Genetics of host-pathogen interactions in the wheat-*Stagonospora nodorum* pathosystem

Faris JD[1], Lu HJ [2], Zhang Z[3], Reddy L[3], Liu ZH[2], Xu SS[1], Chu CG[3], Abeysekara N[3], Fellers JP[4], Cloutier S[5], Keller B[6], Scofield SR[7], Friesen TL[1]

[1] USDA-ARS Cereal Crops Research Unit, Northern Crop Science Laboratory, Fargo, ND 58105. [2] Department of Plant Pathology, North Dakota State University, Fargo, ND 58105. [3] Department of Plant Sciences, North Dakota State University, Fargo, ND 58105, [4] USDA-ARS Plant Science and Entomology Research Unit, Manhattan, KS 66506, [5] Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, MB R3T 2M9, [6] Institute of Plant Biology, University of Zurich, Switzerland, [7] USDA-ARS Crop Production and Pest Control Research Unit, West Lafayette, IN 47907

ABSTRACT

Stagonospora nodorum causes the disease Stagonospora nodorum blotch (SNB) in wheat. *S. nodorum* produces numerous host-selective toxins (HSTs), all of which interact with dominant host sensitivity genes to cause disease. These host-toxin interactions are mirror images of classical gene-for-gene interactions. The effects of compatible host-toxin interactions in the development of SNB are largely additive, and they play important roles in disease susceptibility of seedlings as well as adult plants. One of the first *S. nodorum* HSTs was SnToxA, which was recently involved in a lateral transfer from *S. nodorum* to the tan spot pathogen. The map-based cloning of the *Tsn1* locus on chromosome 5B, which confers sensitivity to SnToxA, reveals a complex evolutionary history of the locus when compared to rice, *Brachypodium*, and the homoeologous region of chromosome 5A. The isolation of *Tsn1* will allow the molecular characterization of interactions in the wheat-*S. nodorum* pathosystem, which may be an excellent toxin-based model for other necrotrophic pathosystems.

INTRODUCTION

Stagonospora nodorum blotch caused by the necrotrophic fungus *Stagonospora nodorum* (E. Mull.) Hedjar (anamorph; *Phaeosphaeria nodorum*) is a major disease of common wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L. ssp. *durum*), and it occurs in all major wheat growing areas of the world. The fungus has the ability to infect leaves and glumes. Therefore, it has the ability to cause significant yield losses and negatively impact grain quality. Quantitative trait loci (QTLs) associated with resistance to SNB have been identified on many wheat chromosomes using various mapping populations (16).

Host-selective toxins (HSTs) are important in plant-pathogen interactions because of their specificity in causing disease. The wheat tan spot pathogen, *Pyrenophora tritici-repentis,* produces several proteinaceous HSTs including Ptr ToxA, which has been well characterized (15). Host sensitivity to Ptr ToxA is associated with susceptibility to the fungus (8) and conditioned by a single dominant gene designated *Tsn1* on the long arm of chromosome 5B (2). Ptr ToxA has been implicated as the primary determinant of pathogenicity in races producing this toxin (8).

Here, we summarize some of our recent work, which demonstrates that the wheat-*S. nodorum* pathosystem is largely based on host-toxin interactions that follow an inverse gene-for-gene scenario. We also present work toward the map-based cloning of the ToxA sensitivity gene *Tsn1*.

MATERIALS AND METHODS

Identification of *S. nodorum* HSTs and corresponding host sensitivity genes

Four primary wheat mapping populations all with dense marker coverage have been used to identify toxin sensitivity genes and study the genetics of wheat-*S. nodorum* interactions. These four populations include the International Triticeae Mapping Initiative (ITMI) population of recombinant inbred lines (RILs); a population of RILs derived from the hard red spring wheat (HRSW) varieties BR34 and Grandin (BG population); a population of doubled haploids (DH) derived from the synthetic hexaploid TA4152-60 and the HRSW breeding line ND495 (NC60 population); and a population of RILs derived from a cross between the European winter wheat varieties Arina and Forno (AF population).

To identify toxins produced by *S. nodorum*, culture filtrates were obtained as described in (9) and infiltrated into leaves of young wheat plants of a mapping population. Plants were scored 3-5 days after infiltration for the presence or absence of necrosis. Simple linear regression and interval regression analysis is conducted to identify markers and genomic regions associated with reaction to the culture filtrates. When a single genomic region is found to explain most of the variation in necrosis development, this indicates that a single host-toxin interaction involving one toxin and one corresponding host gene is responsible and we can map the host gene as a Mendelian locus. To determine the effects of a compatible host-toxin interaction, we inoculate the individuals of the mapping population with fungal spores and evaluate the development of disease using a 0-5 rating scale as described in (10). The magnitude of a QTL observed at the toxin sensitivity locus is indicative of the role of the compatible host-toxin interaction in conferring disease susceptibility.

BAC-based physical mapping of the *Tsn1* locus

Saturation, high-resolution, and comparative mapping of the *Tsn1* locus has been previously reported (7, 12, 13). BAC-based physical mapping and chromosome walking were conducted using the Langdon (LDN) durum BAC library (1), and a BAC library developed from the HRSW variety Glenlea (14) was used to span a gap in the LDN BAC contig. The BAC contigs were anchored to a genetic map of the *Tsn1* locus developed using an F_2 population consisting of 2,719 ToxA-insensitive plants derived from LDN x LDN/*T. dicoccoides* disomic chromosome 5B substitution line (LDN-DIC 5B). BACs were sequenced and annotated as described (12).

RESULTS AND DISCUSSION

Genetics of host-toxin interactions in the wheat-*S. nodorum* pathosystem

To date, we have reported the identification of four *S. nodorum* HSTs and the chromosomal location of their corresponding host sensitivity genes (Table 1). The first *S. nodorum* HST identified was designated SnTox1 (9). SnTox1 caused extensive cell death in leaves of sensitive wheat genotypes similar to that observed in a compatible *Tsn1*-Ptr ToxA interaction. Sensitivity to SnTox1 is controlled by a single dominant gene designated *Snn1*, which was located to chromosome arm 1BS in common wheat. QTL analysis of reaction to SNB caused by isolate Sn2000 indicated that the *Snn1* locus accounted for a major disease susceptibility QTL explaining as much as 58% of the phenotypic variation (10). Therefore, SnTox1 was determined to play a major role in the development of SNB.

Sensitivity to a second HST was found to cosegregate with sensitivity to Ptr ToxA at the *Tsn1* locus in the BG population (11). QTL analysis of reaction to SNB caused by this toxin-producing isolate indicated that the *Tsn1* locus underlay a major QTL for disease susceptibility, and it explained 62% of the variation in disease. This raised the question whether *Tsn1* was conferring sensitivity to both Ptr ToxA and the new *S. nodorum* toxin, or if sensitivity was governed by two closely linked genes. Evaluation of *Tsn1*-disrupted mutants indicated that, whereas the wild type was sensitive to both Ptr ToxA and the newly identified *S. nodorum* toxin, the *Tsn1*-disrupted mutants were insensitive to both toxins. This demonstrated that *Tsn1* conferred sensitivity to both Ptr ToxA and the newly identified *S. nodorum* toxin. Concomitant with this finding, a gene with 99.7% similarity to the Ptr *ToxA* gene was identified in the *S. nodorum* genome (4). Additional experiments indicated that *P. tritici-repentis* acquired the *ToxA* gene from *S. nodorum* through a horizontal gene transfer event that occurred prior to 1941. This horizontal transfer and the acquisition of the *ToxA* gene by *P. tritici-repentis* is thought to have been a significant event leading to the emergence of tan spot as an economically significant disease. In light of these findings, we designated the ToxA-like protein identified in *S. nodorum* as SnToxA.

The third *S. nodorum* toxin was designated SnTox2 (5). The corresponding host sensitivity gene, designated *Snn2*, mapped to the short arm of chromosome 2D. *Snn2* accounted for a major susceptibility QTL, and further analysis using an isolate that produced both SnToxA and SnTox2 demonstrated that the effects of compatible *Tsn1*-SnToxA and *Snn2*-SnTox2 interactions are completely additive.

The fourth HST identified was designated SnTox3 and the corresponding host sensitivity gene was mapped to the short arm of chromosome 5B and designated *Snn3* (6). Friesen et al. (6) showed that a compatible *Snn3*-SnTox3 interaction played a significant role in disease development. However, when fungal isolates that produced both SnTox2 and SnTox3 were used, the *Snn2*-SnTox2 interaction was epistatic to the *Snn3*-SnTox3 interaction.

Besides the four host-toxin interactions described above, we have identified more than a dozen additional HSTs and their corresponding host sensitivity genes, which are at various levels of characterization. These host sensitivity loci are scattered throughout the genome and like the previously described host-toxin interactions, compatible interactions involving these toxins require the presence of the toxin from the pathogen in addition to the dominant allele of the host gene.

Toxin	Host Gene	Chrom.	Mapping pop.
SnToxA	*Tsn1*	5BL	BG, NC60, AF
SnTox1	*Snn1*	1BS	ITMI, NC60
SnTox2	*Snn2*	2DS	BG
SnTox3	*Snn3*	5BS	BG, ITMI, NC60

Table 1. Reported *Stagonospora nodorum* HSTs and the corresponding host sensitivity genes, their chromosomal locations, and the populations used to map them.

Our preliminary data regarding the roles of the other host-toxin interactions indicate that some have major effects on disease susceptibility whereas others have relatively minor effects. For example, one interaction accounts for nearly 100% of the variation in disease in a diploid wheat population, but several others explain less than 10% of the variation in hexaploid wheat. The effects of compatible interactions are most likely dependent on several factors including the host genetic background, presence of multiple compatible interactions, and the degree of toxin expression by the isolate used for inoculation. Most of our experiments have been conducted on seedling plants under greenhouse conditions. However, we inoculated adult plants in the field in two locations over three years using an that produces both SnTox2 and SnToxA and found that both the *Snn2*-SnTox2 and the *Tsn1*-SnToxA interactions were significantly associated with susceptibility of adult plants under field conditions. Therefore, loci conferring toxin insensitivity tend to underlie SNB resistance QTLs, and it is possible that

many SNB resistance QTLs reported in the literature are due to toxin-host gene interactions.

In the classical gene-for-gene model (3), a compatible interaction that leads to resistance depends on the recognition of a pathogen elicitor (avirulence gene product) encoded by a receptor (resistance gene product) in the plant. If the host lacks a specific resistance gene, the corresponding avirulence gene cannot be detected, which results in a susceptible reaction. In the host-toxin interactions described above, a single gene conditions host sensitivity to a specific toxin, and disease conferred by a specific HST does not occur in the absence of the corresponding dominant toxin sensitivity allele. A compatible interaction occurs only when the dominant toxin sensitivity gene in the host interacts with the specific toxin. Therefore, the wheat-*S. nodorum* toxin interaction model is a mirror image of the classic gene-for-gene model.

Map-based cloning of the *Tsn1* locus

BAC-based physical mapping and identification of the Tsn1 candidate gene region

Because the *ToxA* gene was involved in a lateral transfer from *S. nodorum* to *P. tritici-repentis*, the *Tsn1* gene confers susceptibility to ToxA-producing isolates of the tan spot and SNB pathogens. To gain knowledge of the molecular mechanisms underlying a compatible *Tsn1*-ToxA interaction, we embarked on the map-based cloning of *Tsn1*. Saturation mapping and fine mapping in a population of 930 F_2 plants derived from LDN x LDN-DIC 5B was previously reported (7), and the RFLP markers *Xfcg17* and *Xfcp9* were developed that flanked the *Tsn1* gene at distances of 0.2 and 0.6 cM, respectively. The population size was increased to 2,719 F_2 plants and these two markers were used to screen the LDN BAC library to initiate chromosome walking. On the proximal side of *Tsn1*, we identified two BACs, which formed a 205 kb contig (Figure 1A). The probes P21-1015 and L7-0813, which were developed from the ends of the contig, cosegregated with *Xfcg17* indicating no recombination event occurred in the 205 kb region among 5,438 gametes. These results suggested that recombination was highly suppressed in this small chromosome region. Thus, orientation of this contig was not determined.

On the distal side of the *Tsn1* gene, multiple chromosome walking steps were performed resulting in a contig consisting of nine BACs spanning ~850 kb (Figure 1A). The chromosome 5B BAC contig contained a gap, which was the result of no positive chromosome 5B BACs being detected during one of the chromosome walking steps. This was remedied by using a homoeologous chromosome 5A BAC to continue the chromosome walk and extend the contig, then reverting back to a chromosome 5B BAC in a subsequent walking step. The gap in the 5B contig was recently closed by screening the Glenlea BAC library and identifying 5B BACs.

The 850 kb contig spans the *Tsn1* gene and recombination events delineate the candidate gene region to a segment of about 350 kb. To further narrow the candidate gene region, we developed PCR-based markers for sequences immediately flanking and within the *Tsn1* region and analyzed haplotypes of natural populations. Analysis of over 300 wheat accessions indicated that linkage disequilibrium at the *Tsn1* locus is lower than expected, and it allowed us to reduce the candidate gene region from 350 kb to a segment of about 140 kb (Figure 1B).

Gene density, recombination, and candidate genes

Together, the two 5B contigs constitute over 1 Mb. A total of 40 genes were predicted in the contigs, among which 14 were located in the 205 kb contig and 26 were predicted from the 805 kb contig giving an average gene density of 1 gene per 25 kb. However, the genes were not randomly distributed and density ranged from 1 gene per 6 kb to 1 gene per 200 kb. Genetic recombination frequencies also varied significantly within the contigs with an average recombination frequency of 1.2 Mb/cM and a range of 230 kb/cM to 10 Mb/cM. It is interesting to note that 14 genes were located in the smaller contig where there were no recombination events observed in 5,438 gametes, and no genes were predicted in the distal ~100 kb region of the larger contig, which was one of the recombination hot spots. On the contrary, a 100 kb segment at the 300-400 kb position of the larger contig was another recombination hot spot where there were 10 putative genes. Therefore, within this region, there seemed to be no consistent relationship between gene density and recombination frequency.

The 140 kb candidate gene region contains five genes. These include a hypothetical gene with a putative F-box domain (FB), an NBS-LRR-like gene, a protein kinase (PK), a U2 snRNP auxiliary factor (URAF), and a potassium transporter (PT).

Colinearity of the Tsn1 locus with the homoeologous region of chromosome 5A, Brachypodium, and rice

Comparison of the *Tsn1* candidate gene region of chromosome 5B with the homoeologous region of chromosome 5A revealed a number of unexpected differences. The URAF and the FB genes are colinear, but 5B contains a suite of genes including the NBS-LRR and the PK genes that are not present on chromosome 5A. In comparison, the *Tsn1* candidate region between the URAF and the FB gene on chromosome 5A is about 20 kb, whereas the same interval on 5B is approximately 130 kb. Also, 5A contains a cysteine protease gene that is not present on 5B, and two wall-associated kinase (WAK) genes found on 5A appear to have been involved in an inversion/duplication event on 5B.

Evaluation of colinearity between the *Tsn1* candidate gene region and rice revealed some conservation with rice chromosomes 9 and 11, but multiple disruptions were apparent (Figure 1B). The FB, PT, and DHHC zinc finger genes had similarity to sequences on rice chromosome 9 but were apparently involved in an

inversion in rice. The NBS-LRR, PK, and URAF had similarity to sequences on rice chromosome 11, but they were very far apart from each other in rice.

Colinearity of the *Tsn1* locus was more conserved with *Brachypodium* than it was with rice. We identified a single *Brachypodium* contig that contained orthologues of three WAK genes, the cysteine protease, and the FB URAF, PT and the DHHC zinc finger, and all were colinear with the *Tsn1* locus. However, as with wheat chromosome 5A and rice, the NBS-LRR and PK genes were not present on the *Brachypodium* contig. The fact that the *Tsn1* genomic region is highly rearranged not only relative to rice, but also to the homoeologous region of chromosome 5A, suggests a dynamic and complicated evolutionary history of the *Tsn1* locus.

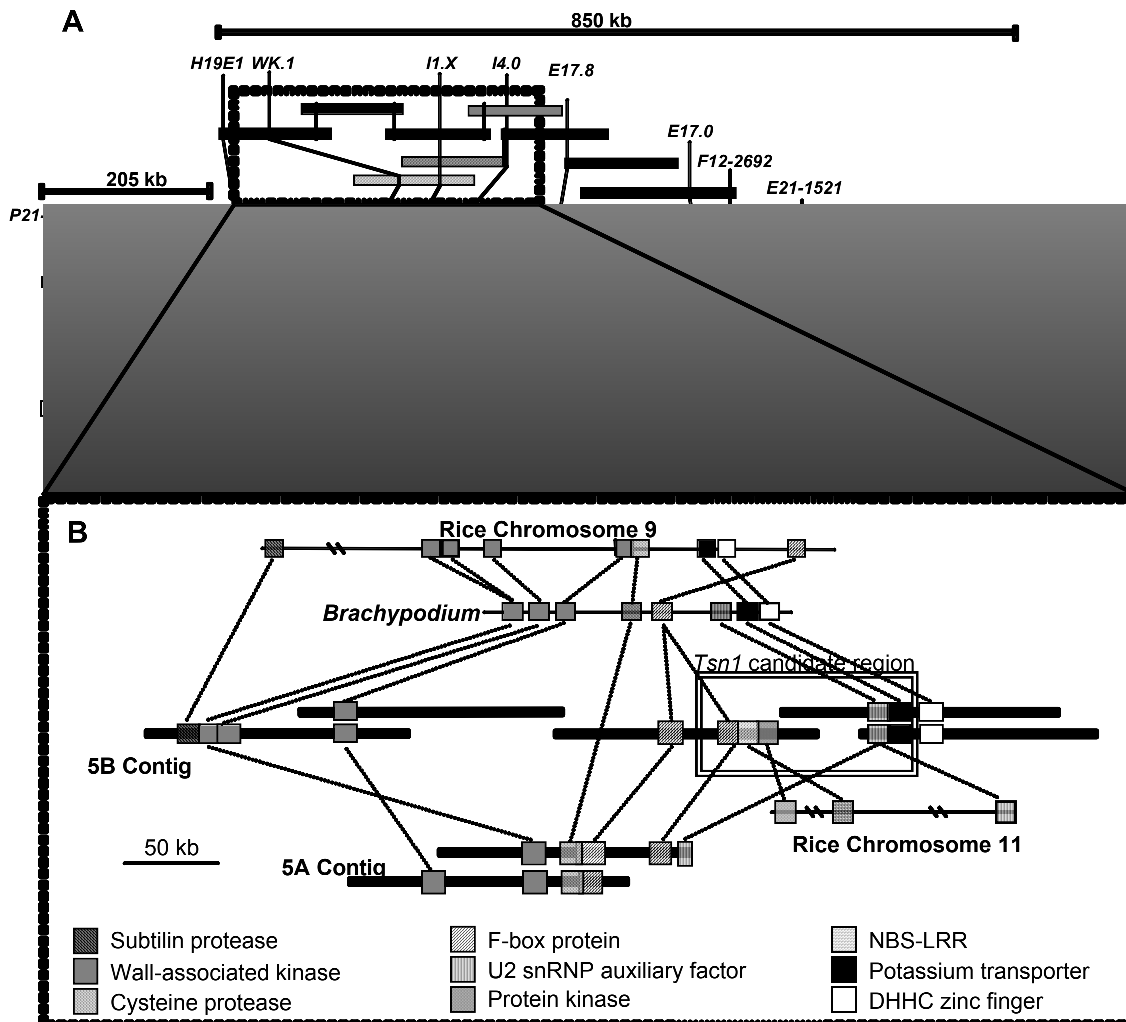

Figure 1. Physical mapping of the *Tsn1* locus on chromosome 5B of wheat. A) Langdon BAC contigs anchored to the genetic map of chromosome 5B derived from LDN x LDN-DIC 5B. Black bars represent LDN 5B BACs the gray bar is a Glenlea 5B BAC, and striped bars represent LDN 5A BACs. The dashed box indicates the region shown in more detail in (B). B) A physical map of the *Tsn1* candidate gene region and its comparison to the homoeologous region of chromosome 5A, *Brachypodium*, and rice. Genes are shown as boxes along the BACs and dotted lines connect homologous gene sequences. The *Tsn1* candidate gene region based on high-resolution mapping and haplotype analysis of natural populations is indicated by the double-lined box.

Analysis of Tsn1 candidate genes

The availability of mutants is invaluable for candidate gene validation and gene characterization. Because insensitivity to ToxA behaves in a recessive manner and the null condition also results in insensitivity, sensitive genotypes exposed to mutagenesis will yield insensitive mutants when *Tsn1* is disrupted. To date, we have developed a total of 10 ethylmethane sulfonate (EMS)-induced *Tsn1*-dirupted mutants in LDN and the ToxA-sensitive hexaploid variety Bobwhite. To validate that each mutant harbored a mutation at the *Tsn1* locus and not another gene essential to the development of necrosis, we crossed each mutant to a sensitive genotype and evaluated corresponding F_2 populations for reaction to ToxA and molecular markers known to be tightly linked to *Tsn1*. We are in the process of conducting comparative sequence analysis of the candidate genes in the wild types with the corresponding mutants to

determine which of the candidate genes is *Tsn1*. We are also in the process of silencing the candidate genes using virus-induced gene silencing (VIGS) as a second line of validation

CONCLUSIONS

Given the extent of the data we have collected on the wheat-*S. nodorum* pathosystem including the identification of multiple HSTs and corresponding host sensitivity genes, it is apparent that the wheat-*S. nodorum* host-pathogen system is reminiscent of a classical gene-for-gene system. However, in the classic gene-for-gene model, an elicitor protein produced by the pathogen interacts directly or indirectly with a host resistance gene leading to a necrotic hypersensitive response (HR). The wheat-*S. nodorum* system differs from the classical model in that the elicitor-like protein (toxin) induces necrosis when interacting with a specific host gene product to induce uninhibited cell death that leads to disease rather than HR-associated resistance. We hypothesize that the *S. nodorum* toxins employ mechanisms that, through evolution, have acquired the ability to commandeer host metabolic pathways to cause disease. It is likely that the host genes serve functions other than to confer toxin sensitivity, but are the target of exploitation by the pathogen to gain entry into the cell. The isolation and functional analysis of *S. nodorum* toxin sensitivity genes such as *Tsn1* will shed light on the molecular mechanisms underlying compatible host-toxin interactions and allow researchers to unravel the signal transduction pathways involved.

ACKNOWLEDGEMENTS

This work was supported by USDA-ARS CRIS projects 5442-22000-037-00D and 5442-22000-033-00D, and by USDA-CSREES NRI grants 2003-35300 and 2008-01013 to JDF.

REFERENCES

1. Cenci, A., N. Chantret, X. Kong, Y. Gu, O.D. Anderson, T. Fahima, A. Distelfeld and J. Dubcovsky (2003) Construction and characterization of a half million clone BAC library of durum wheat (*Triticum turgidum* ssp. *durum*). Theor. Appl. Genet. 107:931-939.
2. Faris, J.D., J.A. Anderson, L.J. Francl and J.G. Jordahl (1996) Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. Phytopathology 86:459-463.
3. Flor, H.H. (1955) Host-parasite interaction in flax rust – its genetics and other implications. Phytopathology 45:680-685.
4. Friesen, T.L., E.H. Stukenbrock, Z.H. Liu, S.W. Meinhardt, H. Ling, J.D. Faris, J.B. Rasmussen, P.S. Solomon, B.A. McDonald and R.P. Oliver (2006) Emergence of a new disease as a result of interspecific virulence gene transfer. Nat. Genet. 38:953-956.
5. Friesen, T.L., S.W. Meinhardt and J.D. Faris (2007) The *Stagonospora nodorum*-wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in a gene-for-gene manner. Plant J. 51:681-692.
6. Friesen, T.L., Z. Zhang, P.S. Solomon, R.P. Oliver and J.D. Faris (2008) Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. Plant Physiol. 146:682-693.
7. Haen K.M., H.-J. Lu, T.L. Friesen and J.D. Faris (2004) Genomic targeting and high-resolution mapping of the *Tsn1* gene in wheat. Crop Sci. 44:951-962.
8. Lamari, L., and C.C. Bernier (1989) Toxin of *Pyrenophora tritici-repentis*: Host-specificity significance in disease, and inheritance of host reaction. Phytopathology 79:740-744.
9. Liu, Z.H., J.D. Faris, S.W. Meinhardt, S. Ali, J.B. Rasmussen and T.L. Friesen (2004a) Genetic and physical mapping of a gene conditioning sensitivity in wheat to a partially purified host-selective toxin produced by *Stagonospora nodorum*. Phytopathology 94:1056-1060.
10. Liu, Z.H., T.L. Friesen, S.W. Meinhardt, S. Ali, J.B. Rasmussen and J.D. Faris (2004b) Quantitative trait loci analysis and mapping of seedling resistance to Stagonospora nodorum leaf blotch in wheat. Phytopathology 94:1061-1067.
11. Liu, Z.H., T.L. Friesen, H. Ling, S.W. Meinhardt, R.P. Oliver, J.B. Rasmussen and J.D. Faris (2006) The *Tsn1*-ToxA interaction in the wheat-*Stagonospora nodorum* pathosystem parallels that of the wheat-tan spot system. Genome 49:1265-1273.
12. Lu, H.J., and J.D. Faris (2006) Macro- and micro-colinearity between the genomic region of wheat chromosome 5B containing the *Tsn1* gene and the rice genome. Funct. Integr. Genomics 6:90-103.
13. Lu, H.J., J.P. Fellers, T.L. Friesen, S.W. Meinhardt and J.D. Faris (2006) Genomic analysis and marker development for the *Tsn1* locus using bin-mapped ESTs and flanking BAC contigs. Theor. Appl. Genet. 112:1132-1142.
14. Nilmalgoda, S.D., S. Cloutier and A.Z. Walichnowski (2003) Construction and characterization of a bacterial artificial chromosome (BAC) library of hexaploid wheat (*Triticum aestivum* L.) and validation of genome coverage using locus-specific primers. Genome 46:870-878.
15. Manning, V.A., and L.M. Ciuffetti (2005) Localization of Ptr ToxA produced by *Pyrenophora tritici-repentis* reveals protein import into wheat mesophyll cells. Plant Cell 17:3203-3212.
16. Xu, S.S., T.L. Friesen and X.W. Cai (2004) Sources and genetic control of resistance to Stagonospora nodorum blotch in wheat. Recent Res. Devel. Genet. Breeding 1:449-469.