*
Xiwa3411	6B	48.03493767	*
Xiwa3410	6B	48.03493767	*
Xiwa7808	6B	49.51677034	*
Xiwa7810	6B	49.51677034	*
Xiwa7809	6B	49.51677034	*
Xiwa7807	6B	49.51677034	*

Marker	Chromosome	Position	Segregation distortion
Xiwa7618	6B	49.51677034	*
Xiwa2219	6B	49.51677034	*
Xiwa5056	6B	50.24670974	*
Xiwa5055	6B	50.24670974	*
Xiwa7937	6B	53.71358541	*
Xiwa7689	6B	53.71358541	*
Xiwa3501	6B	53.71358541	*
Xiwa7897	6B	54.44352481	ns
Xiwa7896	6B	54.44352481	ns
Xiwa2307	6B	54.44352481	ns
Xiwa8011	6B	59.66205327	**
Xiwa4078	6B	59.66205327	**
Xiwa7676	6B	60.76511284	**
Xiwa7783	6B	60.76511284	**
Xiwa7401	6B	60.76511284	**
Xiwa6293	6B	60.76511284	**
Xiwa5748	6B	60.76511284	**
Xiwa4515	6B	60.76511284	**
Xiwa4065	6B	60.76511284	**
Xiwa3971	6B	60.76511284	**
Xiwa2451	6B	60.76511284	**
Xiwa1742	6B	60.76511284	**
Xiwa1545	6B	60.76511284	**
Xiwa685	6B	60.76511284	**
Xiwa4986	6B	60.76511284	**
Xiwa6153	6B	61.86817242	*
Xiwa5504	6B	61.86817242	*
Xiwa5102	6B	61.86817242	*
Xiwa5029	6B	61.86817242	*
Xiwa3917	6B	61.86817242	*
Xiwa2780	6B	61.86817242	*
Xiwa1839	6B	61.86817242	*
Xiwa1838	6B	61.86817242	*
Xiwa3652	6B	62.23312267	*
Xiwa3797	6B	62.23312267	*

Marker	Chromosome	Position	Segregation distortion
Xiwa4169	6B	62.23312267	*
Xiwa4848	6B	62.23312267	*
Xiwa4924	6B	62.23312267	*
Xiwa5966	6B	62.23312267	*
Xiwa6101	6B	62.23312267	*
Xiwa4170	6B	62.23312267	*
Xiwa3459	6B	62.96306206	ns
Xiwa8165	6B	62.96306206	ns
Xiwa5345	6B	62.96306206	ns
Xgwm88	6B	64.00376801	*
Xgwm193	6B	64.00376801	*
Xiwa4484	6B	72.58620812	ns
Xiwa4485	6B	72.58620812	ns
Xiwa5722	6B	73.69744027	ns
Xiwa6329	6B	74.05974615	ns
Xiwa6660	6B	74.05974615	ns
Xiwa457	6B	82.21480653	ns
Xiwa3967	6B	83.31786611	ns
Xiwa6599	6B	83.31786611	ns
Xiwa5170	6B	83.31786611	ns
Xiwa1473	6B	83.31786611	ns
Xiwa1472	6B	83.31786611	ns
Xiwa4202	6B	83.68017198	ns
Xiwa3769	6B	83.68017198	ns
Xiwa1531	6B	83.68017198	ns
Xiwa225	6B	84.41011138	ns
Xiwa2346	6B	84.41011138	ns
Xiwa8566	6B	84.41011138	ns
Xiwa2347	6B	84.41011138	ns
Xiwa3636	6B	84.77241726	ns
Xiwa221	6B	85.13736751	ns
Xiwa4337	6B	85.13736751	ns
Xiwa4959	6B	85.13736751	ns
Xiwa5148	6B	85.13736751	ns
Xiwa967	6B	104.1374867	ns

Marker	Chromosome	Position	Segregation distortion
Xiwa1629	6B	108.8757316	ns
Xiwa1628	6B	108.8757316	ns
Xiwa405	6B	108.8757316	ns
Xiwa404	6B	108.8757316	ns
Xgwm219	6B	111.0991376	ns
Xiwa7500	7A	0	ns
Xiwa6331	7A	8.993402573	ns
Xiwa679	7A	8.993402573	ns
Xiwa8390	7A	32.16903019	ns
Xiwa954	7A	41.16243276	ns
Xcfa2049	7A	42.33895371	ns
Xiwa3505	7A	47.4188189	ns
Xhbg469	7A	53.82557785	ns
Xhbg238	7A	80.83004252	ns
Xiwa7472	7A	109.9851085	ns
Xiwa7724	7A	109.9851085	ns
Xiwa7792	7A	109.9851085	ns
Xiwa7990	7A	109.9851085	ns
Xiwa8171	7A	109.9851085	ns
Xiwa1477	7A	110.3474144	ns
Xiwa8073	7A	122.6419427	ns
Xiwa7090	7A	124.5083753	ns
Xiwa448	7A	124.8706812	ns
Xiwa4996	7A	124.8706812	ns
Xiwa689	7A	125.232987	ns
Xiwa808	7A	125.232987	ns
Xiwa1502	7A	125.232987	ns
Xiwa2385	7A	125.232987	ns
Xiwa2386	7A	125.232987	ns
Xiwa2387	7A	125.232987	ns
Xiwa2437	7A	125.232987	ns
Xiwa4062	7A	125.232987	ns
Xiwa1277	7A	142.0261662	ns
Xiwa3863	7A	142.0261662	ns
Xiwa6124	7A	142.0261662	ns

Marker	Chromosome	Position	Segregation distortion
Xiwa7554	7A	142.0261662	ns
Xiwa8066	7A	142.0261662	ns
Xiwa788	7A	143.1292258	ns
Xiwa1418	7A	143.1292258	ns
Xiwa1871	7A	143.1292258	ns
Xiwa2381	7A	143.1292258	ns
Xiwa2954	7A	143.1292258	ns
Xiwa3693	7A	143.1292258	ns
Xiwa3694	7A	143.1292258	ns
Xiwa4818	7A	143.1292258	ns
Xiwa6183	7A	143.1292258	ns
Xiwa4411	7A	159.2280247	ns
Xiwa4672	7A	159.2280247	ns
Xiwa4735	7A	159.2280247	ns
Xiwa4817	7A	159.2280247	ns
Xiwa5526	7A	159.2280247	ns
Xiwa7650	7A	159.2280247	ns
Xiwa7651	7A	159.2280247	ns
Xiwa7770	7A	159.2280247	ns
Xiwa7917	7A	159.2280247	ns
Xiwa8115	7A	159.2280247	ns
Xiwa8248	7A	159.2280247	ns
Xiwa2176	7A	159.2280247	ns
Xiwa6940	7A	159.5903306	ns
Xiwa2011	7A	165.213555	ns
Xiwa2012	7A	165.213555	ns
Xiwa4288	7A	165.213555	ns
Xiwa614	7A	180.7917635	ns
Xiwa2775	7A	180.7917635	ns
Xiwa2776	7A	180.7917635	ns
Xiwa5489	7A	180.7917635	ns
Xcfa2123	7A	180.7917635	ns
Xiwa1424	7A	184.9675065	ns
Xiwa1425	7A	184.9675065	ns
Xiwa1724	7A	184.9675065	ns

Marker	Chromosome	Position	Segregation distortion
Xiwa1726	7A	184.9675065	ns
Xiwa5790	7A	184.9675065	ns
Xiwa6715	7A	184.9675065	ns
Xiwa7046	7A	184.9675065	ns
Xcfd20.1	7A	223.8504537	ns
Xhbg234	7A	256.0074704	ns
Xiwa7409	7A	262.4052603	ns
Xiwa1526	7B	0	**
Xiwa783	7B	0	**
Xiwa1181	7B	0	**
Xiwa7233	7B	27.70152093	ns
Xiwa3572	7B	27.70152093	ns
Xiwa3508	7B	27.70152093	ns
Xiwa3507	7B	27.70152093	ns
Xiwa518	7B	27.70152093	ns
Xiwa5566	7B	29.58199842	ns
Xiwa5565	7B	29.58199842	ns
Xiwa3958	7B	29.58199842	ns
Xiwa2832	7B	29.58199842	ns
Xiwa3959	7B	29.58199842	ns
Xiwa4873	7B	46.37517761	ns
Xiwa3121	7B	49.84205328	ns
Xiwa5210	7B	49.84205328	ns
Xiwa5661	7B	49.84205328	ns
Xiwa7846	7B	49.84205328	ns
Xiwa7831	7B	49.84205328	ns
Xiwa7830	7B	49.84205328	ns
Xiwa6788	7B	49.84205328	ns
Xiwa5663	7B	49.84205328	ns
Xiwa5662	7B	49.84205328	ns
Xiwa3663	7B	49.84205328	ns
Xiwa8233	7B	51.32388595	ns
Xiwa6401	7B	62.46892294	ns
Xiwa3987	7B	62.46892294	ns
Xiwa1361	7B	62.46892294	ns

Marker	Chromosome	Position	Segregation distortion
Xiwa354	7B	63.5801551	ns
Xiwa355	7B	63.5801551	ns
Xiwa8232	7B	68.75697424	ns
Xiwa6322	7B	68.75697424	ns
Xiwa6667	7B	68.75697424	ns
Xiwa320	7B	91.93260186	ns
Xiwa394	7B	91.93260186	ns
Xiwa395	7B	91.93260186	ns
Xiwa521	7B	93.03566144	ns
Xiwa8519	7B	93.03566144	ns
Xiwa520	7B	93.03566144	ns
Xiwa2369	7B	107.8010081	ns
Xiwa6532	7B	107.8010081	ns
Xiwa130	7B	110.4548581	ns
Xiwa2191	7B	112.7118995	ns
Xiwa4864	7B	112.7118995	ns
Xiwa2193	7B	113.4418389	ns
Xiwa4749	7B	113.4418389	ns
Xiwa6246	7B	113.4418389	ns
Xiwa7907	7B	114.1717783	ns
Xiwa2389	7B	115.2748379	ns
Xiwa7260	7B	116.0047773	ns
Xiwa7964	7B	116.0047773	ns
Xiwa1091	7B	116.0047773	ns
Xiwa1044	7B	120.2779633	ns
Xiwa4522	7B	120.2779633	ns
Xiwa4888	7B	120.2779633	ns
Xiwa7190	7B	120.2779633	ns
Xiwa181	7B	122.1443959	ns
Xiwa1647	7B	124.4014374	ns

* P< 0.05
** P< 0.01
*** P< 0.001
**** P< 0.0001
***** P< 0.00001
ns indicates non-significant markers