IDENTIFICATION AND GENOMIC ANALYSIS OF STAGONOSPORA NODORUM BLOTCH SUSCEPTIBILITY GENES IN WHEAT

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ABSTRACT

Parastagonospora nodorum is a necrotrophic fungal pathogen that causes the disease Stagonospora nodorum blotch (SNB) on wheat. The fungus produces necrotrophic effectors (NEs), that when recognized by corresponding host genes, cause cell death leading to disease. A novel NE, designated SnTox7, was identified from culture filtrates of isolate Sn6 of *P. nodorum*. SnTox7 is a small protein with estimated size less than 30 kDa. The interaction between SnTox7 and its corresponding host sensitivity gene, *Snn7*, explained 33% of the disease variation among a segregating F_2 population. The *Snn7* gene governs sensitivity to SnTox7 and was delineated to a 2.7 cM interval on the long arm of wheat chromosome 2D. Another host sensitivity gene *Snn3-B1*, conferring sensitivity to SnTox3, was previously mapped on the short arm of wheat chromosome 5B. Forty-four molecular markers were added to the genetic map to saturate the *Snn3-B1* gene region. High-resolution mapping of the *Snn3-B1* locus in 5,600 gametes delineated the gene to a 1.5 cM interval. The closely linked markers should be very useful for marker-assisted selection against *Snn3-B1*. A third host gene, *Snn1,* confers sensitivity to the NE Tox1. *Snn1* was isolated through map-based cloning, and its structure, expression and allelic diversity were further characterized. A bacterial artificial chromosome (BAC) contig of about 2.5 Mb in size was identified to span the *Snn1* locus through screening of Chinese Spring chromosome arm 1BS minimum tiling path (MTP) pools. Additional markers developed from BAC end sequences (BESs) delineated the *Snn1* gene to a physical segment consisting of four BAC clones. Sequencing and bioinformatic analysis of these clones led to the identification of seven candidate genes. Six of the seven candidates were excluded through critical recombinants. The seventh

gene, a cell wall-associated kinase (*WAK*), was verified as *Snn1* through comparative sequence analysis with ethylmethane sulfonate (EMS)-induced mutants. The *Snn1* transcription profile showed that it was regulated by light and possibly circadian rhythms. These results demonstrate that *P. nodorum* can hijack multiple host pathways driven by different classes of genes that typically confer resistance to biotrophic pathogens, thus demonstrating the surprisingly intricate nature of plant-necrotrophic pathogen interactions.

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LIST OF ABBREVIATIONS

AAD .... Acidic transcriptional activation domain

AFLP .... Amplified fragment length polymorphism

Avr .... Avirulence

BAC .... Bacteria artificial chromosome

BES .... BAC end sequence

DTT .... Dithiothreitol

ETI .... Effector-triggered immunity

ETS .... Effector-triggered susceptibility

ET .... Ethylene

EGF .... Epidermal growth factor

EMS .... Ethyl methanesulfonate

GUB .... Galacturonan binding

HST .... Host-selective toxins

HR .... Hypersensitive responses

IWGSC .... International wheat genome sequencing consortium

JA .... Jasmonic acid

LRR .... Leucine-rich repeat

MAMPs .... Microbe-associated molecular pattern

MAPKs .... Mitogen activated protein kinases

MTP .... Minimum tiling path

NCBI .... National Center for Biotechnology Information

NE .... Necrotrophic effectors

NETS ....Necrotrophic effector triggered susceptibility

NB..Nucleotide binding

NLS ....Nuclear localization signal

OGs ....Oligogalacturonides

PAMPs ....Pathogen- associated molecular pattern

PTI ....PAMP-triggered immunity

PR ....Pathogenesis related

PRRs ....Pattern recognition receptors

PCD ....Programmed cell death

PKc ....Protein kinase

RACE ....Rapid amplification of cDNA ends

ROS ....Reactive oxygen species

RLKs ....Receptor-like kinase

RLPs ....Receptor-like proteins

RFLP ....Restriction fragment length polymorphism

SA ....Salicylic acid

SSR ....Simple sequence repeat

SNP ....Single nucleotide polymorphism

TTSS ....Type III secretion system

VIGS ....Virus induced gene silencing

WAK ....Wall-associated kinase

CHAPTER I. GENERAL INTRODUCTION

Cereals provide more than 50% of global crop production and are important resources for food, feed and industrial materials (Krattinger et al. 2009). Wheat is the primary cereal and a staple food for 40% of the world's population and provides 20% of the calories consumed worldwide (Bockus et al. 2010). Wheat is the second most widely grown crop, recently surpassed by maize. The major wheat producers include China, the European Union, India, the United States, Australia and Canada. The United States produces about 55-60 million tons per year and is consistently the world's biggest wheat exporter (USDA ERS 2014a). However, due to the world population growth, the amount of wheat production must be doubled by 2030 to feed the ever-increasing population (Dixon et al. 2009). This increase can be achieved only by enhancing the yield per acre because of the limited wheat growing area. Therefore, understanding of plant biology and genetic improvement for yield increase are crucial to attain this goal.

It is estimated that 25 to 30% of the global wheat crop is lost to abiotic and biotic stresses (Bockus et al. 2010). The majority of biotic stresses are caused by multiple kinds of wheat pathogens. Stagonospora nodorum blotch (SNB) is a common component of the fungal leaf and glume blotch complex in most wheat production areas. It can cause 10-20% losses in grain yield and reductions in grain quality (Bockus et al. 2010). *Parastagonospora nodorum*, a necrotrophic fungus, is the causal agent of SNB. During the past decade, numerous studies conducted on the wheat-*P. nodorum* pathosystem have demonstrated that necrotrophic effectors (NEs) are major determinants of SNB, and when an NE is recognized by the corresponding dominant host gene, a compatible reaction occurs leading to disease susceptibility. However, the absence of either the NE or the host

sensitivity gene will result in an incompatible interaction and no disease will occur (Friesen et al. 2008; Friesen and Faris 2010). To date, eight NE-host sensitivity gene interactions have been identified from this pathosystem (Friesen et al. 2006; Faris et al. 2010; Liu et al. 2012; Reddy et al. 2008; Friesen et al. 2007; Liu et al. 2009; Zhang et al. 2011; Abeysekara et al. 2009; Friesen et al. 2012; Gao et al. 2014) and made it a model to study host-necrotrophic pathogen interactions. Further studies will greatly enhance our knowledge and understanding of host-necrotroph interactions and benefit the manipulation of necrotrophic disease through host resistance.

To understanding the biology and molecular mechanisms underlying plant disease resistance, the isolation of the host genes is the first step. However, the 17-gigabase hexaploid bread wheat genome and its genomic complexity make it a challenge to clone genes from wheat without a reference sequence or an extensive physical map (Krattinger et al. 2009; IWGSC 2014). More than 80% of the genome consists of repetitive sequences (IWGSC 2014), which makes chromosome walking and marker development extremely difficult. Despite its large genome size and high content of repetitive sequences, significant progress has been made in map-based cloning of genes with great genetic tools, advances in sequencing technology as well as more genome sequence information (Wang et al. 2014; Brenchley et al. 2012; IWGSC, 2014). Many of the wheat genes cloned to date are genes conferring resistance to diseases.

In this dissertation, I will describe the identification and genetic analysis of a new wheat sensitivity gene and its association with a novel NE produced by *P. nodorum*. I also report the saturation and high-resolution mapping of the sensitivity gene *Snn3-B1,*

and the development of markers suitable for marker-assisted selection. Finally I describe the cloning and characterization of wheat NE sensitivity gene *Snn1*.

References

Abeysekara N, Friesen TL, Keller B, Faris JD (2009) Identification and characterization of a novel host-toxin interaction in the wheat-*Stagonospora nodorum* pathosystem. Theor Appl Genet 120:117-126

Bockus WW, Bowden RL, Hunger RM, Morrill WL, Murray TD, Smiley RW (2010) Compendium of Wheat Diseases and Pests: Third Edition. American Phytopathological Society, St. Paul, MN

Brenchley R, Spannagl M, Pfeifer M, Barker G, D'Amore R, Allen A, McKenzie N, Kramer M, et al (2012) Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature 491:705-710

Dixon J, Braun HJ, Kosina PP, Crouch J (2009) Wheat facts and futures. CIMMMYT, Mexico

Faris JD, Zhang ZC, Lu HJ, Lu SW, Reddy L, Cloutier S, Fellers JP, Meinhardt SW, Rasmussen JB, Xu SS, Oliver RP, Simons KJ, Friesen TL (2010) A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proc Natl Acad Sci USA 107:13544-13549

Friesen TL, Stukenbrock EH, Liu ZH, Meinhardt SW, Ling H, Faris JD, Rasmussen JB, Solomon PS, Mcdonald BA, Oliver RP (2006) Emergence of a new disease as a result of interspecific virulence gene transfer. Nat Genet 38:953-956

Friesen TL, Meinhardt SW, Faris JD (2007) The *Stagonospora nodorum* – wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. Plant J 51:681-692

Friesen TL, Faris JD, Solomon PS, Oliver RP (2008) Host-specific toxins: Effectors of necrotrophic pathogenicity. Cell Microbiol 10:1421-1428

Friesen TL, Faris JD (2010) Characterization of the wheat-*Stagonospora nodorum* disease system: what's the molecular basis of this quantitative necrotrophic disease interaction? Can J Plant Pathol 32:20-28

Friesen TL, Chu C, Xu SS, Faris JD (2012) SnTox5-*Snn5*: a novel *Stagonospora nodorum* effector-wheat gene interaction and its relationship with the SnToxA-*Tsn1* and SnTox3-*Snn3-B1* interactions. Mol Plant Pathol 13:1101-1109

Gao Y, Faris JD, Liu ZH, Xu SS, Friesen TL (2014) Identification and characterization of the SnTox6-*Snn6* interaction in the wheat-*Stagonospora nodorum* pathosystem. Mol Plant-Microbe Interact (accepted)

The International Wheat Genome Sequencing Consortium (IWGSC) (2014) A chromsome-based draft sequence of the hexaploid bread wheat genome. Science DOI: 10.1126/science.1251788

Liu ZH, Faris JD, Oliver RP, Tan KC, Solomon PS, McDonald MC, McDonald BA, Nunez A, Lu S, Rasmussen JB, Friesen TL (2009) SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the *Snn3* gene. PLoS Pathogens 5:e1000581

Liu ZH, Zhang ZC, Faris JD, Oliver RP, Syme R, McDonald MC, McDonald BA, Solomon PS, Lu SW, Shelver WL, Xu SS, Friesen TL (2012) The cysteine rich necrotrophic effector SnTox1 produced by *Stagnospora nodorum* triggers susceptibility of wheat lines harboring *Snn1*. PLoS Pathogens 8:e1002467

Krattinger S, Wicker T, Keller B (2009) Map-based cloning of genes in *Triticeae* (wheat and barley). Plant genetics and genomics: Genetics and genomics of the *Triticeae*. Feuillet C, Muehbauer GJ (Eds) New York, NY Springer

Reddy L, Friesen TL, Meinhardt SW, Chao S, Faris JD (2008) Genomic analysis of the *Snn1* locus on wheat chromosome arm 1BS and the identification of candidate genes. Plant Genome 1: 55-66

USDA ERS (2014a) Wheat outlook: U.S. export, Census and export sales comparison. http://www.ers.usda.gov/publications/whs-wheat-outlook/whs-14i.aspx#.VCGJ593-E1A. Date accessed: 09/23/2014

Wang SC, Wong D, Forrest K, Allen A, Chao S, Huang B, Maccaferri M, et al (2014) Characterizaiton of polyploid wheat genomic diversity using a high-density 90000 single nucleotide polymorphism array. Plant biotech J12: 787-796

Zhang ZC, Friesen TL, Xu SS, Shi GJ, Liu ZH, Rasmussen JB, Faris JD (2011) Two putatively homoeologous wheat genes mediate recognition of SnTox3 to confer effector-triggered susceptibility to *Stagonospora nodorum*. Plant J 65:27-38

CHAPTER II. LITERATURE REVIEW

Introduction

Wheat is one of the earliest domesticated crops from the Middle East and now widely cultivated all over the world. It is a staple food for 40% of the world's population and provides 20% of the calories consumed (Bockus et al. 2010). As the world's most favored staple food, wheat flour is rich in starch and gluten, and a leading source of vegetable protein (Carver 2009). It is grown on 20% of the cultivated land area of the world from 67° north latitude to 45° south latitude. Its annual planting acreage ranks second only to maize, and world trade in wheat is greater than for all the other crops combined. It ranks third in cereal production after maize and rice and about 713 million tonnes of common wheat (*T. aestivum* ssp. *aestivum* L.) and durum wheat (*T. turgidum* ssp. *durum* L.) were produced in 2013 (http://faostat.fao.org). Due to the world's population growth, the amount of wheat production must be doubled by 2030 to feed the ever-increasing population (Dixon et al. 2009). This increase can be achieved only by enhancing the yield per acre because the wheat planting area cannot be increased beyond what is currently available. Therefore, genetic improvement for yield increase is crucial to attain this goal.

Wheat production in the U.S.

The major wheat producers include China, the European Union, India, the United States, Australia and Canada. The United States produces about 55-60 million tons per year and supplies about 40% of the world exports. The U.S. is consistently the world's biggest wheat exporter with more than 32 million tonnes during 2013/2014 (USDA ERS 2014a).

Wheat varieties grown in the U.S. are classified as either winter wheat or spring wheat. Winter wheat is sowed in the fall and requires exposure to cold temperatures for a certain amount of time, or vernalization, before it can enter the reproductive stage and produce seeds. In the spring, plants resume growing and seeds are harvested in summer. In the Northern Plains where the winters are generally too severe for winter wheat to survive, spring wheat and durum wheat are planted in the spring and harvested in late summer or fall.

Five major classes of wheat are grown in the U.S., hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), white, and durum wheat. Forty percent of the total production is HRW, which is grown in the Great Plains and used for making bread. HRS accounts for 20 percent of the total and is mainly grown in the Northern Plains (North Dakota, Montana, Minnesota and South Dakota). HRS contains high protein content and is suitable for specialty bread. SRW wheat accounts for 15 to 20 percent of total production and is grown in states along the Mississippi River and eastern states. SRW is good for making cakes and cookies. White wheat is grown primarily in Washington, Oregon, Idaho, Michigan and New York, accounts for 10 to 15 percent of the total production, and is mainly used for making noodles, cookies and cereals. Durum wheat is mainly used to make pasta and primarily produced in North Dakota and Montana (USDA ERS 2014b).

The origin and domestication of wheat

The origin, evolution and domestication of cereals are principal events involved in the development of human civilization (Riehl et al. 2013; Marcussen et al. 2014). Botanical, genetic, and archaeological evidence has pointed to the Fertile Crescent as the

origin of agriculture, which occurred about 10,000 years ago (Heun et al. 1997; Lev-Yadun et al. 2000; Carver, 2009; Riehl et al. 2013; Salamini et al. 2002).

The *Poaceae* family (grasses) evolved 50-70 million years ago (MYA) (Kellogg 2001; Huang et al. 2002). The *Triticeae* tribe diverged from its progenitor about 35 MYA and the *Triticum* group separated about 11 MYA. The diploid progenitors and close relatives of modern wheat radiated from a common ancestor about 3 MYA and produced *Triticum* and *Aegilops* taxa. All members of the *Triticeae* have a basic chromosome number of 7. The chromosomes (1 to 7) in different diploid groups are considered homoeologous chromosomes (Carver 2009).The *Triticum* group includes the A-genome diploids *T. urartu* Tumanian ex Gandylian ($2n = 2x = 14$, AA) and *T. monococcum* ssp. *aegilopoides* Thell ($2n = 2x = 14$, AA) (Johnson and Dhaliwal 1976). The *Aegilops* species consisted of many species, such as *Ae. tauschii* Coss ($2n = 2x = 14$, DD) and *Ae. speltoides* Tausch ($2n = 2x = 14$, SS).

The only cultivated diploid wheat is einkorn (*T. monococcum* ssp. *monococcum* L., $2n = 2x = 14$, A^mA^m). Cultivated tetraploid wheats include *T. timopheevii* ssp. *timopheevii* Zhuk ($2n = 4x = 28$, AAGG), *T. turgidum* ssp. *dicoccum*, *T. turgidum* ssp. *durum*, *T. turgidum* ssp. *parvicoccum, T. turgidum* ssp. *carthlicum*, *T. turgidum* ssp. *turgidum*, and *T. turgidum* ssp. *polonicum* ($2n = 4x = 28$, AABB) and cultivated hexaploid wheats consist of *T. zhukovskyi* ($2n = 6x = 42$, A^mA^mAAGG), *T. aestivum* ssp. *spelta*, *T. aestivum* ssp. *aestivum*, *T. aestivum* ssp. *compactum*, and *T. aestivum* ssp. *sphaerococcum* ($2n = 6x = 42$, AABBDD) species.

Origin of the A genome

Early cytogenetic studies suggested that *T. monococcum* contributed the A genome of *T. timopheevii* and *T. turgidum* (Sax 1922; Lilienfeld and Kihara 1934). Later research showed that the A genome in *T. turgidum* was contributed by *T. urartu*, and the A genome in *T. timopheevii* by *T. monococcum* (Konarev et al. 1979). Now it is known that *T. urartu* is the donor of the A genome to all polyploid wheat species (Nishikawa et al. 1994; Dvorak et al. 1993). Both *T. urartu* and *T. monococcum* have been found in natural habitats from southwestern Iran, northern Iraq, Transcaucasia, eastern Lebanon, southeastern Turkey, western Syria (Kimber and Feldman 1987).

Origin of the B genome

Massive changes have occurred since divergence and polyploidization in both the B and G genomes of tetraploid wheat, and it is widely considered that they are modified forms of the S genome (Zhang et al. 2002; Kilian et al. 2007; Dvorak and Zhang 1990). *Ae. speltoides* ($2n = 2x = 14$, genome SS) is thought to be the closest relative of B genome progenitor (Dvorak and Zhang 1990; Salse et al. 2008). Cytoplasmic evidence showed that *Ae. speltoides* was the maternal donor for both tetraploid and hexaploid wheat (Wang et al. 1997).

Origin of *Triticum turgidum*

T. turgidum is further divided into several species, including *T. turgidum* ssp. *dicoccoides*, the well-known progenitor of all cultivated polyploid wheat, and *T. turgidum* ssp. durum, widely cultivated durum wheat. *Triticum urartu* is the A genome donor for tetraploid wheat, *T. turgidum* (AABB) and *T. timopheevii* (AAGG) (Nishikawa et al. 1994; Dvorak et al. 1993). It hybridized with the B genome ancestor to produce

wild emmer wheat (*T. turgidum* ssp. *dicoccoides,* $2n = 4x = 28$, AABB) and *T. timopheevii* ssp. *araraticum* ($2n = 4x = 28$, AAGG) (Nishikawa et al. 1994; Dvorak et al. 1993). Wild emmer has a restricted distribution range and grows especially in the western and central parts of the Fertile Crescent. Wild emmer wheat was cultivated for some time and a mutation occurred for a non-brittle rachis, which gradually led to cultivated emmer wheat (Kislev 1984). Free-threshing tetraploid wheat, such as the extinct *T. turgidum* ssp. *parvicoccum*, appeared shortly after domesticated emmer (Kislev 1984). Durum wheat (*T. turgidum* ssp. *durum*) evolved from domesticated emmer possibly through ssp. *parvicoccum* (Hillman 1978).

T. turgidum ssp. *dicoccoides* is found in Israel and Syria (centers of distribution), Jordan, Lebanon, southeast Turkey, northern Iraq, and western Iran (Nevo 1998). *Triticum turgidum* ssp. *dicoccoides* is the only wild ancestor in the genus *Triticum* that crosses compatibly with cultivated wheat, which makes the genetic diversity transferable from wild to cultivated gene pools (Xie and Nevo 2008).

Origin of hexaploid wheat

There are two major forms of hexaploid wheat, including *T. zhukovskyi*, a recent hybridization between *T. timopheevii ssp. timopheevii* and *T. monococcum* (Johnson 1968). The most important hexaploid wheat is *T. aestivum* (AABBDD) derived from a hybridization of an AB genome-containing tetraploid and *Ae. tauschii*, which contributed the D genome (Kihara 1944; McFadden and Sears 1946). The most probable donor of the AB genome is a free-threshing form of a subspecies of tetraploid *T. turgidum* (Matsuoka and Nasuda 2004; Faris et al. 2014). Though the exact site of the origin of hexaploid

wheat is still uncertain, the area of Iran southeast of the Caspian Sea is considered the most likely birthplace of hexaploid wheat (Jaaska 1980; Dvorak et al. 1998)

Domestication of wheat

Domesticated species differ from their wild ancestors in a set of traits known as the domestication syndrome, which includes traits such as growth habit, flowering time, seed size and dispersal, and changes in reproductive shoot architecture (Meyer and Purugganan 2013). It is valuable to study the genetics and genomics of these syndrome traits for wheat breeding of high yield and adaptability. Transitions in the following three major traits resulted in free-threshing fully domesticated bread wheat.

Brittle rachis: Spikelet disarticulation caused by a brittle rachis in wild forms of wheat is important for wild plants to disperse their seeds and further propagate. However, it is detrimental for cultivated plants because the seeds that fall to the ground prematurely are lost and unable to be harvested. Therefore, the transition of a brittle rachis to a non-brittle rachis was a key step toward the domestication of wheat because it allowed early farmers to more easily harvest their crops.

Sharma and Waines (1980) showed that the non-brittle rachis trait in *T. monococcum* was controlled by two complementary recessive genes. However, the chromosome location was not determined. Studies on the transition from wild emmer to cultivated emmer indicated the brittle rachis trait in wild emmer was controlled by two genes designated *Br* (Watanabe and Ikebata 2000). One was located on the short arm of chromosome 3A ($Br1^{3A}$) and the other on the short arm of chromosome 3B ($Br1^{3B}$). These two genes are likely homoeologous (Nalam et al. 2006). A gene homoeologous to $Br1^{3A}$ and $Br1^{3B}$ was also identified on the short arm of chromosome 3D ($Br1^{3D}$), and was

derived from *Ae. tauschii* (Cao et al. 1997; Chen et al. 1998). Other work led to the identification of a *Br* gene on the long arm of chromosome 3D ($Br2^{3D}$) (Li and Gill 2006). Another locus on the long arm of chromosome 2A was also reported to control rachis brittleness in wild emmer (Peng et al. 2003; Peleg et al. 2011).

Tenacious glume: Thick, tenacious glumes of non-domesticated wheat species help protect and encompass the seed tightly during seed dispersal. Wild wheats have tenacious glumes that make it very difficult to liberate the seed. However, domesticated wheat species have soft, papery glumes that render the seed free-threshing.

Threshability studies in einkorn wheat led to the identification of a single recessive gene (*sog*) controlling the soft glume trait, and it was mapped to the short arm of chromosome $2A^m$ (Taenzler et al. 2002; Sood et al. 2009). Kerber and Dyck (1969) were the first to show that the tenacious glume trait in hexaploid wheat was controlled by an incomplete dominant gene (*Tg-D1*), which was mapped on the short arm of chromosome 2D (Nalam et al. 2007; Sood et al. 2009). One major QTL was mapped on the short arm of chromosome 2B associated with the free-threshing trait (Simonetti et al. 1999). Recently *Tg-B1* was identified from wild emmer and mapped on the short arm of chromosome 2B and was shown to be homoeologous to *Tg-D1* (Faris et al. 2014).

Free threshing: The free-threshing trait allows the seed to be liberated from the spike easily and efficiently. The free-threshing trait is governed by two genes, *Q* and *Tg,* and *Tg* is epistatic to *Q* (Kerber and Rowland 1974; Faris 2014). A single amino acid mutation in the q^{5A} allele led to the partially dominant Q^{5A} allele, which result in free-threshing seed (Simons et al. 2006).

The *Q* gene on chromosome arm 5AL inhibits speltoidy and has pleiotropic effects on rachis fragility, glume toughness, spike architecture, flowering time, plant height, and other traits (Simons et al. 2006; Zhang et al. 2011). The *Q* gene was shown to be a member of the *AP2* family of transcription factors. A single amino acid difference between the *Q* and *q* alleles may affect the properties of the transcription factor. In addition, the expression level of Q^{5A} was much higher than that of q^{5A} (Simons et al. 2006).

Map-based cloning of genes in wheat

A long history of wheat genetic research has led to the identification and mapping of a great number of important agronomic traits in wheat (McIntosh et al. 2012). To further understand the biology and molecular mechanisms underlying these traits, the isolation of the genes is needed. However, the 17 Gb hexaploid bread wheat genome and its genomic complexity make it a challenge to clone genes from wheat without a reference sequence or an extensive physical map (Kattinger et al. 2009a; IWGSC 2014). More than 80% of the genome consists of repetitive sequences (IWGSC 2014), which makes chromosome walking and marker development extremely difficult. Despite its large genome size and high content of repetitive sequences, a growing number of genes have been isolated from wheat through positional cloning (Kattinger et al. 2009a, Table 2.1). Many of them are genes conferring resistance to diseases. Following is a brief summary of the steps for map-based cloning and genes cloned through positional cloning.

Table 2.1. List of genes that have been isolated by positional cloning method in wheat.

Gene	Gene class	Population size (gametes)	Size of genetic /physical interval	Candidate gene validation	References
Sr35	CC-NB-LRR	4,575	1 cM/ 307 kb	Transgenic plant, mutant analysis, expression analysis	Saintenac et al. 2013
Sr33	CC-NB-LRR	2,850	1 cM/ MD	Mutant analysis, transgenic plant, expression analysis, VIGS	Periyannan et al. 2013
TaPHS1	Homologue of TaMFT	3,748	2 cM/ 210 kb	RNAi; expression analysis	Liu et al. 2013
Tsn1	S/TPK-NB-LRR	5,438	0.11 cM/ 350 kb	Mutant analysis, allele diversity, expression analysis	Faris et al. 2010
Lr34	*ABC* transporter	8064	0.15 cM/363 kb	Mutant analysis, allele diversity, expression analysis	Krattinger et al. 2009b
Yr36	Kinase-START	4,500	0.14 cM/314 kb	Mutant analysis,Transgenic plant, expression analysis	Fu et al. 2009
Lr1	CC-NB-LRR	7,300	0.075 cM/200 kb	Transgenic plant, VIGS	Cloutier et al. 2007
Gpc-B1	*NAC* transcription factor	9,000	MD/7.4 kb	Allele diversity, RNAi, expression analysis	Uauy et al. 2006
VRN3	Orthologue of *FT*	1,600	0.2 cM/ MD	Transgenic plant, allele diversity, expression analysis	Yan et al. 2006
Ph1	A major chromosome pairing locus	MD	MD/ 2.5 Mb	Deletion mutant analysis	Griffiths et al. 2006
Pm3	CC-NB-LRR	2,680	0.22 cM/ MD	Mutant analysis, expression analysis, transient expression	Yahiaoui et al. 2004 Yahiaoui et al. 2006
VRN2	Dominant repressor of flowering	5,698	0.04 cM/439 kb	RNAi, allele diversity, expression analysis	Yan et al. 2004
Lr21	NB-LRR	1,040	1.7 cM/ MD	Transgenic plant, VIGS	Huang et al. 2003
VRN1	*AP1* like TF	6,190	0.04 cM/550 kb	Allelic diversity, expression analysis	Yan et al. 2003
Q	*AP2* TF	930	0.7 cM/250 kb	Mutant analysis, allele diversity, expression analysis, transgenic plant	Faris et al. 2003; Simons et al. 2006
Lr10	CC-NB-LRR	6,240	0.13 cM/300 kb	Mutant analysis, allelic diversity, transgenic plant	Feuillet et al (2003)

*MD- missing data

Marker development and fine-mapping for map-based cloning

The availability of a high-resolution map is essential for successful cloning of a gene, which is a time-consuming process. Fine mapping requires a large segregating population and the genetic locus of interest saturated with molecular markers. The

mapping population must provide enough recombinants to resolve tightly linked markers and possibly distinguish candidate genes around the gene region. If crossover suppression occurs, it would make chromosome walking much more difficult if not impossible. Therefore, it is beneficial to first evaluate several populations for their utility and feasibility of fine mapping before choosing which population to use.

There are now thousands of molecular markers available for mapping the wheat genome, and they include many different types such as restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), and single nucleotide polymorphism (SNP) markers. These markers can be used to rapidly localize a gene of interest and construct a low-resolution map of the target locus (Song et al. 2005; Peng and Lapitan. 2005; Wang et al. 2014; Kattinger et al. 2009a). Once a low-resolution map is generated, deletion bin-maps can be used to quickly identify SSR or EST markers for saturating the gene region (Qi et al. 2004). Then the two flanking markers closest to the gene can be used to screen a large high-resolution mapping population consisting of >5,000 gametes to identify plants with recombination events between the markers and the target gene. Only those plants having crossovers between the two flanking markers need to be kept for high-resolution mapping and phenotyping.

Mapped EST markers can be used for comparative mapping to identify the collinear regions in the sequenced barley, *Brachypodium* or rice genomes. Once the homologous sequences or genes in the collinear interval are identified in wheat, more markers can be developed and mapped in the high-resolution population. At the same time these homologous genes make good candidates for the target gene.

Recent advances in sequencing technology development have led to the identification of a large number of SNPs through genotyping by sequencing (GBS) (Cavanagh et al. 2013; Wang et al. 2014). These SNP markers can be directly used to screen for polymorphisms between mapping parents. Alternatively, the flanking sequences of SNPs can be used to search the wheat survey sequence database (https://urgi.versailles.inra.fr/blast/blast.php). The chromosome arm-specific assembled survey sequences can be used to identify more SSRs, genes, repeat junctions, and other features for further marker development. Any genes annotated from these survey sequences that cosegregate with the phenotype would also be considered candidate genes.

Draft genome sequences of two wheat progenitors, *T. urartu* and *Ae. tauschii*, are available as well, which will greatly benefit the cloning of genes on the A and D genome chromosomes (Jia et al. 2013; Ling et al. 2013). Moreover, there are more wheat genomic sequences available (Brenchley et al. 2012) and the survey sequence database of all 21 chromosomes is especially valuable to obtain sequences for each individual chromosome. Furthermore, a chromosome-based draft sequence was just released by the International Wheat Genome Sequencing Consortium (IWGSC), which opens a new era for the wheat community (IWGSC, 2014).

Anchoring a physical map to the genetic map

Establishment of a physical region spanning the targeted gene region is crucial for map-based cloning (Krattinger et al. 2009a). The closest flanking markers from the high-resolution map are used to screen a large insert library, such as a bacterial artificial chromosome (BAC) library, to construct a physical contig covering the gene region. Because the ratio of genetic to physical distance varies across the genome, it is difficult to

predict the number of BAC clones needed to span the distance between the flanking markers. Gaps in the physical map can be filled by chromosome walking (Krattinger et al. 2009a).

Today, numerous BAC libraries are available for hexaploid wheat as well as many wheat relatives. In the framework of the IWGSC, BAC libraries were constructed for each individual wheat chromosome or chromosome arm. For many of these libraries, the BAC clones have been fingerprinted, assembled into contigs, used to establish minimum tiling paths (MTPs), and in many cases anchored to genetic maps with molecular markers (Raats et al. 2013). This provides a tremendous resource for greatly enhancing the efficiency of the map-based cloning process in wheat.

Once a BAC contig is constructed covering the target gene region, the next step is to identify and validate candidate genes. If there are several BAC clones representing the candidate gene region, the BACs can be sequenced. Sequencing can help to further delimit the gene region by providing information to develop new markers using bioinformatic analysis. It would be ideal for the two flanking markers to land on the same BAC clone, which would indicate that the target gene was present on that BAC clone. The BAC clone would then be sequenced and all genes on this BAC would be considered as candidates for the target gene.

Another concern is whether or not the target gene is present in the genotype used to develop the chosen BAC library, which depends on the cultivar used to construct the BAC library. If it is absent, selective PCR of regions of interest or specific cosmid or fosmid libraries from the line containing the target gene should be considered (Krattinger et al. 2009a).

Candidate gene validation

The final step for map-based cloning is to analyze all candidate genes lying in the physical interval encompassing the gene. The difficulty of this task depends on the size of this physical segment and gene density in this interval. Numerous tools are available for gene validation in wheat, such as expression data, allelic diversity, mutant analysis, virus-induced gene silencing (VIGS) and gene transformation.

Candidate genes can be sequenced from natural or ethyl methanesulfonate (EMS) -induced mutants and compared with sequences from the wild types to rapidly reveal if the candidate is the target gene or not. Many wheat genes such as *Lr10* (Feuillet et al. 2003), *Pm3* (Yahiaoui et al. 2004), *Sr35* (Saintenac et al. 2013), *Q* (Faris et al. 2003; Simons et al. 2006) and *Tsn1* (Faris et al. 2010) were validated in this way.

Transient assays by particle bombardment were used to validate some genes such as *Pm3* where the host pathogen interaction occurred in epidermal cells (Yahiaoui et al. 2004). Transformed cells can be screened by marker genes and further examined their compatibilities with specific race of the pathogen. However, this transient assay is restricted to young seedlings and epidermal tissue. Stable transformation is now routine, but still time-consuming to obtain results from T1 or T2 Plants. Particle bombardment is the most widely used in wheat. Though the lines are restricted for efficient transformation, the list of genotypes successfully used is expanding (Simons et al. 2006; Cloutier et al. 2007; Saintenac et al. 2013; Periyannan et al. 2013).

Virus-induced gene silencing (VIGS) is another valuable tool for functional gene analysis. VIGS has been successful applied for the functional analysis of *Lr21* (Scofield et al. 2005), *Lr1* (Cloutier et al. 2007) and *Sr33* (Periyannan et al. 2013). RNAi is also an

effective way to silence candidate genes and was successfully used to validate the function of *VRN2* (Yan et al. 2004).

Allelic diversity analysis and expression analysis can further verify the identification of a candidate gene. These forms of analyses are typical and used in nearly all gene-cloning experiments (Yan et al. 2006; Faris et al. 2010; Saintenac et al. 2013; Periyannan et al. 2013).

Host-pathogen interactions

It is estimated that 25 to 30% of a given wheat crop is lost to abiotic and biotic stress (Bockus et al. 2010). Host resistance is the most economically viable, environmentally friendly and sustainable means for disease and pest control. The effectiveness of host resistance prompted early genetic studies that defined the “gene-for-gene” theory between host resistance genes and pathogen avirulence factors (Flor 1971). The interaction between a plant and a pathogen involves two-way communication. Plants are able to recognize the pathogen and initiate defense responses to protect themselves from harm from pathogens, while most pathogens strive to escape host recognition and manipulate plant biology to create a suitable environment for their growth and reproduction (Boyd et al. 2013). Molecular studies have revealed that resistance genes encode components of the plant immune system that allow plants to recognize and respond to specific pathogens. At the same time, studies on pathogen biology have unraveled how these pathogens overcome host immunity and cause disease. Understanding the molecular basis of resistance from both host and pathogen aspects greatly broadens our perspective on host-pathogen interactions and provides us with more approaches for crop protection (Dodds and Rathjen 2010).

Pathogen-associated or microbe-associated molecular pattern (PAMP or MAMP)-triggered immunity and effector-triggered immunity

Plants are subject to attack by a variety of pathogens and completely rely on innate immunity for their defense. Therefore, plants have evolved sophisticated immune systems to protect themselves from biotrophic pathogens. The first recognition is through perception of microbe-associated or pathogen-associated molecular patterns (MAMPs or PAMPs) by pattern recognition receptors (PRRs) (Zipfel 2008; Tsuda and Katagiri 2010). PAMPs or MAMPs are well-conserved molecular structures unique to the pathogen, such as bacteria flagellin or fungal chitin. Therefore, variation of these patterns to escape recognition is limited (Gohre and Robatzek 2008). These PAMPs or MAMPs can be recognized by surface PRRs and trigger defense responses. PRRs usually locate in the plasma membrane and function conservatively across families (Dodds and Rathjen 2010). Missense or nonsense mutations of PRRs will result in failure of recognition resulting in disease. This PAMP-triggered immunity (PTI) is essential for plant defense. Well-characterized PTI interactions include FLS2 for bacteria flagellin (Gomez-Gomez and Boller 2000), EFR for bacterial EF-Tu (Zipfel et al. 2006), and CERK1 for fungal chitin (Miya et al. 2007). The recognition initiates a series of signal cascades including Ca^{2+} fluxes and activation of mitogen activated protein kinases (MAPKs), production of reactive oxygen species (ROS) and antimicrobial products, and accumulation of callose at the infection site to prevent pathogen penetration (Gohre and Robatzek 2008).

Successful pathogens have evolved effectors to suppress PTI, which leads to effector-triggered susceptibility (ETS) (Zipfel 2009). Effectors are often internalized within the plant cell. The best example of an internalization mechanism is the type III

secretion system (TTSS) in gram-negative bacteria. Acquirement of TTSS enables bacteria to directly deliver effectors into plant cell to suppress PTI through different mechanisms (Chisholm et al. 2006). Members of the *Xanthomonas AvrBs3* effector family contain a C-terminal nuclear localization signal (*NLS*) and an acidic transcriptional activation domain (*AAD*), which suggest that these effectors could alter plant nuclear gene transcription during infection to down-regulate host defenses (Zhu et al. 1998; Chisholm et al. 2006).

As a counter measure, plants have evolved resistance (R) genes to recognize the effectors produced by the pathogen and initiate effector-triggered immunity (ETI) (Jones and Dangl 2006; Zipfel 2009; Tsuda and Katagiri 2010). Effectors, encoded by avirulence (*Avr*) genes, are typically variable and dispensable between individuals of a pathogen species, which provides the basis for discrimination of different races/strains/isolates within a pathogen species (Schulze-Lefert and Panstruga 2011). The cognate host resistance genes that recognize these strain-specific pathogen effectors are also polymorphic between individuals of a plant species. This pairwise association between pathogen *Avr* and *R* genes has been described as the gene-for-gene model (Flor 1971). In the presence of a cognate *Avr* and *R* gene association, a defense response will be initiated and lead to resistance. Either the *Avr* gene or *R* gene is absent, the pathogen could escape recognition, colonize the host and cause disease.

This dynamic process will continue between the pathogen and the host. Each one will evolve new molecular weapons to start a new round of battle. So far, numerous effectors produced by biotrophic pathogens and the corresponding *R* genes have been isolated and characterized (Rafiqi et al. 2012). Despite the broad spectrum of resistance

imparted by R proteins, these gene products could be classified into two categories based on their gene structures. The largest group of *R* genes cloned to date is a family of members containing a nucleotide binding (*NB*) and leucine-rich repeat (*LRR*) domains. The NB motif indicates that the protein may require ATP-binding and/or hydrolysis for its function (Tameling et al. 2002). LRRs appear to be involved in protein-protein interactions. The well-characterized members in this *NB-LRR* class include *RPS2*, *RPM1* and *RPS5* in *Arabidopsis*, which confer resistance to *P. syringae* secreting bacterial effectors *AvrRpt2*, *AvrRpm1*/*AvrB* and *AvrPphB*, respectively (Jones and Dangl 2006; Dangl and Jones 2000). The second class of *R* genes encodes extracellular LRR (eLRR) proteins, including receptor-like proteins (RLPs), receptor-like kinase (RLKs) and polygalacturonase-inhibiting proteins (PGIPs) (Chisholm et al. 2006). The best-characterized *RLPs* are the tomato *Cf* genes, which confer resistance to the leaf mold pathogen *C. fulvum* (Jones et al. 1994). Analysis of apoplast proteins secreted by *C. fulvum* during its growth within the tomato leaves led to identification of the race-specific effectors, *Avr2*, *Avr4* and *Avr9*, which could be recognized by their corresponding host resistance gene *Cf-2*, *Cf-4* and *Cf-9*, respectively (de Wit 1995; Joosten et al. 1994; van Esse et al. 2008). Though these *Cf* genes lack a signaling domain, it is believed that the signal is passed down through interaction with other proteins.

Comparing signaling mechanisms between PTI and ETI

An oxidative burst, hormonal changes and transcriptional variations occur in both PTI and ETI systems, which indicates that both systems share overlapping signaling pathways (Zipfel et al. 2006; Tao et al. 2003; Navarro et al. 2004). There are also observations that the ETI signaling network is more robust than PTI signaling (Navarro et

al 2008; de Torres-Zabala et al. 2007; Rosebrock et al. 2007). Some differences and similarities between PTI and ETI signaling responses are described below.

Whole genome DNA microarray analysis revealed that transcriptome responses triggered by different PAMPs are very similar at early stages but diverge at later stages, suggesting that a common downstream pathway is shared at the initial period but different late responses are required to combat each specific pathogen (Gust et al. 2007; Lu et al. 2009). There is also a significant overlap of genes induced by PAMPs and effectors (Navarro et al. 2004). It is possible that ETI inherits most of the PTI system based on the assumption that PTI evolved prior to ETI (Jones and Dangl 2006).

ETI is often associated with a hypersensitive response (HR), a rapid cell death triggered by the effectors to restrict the pathogen growth (Jones and Dangl 2006). Naito et al. (2008) also found that cell death was induced by flagellin derived from *P. syringae* pv. *Tabaci* 6605. Thus, plant cell death responses can be mediated through both ETI and PTI pathways.

An oxidative burst, or ROS production, is another feature in plant defense response, which can act as a secondary messenger or to directly neutralize the pathogen (Torres et al. 2006). Recognition of PAMPs by PRRs will trigger a rapid and transient ROS production through NADPH oxidase *AtRbohD* (Zipfel et al. 2006; Zhang et al. 2007). An *AtRbohD*-dependent ROS accumulation was also observed in the recognition of effectors by *R* genes (Torres et al. 2006)

MAPK-induced signaling is often reported to be involved in defense responses as well. *MAPK* activation by PAMPs occurs at very early stages of pathogen infection and lasts for a short period (Zipfel et al. 2006; Gust et al. 2007). However, the *MAPK* activity

is generally more robust after being activated by effectors (Underwood et al. 2007). The duration of *MAPK* activation may differentiate PTI from ETI.

Salicylic acid (SA), jamonic acid (JA) and ethylene (ET) are often associated with plant immunity. Many cases have reported their involvement in the signaling pathways triggered by both PAMPs and effectors (Tsuda et al. 2008; Katagiri 2004). However, the relationship between these hormones is different in PTI and ETI, a synergistic relationship in PTI and a compensatory one in ETI, which could explain the robustness of ETI against the pathogenic perturbations (Tsuda et al. 2009).

Stagonospora nodorum blotch in wheat

Stagnospora nodorum blotch is a common component of the fungal leaf and glume blotch complex in most wheat production areas. It can cause 10-20% grain yield losses and nearly 2% of the world wheat annually (Bockus et al. 2010). *P. nodorum* is considered to be one of the most destructive pathogens in Australia (Oliver et al. 2012) and North America (Crook et al. 2012), and it has been reported in South Asia, North Africa and Europe as early as 1889 (Bearchell et al. 2005). Therefore, SNB is found in most wheat-growing areas and ranked as one of the top three diseases in terms of economic losses worldwide (Oliver et al. 2012).

P. nodorum initially causes water-soaked and small chlorotic lesions on the lower leaves of the plant. The lesions become yellow and red-brown, sometimes with a yellow halo. Mature lesions are generally lens-shaped and expand into a gray-brown center containing brown specks (Friesen and Faris 2010). On wheat heads, the lesions are often dark brown or purple. Lesions are more intense on the upper third of the glume, with brown streaks or blotches extending towards the base of the spikelet. Severe SNB can

cause lightweight, shriveled kernels. The presence of small, honey-colored fungal reproductive structures is diagnostic for SNB (McMullen and Adhikari 2009).

The fungi can survive on wheat stubble, other wheat residues and volunteer wheat crops. It can live up to three years on wheat stubble. Inoculum from pathogen infected seeds can be a sufficient source for disease development. Ascospores released from the pseudothecia can be dispersed by wind and cause the first lesions on leaves. The infection usually requires 12 to 18 hr of wetness and the disease develops most rapidly between 20 and 27°C. After initial infection, 10 to 20 days are needed before new spores are released from pycnidia. Conidia released from pycnidia are water-splash dispersed and enable a polycyclic infection cycle through the growing season. The wheat plant is more susceptible to infection at later growth stages. It becomes more prevalent with dense foliage and heavy fertilization (McMullen and Adhikari 2009).

Host gene-necrotrophic effector interactions identified in the wheat-*P. nodorum* system

Necrotrophic pathogens are known to produce a number of necrotrophic effectors (NEs) (also known as host-selective toxins) as essential determinants for pathogenicity or virulence factors (Scheffer and Livingston 1984; Wolpert et al. 2002). These NEs cause disease when recognized by specific genes in the host (Friesen et al. 2007). In contrast to PTI/ETI in the classic gene-for-gene model, the recognition of NEs follows an inverse gene-for-gene model, in which the recognition of NEs results in susceptibility. However, the absence of either the NE or the corresponding host sensitivity gene results in an incompatible interaction and no disease occurs (Friesen and Faris 2010).

During the past decade, numerous studies have been conducted on the wheat-*P. nodorum* pathosystem and demonstrated that NEs are major determinants of SNB. When an NE is recognized by the corresponding dominant host gene, a compatible interaction occurs and leads to disease susceptibility (Friesen et al. 2008a; Friesen and Faris 2010). To date, seven proteinaceous NEs have been identified from different isolates of *P. nodorum* and eight host gene-NE interactions have been characterized in this pathosystem (Table 2.2) (Friesen and Faris 2010). Both additive and epistatic effects were observed when multiple compatible interactions exist in the same segregating population (Friesen et al. 2008a; 2008b).

Table 2.2 Currently identified NE-wheat gene interactions in the wheat-*Parastagonospora nodorum* pathosystem.

NEs	Host gene	Markers	Maximum disease significance	Host gene chromosome arm location	Reference
SnToxA	*Tsn1*	*Xfcp1, Xfcp2, Xfcp394, Xfcp620, Xfcp623*	95%	5BL	Friesen et al. 2006 Liu et al. 2006 Friesen et al. 2009 Zhang et al. 2009 Faris and Friesen, 2009 Faris et al. 2010
SnTox1	*Snn1*	*Xfcp618, Xpsp3000*	58%	1BS	Liu et al. 2004a Liu et al. 2004b Liu et al. 2012 Reddy et al. 2008
SnTox2	*Snn2*	*XTC253803, Xcfd51*	47%	2DS	Friesen et al. 2007 Friesen et al. 2009 Zhang et al. 2009
SnTox3	*Snn3-B1*	*Xcfd20*	18%	5BS	Friesen et al. 2008b Liu et al. 2009
	Snn3-D1	*Xcfd18, Xhbg337*	100%	5DS	Zhang et al. 2010
SnTox4	*Snn4*	*XBG262267, XBG262975, Xcfd58*	41%	1AS	Abeysekara et al. 2009
SnTox5	*Snn5*	*Xwmc349, Xcfd22*	63%	4BL	Friesen et al. 2012
SnTox6	*Snn6*	*XBE424987, XBE403326*	27%	6AL	Gao et al. 2014

SnTox1-*Snn1*

The SnTox1-*Snn1* interaction was the first NE–host interaction to be identified in the wheat-*P. nodorum* pathosystem (Liu et al. 2004a). SnTox1, a proteinaceous NE, was

partially purified from culture filtrates of isolate Sn2000 of *P. nodorum*. The associated host gene, *Snn1*, was mapped to the distal end of the short arm of chromosome 1B (Liu et al. 2004a). A compatible SnTox1-*Snn1* interaction explained as much as 58% of the variation in the development of SNB caused by Sn2000 (Liu et al. 2004b).
SnTox1 was cloned using bioinformatics tools and verified through heterologous expression in *Pichia pastoris* (Liu et al. 2012). SnTox1 encodes a protein of 117 amino acids, consisting of a 17 amino acid signal peptide and 16 cysteine residues in the mature protein with an estimated size of 10.3 kDa (Liu et al. 2012). Rich cysteine content is a common feature for apoplastic fungal effectors, which indicates that SnTox1 may function in the apoplast (Liu et al. 2012). The chitin-binding domain of SnTox1 was different from that in the effector *Avr4* from the tomato pathogen *C. fulvum* (Stergiopoulos and de Wit, 2009). The chitin-binding function needs to be further verified (Liu et al. 2012). SnTox1 was present in 85% of the global collection of *P. nodorum* isolates and 11 protein isoforms were identified. There is evidence that SnTox1 was under strong diversifying selection (Liu et al. 2012). Typical disease defense responses were observed after the SnTox1-*Snn1* recognition, including induction of an oxidative burst, DNA laddering and induction of pathogenesis related (PR) protein expression (Liu et al. 2012).

For the host sensitivity gene *Snn1*, comparative, saturation and high-resolution mapping was conducted on a population derived from a cross between Chinese Spring (CS) and a CS-dicoccoides 1B substitution line (Reddy et al. 2008). Wheat EST markers were used for the saturation mapping and a detailed comparison showed that colinearity was poorly conserved between the *Snn1* genomic region of wheat and syntenic regions of

rice. High-resolution mapping of the locus led to the delineation of *Snn1* to a 0.46 cM interval. Two ESTs that cosegregated with *Snn1* showed high similarity to known NB-LRR disease resistance-like genes and were proposed to be candidates for *Snn1*.

SnToxA-*Tsn1*

The SnToxA-*Tsn1* interaction is the best characterized interaction of this pathosystem, in which both host and pathogen genes have been cloned and characterized (Ciuffetti et al. 1997; Friesen et al. 2006; Faris et al. 2010). The ToxA gene encodes a 13 kDa polypeptide and was involved in a horizontal transfer from *P. nodorum* to *Pyrenophora tritici-repentis*, the casual pathogen for tan spot in wheat (Friesen et al. 2006). Sensitivity to ToxA is conferred by the dominant gene *Tsn1*, which was mapped to the long arm of chromosome 5B (Faris et al. 1996). A compatible ToxA-*Tsn1* interaction has been shown to account for up to 95% of the disease variation demonstrating the importance of the ToxA-*Tsn1* interaction for disease development in both common wheat and durum wheat (Faris and Friesen 2009).

Sequence analysis showed that *SnToxA* contained three exons and two introns, sharing high sequence and structural similarities between *P. nodorum* and *P. tritici-repentis*. *SnToxA* was detected in 24% of the worldwide collection of *P. nodorum* and 80% of *P. tritici-repentis*. Eleven protein isoforms were identified in *P. nodorum* and only one in *P. tritici-repentis*, which led to the conclusion that ToxA was horizontally transferred from the former to the latter fairly recently (Friesen et al. 2006).

As for its cognate host gene *Tsn1*, a BAC contig spanning the *Tsn1* locus was assembled through saturation, comparative, and physical mapping (Haen et al. 2004; Lu et al. 2006; Lu and Faris 2006). Six candidate genes were identified and two of them

were excluded as candidates for *Tsn1* through association mapping. Sequence analysis of the remaining four candidate genes from EMS-induced mutants indicated that a serine/threonine protein kinase(S/T)-NB-LRR-like gene was *Tsn1* (Faris et al. 2010). *Tsn1* had no significant similarity to any known genes in the National Center for Biotechnology Information (NCBI) non-redundant database. The S/TPK domain of *Tsn1* had similarity to the barley stem rust R gene *Rpg1* (Brueggeman et al. 2002) and the NB domain to that of the maize *Rp3* rust R gene (Webb et al. 2002; Faris et al. 2010). Although *Tsn1* has typical resistance gene-like features, it confers sensitivity to SnToxA leading to SNB susceptibility, which suggests that necrotrophic pathogens can hijack ETI resistance mechanisms to gain nutrients and cause disease.

Phylogenetic analysis indicated that *Tsn1* arose from a B-genome diploid ancestor. Its transcription was tightly regulated by light and light exposure patterns, which suggested that photosynthesis was involved in the *Tsn1*-SnToxA interaction (Faris et al. 2010). Yeast-two-hybrid showed that the Tsn1 protein did not interact directly with SnToxA. Tsn1 may monitor another protein targeted by SnToxA and the perception may initiate downstream signal transduction.

SnTox2-*Snn2*

SnTox2 was the third proteinaceous NE identified that causes necrosis on wheat genotypes harboring *Snn2*, the wheat gene mapped to the short arm of chromosome 2D that confers sensitivity to SnTox2 (Friesen et al. 2007). SnTox2 was partially purified from isolate Sn6 and shown to be between 7 and 10 kDa in size (Friesen et al. 2007). The SnTox2-*Snn2* interaction accounted for as much as 47% of the disease variation in a

population of wheat recombinant inbred lines derived from the hard red spring wheat varieties BR34 and Grandin (BG population).

Using the BG population, Friesen et al. (2007) studied the combined role of both the SnTox2-*Snn2* and the SnToxA-*Tsn1* interactions on disease development. Results showed that the effects of compatible ToxA-*Tsn1* and SnTox2-*Snn2* interactions were almost completely additive. Subsequent genome analysis and mapping studies led to the identification and development of PCR-based markers that delimited *Snn2* to a 4.0 cM interval (Zhang et al. 2009).

SnTox3-*Snn3*

SnTox3 induces programmed cell death (PCD) on wheat lines carrying the *Snn3* gene. The *SnTox3* gene was cloned and found to be an intron-free gene of 693 bp that produces an immature protein of 330 amino acids (Liu et al 2009). The *SnTox3* gene produces a 25.8 kDa immature protein and the mature SnTox3 contains six cysteine residues. No sequences with homology to SnTox3 were found in other microbes. However, *SnTox3* was shown to be prevalent among *P. nodorum* isolates worldwide. There were 11 nucleotide haplotypes resulting in four amino acid haplotypes of SnTox3 (Liu et al. 2009).

Friesen et al. (2008b) showed that the *Snn3* gene was located on chromosome arm 5BS and a compatible SnTox3-*Snn3* interaction accounted for 18% of the disease variation. Unlike the other four NE-host gene interactions, the SnTox3-*Snn3* interaction is light independent. Friesen et al. (2008b) also showed that the SnTox2-*Snn2* interaction is epistatic to the SnTox3-*Snn3* interaction.

More recently, a gene was identified in *Aegilops tauschii* (Coss.) ($2n = 2x = 14$, DD genomes), the D-genome donor of hexaploid wheat, that also mediated recognition of SnTox3 to confer SNB susceptibility (Zhang et al. 2011). The *Ae. tauschii* gene was located at the distal end of chromosome arm 5DS and shown to be likely homoeologous with *Snn3* on 5BS through comparative mapping experiments (Zhang et al. 2011). Therefore, Zhang et al. (2011) proposed to designate *Snn3* on 5BS and the newly discovered gene on *Ae. tauschii* 5DS as *Snn3-B1* and *Snn3-D1*, respectively. Further saturation and high-resolution mapping experiments led to the identification of markers *Xcfd18* and *Xhbg337*, which delineated *Snn3-D1* to a 1.44 cM interval. Comparative analysis indicated that good levels of colinearity exist among the *Snn3-B1*, *Snn3-D1* region, rice chromosome 12 and *Brachypodium* chromosome 4, which would be valuable for marker development using the rice and *Brachypodium* genome sequences in the collinear region. Because *Snn3-B1* and *Snn3-D1* are homoeoalleles, the genomic analysis and/or isolation of one will greatly expedite the isolation of the other gene.

SnTox4-*Snn4*

The fifth NE, SnTox4, was partially characterized from the Swiss isolate Sn99CH 1A7a (Abeysekara et al. 2009). SnTox4 was estimated to be 10–30 kDa in size and proteinaceous in nature. The associated host sensitivity gene was designated *Snn4*, and it was mapped to the short arm of wheat chromosome 1A. The SnTox4-*Snn4* interaction led to a mottled necrotic reaction, different from severe necrosis of other interactions. The *Snn4* locus was delineated to a 2.5 cM interval flanked by the EST-based markers *XBG262267* and *XBG262975* on the distal side and the microsatellite marker *Xcfd58* on

the proximal side (Abeysekara et al. 2009). The SnTox4- *Snn4* interaction accounted for as much as 41% of disease variation.

SnTox5-*Snn5*

SnTox5 was another proteinaceous NE identified from isolate Sn2000 (Friesen et al. 2012). Initial characterization of SnTox5 showed that it was between 10 and 30 kDa in size. It is recognized by wheat sensitivity gene *Snn5*, which was mapped on the long arm of wheat chromosome 4B. The SnTox5-*Snn5* interaction was light dependent like most of other interactions and explained 37-63% of the disease variation, demonstrating that it plays a significant role in disease development. It showed strong additive effects with the SnToxA-*Tsn1* interaction when both were present in the same population (Friesen et al. 2012).

SnTox6-*Snn6*

The *P. nodorum* isolate Sn6 was found to produce a novel NE SnTox6, which was about 6.5-12.3 kDa in size. SnTox6 is recognized by the host sensitivity gene *Snn6,* which lies on wheat chromosome arm 6AL. This interaction accounted for 27% of the SNB disease variation. Initial characterization showed that this SnTox6-*Snn6* recognition is also light-dependent (Gao et al. 2014).

The mode for NEs-host gene interactions

In most cases, the responses resulting from both PTI and ETI involve programmed cell death (PCD), activation of MAPK signaling, an oxidative burst and pathogenesis related (PR) protein expression (Tsuda and Katagiri 2010). The HR leads to rapid and localized cell death, which would halt the further growth of a biotrophic pathogen (Liu et al. 2012). However, the death of host cells would benefit necrotrophic

pathogens, which feed on the nutrients leaking from the dead cells. Consequently, the recognition of PAMPs/effectors elicited from necrotrophic pathogens will lead to PAMP-triggered susceptibility (PTS) or necrotrophic effector-triggered susceptibility (NETS) (Jones and Dangl 2006; Liu et al. 2009; Faris et al. 2010).

Though the molecular mechanisms for NETS are largely unknown, typical disease resistance responses have been observed including induction of an HR, DNA laddering, heterochromatin condensation, cell shrinkage, an oxidative burst and PR protein expression (Wolpert et al. 2002; Liu et al. 2012). To date, three NE sensitivity genes, *LOV1*, *Pc*-2 and *Tsn1*, have been cloned and are all members of the NB-LRR class of genes (Lorang et al. 2007; Nagy and Bennetzen 2008; Faris et al. 2010). The *LOV1* gene governs susceptibility to victorin produced by *Cochliobolus victoriae* in *Arabidopsis* (Lorang et al. 2007), the *Pc-2* gene of sorghum controls sensitivity to Pc toxin produced by *Periconia circinata* (Nagy and Bennetzen 2008), and as mentioned above, *Tsn1* confers sensitivity to ToxA produced by both *P. nodorum* and *P. tritici-repentis* (Faris et al. 2010). ToxA is a small protein with a signal peptide for secretion and an arginine-glycine-aspartate (RGD) solvent exposed loop for internalization (Ciuffetti et al. 1997; Manning et al. 2008). Previous work demonstrated that ToxA is internalized into the host cell in lines with *Tsn1* and then localized to chloroplasts (Manning et al. 2007). Yeast-two-hybrid analysis showed that SnToxA interacted directly with plastocyanin (Tai et al. 2007), ToxABP1 (Manning et al. 2007) and PR1 (Lu et al. 2014), but *Tsn1* did not interact directly with any of them (Faris et al. 2010). It is possible that *Tsn1* monitors a target of SnToxA leading to internalization. Once internalized, ToxA may disturb the photosynthesis pathway after binding directly with plastocyanin, a vital component of

electron transport in photosystem II and ToxABP1 (Manning et al. 2007; Tai et al. 2007). The recognition of ToxA strongly activated wheat defense responses and affected numerous cellular processes (Manning et al. 2004; Rasmussen et al. 2004). Transcriptome analysis revealed that a large number of genes associated with plant defense responses were differentially expressed between ToxA-infiltrated and mock-infiltrated wheat plants, such as *PR* genes, *WRKY* transcription factors, and receptor-like kinases (Pandelova et al. 2009).

Therefore, the host defense responses in NETS to necrotrophic pathogens are very similar to those in ETI to biotrophic pathogens. The difference arises from the biology of two types of pathogens, in which necrotrophic pathogens may hijack plant resistance pathways for their own good. The induction of cell death by a necrotroph creates an environment favorable for its survival and sporulation, which is detrimental to biotrophic pathogens. Studies have shown that global climate changes have multiple effects on plant growth and biology (Chakraborty et al. 2000). Reduced net photosynthesis and premature ripening and senescence could benefit the colonization of plants by necrotrophic pathogens (Manning and Tiedemann 1995). Therefore, further study on necrotrophic pathosystems will greatly enhance our knowledge and understanding of host-necrotroph interactions and benefit the manipulation of necrotrophic disease through host resistance.

References

Abeysekara N, Friesen TL, Keller B, Faris JD (2009) Identification and characterization of a novel host-toxin interaction in the wheat-*Stagonospora nodorum* pathosystem. Theor Appl Genet 120:117-126

Bearchell SJ, Fraaije BA, Shaw MW, Fitt BD(2005) Wheat archive links long-term fungal pathogen population dynamics to air pollution. Proc Natl Acad Sci USA 102:5438-5442

Bockus WW, Bowden RL, Hunger RM, Morrill WL, Murray TD, Smiley RW (2010) Compendium of Wheat Diseases and Pests: Third Edition. American Phytopathological Society, St. Paul, MN

Boyd LA, Ridout C, O'Sullivan DM, Leach JE, Leung H (2013) Plant-pathogen interactions: disease resistance in modern agriculture. Trends Genet 29:233-240

Brenchley R, Spannagl M, Pfeifer M, Barker G, D'Amore R, Allen A, McKenzie N, Kramer M, et al (2012) Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature 491:705-710

Brueggeman R, Rostoks N, Kudrna D, Killian A, Han F, Chen J, Druka A, Steffenson B, Kleinhofs A (2002). The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci USA 99:9328-9333

Cao W, Scoles GJ, Hucl P (1997) The genetics of rachis fragility and glume tenacity in semi-wild wheat. Euphytica 94:119-124

Carver B (2009) Wheat science and trade. Wiley-blackwell, Ames, Iowa
Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunoca A, et al (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proc Natl Acad Sci USA 110:8057-8062

Chakrabory S, Tiedemann AV, Teng PS (2000) Climate change: potential impact on plant disease. Environ Pollut 108:317-326

Chen QF, Yen C, Yang JL (1998) Chromosome location of the gene for brittle rachis in the Tibetan weedrace of common wheat. Genet Res Crop Evol 45:21-25

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124:803-814

Ciuffetti LM, Tuori RP, Gaventa JM (1997) A single gene encodes a selective toxin causal to the development of tan spot of wheat. Plant cell 9:135-144

Cloutier S, McCallum BD, Loutre C, Banks TW, Wicker T, et al (2007) Leaf rust resistance gene *Lr1*, isolated from bread wheat is a member of the large *psr567* gene family. Plant Mol Biol 65:93-106

Crook AD, Friesen TL, Liu ZH, Ojiambo PS, Cowger C (2012) Novel necrotrophic effectors from *Stagonospora nodorum* and corresponding host genes in winter wheat germplasm in the southeastern US. Phytopathology 102:498-505

Dangl JL, Jones JD (2000) Plant pathogens and integrated defence responses to infection. Nature 411:826-833

De Torres-Zabala M, Truman W. Bennett MH, Lafforgue G, Mansfield JW, Rodriguez Egea P, Bogre L, Grant M (2007) *Pseudomonas syringae* pv. tomato hijacks the Arabidopsis abscisic acid signaling pathway to cause disease. EMBO J 26:1434-1443
de Wit PJ (1995) *Cf9* and *Avr9*, two major players in the gene-for-gene game. Trends Microbiol 3:251-252

Dixon J, Braun HJ, Kosina PP, Crouch J (2009) Wheat facts and futures. CIMMMYT, Mexico

Dodds P N, Rathjen J P (2010) Plant immunity: towards an integrated view of plant-pathogen interactions. Nature Rev Genet 11:539-548

Dvorak J, Luo MC, Yang Zl, Zhang HB (1998) The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat. Theor Appl Genet 97:657-670

Dvorak J, Diterlizzi P, Zhang HB, Resta P (1993) The evolution of polyploidy wheat: identification of the A genome donor species. Genome 36:21-31

Dvorak J, Zhang HB (1990) Variation in repeated nucleotide sequences sheds light on the origin of the wheat B and G genomes. Proc Natl Acad Sci USA 87:9640-9644

Faris JD, Fellers JP, Brooks SA, Gill BS (2003) A bacterial artificial chromosome contig spanning the major domestication locus *Q* in wheat and identification of a candidate gene. Genetics 164:311-321

Faris JD, Friesen TL (2009) Reevaluation of a tetraploid wheat population indicates that the *Tsn1*-ToxA interaction is the only factor governing Stagonospora nodorum blotch susceptibility. Phytopathology 99:906-912

Faris JD, Zhang ZC, Lu HJ, Lu SW, Reddy L, Cloutier S, Fellers JP, Meinhardt SW, Rasmussen J B, Xu SS, Oliver RP, Simons KJ, Friesen TL (2010) A unique wheat disease resistance-like gene governs effector-induced susceptibility to necrotrophic pathogens. Proc Natl Acad Sci USA107:13544-13549

Faris JD, Zhang ZC, Chao S (2014) Map-based analysis of the tenacious glume gene *Tg*-B1 of wild emmer and its role in wheat domestication. Genetics 542:198-208

Faris JD. 2014. Genomics of plant genetic resources. Volume 1. Managing, sequencing and mining genetic resources. Tuberosa R, Graner A, Frison E (Eds). New York, NY: Springer

Feuillet C, Travella S, Stein N, Albar L, Nublat A and Keller B (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat genome. Proc Natl Acad Sci USA 100:15253-15258

Feulliet C, Travella S, Stein N, Albar L, Nublat A, Keller B (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat genome. Proc Natl Acad Sci USA 100:15253-15258

Feuillet C, Muehlbauer GJ (eds) (2009) Plant genetics and genomics: Genetics and genomics of the *Triticeae*. Springer, New York, NY

Flor HH (1971) Current status of gene-for-gene concept. Annu Rev Phytopathol 9:275-296

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

Friesen TL, Stukenbrock EH, Liu ZH, Meinhardt SW, Ling H, Faris JD, Rasmussen JB, Solomon PS, Mcdonald BA, Oliver RP (2006) Emergence of a new disease as a result of interspecific virulence gene tranfer. Nat Genet 38:953-956

Friesen TL, Meinhardt SW, Faris JD (2007) The *Stagonospora nodorum* – wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. Plant J 51:681-692

Friesen TL, Faris JD, Solomon PS, Oliver RP (2008a) Host-specific toxins: Effectors of necrotrophic pathogenicity. Cell Microbiol 10:1421-1428

Friesen TL, Zhang ZC, Solomon PS, Oliver RP, Faris JD (2008b) Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. Plant Physiol 146:682-693

Friesen TL, Chu CG, Liu ZH, Halley S, Faris JD (2009) Host-selective toxins produced by *Stagonospora nodorum* confer disease susceptibility in adult wheat plants under field conditions. Theor Appl Genet 118:1489-1497

Friesen TL, Faris JD (2010) Characterization of the wheat-*Stagonospora nodorum* disease system: what's the molecular basis of this quantitative necrotrophic disease interaction? Can J Plant Pathol 32:20-28

Friesen T, Chu C, Xu SS, Faris J (2012) SnTox5-*Snn5*: a novel *Stagonospora nodorum* effector-wheat gene interaction and its relationship with the SnToxA-*Tsn1* and SnTox3-*Snn3*-B1 interactions. Mol Plant Pathol 13:1101-1109

Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen X, Sela H, Fahima T, Dubcovsky J (2009) A kinase-*START* gene confers temperature-dependent resistance to wheat strip rust. Science 323:1357-1360

Gao Y, Faris JD, Liu ZH, Xu SS, Friesen TL (2014) Identification and characterization of the SnTox6-*Snn6* interaction in the wheat-Stagonospora nodorum pathosystem. Mol Plant-Microbe Interact (accepted)

Giles RG, Brown TA (2006) *GluDy* allele variations in *Aegilops tauchii* and *Triticum aestivum*: implications for the origins of hexaploid wheats. Theor Appl Genet 112:1563-1572

Gill BS, Li W, Sood S, Kuraparthy V, Friebe Simons KJ, Zhang Z, Faris JD (2007) Genetics and genomics of wheat domestication-driven evolution. Isr J Plant Sci 55:223-229

Gohre V, Robatzek S (2008) Breaking the barriers: microbial effector molecules subvert plant immunity. Annu Rev Phytopathol 46:189-215

Gomez-Gomez L, Boller T (2000) *FLS2*: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Mol Cell 5:1003-1011

Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, Reader S, Colas I, Moore G (2006) Molecular characterization of Ph1 as a major chromosome pairing locus in polyploid wheat. Nature 439:749-752

Gust AA, Biswas R, Lenz HD, Rauhut T, Ranf S, Kemmerling B, Gotz F, Glwischnig E, Lee J, Felix G (2007). Bacteria-derived peptidoglycans constitute pathogen-associated molecular patterns triggering innate immunity in Arabidopsis. J Biol Chem 282:32338-32348

Heun M, Schafer-Pregl R, Klawan D, Castagna R, Accerbi M, Borghi B, Salamini F (1997) Site of Einkorn wheat domestication identified by DNA fingerprinting. Science 278:1312-1314 DOI: 10.1126/science.278.5341.1312

Hillman GC (1978) On the origins of domestic rye- Secale cereal: the finds from Aceramic Can Hasan III in Turkey. Anatolian Stud 28:157-174

Huang L, Brooks SA, Li W, Fellers JP, Trick HN and Gill BS (2003) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics 164:655-664

Huang S, Sirikhachornkit A, Su X, Faris J, Gill B, Haselkorn R, Gornicki P (2002) Genes endoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the *Triticum*/*Aegilops* complex and the evolutionary history of polyploid wheat. Proc Natl Acad Sci USA 99:8133-8138

The International Wheat Genome Sequencing Consortium (IWGSC) (2014) A chromsome-based draft sequence of the hexaploid bread wheat genome. Science DOI: 10.1126/science.1251788

Jaaska V (1980) Electrophoretic survey of seedling esterases in wheats in relation to their phylogeny. Thero Appl Genet 56:273-284

Jia J, Zhao S, Kong X, Li Y, Zhao G, He W, Appels R, Pfeifer M, Tao Y, Zhang X, et al (2013) *Aegilops tauschii* draft genome sequence reveals a gene repertoire for wheat adaptation. Nature 496:91-95

Johnson BL, Dhaliwal HS (1976) Reproductive isolation of *Triticum boeoticum* and *Triticum uratum* and the origin of the tetraploid wheats. Am J Bot 63:1088-1094

Johnson BL (1968) Electrophoretic evidence on the origin of *Triticum zhukovskyi*. In: Finlay KW, Shepherd KW (eds) Proceedings of the Third International Wheat Genetics Symposium. Canberra, Australia 105-110

Jones DA, Takemoto D (2004) Plant innate immunity-direct and indirect recognition of general and specific pathoen-associated molecules. Curr Opin Immunol 16:48-62
Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323-329

Joosten MH, Cozijnsen TJ, de Wit PJ (1994) Host resistance to a fungal tomato pathogen lost by a single base-pair change in an avirulence gene. Nature 367:384-386

Katagiri F (2004) A global view of defense gene expression regulation- a highly interconnected signaling network. Curr Opin Plant Biol 7:506-511

Kellogg EA (2001) Evolutionary history of the grasses. Plant Physiol 125:1198-1205

Kerber ER, Dyck PL (1969) Inheritance in hexaploid wheat of leaf rust resistance and other characters derived from *Aegilops squarossa*. Can J Genet Cytol 11:639-647

Kerber ER, Rowland GG (1974) Origin of the free threshing character in hexaploid wheat. Can J Genet Cytol 16:145-154

Kihara H (1944) Discovery of the DD-analyzer, one of the ancestors *Triticum vulgare*. Agr Horticulture 19:13-14

Kilian B, Ozkan H, Deusch O et al (2007) Independent wheat B and G genome origins in outcrossing *Aegilops* progenitor haplotypes. Mol Biol Evol 24:217-227

Kimber G, Feldman M (1987) Wild wheats, an introduction. P. 1-142. Special report 353. College of agricultural, University of Missouri, Columbia, MO

Kislev ME (1984) Emergence of wheat agriculture. Paleorient 10:61-70

Konarev VG, Gavrilyuk IP, Gubareva NK, Peneva TI (1979) About nature and origin of wheat genomes on the data of biochemistry and immunochemistry of grain proteins. Cereal Chem 56:272-278

Konishi S, Izawa T, Lin Sy, Ebana K, Fukuta Y, Sasaki T, Yano M (2006) An SNP caused loss of seed shattering during rice domestication. Science 312:1392-1396

Krattinger S, Wicker T, Keller B (2009a) Map-based cloning of genes in *Triticeae* (wheat and barley). Plant genetics and genomics: Genetics and genomics of the *Triticeae*. Feuillet C, Muehbauer GJ (Eds) New York, NY Springer

Krattinger S, Lagudah E, SPielmeyer W, Singh R, Huerta-Espino J, McFadden H, Bossolini E, Selter L, Keller B (2009b) A putative *ABC* transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360-1363

Lev-Yadun S, Gopher A, Abbo S (2000) The cradle of agriculture. Science 288:1602-1603

Lilienfeld F, Kihara H (1934) Genomanalyse bei *Triticum* and *Aegilops* :V. *Triticum timopheevii* Zhuk. Cytologia 6:87-122

Li W, Gill BS (2006) Multiple genetic pathways for seed shattering in the grasses. Funct Integrative Genomics 6:300-309

Ling H, Zhao S, Liu D, Wang J, Sun H, Zhang C, Fan H, Li D, Dong L, Tao Y, et al (2013) Draft genome of the wheat A-genome progenitor *Triticum urartu*. Nature 496:87-90

Liu S, Sehgal S, Li J, Lin M, Trick H, Yu J, Gill B, Bai G (2013). Cloning and characterization of a critical regulator for preharvest sprouting in wheat. Genetics doi: 10.1534/genetics.113.152330

Liu ZH, Faris JD, Meinhardt SW, Ali S, Rasmussen JB, Friesen TL (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

Liu ZH, Friesen TL, Meinhardt SW, Ali S, Rasmussen JB, Faris JD (2004b) Quantitative trait loci analysis and mapping of seedling resistance to Stagonospora nodorum leaf blotch in wheat. Phytopathology 94:1061-1067

Liu ZH, Friesen TL, Ling H, Meinhardt SW, Oliver RP, Rasmussen JB, Faris JD (2006) The *Tsn1*-ToxA interaction in the wheat-*Stagonospora nodorum* pathosystem parallels that of the wheat-tan spot system. Genome 49:1265-1273

Liu ZH, Faris JD, Oliver RP, Tan KC, Solomon PS, McDonald MC, McDonald BA, Nunez A, Lu S, Rasmussen JB, Friesen TL (2009) SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the *Snn3* gene. PLoS Pathogens 5:e1000581. doi:10.1371/journal.ppat.1000581

Liu ZH, Zhang ZC, Faris JD, Oliver RP, Syme R, McDonald MC, McDonald BA, Solomon PS, Lu SW, Shelver WL, Xu SS, Friesen TL (2012) The cysteine rich necrotrophic effector SnTox1 produced by *Stagnospora nodorum* triggers susceptibility of wheat lines harboring *Snn1*. PLoS Pathogens 8:e1002467 Doi:10.1371/journal.ppat.1002467

Lorang JM, Sweat TA, Wolpert TJ (2007) Plant disease susceptibility conferred by a resistance gene. Proc Natl Acad Sci USA 104:14861-14866

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

Lu HJ, Faris JD (2006) Macro- and microcolinearity between the genomic region of wheat chromosome 5B containing the *Tsn1* gene and the rice genome. Funct Integrative Genomics 6:90–103.

Lu X, Tintoe N, Mentzel T, Kombrink E, Boller T, Robatzek S, Schulze-Lefert P, Saijo Y (2009). Uncoupling of sustained MAMP receptor signaling from early outputs in an Arabidopsis *ER* glucosidase II allele. Proc Natl Acad Sci USA 106:22522-22527

Manning VA, Andrie RM, Trippe AF, Ciuffetti LM (2004). Ptr ToxA requires multiple motifs for complete activity. Mol Plant Microbe Interact 17:491-501

Manning VA, Hamilton SM, Ciuffetti LM (2007) PtrToxA interacts with a chloroplast-localized protein. Mol Plant Microbe Interact 20:168-177

Manning VA, Hamilton SM, Karplus PA, Ciuffetti LM (2008) The Arg-Gly-Asp-containing, solvent-exposed loop of Ptr ToxA is required for internalization. Mol Plant Microbe Interact 21:315-325

Manning WJ, Tiedemann AV (1995) Climate change: potential effects of increased atmospheric carbon dioxide, ozone, and ultraviolet-B radiation on plant diseases. Environ Pollut 88:219-245

Marcussen T, Sandve SR, Heier L, Spannagl M, Pfeifer M, IWGSC, Jakobesn KS, Wulff BBH, Steuernagel B, Mayer KFX, Olsen OA (2014) Ancient hybridizations among the ancestral genomes of bread wheat. Science 345:1250092-1-4

Matsuoka Y, Nasuda S (2004) Durum wheat as a candidate for the unknown female progenitor of bread wheat: an empirical study with a highly fertile F_1 hybrid with *Aegilops tauchii* Coss. Theor Appl Genet 109:1710-1717

McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered 37:81-89, 107-116

McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers J, Morris C, Somers DJ, Appels R, Devos KM (2012). Catalogue of gene symbols for wheat

McMullen M, Adhikari T (2009) Fungal leaf spot diseases of wheat: Tan spot, Stagonospora nodorum blotch and Septoria tritici blotch. Plant Disea Manag. NDSU extension service

Meyer RS, Purugganan MD (2013) Evolution of crop species: genetics of domestication and diversification. Nature Rev Genet 14:840-852

Miya A, Albert P, Shinya T, Desaki Y, Ichimura K, et al (2007) *CERK1*, a *LysM* receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. Proc Natl Acad Sci USA 104:19613-19618

Nagy ED, Bennetzen JL (2008) Pathogen corruption and site-directed recombination at a plant disease gene cluster. Genome Res 18:1918-1923

NaitoK, Taguchi F, Suzuki T Inagaki Y, Toyoda K, Shiraishi T (2008) Amino acid sequence of bacterial microbe-associated molecular pattern *flg22* is required for virulence. Mol Plant Microbe Interact 21:1165-1174

Nalam V, Vales MI, Watson CJW, Kianian SF, Riera-Lizarazu O (2006) Map based analysis of genes affecting the brittle rachis character in tetraploid wheat. Theor Appl Genet 112:373-381

Nalam V, Vales MI, Watson CJW, Johnson EB, Riera-Lizarazu O (2007) Map based analysis of gentic loci on chromosome 2D that affect glume tenacity and threshability, components of the free-threshing habit in common wheat. Theor Appl Genet 116:135-145

Navarro L, Zipfel C, Rowland O, Keller I, Robatzek S, Boller T, Jones JD (2004) The transcriptional innate immune response to *flg22*, interplay and overlap with *Avr* gene-dependent defense responses and bacterial pathogenesis. Plant Physiol 135:1113-1128

Navarro L, Jay F, Nomura K, He SY, Voinnet O (2008) Suppression of the microRNA pathway by bacterial effector proteins. Science 321:964-967

Nevo E (1998) Genetic diversity in wild cereals: regional and local studies and their bearing on conservation ex-situ and in-situ. Genet Crop Evol 45:355-370

Nishikawa K, Mizuno S, Furuta Y (1994) Identification of chromosomes involved in translocations in wild emmer. Jpn J Genet 69:371-376

Oliver RP, Friesen TL, Faris JD, Solomon PS (2012) *Stagnospora nodorum*: from pathology to genomics and host resistance. Annu Rev Phytopathol 50:2.1-2.21

Pandelova I, Betts MF, Manning VA, Wilhelm LJ, Mockler TC, Ciuffetti LM (2009) Analysis of transcriptome changes induced by PtrToxA in wheat provides insights into the mechanisms of plant susceptibility. Mol Plant 2:1067-1083

Peleg Z, Fahima T, Korol AB et al (2011) Genetic analysis of wheat domestication and evolution under domestication. J Exp Bot 62:5051-5061

Peng JH, Ronnin Y, Fahima T, Roder MS, Li YC, Nevo E, Korol A (2003) Domestication quantitative trait loci in *Triticum dicoccoides*, the progenitor of wheat. Proc Natl Acad Sci USA 100:2489-2494

Peng JH, Lapitan NLV (2005) Characterizaion of EST-derived microsatellites in the wheat genome and development of eSSR markers. Funct Integrative Genomics 5:80-96

Peng JH, Sun DF, Nevo E (2011) Domestication evolution, genetics and genomics. Mol Breeding 28:281-301

Periyannan S, Moore J, Ayliffe M, Bansal U, Wang X, Huang L, Deal K, Luo M, Kong XY, Bariana H, Mago R, McIntosh R, Dodds P, Dvorak J, Lagudah E (2013) The gene *Sr33*, an ortholog of barley *Mla* genes, encodes resistance to wheat stem rust race Ug99. Science DOI: 10.1126/science.1239028

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Gill BS (2004) A chromosome bin map of 16000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. Genetics 168:701-712

Rasmussen JB, Kwon CY, Meinhardt SW (2004) Requirement of host signaling mechanisms for the action of PtrToxA in wheat. Eur J Plant Pathol 110:333-335

Raats D, Frenkel Z, Krugman T, Dodek I, Sela H, Sinkov H, Magni F, Cattonaro F, Vautrin S,Berges H, Wicker T, Keller B, Leroy P, Philipe R, Paux E, Dolezel J, Feuillet C, Korol A, Fahima T (2013) The physical map of wheat chromosome 1BS provides insights into its gene space organization and evolution. Genome Biol 14:R138

Rafiqi M, Ellis JG, Ludowici VA, Hardham AR, Dodds PN (2012) Challenges and progress towards understanding the role of effectors in plant-fungal interactions. Curr Opin Plant Biol 15:477-482

Reddy L, Friesen TL, Meinhardt SW, Chao S, Faris JD (2008) Genomic analysis of the *Snn1* locus on wheat chromosome arm 1BS and the identification of candidate genes. Plant Genome 1:55-66

Riehl S, Zeidi M, Conard NJ (2013) Emergence of agriculture in the foothills of the Zagros Mountains of Iran. Science 341:65-67

Rosebrock TR, Zeng L, Brady JJ, Abramovitch RB, Xiao F, Martin GB (2007) A bacterial E3 ubiquitin ligase targets a host protein kinase to disrupt pant immunity. Nature 448:370-374

Saintenac C, Zhang W, Salcedo A, Rouse M, Trick H, Akhunov E, Dubcovsky J (2013) Identification of wheat gene *Sr35* that confers resistance to Ug99 stem rust race group. Science 341:783-786

Salamini F, Ozkan H, Brandolini A, Schafer-Pregl R, Martin W (2002) Genetics and geography of wild cereal domestication in the near east. Nat Rev Genet 3:429-441

Salse J, Chague V, Bolot S, Magdelenat G, Huneau C, Pont C, Belcram H, Couloux A, Gardais S, Evrard A, Segurens Bm Charles M, Ravel C, Samain S, Charmet G, Boudet N, Chalhoub B (2008) New insights into the origins of the B genome of hexaploid wheat: evolutionary relationships at the *SPA* genomic region with the S genome of the diploid relative *Aegilops speltoides*. BMC Genomics 9:555

Sax K (1922) Sterility in wheat hybrids: II. Chromosome behavior in partially sterile hybrids. Genetics 7:513-552

Scofield SR, Huang L, Brandt AS and Gill BS (2005) Development of a virus-induced gene-silencing system for hexaploid wheat and its use in functional analysis of the *Lr21*-mediated leaf rust resistance pathway. Plant Physiol 138:2165-2173

Scheffer RP, Livingston RS (1984) Host-selective toxins and their role in plant diseases. Science 223:17-21

Schulze-Lefert P, Panstruga R (2011) A molecular evolutionary concept connecting non-host resistance, pathogen host range, and pathogen speciation. Trends Plant Sci 16:117-125

Simonetti MC, Bellomo MP, Laghetti G et al (1999) Quantitative trait loci influencing free-threshing habit in tetraploid wheats. Genet Res Crop Evol 46:267-271

Simons K, Fellers JP, Trick HN, Zhang ZC, Tai YS, Gill BS, Faris JD (2006) Molecular characterization of the major wheat domestication gene *Q*. Genetics 172:547-555

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite markers in wheat. Thero Appl Genet 10.1007/s00122-004-1871-x

Sood S, Kuraparthy V, Bai GH, Gill BS (2009) The major threshability genes soft glume (*sog*) and tenacious glume (*tg*), of diploid and polyploid wheat, trace their origin to independent mutations at non-orthologous loci. Thero Appl Genet 119:341-351

Tameling WI, Elzinga SD, Darmin PS, Vossen JH, Takken FL, Haring MA, Cornelissen BJ (2002) The tomato *R* gene products I-2 and MI-1 are functional ATP binding proteins with ATPase activity. Plant cell 14:2929-2939

Taenzler B, Esposti RF, Vaccino P et al (2002) Molecular linkage map of einkorn wheat: mapping of srorage-protein and soft-glume genes and bread-making quality QTLs. Genet Res Camb 80:131-143

Tai YS, Bragg J, Meinhardt SW (2007) Functional characterization of ToxA and molecular identification of its intracellular targeting protein in wheat. Am J Plant Physiol 2:76-89

Tao Y, Xie Z, Chen W, Glazebrook J, Chang HS, Han B, Zhu T, Zou G, Katagiri F (2003) Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen *Psedomonas syringae*. Plant cell 15:317-330

Torres MA, Jones JD, Dangl JL (2006) Reactive oxygen species signaling in response to pathogens. Plant Physiol 141:373-378

Tsuda K, Sato M, Glazebrook J, Cohen JD, Katagiri F (2008) Interplay between MAMP-triggered and SA-mediated defense response. Plant J 53:763-775

Tsuda K, Sato M, Stoddard T, Glazebrook J, Katagiri F(2009) Network properties of robust immunity in plants. PLoS Genetics 5:e1000772

Tsuda K, Katagiri F(2010) Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity. Curr Opin Plant Biol 13:459-465

Uauy C, Distelfeld A, Fahima T, Blechl A and Dubcovsky J (2006) A *NAC* gene regulating senescence improving grain protein, zinc and iron content in wheat. Science 314:1298-1301

Underwood W, Zhang S, He SY (2007) The *Pseudomonas syringae* type III effector tyrosine phosphatase HopAO1 suppresses innate immunity in Arabidopsis thaliana. Plant J 52:658-672

USDA ERS (2014a) Wheat outlook: U.S. export, Census and export sales comparison. http://www.ers.usda.gov/publications/whs-wheat-outlook/whs-14i.aspx#.VCGJ593-E1A. Date accessed: 09/23/2014

USDA ERS (2014b) Wheat background. http://www.ers.usda.gov/topics/crops/wheat/background.aspx#.U9up4d3-E1A). Date accessed: 09/20/2014

Van Esse HP, Van't klooster JW, Bolton MD, Yadeta KA, van Baarlen PV, Boeren S, Veroort J, de Wit P, Thomma B (2008) The *Cladosporium fulvum* virulence protein Avr2 inhibits host proteases required for basal defense. Plant cell 20:1948-1963

Wang G, Miyashita NT, Tsunewaki KT (1997) Plasmon analyses of *Triticum* and *Aegilops*: PCR-single-strand conformational polymorphism (PCR-SSCP) analyses of organeller DNAs. Proc Natl Acad Sci USA 94:14570-14577

Wang SC, Wong D, Forrest K, Allen A, Chao S, Huang B, Maccaferri M, et al (2014) Characterization of polyploid wheat genomic diversity using a high-density 90000 single nucleotide polymorphism array. Plant Biotechnol J12:787-796

Watanabe N, Ikebata N (2000) The effects of homoeologous group 3 chromosomes on grain color dependent seed dormancy and brittle rachis in tetraploid wheat. Euphytica 115:215-220

Webb CA., Richter TE, Collins NC, Nicolas M, Trick HN, Pryor T, Hulbert SH (2002) Genetic and molecular characterization of the maize *rp3* rust resistance locus. Genetics 162:381-394

Wolpert TJ, Dunkle LD, Ciuffetti LM (2002) Host-selective toxins and avirulence determinants: what's in a name? Annu Rev Phytopathology 40:251-285

Xie W, Nevo E (2008) Wild emmer: genetic resources, gene mapping and potential for wheat improvement. Euphytica 164:603-614

Yahiaoui N, Srichumpa P, Dudler R and Keller B (2004) Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3* from hexaploid wheat. Plant J 37:528-538

Yahiaoui N, Brunner S and Keller B (2006) Rapid generation of new powdery mildew resistance genes after wheat domestication. Plant J 47:85-98

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T and Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *Vrn1*. Proc Natl Acad Sci USA 100:6263-6268

Yan L, Lousoianov A, Blechl A, Tranquilli G, Ramakrishna W, et a(2004) The wheat *Vrn2* gene is a flowering repressor down-regulated by vernalization. Science 303:1640-1644

Yan L, Fu D, Li C, Blechl A, Tranquili G, Bonafede M, Sanchez A, Valarik M, Yasuda S, Dubcovsky J (2006) The wheat and barley vernalization gene *Vrn3* is an ortholog of *FT*. Proc Natl Acad Sci USA 103:19581-19586

Zhang J, Shao F, Li Y, Cui H, Chen L, Li H, Zou Y, Long C, Lan L (2007) A *Pseudomonas syringae* effector inactivates MAPKs to suppress PAMP-induced immunity in plants. Cell Host Microbe 1:175-185

Zhang P, Friebe B, Gill BS (2002) Variation in the distribution of a genome-specific DNA sequence on chromosomes reveals evolutionary relationships in the *Triticum* and *Aegilops* complex. Plant Syst Evol 235:169-179

Zhang ZC, Friesen TL, Simons KJ, Xu SS, Faris JD (2009) Identification, development and validation of markers for marker-assisted selection against the *Stagonospora nodorum* toxin sensitivity genes *Tsn1* and *Snn2* in wheat. Mol Breeding 23:35-49

Zhang Z, Friesen TL, Xu SS, Shi GJ, Liu Z, Rasmussen JB, Faris JD (2011) Two putatively homoeologous wheat genes mediate recognition of SnTox3 to confer effector-triggered susceptibility to *Stagonospora nodorum*. Plant J 65:27-38

Zhang ZC, Belcram H, Gornicki P, Charles M, Just J, Huneau C, Faris JD, Chahoub B (2011) Duplication and partitioning in evolution and function of homoeologous *Q* loci governing domestication characters in polyploid wheat. Proc Natl Acad Sci USA 108:18737-18742

Zhu W., Yang B, Chittoor JM, Johnson LB, White FF (1998) *AvrXa10* contains an acidic transcriptional activation domain in the functionally conserved C terminus. Mol Plant Microbe Interact 11:824-832

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, Felix G (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts agrobacterium-mediated transformation. Cell 125:749-760

Zipfel C (2008) Pattern-recognition receptors in plant innate immunity. Curr Opin Immunol 20:10-16

Zipfel C (2009) Early molecular events in PAMP-triggered immunity. Curr Opin Plant Biol 12:414-420

CHAPTER III. IDENTIFICATION AND GENETIC ANALYSIS OF THE *SNN7*-SNTOX7 INTERACTION IN THE WHEAT-*PARASTAGONOSPORA NODORUM* PATHOSYSTEM

Abstract

Parastagonospora nodorum is a necrotrophic fungal pathogen that causes the disease Stagonospora nodorum blotch (SNB) on wheat. The fungus produces necrotrophic effectors (NEs), that when recognized by corresponding host genes, cause cell death, which ultimately leads to disease. To date, eight host gene-NE interactions have been described in the wheat-*P. nodorum* system. Here, I report the identification and partial characterization of a ninth interaction involving a *P. nodorum*-produced NE designated SnTox7 and a wheat gene designated *Snn7*. SnTox7 is small protein with estimated size less than 30 kDa and largely resistant to heat and chemical treatment. The *Snn7* gene governs sensitivity to SnTox7 and was delineated to a 2.7 cM interval on the long arm of wheat chromosome 2D. The *Snn7*-SnTox7 interaction explained 33% of the variation in disease among 406 segregating F_2 plants indicating the interaction plays a prominent role in the development of SNB. The *Snn7* sensitivity allele was identified in the hexaploid wheat cultivar Timstein, but evaluation of a set of 52 hexaploid wheat lines of diverse origin indicated that few genotypes harbored a functional *Snn7* allele, thus indicating that *Snn7* is relatively rare. The identification of the *Snn7*-SnTox7 interaction adds to our knowledge of the wheat-*P. nodorum* pathosystem, which is becoming a model for necrotrophic specialist fungal pathogens and their interactions with plants leading to necrotrophic effector-triggered susceptibility (NETS).

Introduction

The fungus *Parastagonospora* (syn. *ana, Stagonospora; teleo, Phaeosphaeria*) *nodorum* (Berk.) Quaedvleig, Verkley & Crous is an important foliar and glume pathogen of wheat (*Triticum aestivum* ssp. *aestivum* L., 2*n* = 6*x* = 42, AABBDD genomes) and other cereals. The disease, known as Stagonospora nodorum blotch (SNB) can cause yield losses up to 50% and significantly reduce quality. The inheritance of SNB resistance is usually quantitative and additive effects are observed in many cases (Xu et al. 2004; Zhang et al. 2009; Friesen et al. 2012).

P. nodorum is a necrotrophic pathogen, which by definition lives and feeds on dead or dying tissue as opposed to biotrophs, which require living tissue to gain nutrients and sporulate. Plants have evolved sophisticated immune systems to combat biotrophic pathogens. One layer of immunity involves the detection of pathogen-produced effectors by cognate host resistance genes, which are usually members of the nucleotide binding (NB) leucine-rich repeat (LRR) class of genes. Direct or indirect recognition of an effector by the corresponding host resistance gene in a gene-for-gene manner (Flor 1956) leads to the activation of a defense response including an oxidative burst, up-regulation of pathogenesis-related (PR) genes, and localized programmed cell death (PCD) or hypersensitive response (HR), which restricts biotrophic growth and proliferation ultimately resulting in a resistant outcome known as effector-triggered immunity (ETI).

Whereas ETI has been well studied in plant-biotroph interactions, less work has been done to discover the mechanisms involved in plant interactions with nectrophic specialist pathogens. However, dissection of wheat-*P. nodorum* interactions over the past decade has revealed the system involves numerous pathogen-produced necrotrophic

effectors (NEs) (Formerly called host-selective toxins) and cognate dominant host genes (Friesen and Faris 2010 for review). Contrary to the classical gene-for-gene theory (Flor 1956), this system follows an inverse gene-for-gene model. When a specific NE is recognized by a corresponding host gene, a compatible interaction occurs, which results in necrtrophic effector-triggered susceptibility (NETS). The absence of either the NE or its corresponding host gene will result in an incompatible reaction and lead to resistance.

To date, eight interactions between NEs and their cognate host genes have been identified from the wheat-*P. nodorum* pathosystem, and they include *Tsn1*-SnToxA (Liu et al. 2006; Zhang et al. 2009; Faris and Friesen, 2009; Friesen et al. 2006; Friesen et al. 2009; Faris et al. 2010), *Snn1*-SnTox1 (Liu et al. 2004a; Liu et al. 2004b; Liu et al. 2012; Reddy et al. 2008; CHAPTER V), *Snn2*-SnTox2 (Friesen et al. 2007; Friesen et al. 2009; Zhang et al. 2009), *Snn3-B1*-SnTox3 (Friesen et al. 2008; Liu et al. 2009; CHAPTER IV), *Snn3-D1*-SnTox3 (Zhang et al. 2011), *Snn4*-SnTox4 (Abeysekara et al. 2009), *Snn5*-SnTox5 (Friesen et al. 2012), and *Snn6*-SnTox6 (Gao et al. 2014). In each case, the NEs were shown to be small secreted proteins ranging in size from 10 to 30 kDa. The interactions have all been shown to play substantial roles in disease development.

To date three NE-encoding genes, *SnToxA*, *SnTox1* and *SnTox3*, have been cloned from *P. nodorum* (Friesen et al. 2006; Liu et al. 2009, 2012). Manning and Ciuffetti (2005) showed that the ToxA protein was internalized in the plant cell but only in sensitive wheat genotypes. Once internalized, ToxA was localized to cytoplasmic compartments and the chloroplast. ToxA has been shown to directly interact with plastocyanin (Tai et al. 2007), an important component of electron transport in photosystem II, and another chloroplast localized protein known as ToxABP1 (Manning

et al. 2007). More recently, ToxA was shown to interact directly with a *PR-1* gene (Lu et al. 2014). Work by Liu et al. (2012) showed that SnTox1 does not enter the cell, but likely plays a role in fungal penetration. Both *Snn1*-SnTox1 and *Tsn1*-ToxA interactions have been shown to induce hallmarks of defense response including an oxidative burst, DNA laddering, and PR gene expression ultimately leading to PCD (Adhikari et al. 2009; Pandelova et al. 2009; Liu et al. 2012; Winterberg et al. 2014).

To date, two wheat genes governing NE sensitivity have been cloned (Faris et al. 2010, Chapter V). The cloning of the *Tsn1* gene revealed that it harbors features typically observed in 'classic' plant disease resistance genes including protein kinase, NB and LRR domains. However, a rather unique characteristic of *Tsn1* is that all three major domains are transcribed in the same open reading frame. The resulting protein harbors the protein kinase at the N-terminus, the NB in the middle, and the LRR at the C-terminus. The cloning of *Snn1* revealed that it is a member of the wall-associated kinase class of receptor kinases, which are known to act as pattern recognition receptors (PRRs) in the recognition of damage-associated molecular patterns (DAMPs) leading to the activation of PTI (CHAPTER V). The fact that *Tsn1* and *Snn1* resemble a disease resistance gene and a PRR, respectively, and that compatible interactions in both cases lead to the induction of defense responses including MAPK gene expression, *PR* gene up-regulation, an oxidative burst, and PCD (Adhikari et al. 2009; Pandelova et al. 2009; Liu et al. 2012; Lu et al. 2014) reveals that NE-producing necrotrophic specialist fungal pathogens such as *P. nodorum* hijack the plant's own defense mechanisms to gain nutrients and sporulate (Faris et al. 2010; Liu et al. 2012).

The wheat-*P. nodorum* pathosystem has become a model system to investigate interactions between plants and necrotrophic fungal pathogens. Efforts to discover and characterize additional NEs and their cognate host genes are ongoing to further characterize this system. Here, I describe the identification of the ninth interaction between a wheat gene and a *P. nodorum*-produced NE, the partial characterization of the NE, and the genetic analysis and mapping of the wheat gene.

Materials and methods

Plant materials

The complete set of Chinese Spring (CS)-Timstein (CS-Tm) disomic chromosome substitution lines was obtained from the Wheat Genetics Resource Center, Kansas State University, Manhattan, KS and screened for reaction to culture filtrates of the *P. nodorum* isolate Sn6 (Friesen et al. 2007). Chinese Spring is a hexaploid common wheat landrace and Timstein is a hard red spring wheat cultivar released by the University of Minnesota, St. Paul, MN, USA, in 1939. The disomic chromosome substitution lines consist of individual pairs of Timstein chromosomes substituted for homologous pairs of CS chromosomes in the CS background. The set of substitution lines was screened to determine if Timstein harbored any NE sensitivity genes to Sn6 cultures other than the *Snn3-B1* gene as described in Zhang et al. (2011).

An F_2 population, designated as the CT population, was developed by crossing CS with CS-Tm 2D (substitution of CS chromosome 2D by Timstein chromosome 2D), and 406 F_2 individuals were used for disease evaluation, culture filtrate infiltration, and to quantify the role of the wheat gene-NE interaction in causing disease. A total of 87 F_2

plants that were insensitive to the culture filtrates were used to develop the genetic linkage map of chromosome 2D.

The wheat lines BG220, BG223, BG261, which serve as differentials for SnTox3, SnTox2 and SnToxA reactions, respectively (Friesen et al. 2006, 2007, 2008), were included in all culture filtrate infiltration experiments. One F_2 plant, designated as line CTm208, was chosen as a differential line for the initial characterization of the new NE. A set of 52 diverse hexaploid wheat lines was infiltrated with culture filtrates of *P. nodorum* isolate Sn6 and also with SnTox3 cultures in an attempt to evaluate the frequency of sensitivity to the cultures (Table 3.1).

Disease evaluations and NE bioassays

The *P. nodorum* isolate Sn6 (Friesen et al. 2007) was used for the production of NE-containing cultures and inoculum for disease evaluations. Culture filtrates were produced and assayed as described in Friesen and Faris (2012). For infiltrations, plants were grown in the greenhouse at a temperature of 20-25°C and a 16-hr photoperiod. The fully expanded secondary leaf of the parents and F_2 lines was infiltrated with approximately 25 μl of crude culture filtrate using a 1-ml syringe with the needle removed. Reactions were evaluated three days after infiltration and scored based on the presence or absence of necrosis.

The 52 wheat lines evaluated with Sn6 culture filtrates were also infiltrated with SnTox3. SnTox3-containing cultures were obtained by heterologous expression of the *SnTox3* gene in *Pichia pastoris* as described in Liu et al. (2009). Plants were infiltrated and reactions scored as for Sn6 culture filtrates.

Table 3.1. Evaluation of common wheat varieties to SnTox7-containing culture of Sn6.

Wheat line	Accession	Isolate Sn6[a]	SnTox3[b]
6B365	NA	-	-
Alsen	NA	-	-
Amery	NA	+	+
Arina	NA	-	-
Atlas 66	CItr 12561	-	-
Bobwhite	PI 520554	-	-
Boston	NA	+	+
BR34	NA	-	-
Briggs	PI 632970	+	+
Bulk 84-4-12	PI 519204	-	-
Cheyenne	PI 192268	-	-
Chinese Spring	CItr 14108	-	-
Dapps	PI 633862	+	+
Erik	PI 476849	-	-
Fielder	CItr 17268	-	-
Forno	NA	-	-
Genial	PI 573751	-	-
Glenn	PI 639273	-	-
Grandin	PI 531005	+	+
Granger	PI 636134	-	-
Hanna	NA	+	-
Hope	CItr 8178	+	+
Katepwa	NA	+	+
Kulm	PI 590576	-	-
Maris Huntsman	PI 404008	-	-
Mironovskaja 808	PI 410430	-	-
ND495	NA	+	+
Newton	CItr 17715	-	-
Norstar	CItr 17735	+	+
Novo	NA	-	-
Oklee	PI 634553	+	+
Opata 85	PI 591776	+	+
Parshall	PI 613587	+	+
Pitoma	NA	-	-
Red Egyptian	PI 45403	-	-
Renan	PI 564569	-	-
Rescue	CItr 12435	+	+
Roazon	PI 422330	-	-
Rurik	NA	-	-
Salamouni	PI 182673	-	-
Selkirk	CItr 13100	+	+
Siu Mak	NA	+	-
Skater	NA	-	-
Steele-ND	PI 634981	-	-
Sumai3	PI 481542	+	+
TAM105	CItr 17826	-	-
Thatcher	N/A	+	+
Timstein	PI 168688	+	+
T-spelta(P78-81-1)	N/A	-	-
UC1041GPC	NA	-	-
VPM 1	NA	-	-
WA7690	PI 597665	-	-

[a]'+' and '-' symbols indicate sensitivity and insensitivity, respectively to culture filtrates of Sn6; [b]'+' and '-' symbols indicate sensitivity and insensitivity to SnTox3.

For spore inoculations and disease analysis, plants were grown in cones and placed in racks of 98 (Stuewe and Sons) with the outside border of each rack planted with the SNB susceptible wheat variety ‘Grandin’ to reduce any edge effect. Conidia were produced and harvested as described in Liu et al. (2004b). Plants were inoculated at the 2-3 leaf stage until runoff using conidial suspensions containing 1×10^6 spores/ml. Plants were then placed in a mist chamber at 100% relative humidity at 21°C for 24 hr followed by 6 days of incubation in the growth chamber under a 12-hr photoperiod. The second leaf of inoculated plants was scored 7 days post-inoculation using a 0-5 lesion type scale (Liu et al. 2004b), where ‘0’ is highly resistant and ‘5’ is highly susceptible.

Characterization of the NE

All treated cultures and controls described below to characterize the NE were infiltrated into leaves of differential line CTm208 and evaluated in three replications. All plants were grown in a growth chamber at 21 °C, and the reactions were evaluated three days after infiltration.

Pronase (EMD biosciences, Billerica, MA, USA) treatment was used to determine if the NE was a protein. Sn6 culture filtrates were treated with 1 mg/ml pronase at room temperature for 4 hrs. Pronase alone and cultures treated with water alone were used as controls. Sn6 culture filtrates treated with 0.01% SDS for 2 hrs at room temperature were also infiltrated to investigate if protein folding structure was necessary for activity.

Culture filtrates of Sn6 were also treated with dithiothreitol (DTT) and heat to evaluate the stability of the NE. Culture filtrate was treated with DTT with a final concentration of 0 and 20 mM at room temperature for 2 hrs before infiltration. To

evaluate the effects of boiling, culture filtrates were heated for 30 min and 1 hr on a hot plate at 100°C before allowing to cool and then infiltrated into leaves of CTm208.

Centricon ultafiltration devices (Millpore, Billerica, MA, USA) were used to estimate the size of the NE. Cultrate filtrates were subjected to ultrafiltration using 10 kDa and 30 kDa Mt cutoff filters. Filtrates and concentrates were tested for activity by infiltration on CTm208 to estimate the size of the NE.

Light dependence of the host gene-NE interaction was evaluated as described in Friesen et al. (2012). Three plants were infiltrated per replicate per treatment and placed in either 24 h darkness or a 16 h photoperiod in a growth chamber for two days.

Molecular mapping and estimating the effects of the wheat gene-NE interaction

PCR primers for 52 microsatellite markers selected from previously published wheat chromosome 2D maps (Song et al. 2005; Sourdille et al. 2004; Somers et al. 2004; Pestsova et al. 2000; Roder et al. 1998) were surveyed for polymorphism between CS and CS-Tm 2D. Primer pairs were selected from BARC (Song et al. 2005), CFA, CFD (Sourdille et al. 2004), GDM (Pestsova et al. 2000), GWM (Roder et al. 1998) and WMC (Somers et al. 2004) sets. Those revealing polymorphisms between the parents were then used to genotype the 87 F_2 plants that were insensitive to the NE to develop a linkage map of chromosome 2D. DNA isolation and PCR conditions were as described in Lu et al. (2006) and PCR products were separated and visualized as described in Zhang et al. (2009). The computer program MapDisto v.1.8 (Lorieux 2012) was used for the linkage analysis using the Kosambi mapping function (Kosambi 1944) with a logarithm of odds (LOD) threshold of 3.0.

Five microsatellite markers including four linked to the NE sensitivity gene and one (*xcfd51*) known to be tightly linked to the *Snn2* gene (Zhang et al. 2009) were then used to genotype all 406 F_2 plants to develop a linkage map of the region for QTL analysis. Composite interval mapping (CIM) was used to determine associations between disease reaction types and chromosome 2D markers including the NE sensitivity locus and linked microsatellite markers using the computer software Qgene 4.0 (Joehanes and Nelson 2008). A permutation test with 1,000 iterations indicated that an LOD of 2.1 provided a significance threshold at the $P < 0.05$ level of probability.

Statistical analysis

Mean separation for the disease reaction type means was determined by the use of Fisher's protected least significant difference (LSD) at $\alpha = 0.05$. Chi-squared tests were conducted using the program Graphpad.

Results

Identification of a novel NE produced by *P. nodorum* isolate Sn6

Screening of CS, Timstein, and the CS-Tm disomic chromosome substitution lines with Sn6 culture filtrates revealed that only Timstein and the CS-Tm 5B and CS-Tm 2D substitution lines displayed strong necrosis after infiltration (Figure 3.1), which indicated that isolate Sn6 produced at least two NEs interacting with genes on Timstein chromosomes 5B and 2D to induce necrosis. So far, three NEs have been identified to interact with wheat sensitivity genes on chromosomes 5B and 2D, including SnToxA which interacts with *Tsn1* on the long arm of chromosome 5B, SnTox3 which interacts with *Snn3-B1* on the short arm of chromosome 5B, and SnTox2 which interacts with *Snn2* on the short arm of chromosome 2D (Friesen and Faris 2010 for review).

Evaluation of the SnToxA, SnTox2, and SnTox3 differential lines BG261, BG223, and BG220, respectively, revealed that BG261 and BG223 were insensitive but BG220 was sensitive indicating that the Sn6 culture filtrate contained SnTox3 but not SnToxA or SnTox2.

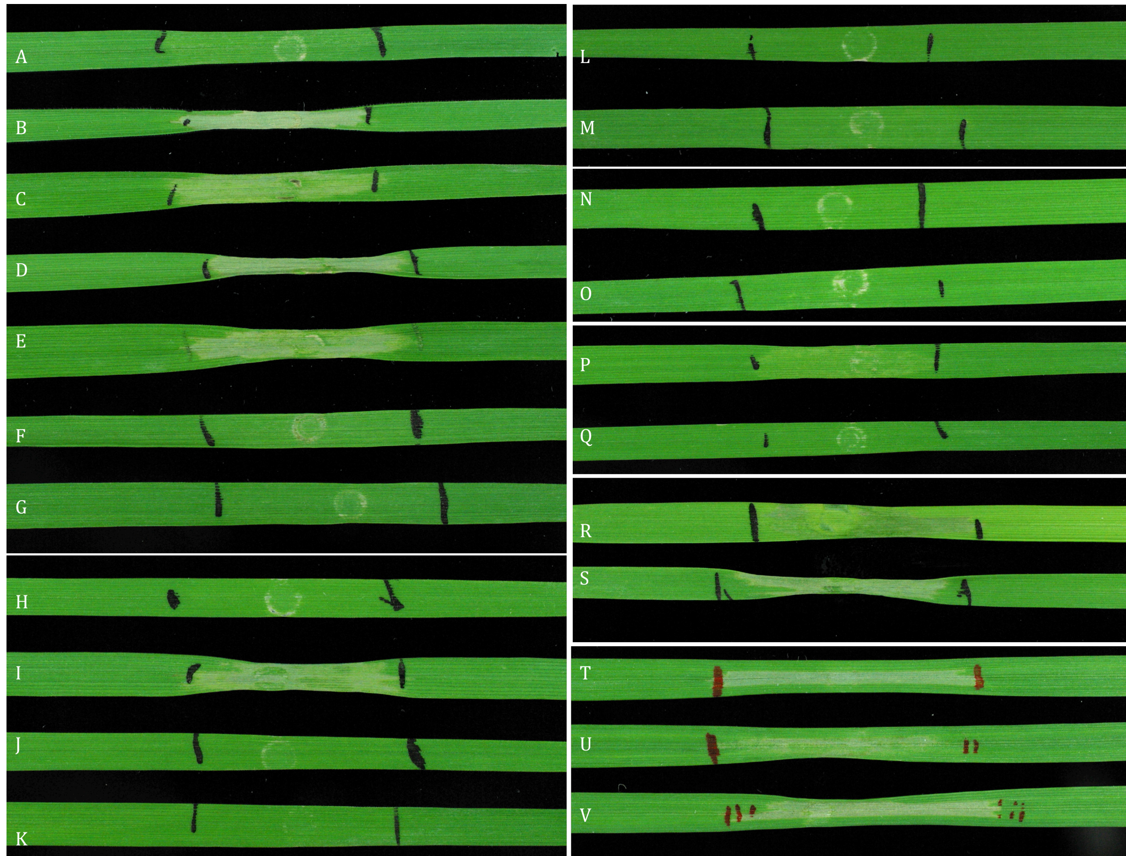


Figure 3.1. Infiltration of wheat leaves with culture filtrates of isolate Sn6. (A. CS; B. Timstein; C. CS-Tm 5B; D. CS-Tm 2D; E. *Snn3-B1* differential line BG220; F. *Snn2* differential line BG223; G. *Tsn1* differential line BG261. Infiltration on *Snn7* differential line CTm208 with: H. Culture filtrates with pronase at 1 mg/ml final concentration; I. culture filtrate diluted to the same concentration as leaf H with water; J. Pronase alone (1 mg/ml); K. water alone; L. Culture filtrates with 0.01% SDS; M. 0.01% SDS only; N. Culture filtrates with 20 mM DTT; O. 20 mM DTT alone; P. Culture filtrates subjected to boiling for 30 mins; Q. Culture filtrates subjected to boiling for 60 mins; R. 48 hr darkness after infiltration; S. 16-hr photoperiod for 48 hr after infiltration; T. 30 kDa concentrates; U. 30 kDa filtrates; V. 10 kDa concentrates.

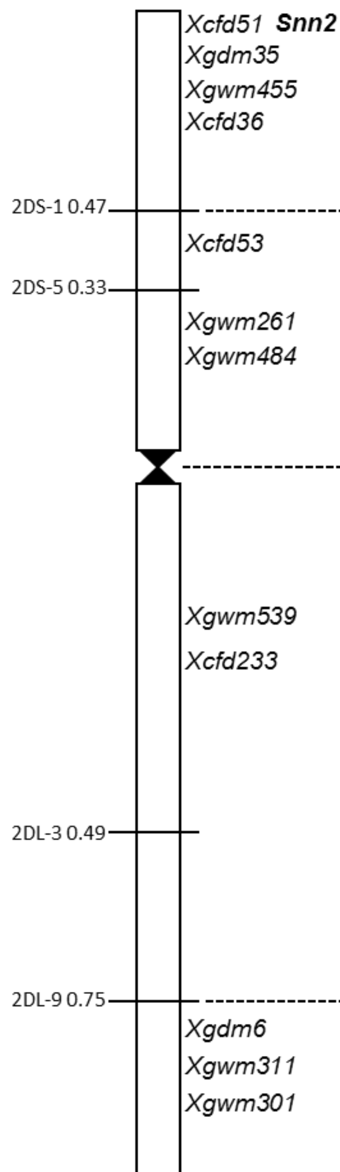
Therefore, the sensitivity of CS-Tm 5B was due to the interaction between the *Snn3-B1* gene in CS-Tm 5B (Zhang et al. 2011) and SnTox3. However, *Snn2* is the only NE sensitivity gene to be identified on chromosome 2D to date and because the Sn6 culture filtrates did not contain SnTox2, this indicated that a novel NE was present in the culture and that it was recognized by a previously unidentified sensitivity gene on wheat chromosome 2D. I propose to designate the newly identified NE and corresponding wheat sensitivity gene as SnTox7 and *Snn7*, respectively.

The inheritance of sensitivity to SnTox7

The 406 F_2 plants of the CT population segregated in a ratio of 319 sensitive plants: 87 insensitive plants. This ratio fit the expected 3:1 (sensitive:insensitive) ratio ($\chi^2_{df=1}$=2.76, 0.10 > P > 0.05) for a single dominant gene governing sensitivity to SnTox7 in the Sn6 culture filtrate. Therefore, like the other eight wheat gene-NE interactions in the wheat-*P. nodorum* system described to date, the *Snn7*-SnTox7 interaction fits the inverse gene-for-gene model.

Molecular mapping of *Snn7*

Twenty-two microsatellite markers revealed polymorphisms and were subsequently used to genotype the 87 insensitive F_2 plants and develop a linkage map of 2D (Figure 3.2). The *Snn7* locus mapped to the long arm of chromosome 2D. The marker *Xcfd44* co-segregated with *Snn7*, and the microsatellite markers *Xgdm6* and *Xgwm349* flanked *Snn7* at distances of 0.9 and 1.8 cM, respectively (Figure 3.2).



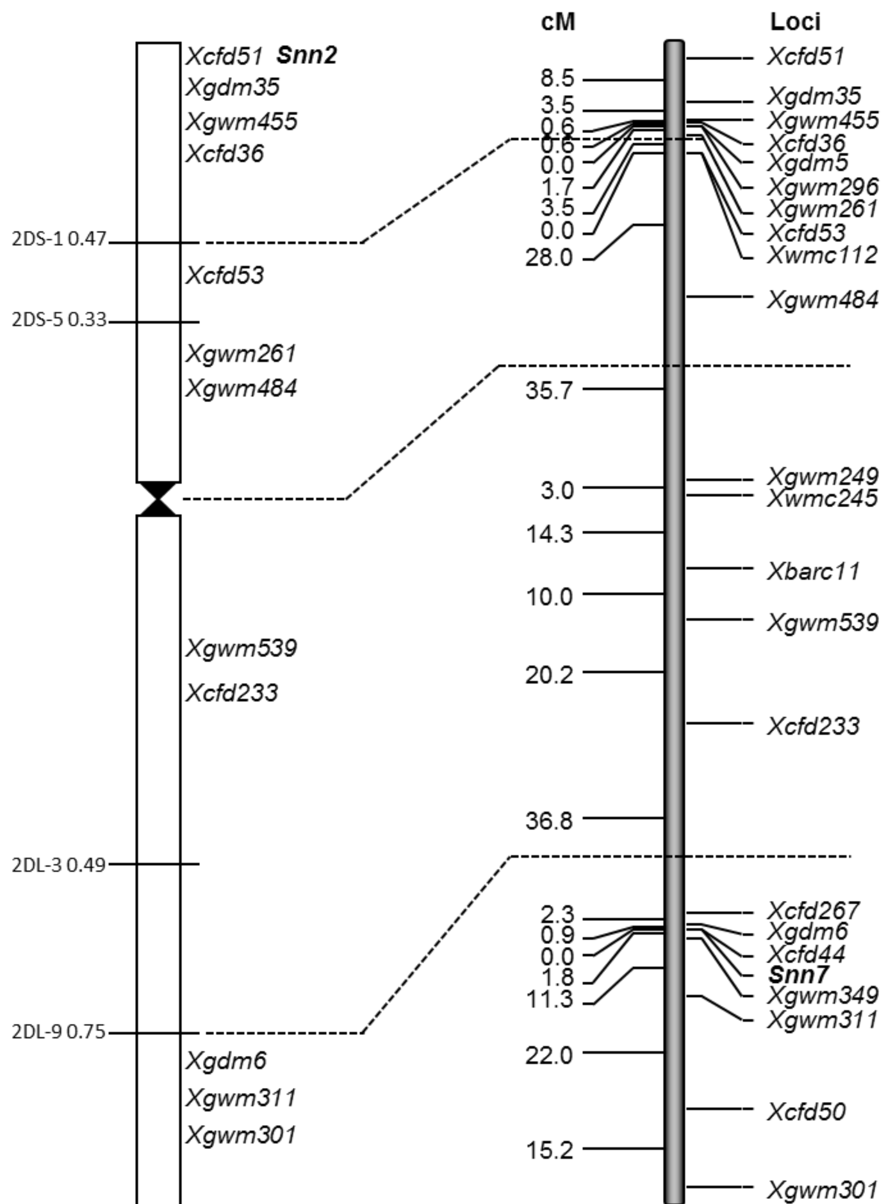


Figure 3.2. Deletion-based physical and genetic linkage maps of wheat chromosome 2D. The deletion-based physical map (left) shows the deletion fraction breakpoints along the left and bin locations of microsatellite markers in common with those mapped in the CT population in this research along the right. Bin locations of microsatellite markers are according to Sourdille et al. (2004), and the bin location of *Snn2* is from Friesen et al. (2007). The genetic linkage map developed in the 87 SnTox7-insensitive F_2 plants of the CT population (right) shows the centiMorgan (cM) distances between markers along the left side of the map and markers along the right. The *Snn7* locus is shown in bold.

Determination of the role of a compatible SnTox7-*Snn7* interaction in causing disease

The 406 F_2 plants of CT population inoculated with conidia of Sn6 had reaction types that ranged from 0.6 to 4.0 with an overall mean of 2.4 (Table 3.2). Disease reaction types for homozygous SnTox7 insensitive plants averaged 1.3. However, the average disease reaction type for those sensitive to SnTox7 was 2.7. The average disease reaction types for CS and CS-Tm 2D were 1.6 and 3.2, respectively (Figure 3.3).

Table 3.2. Average and range of disease reaction types of parents and F_2 plants of the CT population after inoculation with conidia of *Parastagonospora nodorum* isolate Sn6.

Genotype	Average disease reaction type[a]	Reaction type range
CS	1.6	1.0-2.0
CS-Tm 2D	3.2	2.5-4.0
CT (*Snn7_*)	2.7*	0.6-4.0
CT (*snn7snn7)*	1.3*	0.6-2.5

[a]Asterisks indicate the average lesion types for the *Snn7_* and *snn7snn7* genotypic classes were significantly different at the $P < 0.05$ level of probability

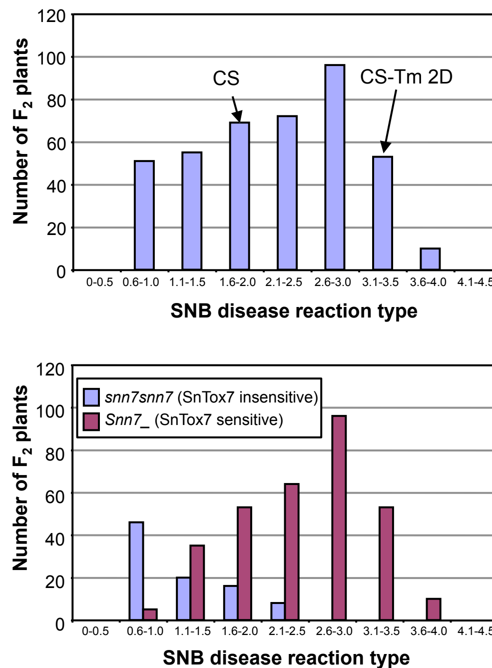


Figure 3.3. Histograms of average disease reaction types caused by *Parastagonospora nodorum* isolate Sn6 in the CT population. (a) Average disease reaction types of the entire CT population. (b) Average disease reaction types of SnTox7 sensitive and insensitive lines.

CIM analysis of the disease reaction types collected from the 406 F_2 plants and the genomic region harboring the *Snn7* locus revealed that significance peaked at the *Snn7* locus with a LOD value of 36.0 (Figure 3.4). The locus had an R^2 value of 0.33 indicating that a compatible *Snn7*-SnTox7 interaction explained 33% of the variation in disease in the CT population, and thus played a major role in the development of SNB.

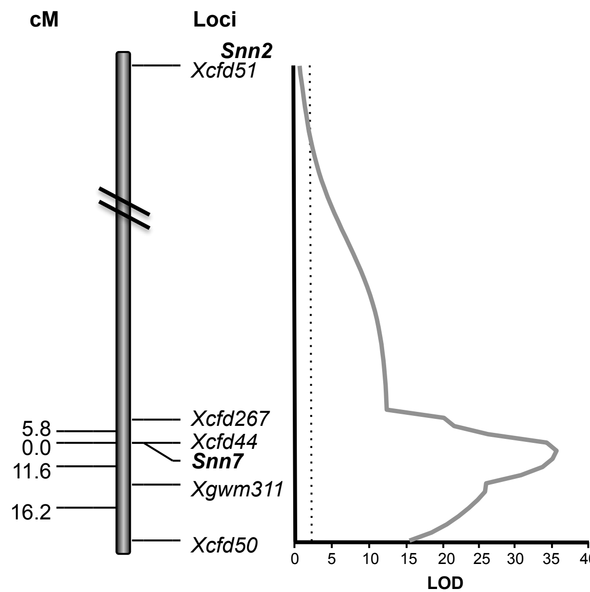


Figure 3.4. Interval regression map of the *Snn7* region of chromosome 2D using the 406 F_2 plants of the CT population. CentiMorgan distances are to the left and marker loci to the right of the genetic linkage map. The *Snn2* locus near marker *Xcfd51* is shown in its inferred location. The double diagonal lines on the linkage map indicate greater than 50% recombination frequency between markers. A LOD scale is shown along the *x* axis of the regression map and the dotted line indicates the LOD significance threshold of 2.1.

Partial characterization of SnTox7

The selected *Snn7* differential line CTm208 was used to further characterize SnTox7. CTm208 is sensitive to SnTox7 but insensitive to all other NEs known to be produced by isolate Sn6 including SnToxA, SnTox2, SnTox3, SnTox5, and SnTox6 (Friesen et al. 2007; Gao et al. 2014).

Infiltration of Sn6 culture filtrates containing SnTox7 treated with Pronase, infiltration of Pronase alone, and infiltration of water alone had no activity on the differential line CTm208 (Figure 3.1H, 1J, 1K), which indicated that SnTox7 is most likely a protein. Dilution of the culture filtrates with water to the same degree as the pronase-treated cultures did not affect activity (Figure 3.1I).

Treatment on the culture filtrates with 0.01% SDS for 2 hrs or with 20 mM DTT for 2 hrs also negated NE SnTox7 activity suggesting that proper protein folding and disulfide bonds are necessary for activity (Figure 3.1L, 1N). Additionally, the stability of SnTox7 was tested by directly heating the culture filtrates on a hot plate. Boiling the cultures on hot plate for 0.5 hr did not completely abolish SnTox7 activity on CTm208 (Figure 3.1P), which indicates that SnTox7 is a relatively stable protein with the ability to resist physical and chemical degradation.

The size of SnTox7 was estimated by using the ultrafiltration system. Both the 30 kDa flow-through and concentrates showed toxin sensitivity on CTm208 (Figure 3.1U, 1T). However, only the concentrates and not the flow-through of the 10 kDa filter had the ability to cause necrosis (Figure 3.1V). This revealed that SnTox7 was likely between 10 kDa and 30 kDa in size. CTm208 plants infiltrated with Sn6 culture filtrates and kept in dark for 48 hrs developed some necrosis but had a weaker reaction comparing to plants kept in a 16 hr photoperiod (Figure 3.1R, 1S) indicating that the interaction was not completely light dependent.

Prevalence of *Snn7*

Nineteen of the 52 (36%) common wheat lines infiltrated with culture filtrates of Sn6 showed a sensitive reaction (Table 3.1). Of these, 17 were also sensitive to SnTox3.

Only two wheat lines, Hanna and Siu Mak, were sensitive to Sn6 culture filtrates but insensitive to SnTox3.

Discussion

Here I described the ninth interaction between a wheat gene and a *P. nodorum*-produced NE. Like the previously described interactions, sensitivity to the NE is conferred by a dominant gene, and thus fits the inverse gene-for-gene model where the recognition of SnTox7 by *Snn7* leads to sensitivity and ultimately NETS. However, if either the wheat *Snn7* allele or the SnTox7 NE is not present, then an incompatible interaction occurs resulting in NE insensitivity. It is now known that compatible host-NE interactions lead to the activation of a host response that strongly resembles a classic 'resistance' response to biotrophic pathogens and includes the activation of MAPK genes and defense response genes, an increase in reactive oxygen species, DNA laddering, and PCD (Faris et al. 2010; Liu et al. 2012) suggesting that necrotrophic specialist pathogens such as *P. nodorum* secrete NEs to effectively hijack the plant's own innate immune system to gain nutrients and proliferate. It is most likely that SnTox7 is yet another weapon employed by *P. nodorum* for this purpose.

The initial characterization of SnTox7 indicated that it has properties similar to the other NEs described to date. For example, SnTox7 is a protein smaller than 30 kDa as are the other seven *P. nodorum* NEs identified so far. However, with the exception of the *Snn3*-SnTox3 interaction, all other host gene-NE interactions described to date in the wheat-*P. nodorum* pathosystem have been shown to be completely dependent on light (Friesen and Faris 2010; Friesen et al. 2012; Gao et al. 2014). Like the *Snn3*-SnTox3 interaction, *Snn7*-SnTox7 interactions still resulted in the development of some necrosis

even in the absence of light. This would suggest that perhaps the *Snn3*-SnTox3 and *Snn7*-SnTox7 interactions might exploit a pathway different from the other host gene-NE interactions.

The *Snn7*-SnTox7 interaction accounted for 33% of the variation in SNB development in the CT population of 406 F_2 plants indicating that this interaction plays a significant role in the development of disease, at least in the CT population. Other wheat gene-NE interactions in the wheat-*P. nodorum* system have been shown to account for between 18 and 95% of the variation, depending on the genetic background and the number of wheat gene-NE interactions operating in the system (Friesen and Faris 2010). Prior to this study it could be concluded that the CT population did not segregate for the NE sensitivity genes *Tsn1*, *Snn1*, *Snn3-B1, Snn3-D1, Snn4, Snn5*, or *Snn6* because these genes all reside on chromosomes other than 2D. The CT population segregated only for chromosome 2D, which is known to harbor *Snn2* and *Snn7* (Friesen et al. 2007; and this research). It was known that CS does not possess *Snn2* (Zhang et al. 2009) but unknown whether Timstein contains the gene or not. The isolate Sn6 is known to produce SnTox2 under artificial inoculation conditions (Friesen et al. 2007), but the QTL analysis of the 406 F_2 plants indicated no significant association between the disease reaction types and marker *Xcfd51*, which is tightly linked to *Snn2* (Zhang et al. 2009). It is most likely that this is because Timstein does not possess *Snn2*, but it is also possible that Timstein does possess *Snn2* and the *Snn2*-SnTox2 interaction is not important for SNB development in this population.

Others have reported the presence of SNB resistance QTLs on chromosome arm 2DL in wheat (Aguilar et al. 2005; Uphaus et al. 2007; Shankar et al. 2008). Aguilar et

al. (2005) evaluated a population of recombinant inbred lines (RILs) derived from a cross between the Swiss winter wheat variety Forno and an accession of *T. aestivum* ssp. *spelta* known as Oberkulmer. They reported a QTL on 2DL that explained 20.8% of the variation in SNB on leaves of adult plants. However, Forno contributed the susceptibility allele at this QTL, and in my work, I found that Forno was insensitive to SnTox7. Therefore, this would suggest that the 2DL QTL reported by Aguilar et al. (2005) is not the same as the *Snn7*-SnTox7 interaction described here.

Uphaus et al. (2007) evaluated a population of RILs derived from two Purdue University breeding lines, P91193D1 and P92201D5, for reaction to SNB on glumes and identified two QTLs on 2D associated with resistance. One of the two QTLs was associated with marker *Xcfd50* indicating that it might be in the vicinity of *Snn7*. However, I screened the lines P91193D1 and P92201D5 (seed kindly provided by Dr. Steve Goodwin, USDA-ARS, West Lafayette, IN) with Sn6 culture filtrates and found that both lines were completely insensitive to the cultures indicating that the P91193D1 × P92201D5 population did not segregate for *Snn7*. Therefore, the QTL associated with glume blotch on 2DL in the P91193D1 × P92201D5 population is not due to the *Snn7*-SnTox7 interaction.

Shankar et al. (2008) reported a QTL on 2DL associated with resistance to SNB on leaves of seedlings in a doubled haploid population derived from a cross between a CIMMYT-bred wheat line 6HRWSN125 (resistant) and the Australian breeding line WAWHT2074 (susceptible). The QTL was located near marker *Xcfd50* on 2DL indicating that it could be the same as *Snn7*, but further work is needed to verify this possibility.

Of the 19 wheat lines that showed sensitivity to Sn6 culture filtrates, 17 were found to harbor the *Snn3* gene because they were sensitive to SnTox3. It is unknown whether or not these 17 lines may contain *Snn7*. Because isolate Sn6 produced SnTox3 in the culture filtrates, evidenced by the development of necrosis on the *Snn3* differential line BG220, I cannot conclude whether the necrosis was conferred by the *Snn3*-SnTox3 interaction alone or if it was due to a combination of *Snn3*-SnTox3 and *Snn7*-SnTox7.

Two wheat lines, Hanna and Siu Mak, were sensitive to Sn6 culture filtrates but insensitive to SnTox3. It is possible that these two lines possess *Snn7*, but I cannot rule out the possibility that other, yet unidentified, NEs are produced by Sn6 for which Hanna and Siu Mak contain cognate sensitivity genes. Therefore, Timstein is the only variety identified so far that is verified to harbor a functional *Snn7* allele conferring sensitivity to SnTox7. Hanna is a spring wheat variety developed by AgriPro in the USA and Siu Mak is a Chinese spring wheat variety. It is unknown if they may be related with each other, or with Timstein, by pedigree.

The *P. nodorum* isolate Sn6 was previously reported to produce the NEs SnToxA, SnTox2 (Friesen et al. 2007), SnTox3 and SnTox6 (Gao et al. 2014). It is interesting to note that in the research presented here, Sn6 produced only SnTox3 and SnTox7. Frequent observations of the inconsistent production of different NEs were noticed by various *P. nodorum* isolates maintained in the laboratory over time (Friesen et al. unpublished). The reasons for this phenomenon are unknown; however, one might speculate that slight variations in conditions used to grow the liquid cultures could affect NE expression. Epigenetic modification may contribute to the non-detectable level of SnTox2 and SnToxA in Sn6 culture. Epigenetic chromatin modifications are known to

affect gene expression in eukaryotes and might thus tune in or out effector gene expression (Schulze-Lefert and Panstruga, 2011). Epigenetic gene silencing is reversible and potentially activates or inactivates effector gene expression in a dynamic manner. Whatever the reason, the alterations in NE expression seem to be specific to growth in liquid Fries media for culture filtrate production because the isolates appear to retain the ability to produce all their NEs when the fungus is inoculated onto plants using spores.

In conclusion, the *Snn7*-SnTox7 interaction plays an important role in the development of SNB on leaves of seedlings. However, the data suggest that the prevalence of *Snn7* among wheat varieties may be relatively low compared to other NE sensitivity genes. Nevertheless, it is important to continue to identify NEs and their corresponding wheat sensitivity genes to further characterize the wheat-*P. nodorum* pathosystem, which has become a model to study the functional genomics and evolution of necrotrophic pathogens and the inverse gene-for-gene system. This work also provides tools for breeders in the way of another NE useful for characterizing germplasm and breeding lines, and molecular markers useful for marker-assisted selection against the dominant allele conferring NE sensitivity.

References

Abeysekara N, Friesen TL, Keller B, Faris JD (2009) Identification and characterization of a novel host-toxin interaction in the wheat-*Stagonospora nodorum* pathosystem. Theor Appl Genet 120:117-126

Adhikari TB, Bai J, Meinhardt SW, Gurung S, Myrfield M, Patel J, Ali S, Gudmestad NC, Rasmussen JB (2009) *Tsn1*-mediated host responses to ToxA from *Pyrenophora tritici-repentis*. Mol Plant-Microbe Interact 22:1056-1068

Aguilar V, Stamp P, Winzeler M, Winzeler H, Schachermayr G, Keller B, Zanetti S, Messmer MM (2005) Inheritance of field resistance to *Stagonospora nodorum* leaf and glume blotch and correlations with other morphological traits in hexaploid wheat. Theor Appl Genet 111:325-336

Faris JD, Friesen TL (2009) Reevaluation of a tetraploid wheat population indicates that the *Tsn1*-ToxA interaction is the only factor governing Stagonospora nodorum blotch susceptibility. Phytopathology 99:906-912

Faris JD, Zhang ZC, Lu HJ, Lu SW, Reddy L, Cloutier S, Fellers JP, Meinhardt SW, Rasmussen JB, Xu SS, Oliver RP, Simons KJ, Friesen TL (2010) A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proc Natl Acad Sci USA 107:13544-13549

Flor HH (1956) The complementary genetic systems in flax and flax rust. Adv Genet 8:29-54

Friesen TL, Stukenbrock EH, Liu ZH, Meinhardt SW, Ling H, Faris JD, Rasmussen JB, Solomon PS, Mcdonald BA, Oliver RP (2006) Emergence of a new disease as a result of interspecific virulence gene transfer. Nat Genet 38:953-956

Friesen TL, Meinhardt SW, Faris JD (2007) The *Stagonospora nodorum* – wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. Plant J 51:681-692

Friesen TL, Zhang Z, Solomon PS, Oliver RP, Faris JD (2008) Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. Plant Physiol 146:682-693

Friesen TL, Chu CG, Liu ZH, Halley S, Faris JD (2009) Host-selective toxins produced by *Stagonospora nodorum* confer disease susceptibility in adult wheat plants under field conditions. Theor Appl Genet 118:1489-1497

Friesen TL, Faris JD (2010) Characterization of the wheat-*Stagonospora nodorum* disease system: what's the molecular basis of this quantitative necrotrophic disease interaction? Can J Plant Pathol 32:20-28

Friesen TL, Chu C, Xu SS, Faris JD (2012) SnTox5-*Snn5*: a novel *Stagonospora nodorum* effector-wheat gene interaction and its relationship with the SnToxA-*Tsn1* and SnTox3-*Snn3-B1* interactions. Mol Plant Pathol 13:1101-1109

Gao Y, Faris JD, Liu ZH, Xu SS, Friesen TL (2014) Identification and characterization of the SnTox6-*Snn6* interaction in the wheat-*Stagonospora nodorum* pathosystem. Mol Plant-Microbe Interact (accepted)

Joehanes R, Nelson JC (2008) QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 24:2788-2789

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172-175

Liu ZH, Faris JD, Meinhardt SW, Ali S, Rasmussen JB, Friesen TL (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

Liu ZH, Friesen TL, Meinhardt SW, Ali S, Rasmussen JB, Faris JD (2004b) Quantitative trait loci analysis and mapping of seedling resistance to *Stagonospora nodorum* leaf blotch in wheat. Phytopathology 94:1061-1067

Liu ZH, Friesen TL, Ling H, Meinhardt SW, Oliver RP, Rasmussen JB, Faris JD (2006) The *Tsn1*-ToxA interaction in the wheat-*Stagonospora* nodorum pathosystem parallels that of the wheat-tan spot system. Genome 49:1265-1273

Liu ZH, Faris JD, Oliver RP, Tan KC, Solomon PS, McDonald MC, McDonald BA, Nunez A, Lu S, Rasmussen JB, Friesen TL (2009) SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the *Snn3* gene. PLoS Pathogens 5:e1000581

Liu ZH, Zhang ZC, Faris JD, Oliver RP, Syme R, McDonald MC, McDonald BA, Solomon PS, Lu SW, Shelver WL, Xu SS, Friesen TL (2012) The cysteine rich necrotrophic effector SnTox1 produced by *Stagnospora nodorum* triggers susceptibility of wheat lines harboring *Snn1*. PLoS Pathogens 8:e1002467

Lorieux M (2012) MapDisto: fast and efficient computation of genetic linkage maps. Mol Breeding 30:1231-1235

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

Lu SW, Faris JD, Sherwood R, Friesen TL, Edwards MC (2014) A dimeric PR-1-type pathogenesis-related protein interacts with ToxA and potentially mediates ToxA-induced necrosis in sensitive wheat. Mol Plant Pathol 15:650-663

Manning VA, Ciuffetti LM (2005) Localization of Ptr ToxA produced by *Pyrenophora tritici-repentis* reveals protein import into wheat mesophyll cells. Plant Cell 17:3203-3212

Manning VA, Hamilton SM, Ciuffetti LM (2007) PtrToxA interacts with a chloroplast-localized protein. Mol Plant-Microbe Interact 20:168-177

Pandelova I, Betts MF, Manning VA, Wilhelm LJ, Mockler TC, Ciuffetti LM (2009) Analysis of transcriptome changes induced by Ptr ToxA in wheat provides insights into the mechanisms of plant susceptibility. Mol Plant 2:1067-1083

Pestsova E, Ganal MW, Roder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689-697

Reddy L, Friesen TL, Meinhardt SW, Chao S, Faris JD (2008) Genomic analysis of the *Snn1* locus on wheat chromosome arm 1BS and the identification of candidate genes. Plant Genome 1: 55-66

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

Schulze-Lefert P, Panstruga R (2011). A molecular evolutionary concept connecting nonhost resistance, pathogen host range, and pathogen speciation. Trends Plant Sci 16:117-125

Shankar M, Walker E, Golzar H, Loughman R, Wilson RE, Francki MG (2008) Quantitative trait loci for seedling and adult plant resistance to *Stagonospora nodorum* in wheat. Phytopathology 98:886-893

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

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550-560

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.) Funct Integrative Genomics 4:12-25

Tai Y-S, Bragg J, Meinhardt SW (2007) Functional characterization of ToxA and molecular identification of its intracellular targeting protein in wheat. Am J Plant Physiol 2:76-89

Uphaus J, Walker E, Shankar M, Golzar H, Loughman R, Francki M, Ohm H (2007) Quantitative trait loci identified for resistance to Stagonospora glume blotch in wheat in the USA and Australia. Crop Sci 47:1813-1822

Winterberg B, Du Fall LA, Song XM, Pascovici D, Care N, Molloy M, Ohms S, Solomon PS (2014) The necrotrophic effector protein SnTox3 re-programs metabolism and elicits a strong defence response in susceptible wheat leaves. BMC Plant Biol 14:215
Xu SS, Friesen TL, Mujeeb-Kazi A (2004) Seedling resistance to tan spot and Stagnospora nodorum blotch in synthetic hexaploid wheats. Crop Sci 44:2238-2245

Zhang ZC, Friesen TL, Simons KJ, Xu SS, Faris JD (2009) Identification, development and validation of markers for marker-assisted selection against the *Stagonospora nodorum* toxin sensitivity genes *Tsn1* and *Snn2* in wheat. Mol Breeding 23: 35-49

Zhang ZC, Friesen TL, Xu SS, Shi GJ, Liu ZH, Rasmussen JB, Faris JD (2011) Two putatively homoeologous wheat genes mediate recognition of SnTox3 to confer effector-triggered susceptibility to *Stagonospora nodorum*. Plant J 65:27-38

CHAPTER IV. HIGH RESOLUTION MAPPING AND MARKER DEVELOPMENT FOR THE STAGONOSPORA NODORUM BLOTCH SUSCEPTIBILITY GENE *SNN3-B1*

Abstract

Stagonospora nodorum blotch (SNB) is a severe foliar and glume disease on durum and hexaploid wheat. Necrotrophic effectors (NEs) are the major determinants for the disease. One such NE is SnTox3, which can be recognized by the host sensitivity gene *Snn3-B1* to induce cell death and disease. In this work, I showed that different *Snn3-B1* alleles can lead to different levels of NE sensitivity and SNB susceptibility. I also developed saturated and high-resolution linkage maps of the *Snn3-B1* region, evaluated colinearity of the gene region with rice and *Brachypodium distachyon,* and evaluated the utility of markers for marker-assisted selection. Two F_2 populations derived from Sumai3 crossed with BR34 and with a Chinese Spring – *Triticum dicoccoides* chromosome 5B disomic substitution line were used to map 44 markers consisting of restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), bin mapped expressed sequence tags (ESTs) and single nucleotide polymorphisms (SNPs). Micro-colinearity of the *Snn3-B1* region was highly disrupted compared to rice and *B. distachyon*. High-resolution mapping of the *Snn3-B1* locus in 5,600 gametes delineated the gene to a 1.5 cM interval. Finally, closely linked markers were used to screen a collection of wheat cultivars to evaluate their utility. Results showed that these markers should be useful for marker-assisted selection, however they were not diagnostic for predicting SnTox3 sensitivity.

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important food crops, providing 20% of the world's caloric intake. However, numerous diseases constantly threaten wheat production. Stagonospora nodorum blotch (SNB), caused by the necrotrophic fungus *Parastagonospora* (syn. *ana, Stagonospora; teleo, Phaeosphaeria*) *nodorum* (Berk.) Quaedvleig, Verkley & Crous, is a severe foliar and glume disease in most wheat production areas. SNB affects both common wheat and durum wheat (*T. turgidum* L.), and it can cause significant yield losses and reductions in quality.

Whereas biotrophic pathogens require living host cells on which to feed and proliferate, necrotrophic pathogens gain their nutrients from dead, or dying, cells. Over the past decade, the wheat-*P. nodorum* pathosystem has emerged as a model system for interactions between a plant and a necrotrophic specialist pathogen (Friesen and Faris 2010). Numerous studies to dissect the components of the system have revealed that *P. nodorum* produces numerous necrotrophic effectors (NEs, also known as host-selective toxins) that induce cell death and necrosis when recognized by cognate host sensitivity genes in an inverse gene-for-gene manner (Friesen and Faris 2010). Compatible interactions between the dominant host gene and the corresponding pathogen-produced NE have been shown to invoke classic defense responses including transcriptional activation of defense response genes, an increase in the production of reactive oxygen species, DNA laddering, and programmed cell death (PCD) (Liu et al. 2009, 2012; Winterberg et al. 2014). The molecular cloning of the wheat gene *Tsn1*, which mediates sensitivity to the *P. nodorum* NE known as SnToxA, revealed that it contained features typically observed in genes that confer resistance to biotrophic pathogens such as

nucleotide binding (NB) and leucine-rich repeat (LRR) domains (Faris et al. 2010). This work and that of Liu et al. (2009, 2012) indicated that necrotrophic specialists such as *P. nodorum* use NEs to essentially hijack the plant's own defense mechanisms to induce PCD. The induction of PCD leads to necrosis, which allows the pathogen, being a necrotroph, to gain nutrients and proliferate. This phenomenon is referred to as necrotrophic effector-triggered susceptibility (NETS).

To date, nine NE-host sensitivity gene interactions have been identified in the wheat-*P. nodorum* pathosystem including *Tsn1*-SnToxA (Friesen et al. 2006; Liu et al. 2006; Faris et al. 2010), *Snn1*-SnTox1 (Liu et al. 2004, 2004b, 2012; Reddy et al. 2008), *Snn2*-SnTox2 (Friesen et al. 2007; Zhang et al. 2009), *Snn3-B1*-SnTox3 (Friesen et al. 2008; Liu et al. 2009), *Snn3-D1*-SnTox3 (Zhang et al. 2011), *Snn4*-SnTox4 (Abeysekara et al. 2009), *Snn5*-SnTox5 (Friesen et al. 2012), *Snn6*-SnTox6 (Gao et al. 2014), and *Snn7*-SnTox7 (CHAPTER III). The pathogen genes encoding three of the NEs, including SnToxA (Friesen et al. 2006), SnTox1 (Liu et al. 2012), and SnTox3 (Liu et al. 2009), have been cloned. In the host, only the *Tsn1* and *Snn1* genes have been isolated so far (Faris et al. 2010; CHAPTER V).

The *Snn3-B1*-SnTox3 interaction plays a significant role in the development of SNB explaining up to 17% of the phenotypic variation (Friesen et al. 2008). More interestingly, *Snn3-B1* lies on the short arm of wheat chromosome 5B and the homoeologous copy of the gene on 5DS, designated *Snn3-D1*, also recognizes SnTox3 to confer NETS (Zhang et al. 2011) even though *Snn3-B1* and *Snn3-D1* diverged from a common ancestor approximately 2.5 million years ago (Chalupska et al. 2008). Furthermore, it is interesting to note that, while the *Tsn1*-SnToxA, *Snn1*-SnTox1, *Snn2*-

SnTox2, *Snn4*-SnTox4, *Snn5*-SnTox5 and *Snn6*-SnTox6 wheat gene-NE interactions are completely dependent on light for the development of necrosis, the *Snn3*-SnTox3 interactions are much less dependent and develop some necrosis even in the absence of light (Friesen et al. 2008; Zhang et al. 2011) suggesting a unique aspect to these interactions and perhaps the exploitation of pathway(s) different from those involved with necrosis resulting from the other interactions.

The isolation and characterization of the homoeologous *Snn3* genes and analysis of their interactions with SnTox3 will shed more light on the molecular basis of wheat-*P. nodorum* host-NE interactions and will further our understanding of evolution of the components involved in this pathosystem (Zhang et al. 2011). *Snn3-B1* was first identified in a population of wheat recombinant inbred lines and mapped on the short arm of wheat chromosome 5B (Friesen et al. 2008). The closest marker to *Snn3-B1* was *Xcfd20*, a microsatellite marker 1.4 cM on the proximal side of the gene. Toward the goal of isolating the *Snn3-B1* gene, we describe here the development of saturated and high-resolution genetic linkage maps of the *Snn3-B1* locus. We also describe the development and analysis of closely linked markers suitable for marker-assisted selection against *Snn3-B1* alleles for use in germplasm development and wheat breeding programs to rid lines of SnTox3 sensitivity and thus enhance resistance to SNB.

Materials and Methods

Plant materials

The hexaploid bread wheat lines Sumai 3 and BR34, and the Chinese Spring-*T. turgidum* ssp. *dicoccoides* chromosome 5B disomic substitution line (CS-DIC 5B), where a pair of *T. turgidum* ssp. *dicoccoides* 5B chromosomes were substituted for the native

pair of 5B chromosomes, were used to generate segregating populations for mapping the *Snn3-B1* locus. Sumai 3 is a landrace from China, and BR34 is a variety from Brazil. Previous results indicated that Sumai 3 is sensitive to SnTox3 whereas BR34 and CS-DIC 5B are insensitive (Zhang et al. 2011). Two segregating F_2 populations were developed by crossing Sumai 3 with BR34 (BS population) and with CS-DIC 5B (CS population). A total of 115 and 85 SnTox3-insensitive F_2 plants from the BS and CS populations, respectively, were used for saturation mapping of the *Snn3-B1* locus. A population consisting of 2,800 F_2 plants (5,600 gametes) of the BS population (hereafter referred to as the BS-HR population) was used for high-resolution mapping. All plants were grown in the greenhouse at an average temperature of 21°C with a 16-h photoperiod. A set of 48 tetraploid wheat and 48 hexaploid wheat lines were deployed to evaluate the utility and diagnostic capabilities of closely linked markers.

Disease evaluations and necrotrophic effector bioassays

SnTox3 was obtained from SnTox3-expressing *Pichia pastoris* cultures as described by Liu et al. (2009). The fully expanded secondary leaves of the parental lines and F_2 plants were infiltrated with approximately 25 µl of the SnTox3 cultures using a 1-ml syringe with the needle removed. Reactions were evaluated three days after infiltration and scored based on the presence or absence of necrosis.

For spore inoculations and disease analysis, parental lines were grown in cones and placed in racks of 98 (Stuewe and Sons) with the outside border of each rack planted with the SNB susceptible variety ‘Grandin’ to reduce any edge effect. Conidia were produced and harvested from isolate Sn1501 as described in Liu et al. (2004b). Plants were inoculated at the 2-3 leaf stage until runoff using conidial suspensions containing

1×10^6 spores/ml. Plants were then placed in a mist chamber at 100% relative humidity at 21°C for 24 hr followed by 6 days of incubation in the growth chamber under a 12-hr photoperiod. The second leaf of inoculated parental lines was scored and recorded 7 days post-inoculation.

PCR amplification and electrophoresis

DNA from the parents and the F_2 plants was isolated according to Faris et al. (2000). All polymerase chain reaction (PCR) experiments were conducted using a total volume of 10 μl and consisted of 100 ng of template DNA, 1.5 mM $MgCl_2$, 0.125 mM dNTPs, 4 pmol of each primer and 1 unit of DNA polymerase. The PCR conditions were 94°C for 5 min., followed by 35 cycles of 94°C for 30s, the appropriate annealing temperature for 30s, and 72°C for 90s, followed by a final extension of 72°C for 7 min. Amplicons were electrophoresed on 6% polyacrylamide gels, stained with SYBR Green, and visualized with a Typhoon 9410 variable mode imager (GE Healthcare).

Marker development and molecular mapping of the *Snn3-B1* region

Markers were developed for the saturation mapping of *Snn3-B1* using multiple resources. First, the sequences of 53 expressed sequence-tags (ESTs) previously mapped to the 5BS deletion bin 5BS6-0.81-1.00 (Qi et al 2004) were obtained from the Graingenes database (http://wheat.pw.usda.gov/GG2/index.shtml). Primers for each TC or EST (if a corresponding TC was not available) were designed using Primer3 (Rozen and Skaletsky 2000) and tested for polymorphism between the parents as sequence tagged-site (STS) markers as described in Lu et al. (2006b). If no polymorphism was observed between the parents, then I PCR-amplified the EST sequences from plasmids and used the amplified products as probes in restriction fragment length polymorphism

(RFLP) assays as described by Lu et al. (2006b). The plasmids containing the cloned EST fragments were provided by Dr. B.S. Gill (Kansas State University, Manhattan, KS, USA). Probe preparation, restriction digestion, and Southern hybridization were carried out as described in Faris et al. (2000) and Lu et al. (2006b).

Second, 52 simple sequence repeat (SSR) markers on wheat chromosome arm 5BS from previously reported physical and genetic maps were surveyed as well (Song et al. 2005; Roder et al. 1998; Sourdille et al.2004; Torada et al. 2006; Alfares et al. 2009). The SSR markers were obtained from BARC (Song et al. 2005), GWM (Roder et al. 1998), HBD, HBG, HBE (Torada et al. 2006), WMC (Somers et al. 2004), and MAG (Xue et al. 2008) primer libraries.

Third, the marker *Xcfb306* described in Alfares et al. (2009) linked to the *Kr* locus on 5BS was also tested in this research as well to determine if it was linked to *Snn3*. Fourth, discovery sequences of SNP markers on wheat 5BS from the 9 K and 90 K SNP arrays (Cavanagh et al. 2013; Wang et al. 2014) were downloaded and used as queries in BLASTn searches of the wheat 5BS survey sequences (IWGSC, 2014) at https://urgi.versailles.inra.fr/blast/blast.php. Primers were designed based on the SNP discovery sequences or adjacent regions in the corresponding survey sequences.

Fifth, we used all the rice and *B. distachyon* gene sequences within the region between genomic positions 27,063,000 and 27,355,000 bp of rice chromosome 12 and between 233,642 and 447,045 bp *B. distachyon* chromosome 4 as queries in BLAST searches of the wheat gene indices database (URL) to identify homologous TC sequences. These segments were reported by Zhang et al. (2011) to correspond to the *Snn3-B1* and *Snn3-D1* regions of 5BS and 5DS, respectively. In total, there were 46 and

39 putative genes within these regions of rice and *B. distachyon*, respectively, that were evaluated. PCR primers were developed for the TC sequences by targeting the 3' ends, and used to screen the mapping population parents for polymorphism.

Sixth, 36 survey sequences found to harbor NB-LRR-like gene sequences on wheat chromosome arm 5BS (Thomas Wicker, personal communication) were downloaded and used for primer design and subsequent marker development. Finally, sequences of markers from the homoeologous region of chromosome arm 5DS containing *Snn3-D1* (Zhang et al. 2011) were also used for primer design and to determine if they detected homoeologous loci on 5BS. All PCR-based markers were amplified and visualized as described for the EST-STS markers above.

Co-dominant PCR markers flanking *Snn3-B1* based on saturation mapping were subsequently used to screen the high-resolution BS population of 2,800 F_2 plants to identify plants with putative recombination events within the *Snn3-B1* interval. The recombinant plants were also screened for reaction to SnTox3. Plants insensitive to SnTox3 were considered homozygous for a recessive *snn3-B1* allele, and plants sensitive to SnTox3 were considered to be either homozygous for the *Snn3-B1* allele or heterozygous. To determine the genotypes of the sensitive F_2 plants, F_3 families consisting of at least 21 plants derived from each sensitive F_2 plant were screened with SnTox3.

Comparative analysis of the *Snn3-B1* region, rice and *Brachypodium distachyon*

To evaluate the level of micro-colinearity between the *Snn3-B1* region of wheat 5BS and rice and *B. distachyon*, the sequences of EST-based markers mapping near *Snn3-B1* (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi) were subjected to

tBLASTx searches of the rice and *B. distachyon* genomic sequences using rice genome annotation project database (http://rice.plantbiology.msu.edu/) and Brachybase (http://www.plantgdb.org) as described by Zhang et al. (2011).

Linkage analysis

The computer program MapDisto v.1.8 (Lorieux 2012) was used for the linkage analysis using the Kosambi mapping function (Kosambi 1944) with a logarithm of odds (LOD) threshold of 3.0 and a maximum recombination frequency of 0.3. The 'find groups' command was used to verify the linkage of markers. The seriation algorithm using SARF (sum of adjacent recombination frequencies) was conducted with the 'order sequence' command. The 'check inversions', 'ripple order', and 'drop locus' were used for local improvement of the order of loci.

Results

Identification of SnTox3-sensitivity in Sumai 3

Zhang et al. (2011) showed that the wheat cultivar Sumai 3 was sensitive to SnTox3. Here, comparisons of SnTox3 infiltrations between the SnTox3 differential BG220 (Friesen et al. 2008) and Sumai 3, indicated that Sumai 3 is more highly sensitive to SnTox3 than BG220 (Figure 4.1C, 1D). Sumai 3 developed much stronger necrosis more rapidly than did BG220. Also, conidial inoculations with Sn1501, an SnTox3-producing isolate, led to stronger disease symptoms on Sumai 3 compared to BG220 (Figure 4.1G, 1H). The wheat lines BR34 and CS-DIC 5B were both insensitive to SnTox3 (Figure 4.1A, 1B, 1E, 1F), and used as parents in crosses with Sumai 3 to develop populations for further saturation and high-resolution mapping of *Snn3-B1*.

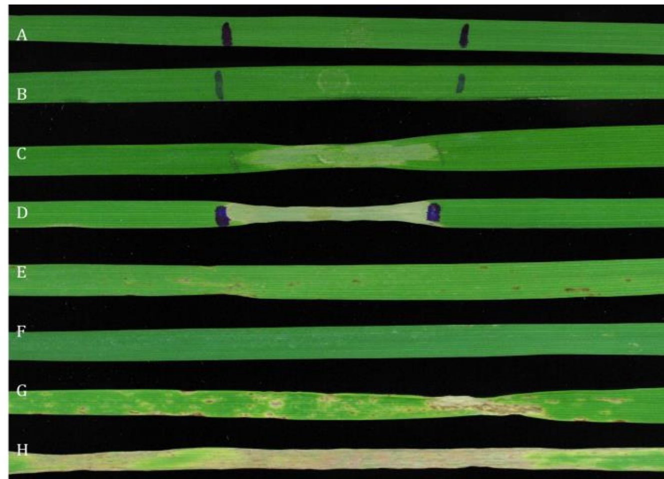


Figure 4.1. Infiltration and inoculation reactions of the parental lines as well as the SnTox3 differential line BG220. Infiltration with purified SnTox3 on CS-DIC 5B (A), BR34 (B), BG220 (C) and Sumai3 (D). Conidial inoculations of the SnTox3-producing isolate Sn1501 showed that Sn1501 was virulent on BG220 (G) and Sumai 3 (H) and avirulent on CS-DIC 5B (E) and BR34 (F).

Saturation mapping of *Snn3-B1*

All markers were tested for polymorphism between BR34 and Sumai 3 and between CS-DIC 5B and Sumai 3. Of the primer sets developed for the 53 ESTs mapping in the 5BS deletion bin 5BS6-0.81-1.00 (Qi et al. 2004), one marker (*XBE446811*) revealed polymorphism between CS-DIC 5B and Sumai 3 and mapped to 5BS in CS F_2 population (Table 4.1, Figure 4.2). The same primer set was monomorphic in the BS population. The remainder of the EST-STS primer sets were either monomorphic or they detected genomic loci other than on chromosome 5BS. Survey of these ESTs for RFLPs between the parental lines indicated that six of them, including BE262914, BE292905, BE443842, BF200555, BE499835, and BF293016 revealed polymorphisms in either the BS or the CS populations and were subsequently mapped (Figure 4.2).

Table 4.1. Markers developed for molecular mapping of *Snn3-B1*.

Marker Type	Marker designation	F Primer	R Primer	Motif[a]	Anneal. Temp.	Source[b]	Survey sequence[c]
SSR	*Xfcp652*	TGTGTTGAGCTCATGTACAAA	CAGCCTTCCTTACTGTGAAA	(TCATC)4	60	90K SNP	2228786
EST-STS	*Xfcp653*	TGTAAGTTGGTACCCGGCTC	TAATCGCCATAACCCTTTGC		60	90K SNP	2255704
SSR	*Xfcp654*	CAACTTTTTCCACATGCTTAT	TACACGCTAATAGCAACCAGT	(GCTA)3	60	90K SNP	1629420
SSR	*Xfcp655*	ACAAAGGTACAATGCACAGAG	AAATCGCTGAAGAGACGAC	(TC)6	60	90K SNP	2274687
SSR	*Xfcp656*	ATTGGGAAAGGTTCAGATTAC	AATAGCAGAGCAAGGAAGC	(CCT)5	60	90K SNP	2297308
SSR	*Xfcp657*	ATTCACGGTTGGTACATATTG	CTTGTATCGCACAAACAGAAC	(GCAGT)4	60	90K SNP	1635726
SSR	*Xfcp658*	CGGTCTTTACGTGACTTGTAT	AGAGAGCCAACTTGAATTTTT	(TGA)6	60	9K SNP	2262359
SSR	*Xfcp659*	GACTCGACTAGGAGAGGGTAA	TAGCTAATACACCAACCATCG	(TGC)6	60	9K SNP	2272737
SSR	*Xfcp660*	GAATTCAACAAATTCTGAAGC	AATGAATCTTGCCTTACAATG	(ATC)9	60	90K SNP	2235368
SSR	*Xfcp661*	TGTGGTGTCTCCTAATTGTTC	CCGTTCTACTTGTTTCTTCCT	(TG)24	60	90K SNP	2294787
EST-STS	*Xfcp662*	CTTGCCGCCGTTAGTTAGAG	GCTACGATCTGGGCATTCAT		60	9K SNP	2293915
SSR	*Xfcp663*	CATAAAATATCTGCTCGGTTG	ACAGATCTAAGTCACCGTCAA	(CCCCCT)3	60	90K SNP	2054789
EST-STS	*Xfcp664*	CATATAGCTTGCGAGGCACATACC	TCAGCCTGCTACAGCCTACAAATC		60	5DS homology	2737650
EST-STS	*Xfcp665*	ATCACCATCTTATTCCGAGTCGTC	CCTTTCCTATCCTTGAGTCCACTGAG		60	5BS NB-LRR	2227605
EST-STS	*Xfcp666*	GGCTTTGAAAACCGAGTGGAC	TGAAGGAAGGACATCCCAAGAAG		60	5BS NB-LRR	2294219
EST-STS	*XBE446811*	CACGACGTTGTAAAACGACTCAGCAC	TAGCCAAAAATCGGTCAAGG		55	5BS6 deletion bin mapped EST	1088662
EST-STS	*XTC266536*	TCGAATCGATTGGCTCTTCT	TCGTCGGTAGCGATGATGTA		60	Rice homology	2228786
EST-STS	*XTC252302*	ACATCTACAATGCCGGTGCT	TGCTCTTGCAGGCATGTTAT		55	Rice homology	2291172
EST-STS	*XTC240301*	CACCGCACTTTAGCTCATCA	AACGACAAGGTTCGCATTTC		55	Rice homology	653474
EST-STS	*XTC249170*	TCGTCGTGTTCCTGTGTGAG	CCAACATCACATCCCAATCC		55	Rice homology	2055544

[a]The repeat motif is shown if the marker is an SSR

[b]9K SNP source: http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=mapdata&name=Wheat,+SynOpDH+GBS+2013;
90K SNP source: http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=mapdata;name=Wheat_2014_90KSNP;show=map;
EST-STS source: https://urgi.versailles.inra.fr/blast/blast.php;
5BS deletion bin map: http://wheat.pw.usda.gov/cgi-bin/westsql/bin_candidates.cgi?bin=5BS6-0.81-1.00;
Rice genome sequences: http://rice.plantbiology.msu.edu/analyses_search_blast.shtml

[c]Chinese Spring survey sequences from https://urgi.versailles.inra.fr/blast/blast.php in survey sequence databanks.

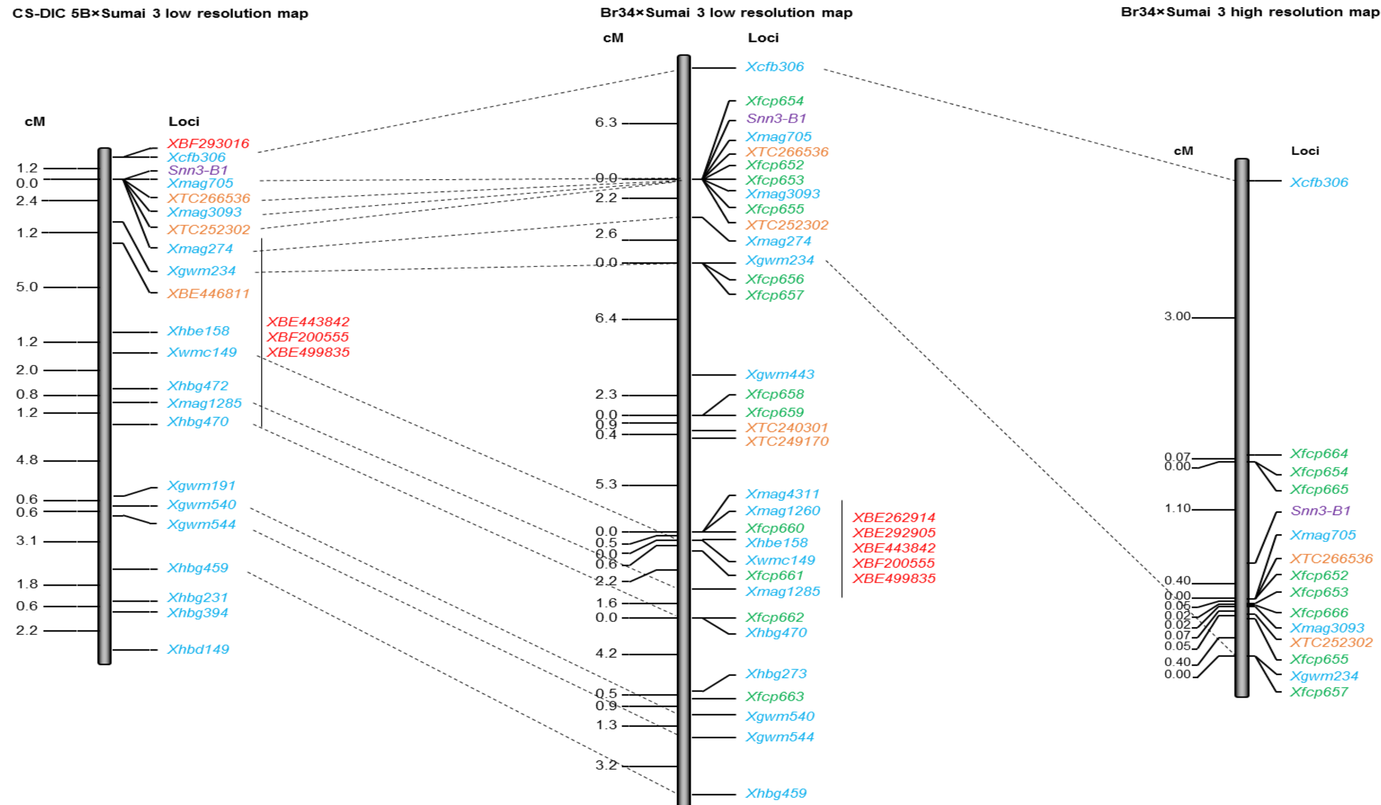


Figure 4.2. Low and high-resolution maps of the *Snn3-B1* region in the CS, BS, and BS-HR F_2 populations. CentiMorgan distances between markers are indicated to the left of the maps and marker loci to the right. Left: Saturation map constructed from CS population of 85 insensitive F_2 plants. Center: Saturation map constructed from BS population of 115 insensitive F_2 plants. Right: High-resolution map constructed from BS-HR population of 2,800 F_2 plants. Markers in blue, green, orange and red represented SSRs, SNP/Survey sequence derived, EST-STS and EST-RFLP markers, respectively. The vertical lines define the region of some RFLP markers mapped and the dash lines connect the same marker mapped in different populations.

Previous macro-colinearity analysis indicated that there was good colinearity between *Snn3-B1*, *Snn3-D1*, rice chromosome 12 and *B. distachyon* chromosome 4 (Zhang et al. 2011). Additional mapping, marker development, and colinearity analysis of the *Snn3-D1* locus compared to rice and *B. distachyon* (data not shown) led to the identification of four wheat 5BS/5DS tentative consensus (TC) sequences homologous to rice genes on chromosome 12 (Table 4.1, 4.2). Markers *XTC266536* and *XTC252302* co-segregated with *Snn3-B1* in both the BS and CS mapping populations, and *XTC240301* and *XTC249170* were located on the proximal side of *Snn3-B1* in the BS population (Figure 4.2).

Of the 52 SSR markers from previously published maps, 15 (29%) and 16 (31%) were polymorphic and subsequently mapped in the BS and CS populations, respectively. Twelve of the SSR markers were mapped in both populations with perfectly conserved order (Figure 4.2).

Discovery sequences were downloaded for 57 SNP markers mapping distal to marker *Xgwm234* from the wheat 9K and 90K consensus maps (Cavanagh et al. 2013; Wang et al. 2014) and used to identify corresponding 5BS survey sequences. This led to the development of twelve markers (Table 4.1), including 10 SSR and 2 STS markers that were mapped in the BS population. Four of the markers, *Xfcp652*, *Xfcp653, Xfcp654*, and *Xfcp655*, cosegregated with *Snn3-B1* (Figure 4.2). Two markers, *Xfcp656* and *Xfcp657,* cosegregated with *Xgwm234* on the proximal side of *Snn3-B1*, and the remaining six markers mapped proximal to *Xgwm234*, which was inconsistent with their locations on the 9 K and 90 K consensus maps (Cavanagh et al. 2013; Wang et al. 2014).

Table 4.2. EST markers mapped to the *Snn3-B1* region with their putative function based on tBLASTx searches against the National Center for Biotechnology Information Database and their homology to rice and *Brachypodium distachyon* genomic sequences.

Marker[a]	NCBI tBLASTx hit	e-value[b]	Rice tBLASTx		*Brachypodium* tBLASTx	
			Hit	e-value	Hit	e-value
XBF293016	**Predicted protein (*Hordeum vulgare*)**	**e-68**	**Os12g44230**	**e-18**	**Bradi4g00430**	**e-38**
XTC270164	**LRR receptor-like S/T Protein kinase (*Brachypodium distachyon*)**	**0**	**Os12g44090**	**e-185**	**Bradi4g00550**	**0**
Xfcp664	Putative disease resistance protein RGA1 (*Aegilops tauschii*)	0	Os11g10610	e-101	Bradi2g36180	e-107
Xfcp665	*Tsn1* gene (*Aegilops speltoides*)	0	Os11g10770	e-104	Bradi2g36180	e-106
Xfcp666	*Tsn1* gene (*Aegilops speltoides*)	0	Os11g10550	e-120	Bradi2g36180	e-165
XTC266536	**Ubiquitin-conjugating enzyme 15-like (*Oryza brachyantha*)**	**e-165**	**Os12g44000**	**e-62**	**Bradi4g00660**	**0**
XTC252302	***BEL1*-like protein 6-like mRNA (*Oryza brachyantha*)**	**0**	**Os12g43950**	**e-280**	**Bradi4g00740**	**0**
Xfcp653	Cysteine-rich receptor-like protein kinase 41 (*Aegilops tauschii*)	e-150	Os11g17380	e-62	Bradi3g01460	e-34
XTC240301	**Pyridine nucleotide-disulfide oxidoreductase like protein (*Brachypodium distachyon*)**	**0**	**Os12g43590**	**e-93**	**Bradi4g00950**	**0**
XTC249170	***whGRP*-1 gene (*Triticum aestivum*)**	**0**	**Os12g43600**	**e-37**	**Bradi4g00940**	**e-110**
XBE446811		NS		NS		NS
XBE262914	Subtilisin like protease protein (*Oryza brachyantha*)	0	Os08g23740	0	Bradi3g20580	0
XBF292905		NS		NS		NS
XBE443842	**GTPase 1 large subunit (*Brachypodium distachyon*)**	**e-38**	**Os12g42370**	**e-25**	**Bradi4g01390**	**e-55**
XBF200555	CYP71C8v1 for P450 (*Triticum aestivum*)	0	Os08g01450	e-124	Bradi1g28860	e-156
XBE449835	N-carbamoylputrescine amidase-like protein (*Brachypodium distachyon*)	0	Os02g33080	e-137	Bradi3g44960	0
Xfcp662	*Tsn1* gene (*Triticum turgidum*)	0	Os011g10550	e-135	Bradi2g36180	e-119

[a]Markers in bold showed colinearity between wheat *Snn3-B1* region and the homologous regions of rice and *Brachypodium distachyon*

[b]NS represents no significant hit

The genetic distances between the most distal marker *Xcfb306* and the most proximal marker *Xhbg459* within the colinear regions of the BS and CS maps were 41.4 cM and 24.1 cM, respectively. Therefore, the recombination frequency near the *Snn3-B1* region of the BS population was nearly twice the level as that of the CS population, which indicated that the BS population was more suitable for high-resolution mapping.

Construction of a high-resolution linkage map of the *Snn3-B1* region

A total of 2,800 F_2 plants of the BS-HR population were genotyped with *Snn3-B1* flanking markers, *Xgwm234* and *Xcfb306*, to identify recombinants for high-resolution mapping. Screening of these plants resulted in the identification of 352 plants with recombination events between *Xgwm234* and *Xcfb306*, which flanked *Snn3-B1* at genetic distances of 1.01 and 4.17 cM, respectively, in the BS-HR population (Figure 4.2).

Markers mapping between *Xgwm234* and *Xcfb306* in the BS population were used to genotype the 352 recombinants of the BS-HR population to place them on the high resolution map. Of the eight markers that cosegregated with *Snn3-B1* on the low resolution map, *Xfcp654* mapped 1.1 cM on the distal side of *Snn3-B1,* and the remaining seven mapped on the proximal side (Figure 4.2). *Xmag705*, *XTC266536,* and *Xfcp652* cosegregated with each other at 0.4 cM proximal to *Snn3-B1*, and the others mapped further to the proximal side. These results delimited *Snn3-B1* to a 1.5 cM interval flanked by *Xfcp654/Xfcp665* and the locus detected by *Xfcp652/ XTC266536/Xmag705*.

Two additional markers, *Xfcp665* and *Xfcp666*, were developed from survey sequences on chromosome 5BS harboring *NB-LRR* genes (Table 4.1, 4.2). *Xfcp665* cosegregated with *Xfcp654* 1.1 cM distal, and *Xfcp666* mapped 0.47 cM proximal to *Snn3-B1* (Figure 4.2). In

addition, one marker, *Xfcp664*, was developed from a chromosome 5D BAC sequence and mapped near *Snn3-D1* (data not shown). This marker also revealed polymorphism between BR34 and Sumai 3 and mapped distal to *Snn3-B1* on 5BS in the BS-HR population (Figure 4.2).

Micro-colinearity between the *Snn3-B1* region, rice and *Brachypodium distachyon*

The marker *XTC266536* was developed based on the colinear segment identified by Zhang et al. (2011) (see above). This and a marker developed from TC270164 that mapped distal to the *Snn3-D1* locus (data not shown), delineated the corresponding *Snn3-B1* region to position 27,264,000-27,308,000 bp in rice and to 260,000-318,000 in *B. distachyon,* which contained eight and ten genes, respectively (Table 4.3).

Table 4.3. Putative genes or gene fragments identified in rice and *Brachypodium* genome between markers *XTC266536* and *XTC270164.*

Markers	Locus	Description	Locus	Description
XTC266536	Os12g44000	Ubiquitin-conjugating enzyme	Bradi4g00660	Ubiquitin-conjugating enzyme
			Bradi4g00650	Starch synthase
			Bradi4g00640	GTP-binding protein
			Bradi4g00630	Hypothetical protein
			Bradi4g00620	HOPZ activated resistance 1
			Bradi4g00610	HOPZ activated resistance 1 protein
			Bradi4g00600	NB-LRR gene
	Os12g44010	Purple acid Phosphate precursor (PAPP)	Bradi4g00590	PAPP
	Os12g44020	S/T protein phosphatase	Bradi4g00580	S/T protein phosphatase
	Os12g44030	PAPP		
	Os12g44040	Transposon		
	Os12g44050	PAPP		
	Os12g44060	Nodulin		
	Os12g44070	Nodulin		
			Bradi4g00570	Hypothetical protein
	Os12g44080	CHCH domain containing protein	Bradi4g00560	CHCH domain containing protein
XTC270164	Os12g44090	Leucine-rich repeat family protein	Bradi4g00550	Leucine-rich repeat family protein

Analysis of these sequences indicated that either there were no homologous wheat sequences, or there were wheat TCs with homology identified but they mapped to locations other than 5BS. Furthermore, using the sequences for *Xfcp653*, *Xfcp662*, *Xfcp664*, *Xfcp665* and *Xfcp666* as queries in BLAST searchers revealed significant hits on rice chromosome 11 and on *B. distachyon* chromosomes 3 and 2 (Table 4.2). These findings indicated that the degree of micro-colinearity within this region was poorly conserved.

Evaluation of markers tightly linked to *Snn3-B1* on a panel of wheat lines

To evaluate the diagnostic capabilities of flanking markers *Xfcp652*, *Xfcp653*, *Xfcp654*, *Xfcp664*, *Xfcp665*, *Xmag705* and *XTC266536*, they were tested on 48 tetraploid and 48 hexaploid wheat varieties and landraces originating from different regions of the world. Among the 48 tetraploid lines, only three (6%) were sensitive to SnTox3 whereas 16 (33%) of the hexaploid lines were sensitive. Among the 96 accessions, *Xfcp654* detected two different alleles with the size of 157 bp and 162 bp plus a null allele (Figure 4.3, Appendix A). *Xmag705* detected a 230 bp allele in some wheat lines and a null allele for the rest (Figure 4.3). *XTC266536* detected a null allele and a 215 bp allele in hexaploid wheat and an additional 220 bp allele in tetraploid wheat (Figure 4.3). The co-dominant marker *Xfcp652* amplified 130 bp and 138 bp fragments. *Xfcp653* was also co-dominant and detected a 215 bp allele, a 231 bp allele, and a null allele. The dominant markers *Xfcp664* and *Xfcp665* amplified 760 bp and 490 bp fragments, respectively. There was no indication of correlation between any of these markers and SnTox3 sensitivity (Appendix A; Figure 4.3), which indicated that none of the markers tightly linked to *Snn3-B1* were diagnostic for presence of the *Snn3-B1* gene.

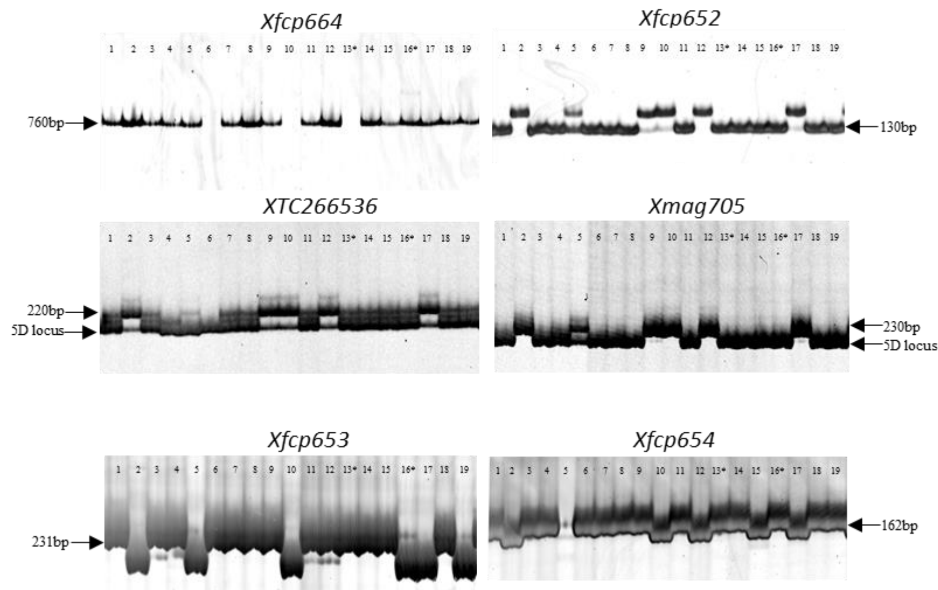


Figure 4.3. Molecular profiles of 19 wheat varieties revealed using markers *Xfcp664*, *Xfcp652*, *XTC266536*, *Xmag705*, *Xfcp653* and *Xfcp654*. Fragments are separated on 8% polyacrylamide gels. Numbers on top represent different cultivars. 1-19 are Rascon 37, Durati, Aconhci 89, Atlas 66, Alsen, Ajaia 9, Bidi 17, Altar 84, D211, Dverd 2, D304, Scaup, Giorgio 331, Scoop 1, Cotrone, Trinakria, Castiglione Pubescente, Anedj, Sincape 90, respectively. * means this line is sensitive to SnTox3.

Discussion

During the past decade, great progress has been achieved in characterizing the wheat-*P. nodorum* pathosystem (Friesen and Faris 2010). However, many of the molecular mechanisms underlying interactions in this system are unknown. The isolation of NE sensitivity genes from the host is needed to gain further understanding of how *P. nodorum* hijacks the cell death-inducing pathways to cause disease. So far, *Tsn1* and *Snn1* are the only wheat host sensitivity genes cloned, and the cloning of these genes has indicated that *P. nodorum* hijacks the PAMP/DAMP- and effector-triggered immunity pathways to gain nutrients and cause disease (Faris et al. 2010; CHAPTER V). Without a reference genome sequence and efficient transformation system in wheat, it makes map-based cloning quite difficult. Here, I developed

saturated and high-resolution maps of the *Snn3-B1* locus. The markers developed in this research delimited *Snn3-B1* to a 1.5 cM interval, which serves as a foundation for the map-based cloning of *Snn3-B1*.

Saturation of the target gene region with molecular markers is a prerequisite for map-based cloning. The deletion bin-mapped ESTs were a unique resource for marker development, comparative mapping and gene discovery (Qi et al. 2004; Lu et al. 2006b). However, the EST markers from 5BS6-0.81-1.0 deletion bin or markers developed from corresponding TC sequences showed a low level of polymorphism between the mapping parents for *Snn3-B1* and only one marker was mapped using PCR. Although a few other EST-derived markers were mapped using RFLP analysis, such markers are not suitable for high throughput genotyping.

Comparative studied have showed good colinearity between wheat and rice at the macro level (Feuillet and Keller 2002; Sorrells et al, 2003). Zhang et al. (2011) demonstrated good levels of colinearity among the wheat *Snn3-B1*, *Snn3-D1*, rice chromosome 12 and *Brachypodium* chromosome 4. Based on the colinearity, four markers were developed in this study and two of them mapped close to *Snn3-B1* (Figure 4.2, Table 4.2). In an effort to develop more markers to further saturate the gene region, micro-colinearity between the *Snn3-B1* gene region, rice and *Brachypodium* was evaluated. However, the results showed that colinearity was highly disrupted, and indicated that the rice and *Brachypodium* sequences were no longer useful for marker development for *Snn3-B1*.

Fortunately, under the International Wheat Genome Sequencing Consortium, wheat survey sequences of all 21 chromosomes that account for 95% of wheat genes have been released (Brenchley et al. 2012; IWGSC 2014). This and the 9 K and 90 K SNP consensus maps

(Cavanagh et al. 2013; Wang et al. 2014) provided extremely valuable resources for marker development. Here, the 5BS SNP marker information as well as the survey sequence information corresponding to the SNP discovery sequences led to the development of twelve markers that were successfully mapped within the targeted region, and two of them closely flanked the *Snn3-B1* locus. In addition, the NB-LRR-like sequences from 5BS identified by T. Wicker (personal communication) led to the development of two additional markers.

Evaluation of the markers developed in this research on a panel of wheat lines indicated that they were not diagnostic for presence of the *Snn3-B1* gene apparently due to a high degree of recombination that has occurred in the *Snn3-B1* region in natural populations. However, these closely linked PCR markers will be very useful for marker-assisted selection against the dominant *Snn3-B1* alleles. The co-dominant markers and also the dominant markers that are in repulsion with *Snn3-B1* will be especially useful in backcrossing schemes to eliminate the *Snn3-B1* allele from elite germplasm because plants homozygous for *Snn3-B1* alleles cannot be distinguished from the desired heterozygotes based on NE infiltration reactions. It is important for researchers to first screen their lines with SnTox3 to know the reaction of each line, then these user-friendly markers can be deployed for marker-assisted selection in crossing schemes without the need to phenotype.

Several studies have shown that some compatible host gene-NE interactions can result in varying levels of disease depending on different factors. For example, Tan et al. (2012) found that different isoforms of ToxA induced quantitative variation in the level of necrosis that developed on wheat lines carrying identical *Tsn1* alleles. The isoforms that induced necrosis most rapidly led to the most sporulation, which indicated that the variation of different isoforms

resulted from selection favoring increased NE sensitivity. Other research showed that different isolates cause different levels of disease through regulation of the transcription level of the NE under infection conditions (Faris et al. 2011). In this work, I observed that a different level of necrosis was induced on BG220 compared to Sumai 3 when infiltrated with SnTox3 (Figure 4.1). While this difference cannot be attributed to variation in SnTox3 isoforms or transcription levels as in the examples above, it is reasonable to speculate that there are different *Snn3-B1* alleles in BG220 and Sumai 3 conferring different NE sensitivity, among which the isoform of Sumai 3 may have higher affinity than that in BG220 and thus lead to more severe necrosis and a higher level of disease. Another 'weak' recognition has also been reported in the SnTox4-*Snn4* interaction, which resulted in a light, mottled necrosis compared to the relatively severe necrosis observed in the other host-NE interactions (Abeysekara et al. 2009). It may be due to the difference of recognition capacity of the host sensitivity gene. Only after the *Snn3-B1* gene is cloned, the mechanism causing the recognition difference may be deciphered.

To clone a gene, high-resolution mapping is required to reduce the confidence interval for the target locus, to obtain closely linked markers, as well as to screen enough recombinants (ideally one recombination per 100 kb) to resolve a gene genetically (Pellio et al. 2005). However, the degree of polymorphism and the recombination frequency within the target gene region are largely dependent on the parents chosen to develop the population. In this study, I observed that the recombination around the *Snn3-B1* region in the BS population was almost double that in the CS population (Figure 4.2). The differences may be due to the recombination suppression around the *Snn3-B1* region in the CS population or recombination enhancement in the BS population. Faris et al. (unpublished data) noticed drastic reduction in recombination

around the *Snn1* region in an F_2 population derived from a cross between CS and a CS-DIC 1B substitution line, in which there was a deletion near the *Snn1* gene in CS-DIC 1B. Here I deployed a similar substitution line, CS-DIC 5B, to make the CS population and it is possible that deletions exist within the *Snn3-B1* region of CS-DIC 5B compared to Sumai 3, which would cause suppression of recombination.

It is also well known that the more closely related two genomes are, the more recombination that occurs (Saintenac et al. 2009). Genetic maps obtained from interspecific crosses are shorter than those from intraspecific crosses (Luo et al. 2000). Moreover, the recombination is known to be low near introgressed segments of alien material (Ji and Chetelat 2007). For the parental lines used in this study, Sumai 3 and BR34 both are hexaploid cultivars, while CS-DIC 5B has a pair of *T. turgidum* ssp. *dicoccoides* 5B chromosomes. It is likely that more sequence variation and indel polymorphisms exist between the *T. turgidum* ssp. *dicoccoides* 5B chromosomes and the Sumai 3 5B chromosomes as compared to the 5B chromosomes of BR34 and Sumai 3, which would provide an explanation for the occurrence of higher recombination frequency in the BS population compared to the CS population. Therefore, it is wise to start the genetic mapping with several different populations and choose the one with the highest recombination rate within the target region for high-resolution mapping and map-based cloning.

Additionally, it is important to know that there is a trade-off between polymorphism and recombination. A cross between two closely related lines will yield high recombination frequency but the polymorphism level will be very low. I also noticed in this study that none of the 53 EST PCR markers showed polymorphism between Sumai 3 and Br34. So one needs to

find a balance between recombination rate and polymorphism to choose the best population for fine-mapping.

The markers developed in this research and recombinants screened through high-resolution mapping provide a basis for the map-based cloning of *Snn3-B1*. Furthermore, sequence information from the BAC contigs covering *Snn3-D1* region will be extremely useful for marker exploration and candidate gene identification for *Snn3-B1* (Faris et al. unpublished data). Under the International Wheat Genome Sequencing Consortium, the generation of a 5BS chromosome arm-based physical map and its marker information will greatly expedite the positional cloning of *Snn3-B1*.

Once both *Snn3-B1* and *Snn3-D1* are cloned, it will be interesting to study the evolution of these homoelogous genes and determine how they retained their function to recognize SnTox3 since diverging from a common ancestor 2.5 million years ago. A recent report showed that SnTox3-induced host cell death responses were distinctly different from those induced by SnToxA (Winterberg et al. 2014). Therefore, cloning of the *Snn3* genes will further deepen our understanding of necrotrophic effector triggered susceptibility and broaden our knowledge of the complexity of this model system. At the same time this knowledge will help us to devise appropriate strategies for manipulating host resistance to better control SNB and other diseases.

References

Abeysekara N, Friesen TL, Keller B, Faris JD (2009) Identification and characterization of a novel host-toxin interaction in the wheat-*Stagonospora nodorum* pathosystem. Theor Appl Genet 120:117-126

Alfares W, Bouguennec A. Balfourier F, Gay G, Berges H, Vautrin S, Sourdile P, Bernard M, Feuillet C (2009) Mapping and marker development for the crossability gene *SKr* on chromosome 5BS of hexaploid wheat. Genetics 183:469-481

Bennett MD, Leitch IJ (1995) Nuclear DNA amounts in angiosperms. Ann Bot 76:113-176

Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunoca A, See D, Bai GH, Pumphrey M, Tomar L, Wong D, Kong S, Reynolds M, da Silva ML, Bockelman H, Talbert L, Anderson JA, Dreisigacker S, Baenziger S, Carter A, Korzun V, Morrell PL, Dubcovsky J, Morell MK, Sorrells ME, Hayden MJ, Akhunov E (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proc Natl Acad Sci USA 110:8057-8062

Chalupska D, Lee HY, Faris JD, Evrard A, Chalhoub B, Haselkorn R, Gornicki P (2008) *Acc* homoeoloci and the evolution of wheat genomes. Proc Natl Acad Sci USA 105:9691-9696

Faris JD, Zhang ZC, Lu HJ, Lu S.W., Reddy L, Cloutier S, Fellers JP, Meinhardt SW, Rasmussen J. B., Xu SS, Oliver RP, Simons KJ, Friesen TL (2010) A unique wheat disease resistance-like gene governs effector-induced susceptibility to necrotrophic pathogens. Proc Natl Acad Sci USA 107:13544-13549

Faris JD, Zhang ZC, Rasmussen JB, Friesen TL (2011) Variable expression of the Stagonospora nodorum effector SnToxA among isolate is correlated with levels of disease susceptibility in wheat. Mol Plant Microbe Interact 24:1419-1426

Friesen TL, Stukenbrock EH, Liu ZH, Meinhardt SW, Ling H, Faris JD, Rasmussen JB, Solomon PS, Mcdonald BA, Oliver RP (2006) Emergence of a new disease as a result of interspecific virulence gene transfer. Nat Genet 38:953-956

Friesen TL, Meinhardt SW, Faris JD (2007) The *Stagonospora nodorum* – wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. Plant J 51:681-692

Friesen TL, Zhang ZC, Solomon PS, Oliver RP, Faris JD (2008) Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. Plant Physiol 146:682-693

Friesen TL, Faris JD (2010) Characterization of the wheat-*Stagonospora nodorum* disease system: what's the molecular basis of this quantitative necrotrophic disease interaction? Can J Plant Pathol 32:20-28

Friesen T, Chu C. Xu SS, Faris J (2012) SnTox5-*Snn5*: a novel *Stagonospora nodorum* effector-wheat gene interaction and its relationship with the SnToxA-*Tsn1* and SnTox3-*Snn3-B1* interactions. Mol Plant Pathol 13:1101-1109

Gao Y, Faris JD, Liu ZH, Xu SS, Friesen TL (2014) Identification and characterization of the SnTox6-*Snn6* interaction in the wheat-*Stagonospora nodorum* pathosystem. Mol Plant Microbe Interact (accepted)

Gill KS, Gill BS, Endo TR, Boyko EV (1996) Identification and high-density mapping of gene-rich regions in chromosome group 5 of wheat. Genetics 143:1001-1012

The International Wheat Genome Sequencing Consortium (IWGSC) (2014) A chromosome-based draft sequence of the hexaploid bread wheat genome. Science DOI: 10.1126/science.1251788

Ji Y, Chetelat RT (2007) GISH analysis of meiotic chromosome pairing in *Solanum lycopersicoides* introgression lines of cultivated tomato. Genome 50:825-833

Kosambi, DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172-175

Krattinger S, Wicker T, Keller B (2009) Map-based cloning of genes in *Triticeae* (wheat and barley). Plant genetics and genomics: Genetics and genomics of the *Triticeae*. Feuillet C, Muehbauer GJ (Eds) New York, NY Springer

Kumar S, Goyal A, Mohan A, Balyan HS, Gupta PK (2013) An integrated physical map of simple sequence repeats in bread wheat. Aus J Crop Sci 7:460-468

Liu ZH, Faris JD, Meinhardt SW, Ali S, Rasmussen JB, Friesen TL (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

Liu ZH, Friesen TL, Meinhardt SW, Ali S, Rasmussen JB, Faris JD (2004b) Quantitative trait loci analysis and mapping of seedling resistance to Stagonospora nodorum leaf blotch in wheat. Phytopathology 94:1061-1067

Liu ZH, Friesen TL, Ling H, Meinhardt SW, Oliver RP, Rasmussen JB, Faris JD (2006) The *Tsn1*-ToxA interaction in the wheat-*Stagonospora nodorum* pathosystem parallels that of the wheat-tan spot system. Genome 49:1265-1273

Liu ZH, Faris JD, Oliver RP, Tan KC, Solomon PS, McDonald MC, McDonald BA, Nunez A, Lu SW, Rasmussen JB, Friesen TL (2009) SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the *Snn3* gene. PLoS Pathogens 5:e1000581. doi:10.1371/journal.ppat.1000581

Liu ZH, Zhang ZC, Faris JD, Oliver RP, Syme R, McDonald MC, McDonald BA, Solomon PS, Lu SW, Shelver WL, Xu SS, Friesen TL (2012) The cysteine rich necrotrophic effector SnTox1 produced by *Stagnospora nodorum* triggers susceptibility of wheat lines harboring *Snn1*. PLoS Pathogens 8:e1002467 Doi:10.1371/journal.ppat.1002467

Lorieux M (2012) MapDisto: fast and efficient computation of genetic linkage maps. Mol Breeding 30:1231-1235

Lu HJ, Faris JD (2006a) Macro- and microcolinearity between the genomic region of wheat chromosome 5B containing the *Tsn1* gene and the rice genome. Funct Integrative Genomics 6:90–103

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

Luo MC, Yang ZL, Kota RS, Dvorak (2000) Recombination of chromosomes 3A(m) and 5A(m) of Triticum monococcum with homeologous chromosomes 3A and 5A of wheat: the distribution of recombination across chromsomes. Genetics 154:1301-1308

Pellio B, Streng S, Bauer E, Stein N, Perovic D, Schiemann A, Friedt W, Ordon F, Graner A (2005) High-resolution mapping of the *Rym4*/*Rym5* locus conferring resistance to the barley yellow mosaic virus complex in barley. Thero Appl Genet 110:283-293

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD, Akhunov ED, Dvorak J, Linkiewicz AM, Ratnasiri A, Dubcovsky J, Bermudez-Kandianis CE, Greene RA, Kantety R, La Rota CM, Munkvold JD, Sorrells SF, Sorrells ME, Dilbirligi M, Sidhu D, Erayman M, Randhawa HS, Sandhu D, Bondareva SN, Gill KS, Mahmoud AA, Ma XF, Miftahudin, Gustafson JP, Conley EJ, Nduati V, Gonzalez-Hernandez JL, Anderson JA, Peng JH, Lapitan NLV, Hossain KG, Kalavacharla V, Kianian SF, Pathan MS, Zhang DS, Nguyen HT, Choi DW, Fenton RD, Close TJ, McGuire PE, Qualset CO, Gill BS (2004) A chromosome bin map of 16,000 expressed sequenced tag loci and distribution of genes among the three genomes of polyploid wheat. Genetics DOI: 10.1534/genetics.104.034868

Reddy L, Friesen TL, Meinhardt SW, Chao S, Faris JD (2008) Genomic analysis of the *Snn1* locus on wheat chromosome arm 1BS and the identification of candidate genes. Plant Genome 1:55-66

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

Rozen S, Skaletsky HJ (2000) Primer 3 on the WWW for general users and for biologist programmers. In Bioinformatics methods and protocols: methods in molecular biology (Akrawetz S and Misener S eds) Totawa, NJ, Humana 365-386

Saintenac C, Falque M, Martin OG, Paux E, Feuillet C, Sourdille P (2009) Detailed recombination studies along chromosome 3B provide new insights on crossover distribution in wheat. Genetics DOI: 10.1534/genetics.108.097469

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat. Theor Appl Genet 106:530-538

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550-560

Sourdile P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat. Func Integrative Genomics 4:12-25

Tan KC, Ferquson-Hunt M, Rybak K, Waters OD, Stanley WA, Bond CS, Stukenbrock EH, Friesen TL, Faris JD, McDonald BA, Oliver RP (2012) Quantitative variation in effector activity of ToxA isoforms from Stagonospora nodorum and pyrenophora tritici-repentis. Mol Plant Microbe Interact 25:515-522

Torada A, Koike M, Mochida K, Ogihara Y (2006) SSR-based linkage map with new markers using an intraspecific population of common wheat. Theor Appl Genet 112:1042-1051

Wang SC, Wong D, Forrest K, Allen A, Chao S, Huang B, Maccaferri M, et al. (2014) Characterization of polyploid wheat genomic diversity using a high-density 90000 single nucleotide polymorphism array. Plant Biotechnol J 12:787-796

Winterberg B, Du Fall LA, Song XM, Pascovici D, Care N, Molloy M, Ohms S, Solomon PS (2014) The necrotrophic effector protein SnTox3 re-programs metabolism and elicits a strong defence response in susceptible wheat leaves. BMC Plant Biol 14:215

Xue S, Zhang Z, Lin F, Kong Z, Cao Y, Li C, Yi H, Mei M, Zhu H, Wu J, Xu H, Zhao D, Tian D, Zhang C, Ma Z (2008) A high-density intercarietal map of the wheat genome enriched with markers derived from expressed sequence tags. Theor Appl Genet 117:181-189

Zhang ZC, Friesen TL, Simons KJ, Xu SS, Faris JD (2009) Identification, development and validation of markers for marker-assisted selection against the *Stagonospora nodorum* toxin sensitivity genes *Tsn1* and *Snn2* in wheat. Mol Breeding 23:35-49

Zhang Z, Friesen TL, Xu SS, Shi GJ, Liu ZH, Rasmussen JB, Faris JD (2011) Two putatively homoeologous wheat genes mediate recognition of SnTox3 to confer effector-triggered susceptibility to *Stagonospora nodorum*. Plant J 65:27-38

CHAPTER V. MAP-BASED CLONING AND CHARACTERIZATION OF THE *SNN1* GENE IN WHEAT

Abstract

Wheat-*Parastagonospora nodorum* interactions involve pathogen-produced necrotrophic effectors (NEs) that are recognized by corresponding dominant host sensitivity genes to cause disease. The *Snn1* gene confers sensitivity to the NE SnTox1. Here I report the positional cloning of *Snn1* and further characterization of its structure, expression and allelic diversity. A bacteria artificial chromosome (BAC) contig of about 2.5 Mb in size was identified to span the *Snn1* locus through screening of the previously constructed 1BS minimum tiling path (MTP). Additional markers developed from BAC end sequences (BESs) and survey sequences were used to further delineate the *Snn1* gene to a 0.16 cM segment that corresponded to a physical segment spanned by four overlapping BAC clones. Sequencing and bioinformatic analysis of these clones revealed seven putative candidate genes. Marker development and linkage analysis of these genes showed that only one cosegregated with *Snn1* in a population of 17,000 gametes. The cosegregating gene, a member of the wall-associated kinase (WAK) class of receptor kinases, was validated as *Snn1* by comparative sequence analysis of the *Snn1* gene in ethylmethane sulfonate (EMS)-induced SnTox1-insensitive mutants with the wild type *Snn1* sequence. *Snn1* exhibited a diurnal expression pattern and peaked during the subjective morning. Evaluation of a large number of wheat and durum varieties, relatives, and progenitors indicated that the ability of *Snn1* to recognize SnTox1 likely arose in a tetraploid progenitor of common wheat. These results demonstrate that *P. nodorum* can

hijack multiple host pathways driven by different classes of genes that typically confer resistance to biotrophic pathogens.

Introduction

Plants are subject to attack by a variety of pathogens and rely completely on innate immunity for defense. Plants have evolved several layers of protection against invading pathogens. The first is the detection of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) (Jones and Dangl 2006). PRRs are cell surface receptors in the host and PAMPs are usually conserved molecules that serve an essential function within a class of microbes. Some PRRs also recognize damage-associated molecular patterns (DAMPs), which are endogenous compounds released during pathogen infection or wounding. PRR recognition of PAMPs/DAMPs leads to PAMP-triggered immunity (PTI), which results from the activation of a host response (Zipfel 2009; Mohaghan and Zipfel 2012). Well-characterized interactions include *FLS2* for bacteria flagellin (Gomez-Gomez and Boller 2000), *EFR* for bacterial EF-Tu (Zipfel et al. 2006), and *CERK1* for fungal chitin (Miya et al. 2007). Successful pathogens have evolved the ability to suppress PTI by secreting effectors. Accordingly, a second layer of immunity termed effector-triggered immunity (ETI) involves the recognition of pathogen effectors by proteins that usually contain nucleotide binding (NB) and leucine-rich repeat (LRR) domains. ETI results from a programmed response much the same as for PTI (Dodds and Rathjen 2010).

In general, PTI and ETI are mechanisms used by plants to confer resistance to bacteria, viruses, nematodes, insects, and fungi with biotrophic lifestyles. Less is known about how plants combat disease caused by necrotrophic fungal pathogens, which, as

opposed to biotrophs, require dead tissue to survive. It was previously thought that necrotrophs were generalists and killed their hosts by secreting a barrage of cell wall degrading enzymes. However, work in recent years has shown that many necrotrophic pathogens produce necrotrophic effectors (NEs) that, when recognized by a specific gene in the host, induce cell death followed by disease (Oliver and Solomon 2010). The plant NE-sensitivity genes cloned to date harbor NB and LRR domains, features of classic disease 'resistance' genes involved in ETI (Lorang et al. 2007; Nagy and Bennetzen 2008; Faris et al. 2010). Additional studies of downstream events revealed hallmarks of ETI (Faris et al. 2010; Pandelova et al. 2009) demonstrating that some necrotrophs have acquired the ability to hijack the host's own ETI pathway to cause disease.

Parastagonospora nodorum is a necrotrophic fungal pathogen of wheat that causes Stagonospora nodorum blotch (SNB), a devastating disease causing 10-20% grain yield losses and reductions in grain quality (Bockus et al. 2010). NEs produced by the pathogen are the major disease determinants, and are recognized by cognate host sensitivity genes in an inverse gene-for-gene fashion (Friesen and Faris 2010). Recognition of the NE by the host gene leads to a compatible reaction and ultimately susceptibility, whereas the absence of either the NE or the corresponding host sensitivity gene results in an incompatible interaction and a resistance response (Friesen and Faris 2010). To date, nine host gene-NE interactions have been characterized in this pathosystem (Liu et al. 2006; Faris et al. 2010; Liu et al. 2004a; Liu et al. 2004b; Liu et al. 2012; Reddy et al. 2008; Friesen et al. 2007; Zhang et al. 2009; Friesen et al. 2008; Zhang et al. 2011; Abeysekara et al. 2009; Friesen et al. 2012; Gao et al. 2014; CHAPTER III). The genes encoding three NEs, SnToxA, SnTox1 and SnTox3, have

been cloned from *P. nodorum* (Friesen et al. 2006; Liu et al. 2012, 2009) and *Tsn1* is the only sensitivity gene cloned from wheat (Faris et al. 2010). The *Tsn1* gene harbors features typically observed in 'classic' plant disease resistance genes including protein kinase, NB and LRR domains. Compatible *Tsn1*-SnToxA interactions lead to the induction of defense responses including *MAPK* gene expression, *PR* gene up-regulation, an oxidative burst, and programmed cell death (PCD) (Adhikari et al. 2009; Pandelova et al. 2009; Lu et al. 2014).

SnTox1 was the first identified NE from *P. nodorum*, and it is recognized by the host sensitivity gene *Snn1* (Liu et al. 2004a). The SnTox1-*Snn1* interaction explained as much as 58% of the phenotypic variation in SNB susceptibility, indicating that this interaction played an important role in disease (Liu et al. 2004b). *Snn1* was previously mapped to a gene-rich region of the short arm of wheat chromosome 1B (Liu et al. 2004a). Subsequent saturation and high-resolution mapping delineated *Snn1* to a 0.46 cM region (Reddy et al. 2008). Here, I isolated and characterized the *Snn1* gene to gain understanding of the mechanisms associated with compatible interactions in this wheat-*P. nodorum* pathosystem.

Materials and Methods

Plant Materials

A high-resolution mapping population was developed from a cross between the hexaploid common wheat (*Triticum aestivum* ssp. *aestivum* L., $2n = 6x = 42$, AABBDD genomes) landrace Chinese Spring (CS) and a genetic stock where a pair of 1B chromosome from the common wheat variety Hope was substituted for the CS 1B chromosomes in the CS background (CS-Hope 1B). The population consisted of 8,500 F_2

plants (17,000 gametes) and was used to map *Snn1* and anchor the BAC contig to the genetic linkage map. CS was also used for mutagenesis. A total of 826 tetraploid and hexaploid *Triticum* accessions and 123 *Ae. speltoides* accessions were used to determine the prevalence of *Snn1* alleles (Appendix B).

SnTox1 culture production and infiltrations

SnTox1 cultures were obtained from SnTox1-expressing *Pichia pastoris* cultures as described by Liu et al. (2012). SnTox1 culture filtrates were infiltrated into fully expanded secondary leaves of wheat plants using a 1-ml syringe with the needle removed. Immediately after infiltration, the boundaries of the infiltrated sites were marked with a non-toxic felt pen. Reactions were evaluated 3 d after infiltration and scored as either insensitive (no necrosis) or sensitive (necrosis).

Conidial spores of the SnTox1-producing strain Sn2000 were inoculated on CS and CSems lines as previously described (Liu et al. 2012). The inoculum was prepared by diluting the spore suspensions to 1×10^6 spores/ml and adding 2 drops of Tween-20 per 100 ml of inoculum. After applying conidial suspensions by air spray to plants at the 2-leaf stage until runoff, plants were then placed in a mist chamber at 100% relative humidity at 21°C for 24 hr followed by 6 days of incubation in the growth chamber under a 12-hr photoperiod. Disease reactions were scored 7 days post-inoculation using a 0-5 lesion type scale (Liu et al. 2004b).

For the experiment to assess *MAPK* gene expression, I applied SnTox1 cultures to plants by air-spray (Liu et al. 2012) as opposed to infiltration to avoid any response to wounding that might occur when infiltrating with a syringe. Spray applications of SnTox1 were carried out by diluting the SnTox1 yeast culture five times with distilled

water and adding 2 drops of Tween-20 per 100 ml of solution. Spray inoculation was performed by air-spray on 2-wk-old plants until runoff. Then plants were kept in the growth chamber under a 12-hr photoperiod and sampled at specific time points as outlined below.

Genetic linkage and physical mapping

The population of 8,500 F_2 plants was screened with the PCR-based markers *Xpsp3000* and *Xfcp618*, which were previously shown to delineate *Snn1* to a 0.9 cM interval in a different population (Reddy et al. 2008). The *Snn1* phenotypic marker was placed on the linkage map relative to these markers, and the PCR-based markers *XBE498831*, *Xfcp619* and *Xfcp624* were evaluated on the plants with recombination events between *Xpsp3000* and *Xfcp618* using standard PCR conditions and visualization methods as described (Faris et al. 2010). Linkage distances were calculated manually by dividing the number of recombinants by the total number of gametes analyzed (17,000) multiplied by 100, and expressed as map units. A population of this size has a map resolution of 0.006 map units.

Primer sets for markers *Xfcp618*, *Xfcp619*, and *Xfcp624* were used to screen the minimum tiling path (MTP) for the CS chromosome arm 1BS BAC library as described in Raats et al. (2013). A total of 40 primer pairs for BAC end sequences (BESs) of 19 of the MTP BACs generated by Raats et al. (2013) were tested for polymorphism between CS and CS-Hope 1B (Table 5.1). In order to develop additional markers to anchor the BAC contig to the linkage map, I used BESs as queries for searches against the wheat survey sequences (http://wheat-urgi.versailles.inra.fr/) of chromosome arm 1BS to identify the corresponding survey sequence. The survey sequences were evaluated for

presence of simple sequence repeats (SSRs), which were targeted for primer design and marker development (Table 5.1).

Identification of candidate genes

The four BACs (TaaCsp1BS002N12, TaaCsp1BS093D06, TaaCsp1BS106C02, and TaaCsp1BS134D22) flanking the *Snn1* gene region were sequenced using an Ion Torrent™ next generation sequencer (Life Technologies). Enzymatic shearing of 1 μg BAC DNA and generation of barcoded libraries were carried out by using the Ion Plus Fragment Library Kit and Ion Xpress™ Barcode 1-16 Kit. Templates were prepared by using the Ion PGM™ Template OT2 200 Kit and Ion OneTouch™ 2 System. Sequencing was conducted by using Ion PGM™ with an Ion 314™ chip and the Ion PGM™ 200 Sequencing Kit v2. The Torrent Suite 3.4.2 was used for base calling. Sequences were *de novo* assembled by using DNASTAR SeqMan NGen 11.0.0, then subjected to BLASTx searches of the NCBI nr database to identify putative protein coding sequences.

Sequences with similarity to putative proteins were targeted for primer design and marker development (Table 5.2). The gene-based markers were also tested on the four BACs by standard PCR amplification followed by agarose electrophoresis.

Mutagenesis and validation of the WAK gene

About 1,500 seeds of CS were treated with ethyl methane sulfonate (EMS) as described (Faris et al. 2010), and a total of 1,360 M_2 families consisting of 14 plants per family were infiltrated with SnTox1 and scored for the presence/absence of necrosis as described above. M_2 generation plants showing insensitivity to SnTox1 were self-pollinated to obtain M_3 generation plants, which were screened with SnTox1 for confirmation.

Table 5.1. PCR-based molecular markers used to anchor the Chinese Spring chromosome 1BS BAC contig to the genetic linkage map containing the *Snn1* gene.

Marker	Source	CS survey sequence	F primer	R primer	BAC(s) detected
Xfcp618	SSR from previous fine-mapping	2053630	TCTACATACGGACTGAAATG GATAC	GATTGAGACTCTGGTTACAT AAGACTACTC	TaaCsp1BS027H03 TaaCsp1BS009M24
X6A04	Survey sequence corresponding to BES	3475551	GACAGCACAAGACTCGGACA	GTATCTCAAGCGGGGAACAA	TaaCsp1BS095K24
X7O03	Survey sequence corresponding to BES	3442698	GGTCCTACCCGCTTCCTAAC	GCGCTCTCTCCTATGATTGC	TaaCsp1BS095K24 TaaCsp1BS007O03
XBE498831	EST-based STS from Reddy et al. (2008)	3440935	ATTTTCAGGAGTTAGTGTCAT GCTC	GTTAGTGTGCTTGGTAAAATT ACGG	TaaCsp1BS001M21 TaaCsp1BS018B09 TaaCsp1BS115G12
X73H08	Survey sequence corresponding to BES	3446520	GGAAGCGTTTCATGATCACC	TCTAATCCCCCTCCACCTCT	TaaCsp1BS073H08 TaaCsp1BS134D22
X93D06	Survey sequence corresponding to BES	3446364	GCCGAGAAATGGAGGAAGCA	GACATCACCGCCTCGCTCTT	TaaCsp1BS002N12 TaaCsp1BS007P09
Xfcp624	SSR from previous fine-mapping	3433871	GTGCTGCTAAATGGATTCCTA AGC	CCAAACTGGCAAAAGATTGA GC	TaaCsp1BS060E11 TaaCsp1BS084J20
X117L19	Survey sequence corresponding to BES	3438216	CAAATCCAAAGAATGCGACC	CGGCTTTTTAATGACCCTTG	TaaCsp1BS117L19 TaaCsp1BS094B15
Xfcp619	SSR from previous fine-mapping	3482199	TTGAAAATCTGAAGCCCCCA G	TCCGCAAAAATGTTCCACTC AC	TaaCsp1BS121D11 TaaCsp1BS046E02 TaaCsp1BS130O13
X130O13	Survey sequence corresponding to BES	3445768	AGGGACAAGCTTTTTCGGAC	CATGAATGCGAAAGACCAGA	TaaCsp1BS130O13
X28G13	ISBP from BES	3461863	GTGCAGTGGGGCTAACAAAT	TTCATGTATTGCTTGTCACAT CA	TaaCsp1BS028G139

Table 5.2. PCR-based molecular markers developed for the seven candidate gene sequences.

Marker	Predicted protein[a]	CS survey sequence	F primer	R primer	BAC(s)
X3446364	HCBP pathogenesis protein-like	3446364	GCACCCATGAACTCCTCATC	GGTTCGACGTGTATGGGAAC	TaaCsp1BS002N12 TaaCsp1BS007P09
X500019	Leucine rich-repeat receptor-like	3483553	AGGTTGACAAAGCGCTCAAT	CTACAATCCGAGCATCAGCA	TaaCsp1BS134D22 TaaCsp1BS106C02
Xfcp667	Wall-associated kinase	3476283	TGCGTCGATAGGAGTGAGTG	ATGGCGTAGGAGCACGGGTA	TaaCsp1BS134D22
X3446520	RPP8-like	3446520	AGCACCGAGCTTGATTCTGT	ATCCGATGCTCTCTGCTCAT	TaaCsp1BS134D22
X600041	Serine/threonine protein kinase-like	3446520	CCGGAAAAACCACTTTGTGT	GACCGTCGGATTTGATTTTG	TaaCsp1BS134D22
X600222	S-receptor-like	3482641	CTTAATCCGGGCATGTTGAA	TCTGACTCCTTGCATTCTCG	TaaCsp1BS073H08 TaaCsp1BS134D22
X600110	NB-LRR-like	3457459	AGCAGAGCAACCTTTGGTGT	CGGGCGTAAGATTTGACAAT	TaaCsp1BS073H08 TaaCsp1BS134D22

[a] Based on BLASTx searches of the NCBI nr database.

The full-length genomic sequence of the *WAK* gene was obtained from each of the mutants using the primers in Table 5.3. The gene was amplified in four overlapping fragments and three independent PCR reactions for each fragment were sequenced to eliminate PCR errors. Sequences of the mutants were compared to the wild type *WAK* gene sequence of CS using the software Sequencher v4.8 (Gene Codes Corporation). The wild type and mutant sequences were deposited in Genbank under accession numbers KP085710 and KP085734 through KP085749 (Appendix F).

***Snn1* characterization**

Total RNA was isolated from leaf tissue of CS and used for cDNA synthesis as described by Faris et al. (2010). The full-length cDNA was amplified in three overlapping fragments using the primer pairs 1, 2 and 3 in Table 5.3, sequenced, and compared with the genomic sequence to identify the splicing junctions. The 5′ and 3′ rapid amplification of cDNA ends (RACE) was performed using Advantage^R cDNA polymerase mix (Clontech) to determine the 5′ and 3′ UTRs. The 1st round PCR of the 5’ RACE reaction was performed using the primer UPM provided in the kit combined with the gene-specific primer 5′RACE_R4: CAAGCTCGATCGACGAGAAGGACCAATC. The 2nd round PCR of the 5′ RACE was carried out using the primer NUP provided in the kit and the gene-specific primer 5′ RACE_R3: AGGGGGATACGAGGAGTCGTTGCA CA. The 1st round PCR of the 3’ RACE reaction was performed using the primer UPM provided in the kit combined with the gene-specific primer 3′ RACE_F3: CAGCACAA TCAGCGGACGGTCTAGCTT. The 2nd round PCR of the 3′ RACE was carried out using the primer NUP provided in the kit and the gene-specific primer 3′RACE_F2: CACACTGGACAAGTCATCGGCGACA. PCR products of RACE reactions were

purified, subcloned and sequenced using the Sanger method. The full-length cDNA sequence was deposited in Genbank under accession number KP091701 (Appendix F).

Table 5.3. Primers used to amplify fragments for sequencing of *Snn1* from cDNA and genomic DNA.

Pairs	Forward primer	Reverse primer	Annealing temperature
1	5UTRF1: CGGGGAAGTTAGACGATTCCTTG	2ExonF: ATGCGGGAGCTTGCATTCAT	65
2	600015R: TGCGCCAACTCAACACATAC	3ExonF3: CGAGCAGTTGCTCCGCTACC	65
3	3ExonR: AAGGTGGCCCTCAACTTGGA	3UTRR1: GCCTCAGCTTCCCCTTTTTGTAG	65
4	3476283R3: TGCGTCGATAGGAGTG	3476283F9: ATGGCGTAGGAGCACGGGTA	65
5	1ExonR: CGGCTGGAGAGTCGATGCTT	600015F: TAATTTGGCAAGGCAGGAGT	65
6	2IntronR: GGGTGTGCCACATCACCAAA	3ExonF2: GCCAATGGCACCTTGATGG	65
7	3ExonR1: CAAGGTGCCATTGGCGGTAT	3UTRF4: TTGCCTCAGCTTCCCCTTTTT	65

Southern analysis was conducted on the set of CS nullisomic-tetrasomic lines, where a pair of missing chromosomes is compensated for by a pair of homoeologous chromosomes (Sears 1954). Restriction digestion and Southern hybridization were performed according to Reddy et al. (2008). DNA was digested with *Bam*HI and probed with FCG36 derived from the 5′ UTR and coding region of the *Snn1*. Probe FCG36 was amplified from BAC TaaCsp1BS134D22 using primers 3476283F9: ATGGCGTAGGA GCACGGGTA and 3476283R3: TGCGTCGATAGGAGTG.

Coding and deduced amino acid sequences were used in BLAST searches of the NCBI database to identify sequences homologous to the *Snn1* gene. Major domains of the *Snn1* gene were annotated using numerous tools available at the Expasy Bioinformatics Resource Portal (http://www.expasy.org).

Phylogenetic analysis

The full-length genomic sequence of *Snn1* was obtained from 24 accessions of different ploidy levels (shown in bold with asterisks in Appendix B) (Genbank numbers KP085710 through KP085733, Appendix F). Four overlapping fragments representing

the complete gene were amplified using the primers (pairs 4-7) in Table 5.3. Three independent PCR reactions for each fragment were sequenced to eliminate PCR errors. Sequences were assembled using the software Sequencher v4.8. The phylogenetic tree was constructed from CLUSTALW alignments of the *Snn1* sequences using the Neighbor Joining method and multiple distance-based methods available in MacVector v10.6. Confidence values for nodes were calculated using 1000 bootstraps.

Transcriptional expression

The hexaploid wheat CS was used for *Snn1* transcriptional analysis, and the wheat *GAPDH* gene was used as an internal control as described in Faris et al. (2010). Plants were grown in a growth chamber at 21 °C with a 12 hr light/dark cycle (8 am/8 pm) except for the continuous dark treatment, which was performed under the same conditions without light.

To study the tissue specific expression of *Snn1*, samples were collected from leaves, stems and roots at the seedling stage, and immature spikes at Feekes wheat growth stage 8. Total RNA was extracted from plant tissues by using the RNeasy Plant Mini Kit (Qiagen). First-stand cDNA was synthesized from 2 µg of total RNA using TaqMan Reverse Transcription Reagents (Applied Biosystems). RT-PCR was carried out using primers 60015R: TGCGCCAACTCAACACATAC and Snn1_RT_R4: GCCAATG GCACCCACAGC for *Snn1* and GAPDH.F152: CAACGCTAGCTGCACCACTAACT and GAPDH.R338: GCTGCTTGGAATGATGTTGA for *GAPDH* on the cDNAs from the different plant tissues.

Snn1 transcription were investigated on plants grown under a 12 hr light/dark cycle and plants placed under continuous darkness beginning at the time of collection of

the first sample. Samples were collected from 2-wk-old seedlings every 3 hrs for three consecutive days as described (Faris et al. 2010).

Further studies on the effects of light and dark were conducted by collecting samples from 2-wk-old plants at 11 am, then placing plants under complete darkness for 3 hrs and collecting samples at 2 pm, followed by the placement of plants back into the light for 2 hrs and collecting samples at 4 pm. Samples were also taken from control plants grown in 12 hr light/dark conditions at the same time points.

The effects of SnTox1 on *Snn1* transcription were evaluated by infiltration of 2-wk-old plants. Treatments included SnTox1-infiltrated, water-infiltrated, and non-infiltrated plants. Plants were grown in a growth chamber under a 12 hr light/dark cycle and infiltrations were performed as previously described. Samples were collected from infiltrated regions of SnTox1-infiltrated, water-infiltrated plants and non-infiltrated controls at 0-, 3-, 6-, 12-, 24-, 36-, and 48-hr time points for RNA isolation. All transcriptional experiments consisted of three biological replicates.

To avoid wounding effects on the induction of *MAPK* genes, the transcription of *MAPK3* and *MAPK6* was studied by spray inoculation of SnTox1 cultures on 2-wk-old plants as described in Liu et al. (2012), with water-sprayed and non-sprayed plants as controls. RNA samples were collected at 0-, ¼-, ½-, 1-, 3-, 6-, 12-, 24-, 36-, and 48-hr time points. RT-PCR was carried out using primers TaMPK3F: TACATGAGGCACCT GCCGCAGT and TaMPK3R: GGTTCAACTCCAGGGCTTCGTTG for *MAPK3* and TaMPK6F: GAAGATATCCGCCAACTTCCCCG and TaMPK6R: CGCATGCTGCT CGAAGTCAAAGC for *MAPK6*.

Relative quantitative (RQ)-PCR was performed on a 7500 Real-Time PCR System (Applied Biosystems). Each experiment was repeated three times. The 20-µl PCR reactions contained 1X SYBR PCR MasterMix (Applied Biosystems), 0.25 µM each primer, and 5 µL of 10-fold diluted cDNA. The thermal cycler procedure was as follows: 10 min of pre-incubation at 95°C, followed by 40 cycles for 15 sec at 95°C and for 1 min at 60°C. The CS deletion line 1BS-18, which lacks the terminal portion of chromosome arm 1BS containing *Snn1*, was used as negative control.

Efficiencies of the different primers combinations were evaluated using serial dilutions of CS cDNA (1:5, 1:10, 1:20 and 1:40) and only primers with efficiencies higher than 95% were used for the RQ-PCR. Transcript levels were expressed as the ratio between the initial numbers of molecules in the target and the internal control using the $2^{-\Delta\Delta CT}$ method as described (Faris et al. 2010).

Results

High-resolution mapping, physical mapping, and identification of candidate genes

The *Snn1* gene lies on the short arm of wheat chromosome 1B (Figure 5.1A) for which a high-resolution linkage map was previously developed using a population derived from CS and CS that had a pair of 1B chromosomes from wild emmer (*T. turgidum* ssp. *dicoccoides*) substituted for the native pair of 1B chromosomes (Reddy et al. 2008). However, initial chromosome walking steps using a durum wheat BAC library (Cenci et al. 2003) revealed that the wild emmer 1B chromosome harbored a large deletion encompassing the *Snn1* locus and caused drastic suppression of recombination (Faris, unpublished). Therefore, a new population was developed by crossing CS with the CS-Hope disomic chromosome 1B substitution line (CS-Hope 1B).

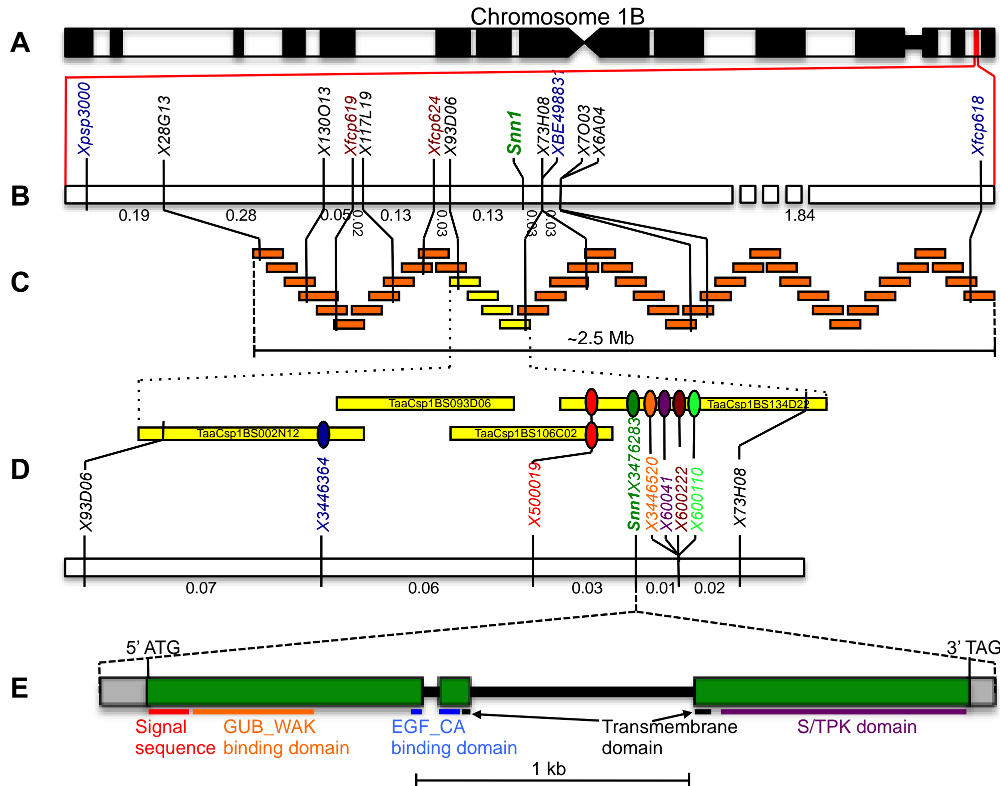


Figure 5.1. Map-based cloning of the *Snn1* gene. A). The genomic region containing the *Snn1* gene on the short arm of chromosome 1B is shown in red. B). The genetic linkage map of the *Snn1* region. Markers in blue are from Reddy et al. (2008), markers in red are from previous unpublished work, and markers in black were developed in this research. C) BAC-based physical map of the *Snn1* region anchored to the genetic linkage map. The four yellow BACs represent the *Snn1* candidate gene region. D) Genetic linkage mapping of the seven candidate genes identified in the four BACs from the candidate gene region in C. E) Gene structure of the *Snn1* (WAK) gene. Exons are in green and UTRs are in gray.

I sampled 17,000 gametes (8,500 F_2 plants) with markers *Xpsp3000* and *Xfcp618*, which were reported in Reddy et al. (2008) to flank the *Snn1* locus, and with markers *Xfcp619* and *Xfcp624*, which were developed from initial chromosome walking efforts. Phenotypic assessment of the 8,500 F_2 plants was conducted by infiltrating the plants with cultures containing the NE SnTox1 as described (Liu et al. 2012) and scoring them as sensitive or insensitive (Figure 5.2). The resulting genetic linkage map spanned 2.73 cM with markers *Xfcp618* and *Xfcp624* flanking *Snn1* at distances of 1.9 and 0.16 cM,

respectively (Figure 5.1B). Screening of the chromosome 1BS BAC-based MTP clones (Raats et al. 2013) revealed a ~2.5 Mb contig consisting of 44 clones containing markers *Xfcp618*, *Xfcp619*, and *Xfcp624*, and thus encompassing *Snn1* (Figure 5.1C). BESs, or whole genome survey sequences (Brenchley et al. 2012) corresponding to BESs (Table 5.1), were used to develop additional markers, and the newly developed markers *X6A04, X7O03, X73H08, X93D06, X117L19*, *X130O13,* and *X28G13* were further anchored to the genetic map (Figure 5.1B). This led to the delineation of *Snn1* to a segment of 0.16 cM spanned by four BAC clones (Figure 5.1C).

The four BACs (TaaCsp1BS002N12, TaaCsp1BS093D06, TaaCsp1BS106C02, and TaaCsp1BS134D22) that defined the *Snn1* candidate gene region were sequenced to 154X coverage using an Ion Torrent™ next generation sequencer. Bioinformatic analyses of these four BAC clones revealed the presence of seven putative genes (Figure 5.1D). A single marker representing each candidate gene was polymorphic and placed on the genetic linkage map. Linkage analysis revealed that only marker, *X3476283*, which represented a wall-associated kinase (WAK) candidate gene, cosegregated with *Snn1*. All other candidate genes were separated from *Snn1* by multiple recombination events and were thereby eliminated as candidates (Figure 5.1D).

Validation and structural characterization of *Snn1*

Seeds of CS were treated with the chemical mutagen EMS and M_2 plants were infiltrated with purified SnTox1 to screen SnTox1-insensitive mutations. Out of 1,360 M_2 families, sixteen independent SnTox1-insensitive/disease resistant mutants were identified (Table 5.4, Figure 5.2).

Table 5.4. Descriptions of induced mutations identified within the *Snn1* gene.

Induced mutant	Mutation type	Position[a]	Exon	Major domain	Codon change	Amino acid change
CSems-231	Missense	119	1	GUB_WAK	TGC→TAC	Cys→Tyr
CSems-237	Missense	149	1	GUB_WAK	GGC→GAC	Gly→Asp
CSems-6107	Missense	2,611	3	PKc	CTC→TTC	Leu→Phe
CSems-6125	Nonsense	988	1	EGF_CA	CAA→TAA	Glu→stop
CSems-6126	Missense	2,556	3	PKc	ATG→ATA	Met→Ile
CSems-6128	Missense	440	1	GUB_WAK	TCG→TTG	Ser→Leu
CSems-6131	Missense	478	1	None	GTC→ATC	Val→Ile
CSems-6132	Missense	2,326	3	PKc	GAG→AAG	Glu→Lys
CSems-6133	Missense	460/2,536	1/3	GUB_WAK/PKc	GTC→ATC/GCG→ACG	Val→Ile/Ala→Thr
CSems-6136	Missense	2,584	3	PKc	CAT→TAT	His→Tyr
CSems-6140	Missense	2,228/2,761	3/3	PKc/PKc	AGT→AAT/GAT→AAT	Ser→Asn/Asp→Asn
CSems-6141[b]	Splice	2056	Intron 2			
CSems-6151	Missense	149	1	GUB_WAK	GGC→GAC	Gly→Asp
CSems-6155	Missense	2,556	3	PKc	ATG→ATA	Met→Ile
CSems-6156	Missense	2,556/2,777	3/3	PKc/PKc	ATG→ATA/GGA→GAA	Met→Ile/Gly→Glu
CSems-6159	Missense	2,342	3	PKc	GCA→GTA	Ala→Val

[a]Nucleotide position counting from the translation start site.

[b]The mutation in CSems-6141 occurred at the acceptor site of intron 2. RT-PCR and sequence analysis indicated that the splice mutation in CSems-6141 results in a product 60 bp shorter than the wild type (see Figure 5.3).

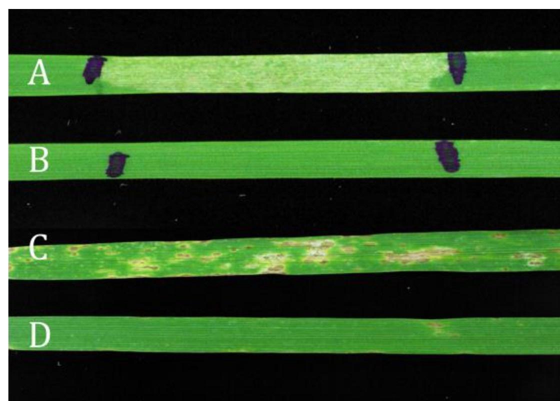


Figure 5.2. Leaves of Chinese Spring (*Snn1*) and CSems6125 (*Snn1* mutant) infiltrated with SnTox1 and inoculated with *Parastagonospora nodorum* A. Infiltration on CS; B. Infiltration on mutant; C. Inoculation on CS; D. Inoculation on mutant.

Comparative sequence analysis of the *WAK* gene from each of the mutants with the *WAK* gene from CS indicated that all but one mutant (CSems-6141) had either missense or nonsense mutations (Table 5.4, Appendix C). There were three CSems lines with two mutations within the *WAK* gene coding region, and the rest of the CSems lines had a single point mutation. CSems-6141 had a point mutation in the acceptor site of intron 2. To determine if the mutation affected splicing, reverse transcriptase (RT)-PCR was conducted using primers 3ExonF3 and 600015R. The amplified fragment in CSems-6141 was 60 bp smaller than in CS (Figure 5.3) indicating the alteration of the splice site. These results verified that the *WAK* gene was *Snn1* and demonstrated that all three major domains (see below) were required for *Snn1* function.

The cDNA sequence was aligned to the genomic sequence to determine the splicing structure. The gene was 3,045 bp from start to stop codon with three exons and a coding sequence of 2,145 bp, and 5' and 3' UTRs of 164 bp and 102 bp, respectively (Figure 5.1D). The predicted protein contained conserved wall-associated receptor kinase galacturonan binding (GUB_WAK), epidermal growth factor – calcium binding

(EGF_CA), transmembrane, and protein kinase (PKc) domains (Figure 5.1E), with the PKc domain predicted to be intracellular and the GUB_WAK and EGF_CA binding domains extracellular.

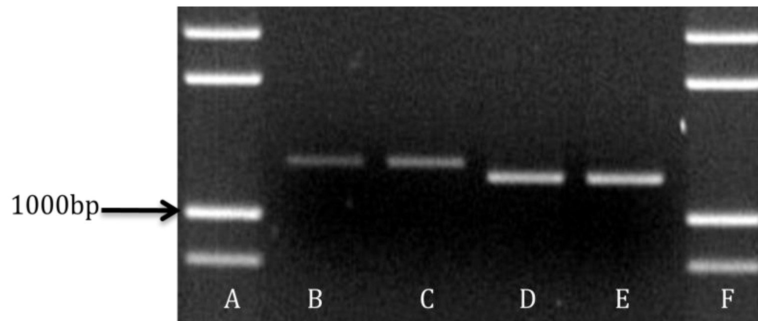
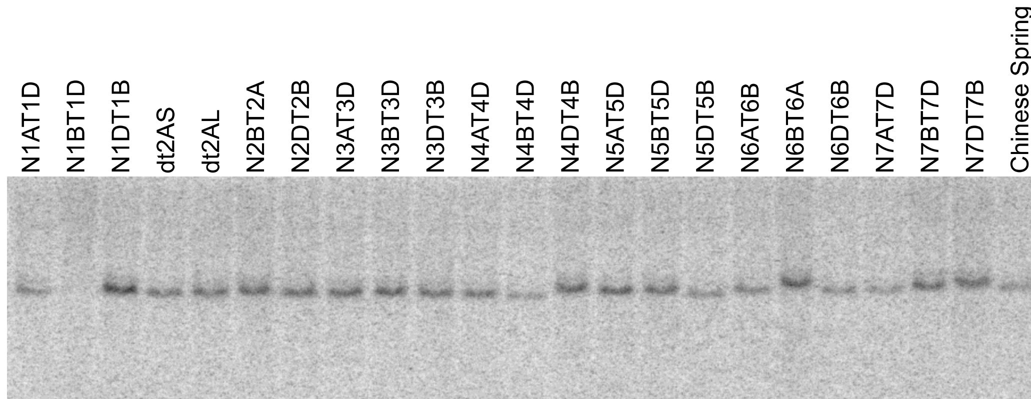


Figure 5.3. Transcription analysis of the splice site mutant CSems-6141. cDNA of CS (Lane B and C) and CSems-6141 (Lane D and E) was amplified with primers 3ExonF3 and 600015R, which flank the point mutant in CSems-6141 at position 2056. Lane A and F were 1kb plus DNA ladder.

To determine the copy number of *Snn1*, Southern analysis was conducted on the set of CS nullisomic-tetrasomic lines with probe FCG36. Absence of the fragment in the nullisomic 1B-tetrasomic 1D line indicated that the gene is located on chromosome 1B, and the lack of hybridizing fragments from chromosomes 1A and 1D showed that homoeoalleles of *Snn1* do not exist in the wheat genome (Figure 5.4).

Comparative and phylogenetic analysis

Further analysis of wheat whole-genome survey sequences revealed that homoeologous chromosome arm 1AS contained no significant matches, but 1DS harbored a gene with 91% nucleotide identity across the entire gene with the exception of the first 800 bp, which had no similarity (Appendix D). Evaluation of CS mRNA indicated that the 1D copy is not transcribed and therefore not functional (Figure 5.5A).



Probe FCG36

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TGCGTCGATAGGAGTGAGTGTTTGCGCGTGTATATATATATGAAAAAAGCATCTACATCCATGATTTTTGCCTAT
TTACTTGTCATCCCGTCTATATATTAAGGGAGGGAGGATGTGCAGCCCAAGTTAGACGATTCCTTGCAACCTGCC
AGACACGATAGAGGGAGTAGAGCAGCATGAGCACCCCAAATTCCCAATTCCATTCCCACCCACTACTGCGGCTGT
GGTTGCCAGTAATCCAACTAGTCATCTTGGCCGCCGCGGCTGAGCAGCAGCAAGGTTGCCGTGACAAGTGCGGCC
ACATCAGCATCCCCTTCCCTTTCGGCATCGGCCGCGGCTGCTTCCGCCATGGCTTCGAGGTCGTGTGCAACGACT
CCTCGTATCCCCCTCGCGCCTTCCTTGCTGGCAAAAGAATATCCAGGTTGTCGTCGTCCCCACTGGATCCCGAGA
ACGGGAGCGATTGGTCCTTCTCGTCGATCGAGCTTGTGAGCATATCAGTCGCCACGGGCAAGGCACGGGTGTACG
CCCCGGTCTCGTACCTCTGCAGCACAAATCTCAACAGGACCTTCTCCGCGATCTACAATATTCACTTGGCCCACA
CGCCGTTCGCCGTGTCGGCGACGCGCAATATTCTCGTCGGCGTCGGCTGGAGAGTCGATGCTTTCGTGACTTTCA
TCGACTTTCCGCTGAGGTGGCCGTATACGGTCATGTTCGCCTGCAGTGCTCGTGCACGCAATCTCCTGCCAAGTG
TGACCCCAACAAACGGGTCGTGCACGGGGAGGGGCTGCTGCCACACCACCATCCCACCGGACAACAACCTCGGCA
ACTTGTTAAGCTTTTCTGTCAAGGGCGAGGTCCCCAACGAGCGGTGGGAAGACTACCCGTGCTCCTACGCCAT
```

Figure 5.4. Southern analysis of probe FCG36 hybridized to DNA of the Chinese Spring nullisomic-tetrasomic lines.The probe sequence is shown below and the primer annealing sites are underlined.

At the amino acid level, matches to predicted proteins from *T. urartu* and *Aegilops tauschii*, the diploid ancestral donors of the A and D genomes of polyploid wheat, respectively, were identified (Table 5.5), but the level of identity (70%) suggested they are not orthologs. Therefore, in polyploid wheat, the ancestral *Snn1* homoeoallele from *T. urartu* was lost from the A genome, and the homoeoallele from *A. tauschii* was involved in a rearrangement and rendered nonfunctional.

The identification of homologs on *Brachypodium* and rice chromosomes 2 and 10, respectively (Table 5.6, 5.7), suggests that these may represent orthologs of *Snn1* based on the syntenic nature among these grasses (The IBI, 2010). Searches of the *Arabidopsis*

genome indicated about 30% identity to each of the five members of the WAK gene family on chromosome 1 (Wagner and Kohorn 2001) (Table 5.8).

Table 5.5. Top five BLASTp hits in the NCBI nr database using the *Snn1* amino acid sequence as a query.

Accession	Description	Score	e-value	Query coverage	Identity
EMT17650.1	Wall-associated receptor kinase 1 (*Ae. tauschii*)	927	0.0	91%	71%
EMT31693.1	Wall-associated receptor kinase 1 (*Ae. tauschii*)	925	0.0	99%	70%
EMS57881.1	Wall-associated receptor kinase 1 (*T. urartu*)	907	0.0	97%	65%
EMT16317.1	Wall-associated receptor kinase 1 (*Ae. tauschii*)	904	0.0	99%	65%
EMT29999.1	Wall-associated receptor kinase 4 (*Ae. tauschii*)	871	0.0	99%	65%

Table 5.6. Top five BLASTp hits in the *Brachypodium dystachyon* genome using the *Snn1* amino acid sequence as a query.

Gene	Description	Score	e-value	Query coverage	Identity
BRADI5G01462.1	Wall-associated kinase	2268	6.9e-221	92%	48%
BRADI1G19290.1	Wall-associated kinase	2267	8.6e-221	96%	45%
BRADI5G03150.1	Wall-associated kinase	2182	2.3e-212	100%	44%
BRADI5G03180.1	Wall-associated kinase	2106	8e-205	92%	46%
BRADI2G03850.1	Wall-associated kinase	2068	4.7e-201	90%	46%

Table 5.7. Top five BLASTp hits in the *Oryza sativa* genome using the *Snn1* amino acid sequence as a query.

Gene	Description	Score	e-value	Query coverage	Identity
Os10g0151500.1	Wall-associated kinase	2094	1.2e-202	99%	42%
Os10g0111400.1	Wall-associated kinase	2050	2.4e-198	98%	42%
Os10g0152000.1	Wall-associated kinase	1958	4.3e-188	98%	40%
Os10g0111900.1	Wall-associated kinase	1937	2.5e-185	98%	41%
Os10g0174548.1	Wall-associated kinase	1934	6.3e-185	86%	45%

Table 5.8. Top five BLASTp hits in the *Arabidopsis thaliana* genome using the *Snn1* amino acid sequence as a query.

Gene	Description	Score	e-value	Query coverage	Identity
AT1G21230.1	WAK5; wall-associated kinase 5	829	2e-88	97%	32%
AT1G21250.1	WAK1; wall-associated kinase 1	828	3e-88	95%	31%
AT1G21240.1	WAK3; wall-associated kinase 3	822	1e-87	97%	33%
AT1G21210.1	WAK4; wall-associated kinase 4	812	2e-86	95%	30%
AT1G21220.1	WAK2; wall-associated kinase 2	781	7e-83	97%	31%

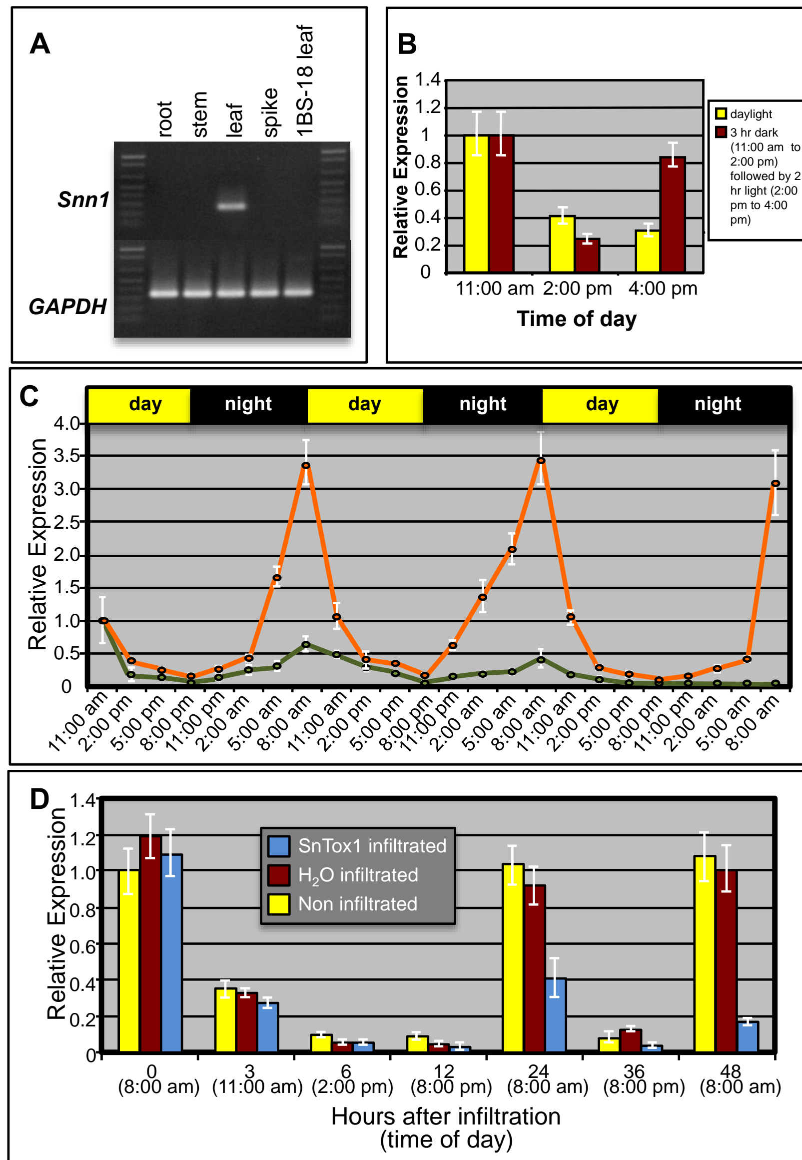


Figure 5.5. Transcriptional expression of *Snn1*. A) *Snn1* expression survey by reverse transcription-PCR with *GAPDH* as an endogenous control. The 1BS-18 line contains a deletion for the terminal portion of chromosome arm 1BS including *Snn1*, but retains the remainder of the genome. Therefore, the absence of an amplicon in 1BS-18 indicates that *Snn1* transcription is unique to the 1B copy and that the putative homoeologous copy on 1D is not expressed. B) RQ-PCR evaluation of *Snn1* expression in two-week old plants entrained with a 12 hr light/dark cycle (control; yellow bars) and plants subjected to three hours of dark followed by two hours of light (red bars). C) *Snn1* expression levels in two-week old plants entrained with a 12 hr light/dark cycle evaluated every three hours over a 72 hr period (orange) and in plants subjected to continuous dark for the same time points (dark green) using relative quantitative (RQ)-PCR. D) RQ-PCR evaluation of *Snn1* expression in SnTox1-challenged plants; blue bars: SnTox1 infiltrated; red bars: H_2O infiltrated; yellow bars: no infiltration.

Compared to other homologs, the GUB_WAK domain of *Snn1* contains an insertion between two conserved cysteine residues (Figure 5.6). Phylogenetic analysis suggested that *Snn1* represents a monocot-specific subgroup within the plant *WAK* family that includes proteins all having the insertion at the same position (Appendix E).

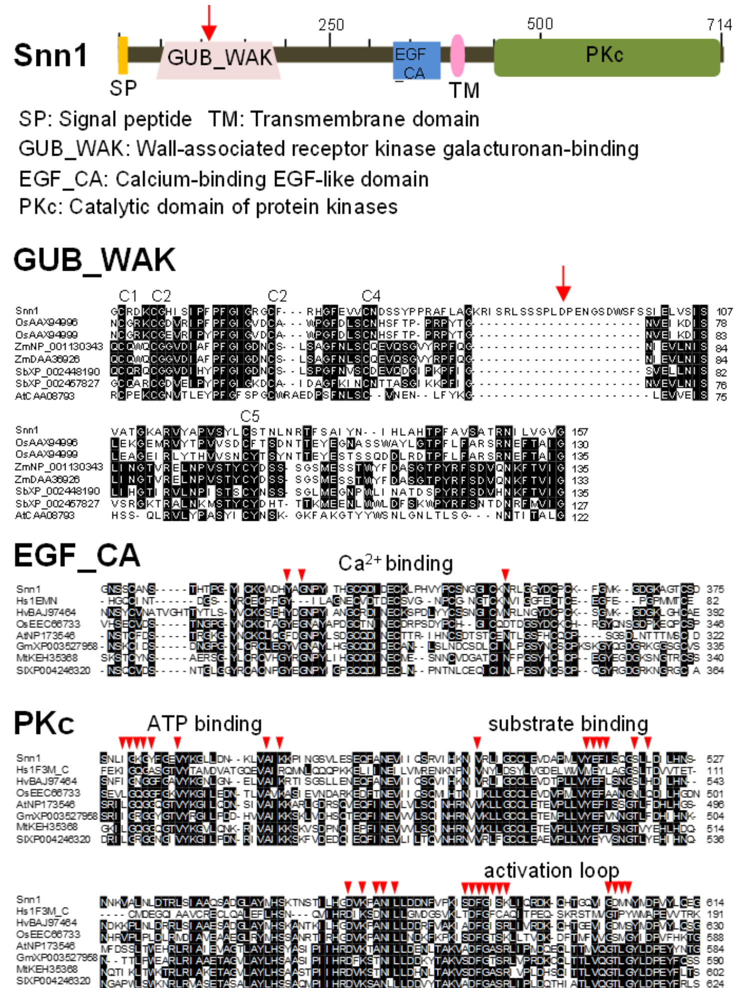


Figure 5.6. Conserved domains and active sites identified in the deduced Snn1 protein. Red arrows indicate the insertion in the GUB-WAK domain of Snn1 with C1-C5 indicating the five conserved cysteine residues. Triangles indicate the active sites in the EGF_CA and PKc domains (as determined in 1EMN and1F3M_C structures of the corresponding human proteins). Species abbreviations (followed by Genbank accession numbers): At, *Arabidopsis thaliana*; Gm, *Glycine max*; Hv, *Hordeum vulgare*; Hs, *Homo sapiens*; Mt, *Medicago truncatula*; Os, *Oryza sativa*; Sb, *Sorghum bicolor*; Sl, *Solanum lycopersicum*; Zm, *Zea mays*.

I evaluated 826 tetraploid and hexaploid *Triticum* accessions and 123 accessions of *Ae. speltoides*, the closest living relative of the diploid B-genome progenitor, for presence of *Snn1* alleles and for sensitivity to SnTox1 (Appendix B, Figure 5.7). While most accessions were positive for harboring an allele of *Snn1*, none of the *Ae. speltoides* or the tetraploid wild emmer accessions was sensitive to SnTox1. Three accessions of cultivated emmer along with 73 and 16% of domesticated durum varieties and hexaploid wheat accessions, respectively, were SnTox1-sensitive.

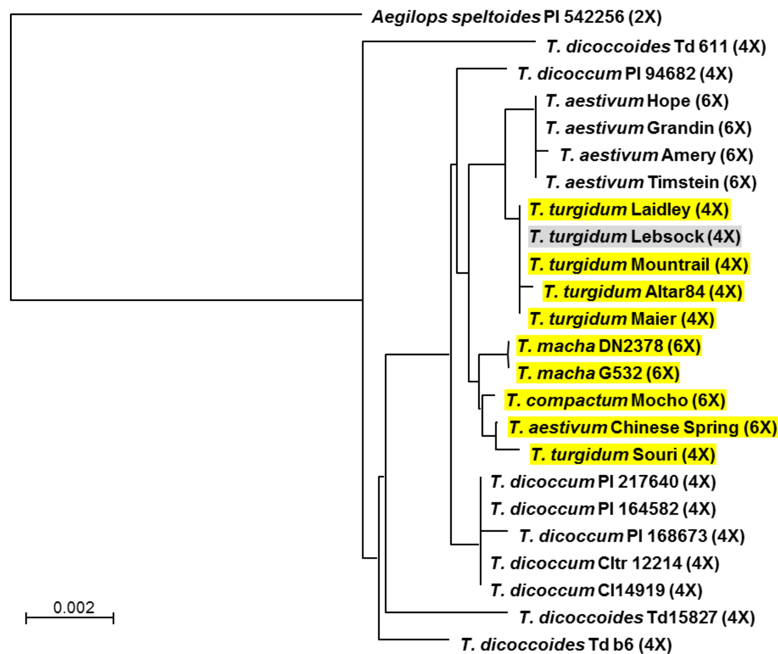


Figure 5.7. Phylogenetic tree of 24 genotypes based on deduced amino acid sequences of the *Snn1* gene. Genotypes sensitive to SnTox1 are highlighted in yellow."*T.*" represent the genus *Triticum*. The ploidy level of each genotype is indicated in parenthesis.

Sequencing and phylogenetic analysis of *Snn1* from 24 accessions including 15 SnTox1-insensitive and 9 SnTox1-sensitive lines (Figure 5.7) indicated that *Snn1* alleles from *Ae. speltoides* and wild emmer wheat were more diverse relative to cultivated emmer, durum and hexaploid wheat as expected. Most of the domesticated emmer was clustered together in one clade, as was the case for durum wheat (Figure 5.7). In the

durum wheat clade, the insensitive cultivar 'Lebsock' had an amino acid sequence identical to sensitive durum wheat cultivars. Further RT-PCR analysis indicated that *Snn1* in 'Lebsock' was not transcribed (Figure 5.8). Sensitive and insensitive hexaploid wheat lines fell into two different clades. Sequence analysis of *Snn1* showed that a single missense mutation in the insensitive hexaploid wheat lines rendered them insensitive to SnTox1.

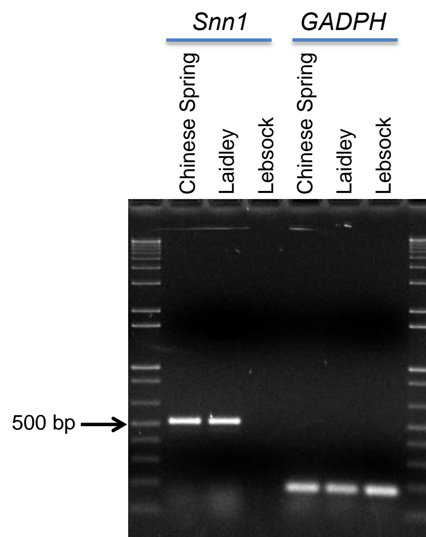


Figure 5.8. Transcription analysis of *Snn1* in the durum wheat variety Lebsock. cDNA of Lebsock, Laidley and CS was amplified with primers 3ExonF4 and 600015R, which produced the 520 bp cDNA fragment in Laidley and CS but not in Lebsock (left), and with wheat *GAPDH* gene primers GAPDH.F152 and GAPDH.R338 (right).

Transcriptional regulation of *Snn1*

Evaluation of *Snn1* expression in different plant tissues of CS by RT-PCR indicated that it is transcriptionally expressed in the leaves, but not in the roots, stems, or spikes (Figure 5.5A). In leaves, *Snn1* expression was significantly down- and then up-regulated when plants were exposed to several hours of darkness followed by light, respectively (Figure 5.5B). In a separate experiment, I evaluated the levels of *Snn1* transcription under 12 hr light/dark regimes and under continuous darkness every three

hours for three days and found that *Snn1* transcription decreased throughout the daylight hours and increased throughout the nighttime hours (Figure 5.5C). *Snn1* expression levels in plants under continuous darkness mimicked those of the light/dark-grown cycle for the first 15 hrs; however, its expression level increased to only 20% of that in light/dark-grown plants at 24 hrs. At 48 hrs, expression level decreased to about 10% of that observed in light/dark-grown plants and it became almost undetectable at 72 hrs (Figure 5.5C). These results suggested that *Snn1* transcription was regulated by light and light exposure patterns.

In addition, transcription of *Snn1* is not up-regulated by SnTox1 infiltration, but expression patterns of *Snn1* in SnTox1-infiltrated samples over time (Figure 5.5D) mimicked those exposed to continuous dark. *Snn1* expression at 24 hrs in both the dark-treated and the SnTox1-infiltrated plants increased to only half the level of the control plants. Expression in both cases was again down regulated at 36 hr just as in the control, and expression levels remained low at subsequent time points.

To determine whether SnTox1 influences *TaMAPKs* gene transcription level, the expression of *TaMAPKs* were evaluated in 2-wk old plants spray-inoculated with SnTox1 and included water spray inoculated and non-inoculated plants as controls. I found that transcription of *TaMAPK3*, but not *TaMAPK6*, was up-regulated within 15 mins and about 10 times higher than that in control plants 30 mins after SnTox1 treatment in compatible *Snn1*-SnTox1 interactions. 3 hrs after treatment the expression level of *TaMAPK3* was back to the same as in control plants. However, expression level of *Snn1* in water spray inoculated and non-inoculated plants did not change (Figure 5.9).

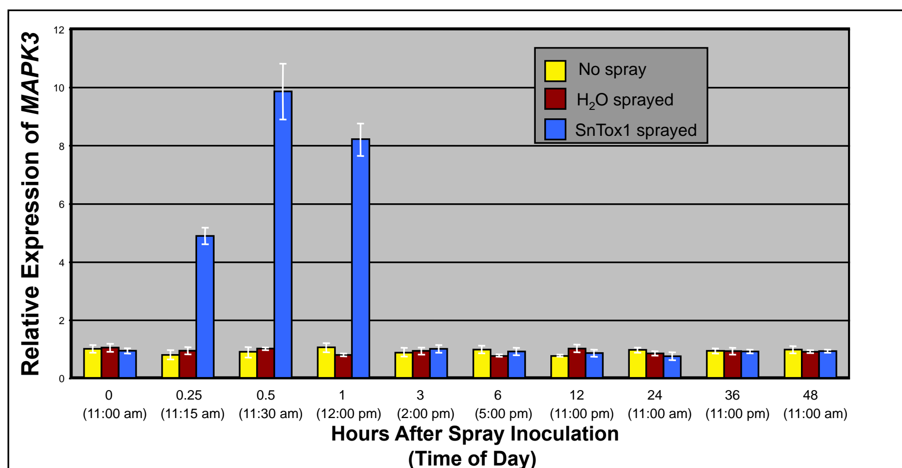


Figure 5.9. *MAPK3* transcription analysis after SnTox1 spray inoculation. RQ-PCR evaluation of *MAPK3* expression was conducted in SnTox1-challenged plants; blue bars: SnTox1 sprayed inoculums; red bars: H_2O sprayed; yellow bars: no spray.

Discussion

Here, I report the cloning and characterization of the gene conferring sensitivity to SnTox1 produced by the necrotrophic pathogen *P. nodorum*. My results demonstrated that *Snn1* is a member of the WAK class of plant receptor kinases.

Tetraploid wild emmer wheat *T. turgidum* ssp. *dicoccoides* arose through hybridization between *T. uratu* and a close relative of *Ae. speltoides*. Wild emmer wheat evolved into cultivated emmer wheat (*T. turgidum* ssp. *dicoccum*), which later gave rise to fully domesticated durum wheat (*T. turgidum* ssp. *durum*). Hexaploid wheat (*T. aestivum*) arose from a second hybridization between an AB-tetraploid and *Ae. tauschii*. Here I found that functional *Snn1* alleles, encoding Snn1 to recognize SnTox1, existed only in cultivated emmer wheat and domesticated tetraploid and hexaploid wheat, and were absent from all diploid and wild emmer wheat accessions tested. This suggests that functional *Snn1* probably arose in cultivated emmer before being passed to domesticated tetraploid and hexaploid wheat.

Plants are continuously threatened by a broad range of pathogens and have evolved an array of response systems to decipher these external signals and initiate a series of defense responses (Antolin-Llovera et al. 2012). Plant receptor kinases play fundamental roles in the perception of these stimuli and activate downstream signaling pathways and defense responses. Plant WAK genes are an important subfamily of plant receptor kinases (Brutus et al. 2010; Kohorn and Kohorn 2012). Five isoforms of WAK genes have been identified from Arabidopsis, and the typical *WAK* gene contains a cytoplasmic Ser/Thr kinase domain and an extracellular epidermal growth factor domain (He et al. 1996, 1999). Plant WAK proteins have been implicated in cell expansion, biotrophic pathogen resistance, and general perception of the extracellular environment (Wagner and Kohorn 2001). WAKs serve as PRRs for pectin fragments known as oligogalacturonides (OGs) (Brutus et al. 2010), which are DAMPs released by the plant cell upon pathogen attack or wounding. Through the N-terminal non-EGF ecodomain, WAK proteins are cross-linked to OGs and pectin. Both *in vivo* and *in vitro* observations demonstrated that WAK is covalently bound to pectin to form a Ca^{2+}-dependent "egg-box" conformation (Cabrera et al. 2008). Moreover, short fragments of pectin compete with longer pectin for WAK binding (Kohorn et al. 2009; Decreux and Messiaen, 2005). WAK-GFP expression showed that WAK is crosslinked with pectin within the golgi, which indicated that the assembly of WAK begins at an early stage inside the cytoplasm rather than in the cell wall (Kohorn et al. 2006).

Recognition of OGs by the WAK PRRs is followed by the activation of a classic biotrophic defense response (Lorenzo et al. 2011). Pectin-treatment induced or repressed the expression of hundreds of genes related to cell wall biogenesis and stress responses.

However, these reactions were shut down in *WAK2*-null cells (Kohorn et al. 2009). Upon stimulation with OGs, a chimeric receptor with the *WAK1* extracellular domain activated ROS accumulation, a rise in ethylene levels and a change in gene expression (Brutus et al. 2010). Another study showed that MPK3 is required for a downstream *WAK2* signaling (Kohorn et al. 2009). Therefore, when a cell wall disturbance generates OGs, WAK will bind preferably with OGs to initiate responses to pathogens and wounding, which then activates downstream defense responses (Kohorn et al. 2006, 2009; Denoux et al. 2008; Brutus et al. 2010).

As a member of the WAK class of receptors, *Snn1* is likely a PRR that activates PTI pathway-induced cell death, a response that is exploited by *P. nodorum* to cause disease. Because SnTox1 is not imported into plant cells (Liu et al. 2012), it is possible that perturbations in the pectin matrix caused by SnTox1 lead to the generation of OGs, which are then recognized by Snn1 to activate the host response. It was demonstrated that compatible *Snn1*-SnTox1 interactions result in an oxidative burst, DNA laddering, and up-regulation of pathogenesis related (PR) gene expression, all of which are hallmarks of a defense response to a biotroph (Liu et al. 2012). Another hallmark is the induction of mitogen-activated protein kinase (MAPK) genes *MAPK3* and *MAPK6* leading to transcriptional reprogramming (Meng and Zhang 2013). Here, I found that transcription of *TaMAPK3* was up-regulated minutes after SnTox1 treatment in compatible *Snn1*-SnTox1 interactions. In PTI signaling, MAPK activation typically occurs within minutes and is transient, whereas it is more prolonged in ETI activation (Tsuda and Katagiri 2010).

Light has long been recognized to be required for plant defense responses (Hua for review 2013). Genetic studies showed that the light-dependent responses were mediated through photosynthesis as well as photoreceptor signaling (Gohre et al. 2012; Jeong et al. 2010). Manning et al. (2009) showed that the ToxA-*Tsn1* interaction was associated with the photosynthesis pathway. In a compatible ToxA-*Tsn1* interaction, photosynthetic pathways were targeted by ToxA and photosynthesis was rendered less effective (Manning et al. 2007). As for the SnTox1-*Snn1* interaction, light is also required for disease (Liu et al. 2004a). Sequence analysis found that there were several light-responsive elements as well as light-regulated transcription factors present in the promoter region of *Snn1* (not shown data). Whether these elements are involved in the SnTox1-*Snn1* recognition or not needs to be tested further.

Snn1 displayed a diurnal regulation for its expression and peaked during the early morning (Figure 5.5). Faris et al. (2010) showed a similar transcriptional pattern for *Tsn1* with a slightly different peak time. Studies have shown that many genes involved in the perception of PAMPs/DAMPs also exhibited peak expression in the morning and it has been proposed that the *Arabidopsis* innate immune system may be primed to respond most strongly to the detection of PAMPs/DAMPs in the subjective morning, when pathogens are most abundant (Bhardwaj et al. 2011).

Kohorn et al. (2006) showed that WAK1 was crosslinked with pectin in a cytoplamic compartment and coordinated with synthesis of surface cellulose. Other research showed that the expression of cell wall synthesizing genes peaked toward the end of the subjective night (Harmer et al. 2000). *Snn1*, as a member of WAK family and one component of the cell wall, is also most highly expressed during the early morning.

The question of whether or not this pattern is related to cell wall synthesis, or if it is just coincidence remains to be addressed.

The hijacking of an ETI pathway was shown by the cloning of the wheat NB-LRR gene *Tsn1* (Faris et al. 2010), whereas the current work suggests *P. nodorum* hijacks a PTI pathway via the recognition of SnTox1 by *Snn1*. Plants carrying both *Snn1* and *Tsn1* experience twice the disease as plants having only one of the genes (Chu et al. 2010). Therefore, the hijacking of both pathways benefits the pathogen in terms of survival and propagation. To better combat this, breeders need to understand that a paradigm shift is needed, i.e. disease susceptibility genes such as *Tsn1* and *Snn1* need to be selected against and removed from germplasm, as opposed to selecting in favor of active resistance genes as is the case for improving resistance to biotrophic pathogens.

References

Abeysekara N, Friesen TL, Keller B, Faris JD (2009) Identification and characterization of a novel host-toxin interaction in the wheat-*Stagonospora nodorum* pathosystem. Theor Appl Genet 120:117-126

Adhikari TB, Bai J, Meinhardt SW, Gurung S, Myrfield M, Patel J, Ali S, Gudmestad NC, Rasmussen JB (2009) *Tsn1*-mediated host responses to ToxA from *Pyrenophora tritici-repentis*. Mol Plant-Microbe Interact 22:1056-1068

Antolin-Llovera M, Ried MK, Binder A, Pamiske M (2012) Receptor kinase signaling pathways in plant-microbe interactions. Annu Rev Phytopathol 50: 451-473

Bhardwaj V, Meier S, Petersen LN, Ingle RA, Roden LC (2011) Defence responses of Arabidopsis thaliana to infection by Pseudomonas syringae are regulated by the circadian clock. PloS One 6:e26968

Bockus WW, Bowden RL, Hunger RM, Morrill WL, Murray TD, Smiley RW (2010) Compendium of Wheat Diseases and Pests: Third Edition. American Phytopathological Society, St. Paul, MN

Brenchley R, Spannagl M, Pfeifer M, Barker GL, D'Amore R, Allen AM, McKenzie N, Kramer M, Kerhomou A, Bolser D, Kay S, Waite D, Trick M, Bancroft I, Gu Y, Huo N, Luo MC, Sehgal S, Gill B, Kinaian S, Anderson O, Kersey P, Dvorak J, McCombie RW,

Hall A, Mayer KFX, Edwards KH, Bevan MW, Hall Neil (2012) Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature 491:705-710

Brutus A, Sicilia F, Macone A, Cervone F, De Lorenzo G (2010) A domain swap approach reveals a role of the plant wall-associated kinase 1(*WAK1*) as a receptor of oligogalacturonides. Proc Natl Acad Sci USA 107:9452-9457

Cabrera JC, Boland A, Messiaen J, Cambier P, Van Cutsem P (2008) Egg box comformation of oligogalacturonides: the time-dependent stabilization of the elicitor-active conformation increases its biological activity. Glycobiology 18:473-482

Cenci A, Chantret N, Kong X, Gu Y, Anderson OD, Fahima T, Distelfeld A, Dubcovsky J (2003) Construction and characterization of a half million clone BAC library of durum wheat (*Triticum turgidum* ssp. *durum*). Theor Appl Genet 107:931-939

Chu CG, Faris JD, Xu SS, Friesen TL (2010) Genetic analysis of disease susceptibility caused by compatible *Tsn1*-SnToxA and *Snn1*-SnTox1 interactions in the wheat-*Stagonospora nodorum* pathosystem. Theor Appl Genet 120:1451-1459

Dangl JL, Jones JD (2000) Plant pathogens and integrated defence responses to infection. Nature 411:826-833

Dexreux A, Messiaen, J (2005) Wall-asociated kinase *WAK1* interacts with cell wall pectins in a calcium-induced conformation. Plant Cell Physiol 46:268-278

Dodds PN, Rathjen JP (2010) Plant immunity: Towards an integrated view of plant-pathogen interactions. Nat Rev Genet 11:539-548

Faris JD, Zhang ZC, Lu HJ, Lu SW, Reddy L, Cloutier S, Fellers JP, Meinhardt SW, Rasmussen JB, Xu SS, Oliver RP, Simons KJ, Friesen TL (2010) A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proc Natl Acad Sci USA 107:13544-13549

Friesen TL, Stukenbrock EH, Liu ZH, Meinhardt SW, Ling H, Faris JD, Rasmussen JB, Solomon PS, Mcdonald BA, Oliver RP (2006) Emergence of a new disease as a result of interspecific virulence gene transfer. Nat Genet 38:953-956

Friesen TL, Meinhardt SW, Faris JD (2007) The *Stagonospora nodorum* – wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. Plant J 51:681-692

Friesen TL, Zhang Z, Solomon PS, Oliver RP, Faris JD (2008) Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. Plant Physiol 146:682-693

Friesen TL, Faris JD (2010) Characterization of the wheat-*Stagonospora nodorum* disease system: what's the molecular basis of this quantitative necrotrophic disease interaction? Can J Plant Pathol 32:20-28

Friesen TL, Chu C, Xu SS, Faris JD (2012) SnTox5-*Snn5*: a novel *Stagonospora nodorum* effector-wheat gene interaction and its relationship with the SnToxA-*Tsn1* and SnTox3-*Snn3*-B1 interactions. Mol Plant Pathol 13:1101-1109

Gao Y, Faris JD, Liu ZH, Xu SS, Friesen TL (2014) Identification and characterization of the SnTox6-*Snn6* interaction in the wheat-*Stagonospora nodorum* pathosystem. Mol Plant-Microbe Interact (accepted)

Gohre V, Jones AME, Sklenar J, Robatzek S, Weber APM (2012) Molecular crosstalk between PAMP-triggered immunity and photosynthesis. Mol Plant Microbe Interact 25: 1083-1092

Gomez-Gomez L, Boller T (2000) FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Mol Cell 5:1003-1011

He ZH, Fujiki M, Kohorn BD (1996) A wall-associated, receptor-like protein kinase. J Biol Chem 271:19789-19793

He ZH, Cheeseman I, He D, Kohorn BD(1999) A cluster of five cell wall-associated receptor kniase genes, *Wak1*-5, are expressed in specific organs of *Arabidospsis*. Plant Mol Biol 39:1189-1196

The International Brachypodium Initiative (2010) Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature 463:763-768

Jeong RD, Kachroo A, Kachroo P (2010) Blue light photoreceptors are required for the stability and function of a resistance protein mediating viral defense in Arabidopsis. Plant Signal Behav 5:1504-1509

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323-329

Kohorn BD, Johansen S. Shishido A, Todorva T, Martinez R, Defeo E, Obregon P (2009) Pectin activation of MAP kinase and gene expression is *WAK2* dependent. Plant J 60:974-982

Kohorn B, Kohorn S (2012) The cell wall-associated kinases, *WAKs*, as pectin receptors. Front Plant Sci 3:88 DOI: 10.3389/fpls.2012.00088

Liu ZH, Faris JD, Meinhardt SW, Ali S, Rasmussen JB, Friesen TL (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

Liu ZH, Friesen TL, Meinhardt SW, Ali S, Rasmussen JB, Faris JD (2004b) Quantitative trait loci analysis and mapping of seedling resistance to Stagonospora nodorum leaf blotch in wheat. Phytopathology 94:1061-1067

Liu ZH, Friesen TL, Ling H, Meinhardt SW, Oliver RP, Rasmussen JB, Faris JD (2006) The Tsn1-ToxA interaction in the wheat-*Stagonospora nodorum* pathosystem parallels that of the wheat-tan spot system. Genome 49:1265-1273

Liu ZH, Faris JD, Oliver RP, Tan KC, Solomon PS, McDonald MC, McDonald BA, Nunez A, Lu S, Rasmussen JB, Friesen TL (2009) SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the *Snn3* gene. PLoS Pathogens 5:e1000581

Liu ZH, Zhang ZC, Faris JD, Oliver RP, Syme R, McDonald MC, McDonald BA, Solomon PS, Lu SW, Shelver WL, Xu SS, Friesen TL (2012) The cysteine rich necrotrophic effector SnTox1 produced by *Stagnospora nodorum* triggers susceptibility of wheat lines harboring *Snn1*. PLoS Pathogens 8:e1002467

Lorang JM, Sweat TA, Wolpert TJ (2007) Plant disease susceptibility conferred by a 'resistance' gene. Proc Natl Acad Sci USA 104:14861-14866

Lu SW, Faris JD, Sherwood R, Friesen TL, Edwards MC (2014) A dimeric *PR*-1-type pathogenesis-related protein interacts with ToxA and potentially mediates ToxA-induced necrosis in sensitive wheat. Mol Plant Pathol 15:650-663

Manning VA, Hamilton SM, Ciuffetti LM(2007) PtrToxA interacts with a chloroplast-localized protein. Mol Plant Microbe Interact 20:168-177

Manning VA, Chu AL, Steeves JE, Wolpert TJ, Ciuffetti LM (2009) A host-selective toxin of Pyrenophora tritici-repentis, PtrToxA, induces photosystem changes and reactive oxygen species accumulation in sensitive wheat. Mol Plant Microbe Interact 22:665-676

Meng X, Zhang S (2013) MAPK cascade in plant disease resistance signaling. Ann Rev Phytopathol 51:245-266

Miya A, Albert P, Shinya T, Desaki Y, Ichimura K, et al (2007) *CERK1*, a *LysM* receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. Proc Natl Acad Sci USA 104:19613-19618

Mohaghan J, Zipfel C (2012) Plant pattern recognition receptor complexes at the plasma membrane. Curr Opin Plant Biol 15:349-357

Nagy E D, Bennetzen JL (2008) Pathogen corruption and site-directed recombination at a plant disease resistance gene cluster. Genome Res 18:1918-1923

Oliver RP, Solomon PS (2010) New developments in pathogenicity and virulence of necrotrophs. Curr Opin Plant Biol 13:415-419

Pandelova I, Betts MF, Manning VA, Wilhelm LJ, Mockler TC, Ciuffetti LM (2009) Analysis of transcriptome changes induced by Ptr ToxA in wheat provides insights into the mechanisms of plant susceptibility. Mol Plant 2:1067-1083

Raats D, Frenkel Z, Krugman T, Dodek I, Sela H, Simkova H, Magni F, Dattonaro F, Vautrin S, Berges H, Wicker T, Keller B, Leroy P, Paux E, Dolezel J, Feuillet C, Korol A, Fahima T (2013) The physical map of wheat chromosome 1BS provides insights into its gene space organization and evolution. Genome Biol 14:R138

Reddy L, Friesen TL, Meinhardt SW, Chao S, Faris JD (2008) Genomic analysis of the *Snn1* locus on wheat chromosome arm 1BS and the identification of candidate genes. Plant Genome 1:55-66

Sears ER (1954) The aneuploids of common wheat. Mo Agr Exp Sta Res Bull 572:1-59
Tsuda K, Katagiri F (2010) Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity. Curr Opin Plant Biol 13:459-465

Wagner TA, Kohorn BD (2001) Wall-associated kinases are expressed throughout plant development and are required for cell expansion. Plant Cell 13:303-318

Zhang ZC, Friesen TL, Simons KJ, Xu SS, Faris JD (2009) Identification, development and validation of markers for marker-assisted selection against the *Stagonospora nodorum* toxin sensitivity genes *Tsn1* and *Snn2* in wheat. Mol Breeding, 23: 35-49

Zhang Z, Friesen TL, Xu SS, Shi GJ, Liu Z, Rasmussen JB, Faris JD (2011) Two putatively homoeologous wheat genes mediate recognition of SnTox3 to confer effector-triggered susceptibility to *Stagonospora nodorum*. Plant J 65:27-38

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, Felix G (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *agrobacterium*-mediated transformation. Cell 125:749-760

Zipfel C (2009) Early molecular events in PAMP-triggered immunity. Curr Opin Plant Biol 12:414-420

CHAPTER VI. GENERAL CONCLUSIONS

Plants have developed multiple layers of defense systems, such as PTI, ETI and also systemic acquired resistance (SAR) to combat the infection of biotrophic pathogens (Zipfel 2008, 2009). However, how plants interact with necrotrophic pathogens is less understood. The wheat-*Parastagonospora nodorum* pathosystem has become a model for studying host-necrotrophic pathogen interactions as well as the underlying molecular mechanisms that lead to disease. Cloning of *Tsn1*, an NB-LRR-like gene, made it clear that *P. nodorum* can hijack an ETI pathway for its own good (Faris et al. 2010). In my research, cloning of *Snn1* indicated that *P. nodorum* can subvert the PTI pathway as well for its growth and proliferation.

WAK genes have been reported to be associated with resistance to biotrophs (Brutus et al. 2010; Kohorn and Kohorn 2012). Here I found direct evidence that a *WAK*-like gene, such as *Snn1*, was a novel class of gene conferring disease susceptibility. It might also confer resistance to an unidentified biotroph, but lead to susceptibility to the necrotrophs. Just like the *Vb* gene in oat controls the sensitivity to a necrotrophic pathogen *Cochliobolus victorae*, at the same time *Vb* locus governs the resistance to a biotrophic pathogen *Puccinia coronate* (Wolpert et al. 2012). If it is the usual case, we should be cautious about the resistance gene transformation work to control biotrophic disease, which has been conducted by seed companies. These resistance genes to biotrophs might be utilized one day by necrotrophs to induce disease.

The ideal strategy for resistance breeding to necrotrophs is to identify as many sensitivity genes as possible and to knock these genes out of plant germplasm. The absence of sensitivity gene will lead to an incompatible interaction with the pathogen NE

and a resistant reaction will occur. The newly identified *Snn7* added a new member to the sensitivity gene family. The markers developed here that are closely linked to *Snn7* and *Snn3-B1* will be helpful for marker-assisted selection against these sensitivity genes.

Furthermore, thorough understanding of the pathways involved in disease would provide us additional methods to control disease. We could block the signal transduction pathway to achieve resistance to necrotrophs as well. For the SnTox1-*Snn1* interaction, there are still many unanswered questions, such as how does recognition between SnTox1 and *Snn1* occur? Does SnTox1 have any effects on pectin, which is reported to covalently bind to *WAK* genes in Arabidopsis? Is calcium involved in the Snn1-SnTox1 pathway since Snn1 has a Ca^{2+} binding domain? To answer all these questions, the signaling pathway induced by SnTox1 needs to be dissected.

Snn1 and *Tsn1* both showed light-regulated and rhythmic expression in the absence of the pathogen (Faris et al. 2010; CHAPTER VI). *Snn1* expression peaked towards the end of subjective night. For biotrophic diseases, it is shown that central genes in PTI were regulated by the circadian clock and expressed at the highest level at the end of the subjective night, which was proposed to combat the highest threat from biotrophs during the early morning (Bhardwaj et al. 2011). Is *Snn1* primed by the circadian clock to combat a potential biotroph? More work need to do to determine whether the light responsive elements in the promoter region of *Snn1* are relevant for conferring NE sensitivity.

For interactions identified in the wheat-*P. nodorum* system, most are light dependent (Friesen and Faris 2010), which means that photosynthesis pathway or photoreceptors are involved in the defense responses. The light independent interactions,

such as *Snn3-B1*-SnTox3 and *Snn3-D1*-SnTox3 (Friesen et al. 2008; Zhang et al. 2011), as well as the partially light dependent interaction Snn7-SnTox7 (CHAPTER IV), add complexity to the wheat-*P. nodorum* pathosystem. Light independence of the SnTox3-*Snn3* interaction suggests it might utilize a different pathway other than photosynthesis to lead to disease. Partial light dependence of the *Snn7*-SnTox7 interaction infers that it may share some common pathways with both light-dependent and light-independent interactions. A deeper understanding of these interactions will broaden our knowledge of this pathosystem.

With global climate change, increased ozone radical levels will have adverse effects on plant growth and promote the colonizing of plants by necrotrophs. At the same time, reduced net photosynthesis and premature ripening and senescence could benefit the colonization of plants by necrotrophic pathogens (Manning and Tiedemann 1995). Therefore, understanding the molecular mechanism for necrothophic pathogens to cause disease will provide us fundamental knowledge to develop resistant cultivars under global climate change, and lead to sustainable agricultural systems and security of our food supply.

References

Bhardwaj V, Meier S, Petersen LN, Ingle RA, Roden LC (2011) Defence responses of Arabidopsis thaliana to infection by Pseudomonas syringae are regulated by the circadian clock. PloS One 6:e26968

Brutus A, Sicilia F, Macone A, Cervone F, De Lorenzo G (2010) A domain swap approach reveals a role of the plant wall-associated kinase 1(*WAK1*) as a receptor of oligogalacturonides. Proc Natl Acad Sci USA 107:9452-9457

Faris JD, Zhang ZC, Lu HJ, Lu S.W., Reddy L, Cloutier S, Fellers JP, Meinhardt SW, Rasmussen J. B., Xu SS, Oliver RP, Simons KJ, Friesen TL (2010) A unique wheat disease resistance-like gene governs effector-induced susceptibility to necrotrophic pathogens. Proc Natl Acad Sci USA 107:13544-13549

Friesen TL, Zhang Z, Solomon PS, Oliver RP, Faris JD (2008) Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. Plant Physiol 146:682-693

Friesen TL, Faris JD (2010) Characterization of the wheat-*Stagonospora nodorum* disease system: what's the molecular basis of this quantitative necrotrophic disease interaction? Can J Plant Pathol 32:20-28

Kohorn B, Kohorn S (2012) The cell wall-associated kinases, *WAKs*, as pectin receptors. Front Plant Sci 3:88 DOI: 10.3389/fpls.2012.00088

Manning WJ, Tiedemann AV (1995) Climate change: potential effects of increased atmospheric carbon dioxide, ozone, and ultraviolet-B radiation on plant diseases. Environ Pollut 88:219-245

Wolpert TJ, Dunkle LD, Ciuffetti LM (2002) Host-selective toxins and avirulence determinants: what's in a name? Annu Rev Phytopathol 40: 251-285

Zhang Z, Friesen TL, Xu SS, Shi GJ, Liu Z, Rasmussen JB, Faris JD (2011) Two putatively homoeologous wheat genes mediate recognition of SnTox3 to confer effector-triggered susceptibility to *Stagonospora nodorum*. Plant J 65:27-38

Zipfel C (2008) Pattern-recognition receptors in plant innate immunity. Curr Opin Immunol 20:10-16

Zipfel C (2009) Early molecular events in PAMP-triggered immunity. Curr Opin Plant Biol 12:414-420

APPENDIX A. ACCESSIONS OF *TRITICUM AESTIVUM* AND *T. TURGIDUM* SUBSPECIES DEPLOYED IN SNTOX3-*SNN3-B1* INTERACTION STUDY

ACCESSIONS OF *TRITICUM AESTIVUM* AND *T. TURGIDUM* SUBSPECIES DEPLOYED IN SNTOX3-*SNN3-B1* INTERACTION STUDY

PI or CI	Cultivar or accession ID	Species	Country or region	Genotype							
				Xfcp653[a]	*XTC266536*[a]	*Xmag705*[a]	*Xfcp652*[a]	SnTox3[b]	*Xfcp654*[a]	*Xfcp665*[a]	*Xfcp664*[a]
Cltr 5122	Huguenot	*T. turgidum subsp. durum*	Australia	215	220	230	138	I	null	null	null
Cltr 13711	Doubbi	*T. turgidum subsp. durum*	Australia	215	215	null	130	I	162	null	null
PI 67342	Laidley	*T. turgidum subsp. durum*	Australia	231	215	null	130	I	162	490	MD
PI 316080	2012 M65	*T. turgidum subsp. durum*	Australia	215	220	230	138	I	157	null	MD
PI 324929	12:61-8T-5T-2aT-2B-2T	*T. turgidum subsp. durum*	Australia	231	null	null	130	I	162	490	null
PI 434645	Durati	*T. turgidum subsp. durum*	Australia	215	220	230	138	I	157	null	760
PI 191645	Timor	*T. turgidum subsp. durum*	Brazil	231	215	null	130	I	null	null	760
PI 519759	D 73121	*T. turgidum subsp. durum*	Brazil	231	215	null	130	I	162	490	null
Cltr 5094	FHB4512	*T. turgidum subsp. durum*	China	215	220	230	138	I	157	null	760
PI 79900	N-85	*T. turgidum subsp. durum*	China	215	220	230	138	I	157	null	760
PI 283853	China 34	*T. turgidum subsp. durum*	China	231	215	null	130	I	162	490	760
4154-39	Rascon 37	*T. turgidum subsp. durum*	CIMMYT	231	215	null	130	I	162	490	760
4154-49	Aconhci 89	*T. turgidum subsp. durum*	CIMMYT	231	215	null	130	I	162	490	760
4154-40	Ajaia 9	*T. turgidum subsp. durum*	CIMMYT	231	null	null	130	I	162	490	760
4154-4	Altar 84	*T. turgidum subsp. durum*	CIMMYT	231	215	null	130	I	162	490	760
4154-5	Dverd 2	*T. turgidum subsp. durum*	CIMMYT	215	220	230	138	I	157	null	null
4154-17	Scaup	*T. turgidum subsp. durum*	CIMMYT	231	220	230	138	I	157	490	760
4154-27	Scoop 1	*T. turgidum subsp. durum*	CIMMYT	231	215	null	130	I	162	490	760
4154-38	Trinakria	*T. turgidum subsp. durum*	CIMMYT	215	215	null	130	S	162	null	null
PI 306640	Anedj	*T. turgidum subsp. durum*	France	231	215	null	130	I	162	490	760
PI 306641	Bidi 17	*T. turgidum subsp. durum*	France	231	215	null	130	I	162	490	760
PI 306647	D211	*T. turgidum subsp. durum*	France	231	220	230	138	I	162	490	760
PI 306648	D304	*T. turgidum subsp. durum*	France	231	215	null	130	I	162	490	760
Cltr 15108	Giorgio 331	*T. turgidum subsp. durum*	Italy	231	215	null	130	S	162	490	760
CItr 17826	TAM105	*T. aestivum*	US Texas	215	null	null	130	I	null	null	null
PI597665	WA7690	*T. aestivum*	Washington	231	null	230	138	I	157	490	760
PI636134	Granger	*T. aestivum*	US SD	215	215	230	138	I	null	null	760

ACCESSIONS OF *TRITICUM AESTIVUM* AND *T. TURGIDUM* SUBSPECIES DEPLOYED IN SNTOX3-*SNN3-B1* INTERACTION STUDY (continued)

PI 157975	Cotrone	*T. turgidum subsp. durum*	Italy	231	215	null	130	I	157	490	760
PI 157981	Castiglione Pubescente	*T. turgidum subsp. durum*	Italy	215	220	230	138	I	157	null	null
PI 422297	Sincape 90	*T. turgidum subsp. durum*	Italy	215	215	null	130	I	162	null	null
NA	Langdon	*T. turgidum subsp. durum*	ND	215	220	230	138	I	157	null	760
NA	Dilse	*T. turgidum subsp. durum*	ND	null	220	230	138	I	null	null	760
NA	Wales	*T. turgidum subsp. durum*	ND	231	215	null	130	I	157	490	760
NA	Ben	*T. turgidum subsp. durum*	ND	215	220	230	138	I	157	null	760
NA	Lebsock	*T. turgidum subsp. durum*	ND	231	215	null	130	I	157	490	null
NA	Maier	*T. turgidum subsp. durum*	ND	null	220	230	138	I	null	null	null
NA	Alkabo	*T. turgidum subsp. durum*	ND	231	215	null	130	I	157	490	null
NA	Divide	*T. turgidum subsp. durum*	ND	215	220	230	138	I	157	null	null
NA	Mountrail	*T. turgidum subsp. durum*	ND	215	220	230	138	I	157	null	null
NA	Pierce	*T. turgidum subsp. durum*	ND	231	215	null	130	S	157	490	null
PI 274671	Erythromelan	*T. turgidum subsp. durum*	Poland	215	220	230	138	I	null	null	760
PI 274675	Leucomelan Biskrei	*T. turgidum subsp. durum*	Poland	231	null	null	130	I	162	490	null
PI 274681	Muriciense	*T. turgidum subsp. durum*	Poland	215	220	230	138	I	157	null	null
Cltr 3984	ICM314	*T. turgidum subsp. durum*	Tunisia	231	220	230	138	I	157	490	null
PI 41035	Medeah	*T. turgidum subsp. durum*	Tunisia	215+231	215	230	138	I	162	490	760
PI 41046	Mahmoudi Ag	*T. turgidum subsp. durum*	Tunisia	215	215	null	130	I	null	null	null
PI 41051	Souri	*T. turgidum subsp. durum*	Tunisia	215	220	230	138	I	null	null	null
NA	Sceptre	*T. turgidum subsp. durum*	Saskatchewan	215	220	230	138	I	157	null	760
NA	Rusty	*T. turgidum subsp. durum*	ND	215	220	230	138	I	157	null	null
NA	Golden Ball	*T. turgidum subsp. durum*	South Africa	215	220	230	138	I	Null	null	760
NA	Isreal A	*T. turgidum subsp. durum*	Isreal	231	220	230	138	I	157	490	null
NA	Amery	*T. aestivum*	Austalia	231	null	null	130	S	162	490	null
NA	BR34	*T. aestivum*	Brazil	215	215	230	138	I	null	null	760
NA	6B365	*T. aestivum*	Canada	231	null	null	130	I	162	490	MD[c]
CItr12435	Rescue	*T. aestivum*	Canada	215+231	215	230	138	S	157	490	MD

ACCESSIONS OF *TRITICUM AESTIVUM* AND *T. TURGIDUM* SUBSPECIES DEPLOYED IN SNTOX3-*SNN3-B1* INTERACTION STUDY (continued)

CItr 17735	Norstar	*T. aestivum*	Canada	231	null	null	130	S	162	490	760
PI 481542	Sumai3	*T. aestivum*	China	231	null	null	130	S	162	490	760
NA	Siu Mak	*T. aestivum*	China	231	null	null	130	I	162	490	760
PI 520554	Bobwhite	*T. aestivum*	CIMMYT	231	215	230	138	I	157	490	760
PI 591776	Opata 85	*T. aestivum*	CIMMYT	215+231	215	230	138	S	157	490	MD
NA	Pitoma	*T. aestivum*	Croatia	215	215	230	138	I	null	null	MD
PI519204	Bulk 84-4-12	*T. aestivum*	France	231	215	230	138	I	157	490	760
NA	Boston	*T. aestivum*	France	215+231	215	230	138	S	157	490	760
PI573751	Genial	*T. aestivum*	France	215	215	230	138	I	null	null	null
PI 564569	Renan	*T. aestivum*	France	231	null	null	130	I	162	490	760
PI422330	Roazon	*T. aestivum*	France	231	215	230	138	I	157	490	760
NA	Rurik	*T. aestivum*	France	231	215	230	138	I	157	490	760
NA	VPM 1	*T. aestivum*	France	231	215	230	138	I	157	490	760
NA	Certo	*T. aestivum*	Germany	231	null	null	130	S	162	490	760
NA	Puseas	*T. aestivum*	India	231	null	null	130	S	162	490	760
PI 182673	Salamouni	*T. aestivum*	Lebanon	215	215	230	138	I	null	null	null
PI45403	Red Egyptian	*T. aestivum*	South Africa	215	null	230	138	I	null	null	MD
NA	Arina	*T. aestivum*	Switzerland	231	null	230	138	I	157	490	MD
NA	Forno	*T. aestivum*	Switzerland	231	215	230	138	I	157	490	null
PI410430	Mironovskaja 808	*T. aestivum*	Ukraine	231	null	null	130	I	162	490	760
PI404008	Maris Huntsman	*T. aestivum*	United Kingdom	231	215	230	138	I	157	490	null
NA	Skater	*T. aestivum*	United Kingdom	215	215	230	138	I	null	null	760
NA	Hanna	*T. aestivum*	US AgriPro	215	215	230	138	I	null	null	null
NA	UC1041GPC	*T. aestivum*	US California	215	215	230	138	I	null	null	760
PI476849	Erik	*T. aestivum*	US Colorado	215	215	230	138	I	null	null	null
CItr 17268	Fielder	*T. aestivum*	US Idaho	null	215	null	130	I	null	null	null

ACCESSIONS OF *TRITICUM AESTIVUM* AND *T. TURGIDUM* SUBSPECIES DEPLOYED IN SNTOX3-*SNN3-B1* INTERACTION STUDY (continued)

PI604224	KS96WGRC39	*T. aestivum*	US Kansas	null	215	230	3	S	157	490	760
CItr17715	Newton	*T. aestivum*	US Kansas	231	215	230	3	I	157	490	null
PI634553	Oklee	*T. aestivum*	US Minnesota	231	215	null	138	S	null	490	760
PI 168688	Timstein	*T. aestivum*	US MN	231	null	null	3	S	162	490	null
PI 192268	Cheyenne	*T. aestivum*	US Nebraska	231	215	230	138	I	157	490	760
CItr 12561	Atlas 66	*T. aestivum*	US NC	231	null	null	130	I	162	490	760
NA	Alsen	*T. aestivum*	US ND	215	220	230	138	I	null	null	null
PI633862	Dapps	*T. aestivum*	US ND	215+231	null	230	138	S	157	490	760
PI 639273	Glenn	*T. aestivum*	US North Dakota	215	null	230	138	I	null	null	760
PI 531005	Grandin	*T. aestivum*	US North Dakota	215+231	null	230	138	S	157	490	null
PI590576	Kulm	*T. aestivum*	US North Dakota	215	null	230	138	I	null	null	null
NA	ND495	*T. aestivum*	US North Dakota	215+231	215	230	138	S	157	490	null
PI613587	Parshall	*T. aestivum*	US North Dakota	215	215	null	130	S	null	null	null
PI 634981	Steele-ND	*T. aestivum*	US North Dakota	215+231	215	230	138	I	157	490	760
PI632970	Briggs	*T. aestivum*	US South Dakota	215+231	215	230	138	S	157	490	null

[a] Fragment sizes detected by the markers given in base pairs; [b] 'I' indicates insensitive to SnTox3 (contains *snn3-b1* allele) and 'S' indicates sensitive to SnTox3 (contains *Snn3-B1* allele); [c] MD: missing data

APPENDIX B. ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY

Accession[a]	PI/CItr	Ploidy	Genus	Species	Subspecies	SnTox1[b]	*Snn1*[c]
52	CItr 8610	6X	*Triticum*	*aestivum*	*sphaerococcum*	+	+
132	PI 42014	6X	*Triticum*	*aestivum*	*sphaerococcum*	+	+
219	PI 70711	6X	*Triticum*	*aestivum*	*sphaerococcum*	+	+
971	PI 278650	6X	*Triticum*	*aestivum*	*sphaerococcum*	+	+
6B365	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	+
6B662	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Alsen	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Amelio	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Amery*	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Arina	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Atlas 66	CItr 12561	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Bobwhite	PI 520554	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Bola Blanca	PI 25970	6X	*Triticum*	*aestivum*	*compactum*	+	+
Boston	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Boval	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
BR34	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Briggs	PI 632970	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Bulk 84-4-12	PI 519204	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Capnord	PI 324529	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Certo	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Cheyenne	PI 192268	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Chinese Spring*	CItr 14108	6X	*Triticum*	*aestivum*	*aestivum*	+	+
Dapps	PI 633862	6X	*Triticum*	*aestivum*	*aestivum*	-	+
DN2378*	PI 361862	6X	*Triticum*	*aestivum*	*macha*	+	+
Erik	PI 476849	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Fielder	CItr 17268	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Forno	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	+
FR 85-6	PI 520525	6X	*Triticum*	*aestivum*	*aestivum*	-	-
G532*	PI 428146	6X	*Triticum*	*aestivum*	*macha*	+	+
Genial	PI 573751	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Glenlea	CItr 17272	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Glenn	PI 639273	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Regent	PI 234831	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Grandin*	PI 531005	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Granger	PI 636134	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Hanna	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Hope*	CItr 8178	6X	*Triticum*	*aestivum*	*aestivum*	-	+
I12	PI 83402	6X	*Triticum*	*aestivum*	*sphaerococcum*	-	-
Jagger	PI 593688	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Katepwa	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Knudson	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	+
KS96WGRC39	PI 604224	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Kulm	PI 590576	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Largo	Cltr 17895	6X	*Triticum*	*aestivum*	synthetic	-	+
Larned	Cltr 17650	6X	*Triticum*	*aestivum*	*aestivum*	-	-
W-7976	NA	6X	*Triticum*	*aestivum*	synthetic	+	+
W-7984	NA	6X	*Triticum*	*aestivum*	synthetic	+	+
Moisson	PI 315998	6X	*Triticum*	*aestivum*	*aestivum*	-	-

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

Maris Huntsman	PI 404008	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Mironovskaja 808	PI 410430	6X	*Triticum*	*aestivum*	*aestivum*	-	+
ND495	NA	6X	*Triticum*	*aestivum*	*aestivum*	+	+
Newton	CItr 17715	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Norstar	CItr 17735	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Novo	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Oklee	PI 634553	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Opata 85	PI 591776	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Orpic	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Parshall	PI 613587	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Pavon F76	PI 519847	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Pitoma	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Puseas	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Red Egyptian*	PI 45403	6X	Triticum	aestivum	aestivum	+	+
Renan	PI 564569	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Roazon	PI 422330	6X	*Triticum*	*aestivum*	*aestivum*	+	+
Rurik	NA	6X	*Triticum*	*aestivum*	*aestivum*	+	+
Salamouni	PI 182673	6X	*Triticum*	*aestivum*	*aestivum*	+	+
Sardona	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Selkirk	CItr 13100	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Sinai No. 3	PI 60740	6X	*Triticum*	*aestivum*	*compactum*	+	+
Skater	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Steele-ND	PI 634981	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Sumai3	PI 481542	6X	*Triticum*	*aestivum*	*aestivum*	-	+
P78-81-1	NA	6X	*Triticum*	*aestivum*	*spelta*	-	-
Sears407a	NA	6X	*Triticum*	*aestivum*	*spelta*	-	-
TA4152-19	NA	6X	*Triticum*	*aestivum*	synthetic	+	+
TA4152-37	NA	6X	*Triticum*	*aestivum*	synthetic	+	+
TA4152-60	NA	6X	*Triticum*	*aestivum*	synthetic	+	+
TAM105	CItr 17826	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Tapidor	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Termok	PI41023	6X	*Triticum*	*aestivum*	*compactum*	+	+
Terza	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Timstein*	PI 168688	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Tincurrin	PI 434642	6X	*Triticum*	*aestivum*	*compactum*	-	-
Type No. 5	PI 40942	6X	*Triticum*	*aestivum*	*sphaerococcum*	+	+
UC1041GPC	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	+
VPM 1	NA	6X	*Triticum*	*aestivum*	*aestivum*	+	+
WA7690	PI 597665	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Zenith	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
TA2601	NA	6X	*Triticum*	*aestivum*	*compactum*	+	+
LDN-2377syn	NA	6X	*Triticum*	*aestivum*	synthetic	-	+
Rescue	CItr 12435	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Ching Feng	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	-
Siu Mak	NA	6X	*Triticum*	*aestivum*	*aestivum*	-	+
Huguenot	CItr 5122	4X	*Triticum*	*turgidum*	*durum*	+	+
Doubbi	CItr 13711	4X	*Triticum*	*turgidum*	*durum*	-	-
Laidley*	PI 67342	4X	*Triticum*	*turgidum*	*durum*	+	+
2012 M65	PI 316080	4X	*Triticum*	*turgidum*	*durum*	+	+
2016 M65	PI 316083	4X	*Triticum*	*turgidum*	*durum*	+	NA

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

12:61-8T-5T-2aT-2B-2T	PI 324929	4X	*Triticum*	*turgidum*	*durum*	+	+
Durati	PI 434645	4X	*Triticum*	*turgidum*	*durum*	+	+
Timor	PI 191645	4X	*Triticum*	*turgidum*	*durum*	-	+
D 73121	PI 519759	4X	*Triticum*	*turgidum*	*durum*	-	+
FHB4512	CItr 5094	4X	*Triticum*	*turgidum*	*durum*	-	+
Tulatai Maitai	PI 70658	4X	*Triticum*	*turgidum*	*durum*	-	NA
2912	PI 74830	4X	*Triticum*	*turgidum*	*durum*	-	NA
N-85	PI 79900	4X	*Triticum*	*turgidum*	*durum*	-	+
China 34	PI 283853	4X	*Triticum*	*turgidum*	*durum*	+	+
Rascon 37	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Aconchi 89	PI 591761	4X	*Triticum*	*turgidum*	*durum*	+	+
Agamia	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Ajaia 9	NA	4X	*Triticum*	*turgidum*	*durum*	-	+
Altar 84*	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Araos	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Botno	CItr 17283	4X	*Triticum*	*turgidum*	*durum*	-	NA
Chen 7	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Croc 1	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Decoy 1	NA	4X	*Triticum*	*turgidum*	*durum*	-	NA
Dverd 2	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Falcin 1	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Gan	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Green 3	NA	4X	*Triticum*	*turgidum*	*durum*	+	NA
Kapude 1	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Laru	NA	4X	*Triticum*	*turgidum*	*durum*	-	NA
Scaup	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Scoop 1	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Shag 22	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Sora	PI 173460	4X	*Triticum*	*turgidum*	*durum*	+	+
Sterna – DW	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Trinakria	PI 428465	4X	*Triticum*	*turgidum*	*durum*	+	+
Yar	NA	4X	*Triticum*	*turgidum*	*durum*	+	NA
Anedj	PI 306640	4X	*Triticum*	*turgidum*	*durum*	-	-
Bidi 17	PI 306641	4X	*Triticum*	*turgidum*	*durum*	+	+
D 115	PI 306642	4X	*Triticum*	*turgidum*	*durum*	+	+
D211	PI 306647	4X	*Triticum*	*turgidum*	*durum*	+	+
D304	PI 306648	4X	*Triticum*	*turgidum*	*durum*	-	-
Giorgio 331	Cltr 15108	4X	*Triticum*	*turgidum*	*durum*	+	+
Lumillo	PI 5996	4X	*Triticum*	*turgidum*	*durum*	+	+
Farro Lungo	PI 157969	4X	*Triticum*	*turgidum*	*durum*	-	NA
Cotrone	PI 157975	4X	*Triticum*	*turgidum*	*durum*	-	+
Vallelunga Glabra	PI 157979	4X	*Triticum*	*turgidum*	*durum*	+	+
Castiglione Pubescente	PI 157981	4X	*Triticum*	*turgidum*	*durum*	+	+
Cappelli	PI 264949	4X	*Triticum*	*turgidum*	*durum*	+	+
Gerardo 624	PI 367224	4X	*Triticum*	*turgidum*	*durum*	-	NA
Sincape 90	PI 422297	4X	*Triticum*	*turgidum*	*durum*	+	+
Langdon*	NA	4X	*Triticum*	*turgidum*	*durum*	-	+
Vallega Zitelli 611	PI 367240	4X	*Triticum*	*turgidum*	*durum*	+	NA
Dilse	NA	4X	*Triticum*	*turgidum*	*durum*	+	+

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

Grande Dora	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Wales	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Westhope	NA	4X	*Triticum*	*turgidum*	*durum*	+	NA
DG Star	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
DG Max	NA	4X	*Triticum*	*turgidum*	*durum*	+	NA
Ben	NA	4X	*Triticum*	*turgidum*	*durum*	-	+
Lebsock*	NA	4X	*Triticum*	*turgidum*	*durum*	-	+
Maier*	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Alkabo	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Grenora	NA	4X	*Triticum*	*turgidum*	*durum*	-	NA
Divide	NA	4X	*Triticum*	*turgidum*	*durum*	-	+
Mountrail	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Pierce	NA	4X	*Triticum*	*turgidum*	*durum*	-	+
Erythromelan	PI 274671	4X	*Triticum*	*turgidum*	*durum*	+	+
Hordeiforme I	PI 274672	4X	*Triticum*	*turgidum*	*durum*	-	NA
Hordeiforme II	PI 274673	4X	*Triticum*	*turgidum*	*durum*	-	NA
Leucomelan Biskrei	PI 274675	4X	*Triticum*	*turgidum*	*durum*	-	-
Muriciense	PI 274681	4X	*Triticum*	*turgidum*	*durum*	-	-
ICM314	CItr 3984	4X	*Triticum*	*turgidum*	*durum*	+	+
Medeah	PI 41035	4X	*Triticum*	*turgidum*	*durum*	+	+
Realforte	PI 41038	4X	*Triticum*	*turgidum*	*durum*	+	NA
Lenah Khetifa	PI 41039	4X	*Triticum*	*turgidum*	*durum*	+	NA
Mahmoudi Ag	PI 41046	4X	*Triticum*	*turgidum*	*durum*	+	+
Allemand	PI 41049	4X	*Triticum*	*turgidum*	*durum*	+	NA
Berbern	PI 41050	4X	*Triticum*	*turgidum*	*durum*	+	NA
Souri	PI 41051	4X	*Triticum*	*turgidum*	*durum*	+	+
Sceptre	PI 584833	4X	*Triticum*	*turgidum*	*durum*	-	-
Rusty	PI 639869	4X	*Triticum*	*turgidum*	*durum*	-	+
Iumillo	PI 210973	4X	*Triticum*	*turgidum*	*durum*	+	NA
Golden Ball*	CItr 11477	4X	*Triticum*	*turgidum*	*durum*	+	+
Cotrone	PI 294571	4X	*Triticum*	*turgidum*	*durum*	-	+
Kronos*	NA	4X	*Triticum*	*turgidum*	*durum*	+	+
Tdom1	CItr 3686	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom2	CItr4013	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom3	CItr7685	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom4	CItr 7686	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom5	CItr7687	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom6	CItr 7779	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom7	CItr 7962	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom8	CItr 7966	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom9	CItr12213	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom10*	CItr12214	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom11	CItr14085	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom12	CItr14086	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom13	CItr14098	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom14	CItr14133	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom15	CItr14135	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom16	CItr14437	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom17	CItr14454	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom18	CItr14592	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom19	CItr14621	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom20	CItr14636	4X	*Triticum*	*turgidum*	*dicoccum*	-	-

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

Tdom21	CItr14637	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom22	CItr14638	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom23	CItr14639	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom24	CItr14751	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom25	CItr14752	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom26	CItr14787	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom27	CItr14822	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom28	CItr14824	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom29	CItr14834	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom30	CItr14838	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom31	CItr14866	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom32	CItr14867	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom33	CItr14868	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom34	CItr14916	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom35	CItr14917	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom36*	CItr14919	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom37	CItr14970	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom38	CItr14971	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom39	CItr14972	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom40	PI2789	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom41	PI41024	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom42	PI41025	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom43	PI56234	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom44	PI57536	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom45	PI58788	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom46	PI58789	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom47	PI60704	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom48	PI60706	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom49	PI73388	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom50	PI74104	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom51	PI74106	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom52	PI74108	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom53	PI79899	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom54	PI94613	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom55	PI94614	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom56	PI94615	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom57	PI94616	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom58	PI94617	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom59	PI94618	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom60	PI94620	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom61	PI94621	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom62	PI94623	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom63*	PI94624	4X	*Triticum*	*turgidum*	*dicoccum*	+	+
Tdom64	PI94625	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom65	PI94626	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom66	PI94627	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom67	PI94628	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom68	PI94630	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom69	PI94631	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom70	PI94632	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom71	PI94633	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom72	PI94634	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom73	PI94635	4X	*Triticum*	*turgidum*	*dicoccum*	-	-

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

Tdom74	PI94636	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom75	PI94637	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom76	PI94638	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom77	PI94640	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom78	PI94641	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom79	PI94642	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom80	PI94648	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom81	PI94649	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom82	PI94650	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom83	PI94654	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom84	PI94655	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom85	PI94656	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom86	PI94657	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom87	PI94659	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom88	PI94660	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom89	PI94661	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom90	PI94662	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom91	PI94663	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom92	PI94664	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom93	PI94665	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom94	PI94666	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom95	PI94667	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom96	PI94668	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom97	PI94669	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom98	PI94670	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom99	PI94671	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom100	PI94673	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom101	PI94674	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom102	PI94675	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom103	PI94676	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom104	PI94677	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom105	PI94678	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom106	PI94679	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom107	PI94680	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom108	PI94681	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom109	PI94682	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom110	PI94683	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom111	PI94738	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom112	PI94747	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom113	PI101971	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom114	PI13961	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom115	PI13963	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom116	PI133134	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom117	PI154582	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom118	PI164578	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom119	PI164582	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom120*	PI168673	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom121	PI168675	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom122	PI168676	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom123	PI168677	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom124	PI168678	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom125	PI168679	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom126	PI190920	4X	*Triticum*	*turgidum*	*dicoccum*	-	+

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

Tdom127	PI190921	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom128	PI190922	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom129	PI190923	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom130	PI190926	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom131	PI191091	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom132	PI191386	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom133	PI191387	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom134	PI191390	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom135*	PI193641	4X	*Triticum*	*turgidum*	*dicoccum*	+	+
Tdom136	PI193642	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom137	PI193643	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom138	PI193644	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom139	PI193873	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom140	PI193877	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom141	PI193878	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom142	PI193879	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom143	PI193880	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom144	PI193882	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom145	PI193883	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom146	PI194041	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom147	PI194042	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom148	PI194375	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom149	PI195721	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom150	PI195722	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom151	PI195723	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom152	PI196099	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom153	PI196100	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom154	PI196101	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom155	PI196904	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom156	PI196905	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom157	PI197259	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom158	PI197260	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom159	PI197481	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom160	PI197482	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom161	PI197483	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom162	PI197484	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom163	PI197485	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom164	PI197486	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom165	PI197487	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom166	PI197488	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom167	PI197489	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom168	PI197490	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom169	PI197491	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom170	PI197492	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom171	PI197493	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom172	PI197494	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom173	PI197495	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom174	PI197496	4X	*Triticum*	*turgidum*	*dicoccum*	-	-
Tdom175	PI217637	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom176	PI217639	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom177	PI217640	4X	*Triticum*	*turgidum*	*dicoccum*	-	+
Tdom178*	PI221400	4X	*Triticum*	*turgidum*	*dicoccum*	+	+
2470	PI 190919	4X	*Triticum*	*turgidum*	*dicoccum*	-	NA

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

	PI 233288	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
DS-9	PI 256029	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
T.d 84	PI 266841	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
I-1-2708	PI 272582	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Arbel	PI 300989	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
	PI 300990	4X	*Triticum*	*turgidum*	*dicoccum*	-	NA
17053 M65	PI 316905	4X	*Triticum*	*turgidum*	*durum*	-	NA
WIR 41965	PI 343446	4X	*Triticum*	*turgidum*	*dicoccum*	-	NA
Nakhichevan	PI 346783	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Kotschyi	PI 352323	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
T-1503	PI 352324	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Spontaneo villosum	PI 352326	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Namuricum	PI 352327	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Schweinfurthii	PI 352328	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
69Z99.2	PI 355455	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
69Z99.3	PI 355456	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
69Z99.4	PI 355457	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
69Z99.6	PI 355458	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
69Z99.7	PI 355459	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-100-1	PI 414718	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-214	PI 414719	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-236	PI 414720	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-267	PI 414721	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-290	PI 414722	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-233	PI 415149	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-278	PI 415150	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-288	PI 415151	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G647	PI 428013	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G1378	PI 428015	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G2995	PI 428094	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3009	PI 428096	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3010	PI 428097	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3011	PI 428098	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3015	PI 428099	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3018	PI 428100	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3022	PI 428104	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
B+15	PI 466949	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
B+31	PI 466950	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
B+32	PI 466951	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
B+34	PI 466952	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
B+39	PI 466953	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
B+40	PI 466954	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
I+4	PI 466955	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
I+5	PI 466956	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
I-6	PI 466957	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
I-31	PI 466968	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
I+32	PI 466969	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
I-33	PI 466970	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
I+34	PI 466971	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
I-35	PI 466972	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
I-37	PI 466974	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
I+38	PI 466975	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

I+42	PI 466977	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
I-55	PI 466980	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
F-19	PI 466981	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
F-20	PI 466982	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
F-25	PI 466983	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
F+26	PI 466984	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
F+36	PI 466985	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
F+54	PI 466986	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
F-73	PI 466988	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
F-77	PI 466989	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
F-83	PI 466990	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
J-12	PI 466996	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
J-21	PI 466997	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
J+23	PI 466998	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
J-27	PI 466999	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
J-38	PI 467000	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
J+39	PI 467001	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
J+44	PI 467002	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
J-47	PI 467003	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+1	PI 467004	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+2	PI 467005	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+6	PI 467006	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+7	PI 467007	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H-10	PI 467008	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+11	PI 467009	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+15	PI 467012	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H-16	PI 467013	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+22	PI 467014	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+23	PI 467015	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+24	PI 467016	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+25	PI 467017	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+27	PI 467018	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+34	PI 467022	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+40	PI 467023	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+45	PI 467024	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+48	PI 467025	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H+51	PI 467026	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H-53	PI 467027	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G 15-1M	PI 467029	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G 32-1M2	PI 467031	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G 79-1	PI 467033	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G 88-1M-5	PI 467034	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G 219	PI 467036	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G 228-M	PI 467037	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G 320-M	PI 467041	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G 353	PI 467043	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G 412-M	PI 467049	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G 451-1	PI 467051	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
52	PI 470951	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
61	PI 470952	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
8	PI 470957	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
13	PI 470958	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
19	PI 470959	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

22	PI 470960	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
26	PI 470961	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
35	PI 470962	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
37	PI 470963	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
19	PI 470966	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
37	PI 470972	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
48	PI 470974	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
69	PI 470976	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
7	PI 470990	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
62	PI 471013	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
6	PI 471017	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
1	PI 471035	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
2	PI 471036	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
4	PI 471037	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
6	PI 471038	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
7	PI 471039	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
9	PI 471040	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
10	PI 471041	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
12	PI 471042	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
13	PI 471043	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
14	PI 471044	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
19	PI 471048	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
31	PI 471056	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
33	PI 471058	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
35	PI 471059	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
36	PI 471060	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
37	PI 471061	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
44	PI 471065	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
46	PI 471067	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
48	PI 471068	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
3	PI 471070	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
4	PI 471071	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
8	PI 471072	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
17	PI 471073	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
18	PI 471074	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
37	PI 471075	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
52	PI 471076	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-522	PI 471602	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-523	PI 471603	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-524	PI 471604	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-525	PI 471605	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-526	PI 471606	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-537	PI 471611	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-543	PI 471616	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-545	PI 471618	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-552	PI 471624	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-562	PI 471633	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-564	PI 471635	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-565	PI 471636	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-566	PI 471637	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-568	PI 471638	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-569	PI 471639	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-570	PI 471640	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

G-571	PI 471641	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-572	PI 471642	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-573	PI 471643	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-574	PI 471644	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-575	PI 471645	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-576	PI 471646	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-579	PI 471649	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-581	PI 471651	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-584	PI 471653	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-585	PI 471654	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-588	PI 471657	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-589	PI 471658	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-590	PI 471659	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-591	PI 471660	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-592	PI 471661	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-593	PI 471662	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-594	PI 471663	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-595	PI 471664	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-596	PI 471665	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-597	PI 471666	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-598	PI 471667	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-600	PI 471669	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-601	PI 471670	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-602	PI 471671	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-603	PI 471672	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-604	PI 471673	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-605	PI 471674	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-606	PI 471675	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-611	PI 471677	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-613	PI 471678	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-614	PI 471679	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-617	PI 471681	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-619	PI 471683	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-621	PI 471684	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-623	PI 471685	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-624	PI 471686	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-632	PI 471690	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-637	PI 471695	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-638	PI 471696	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-652	PI 471705	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-654	PI 471707	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-657	PI 471708	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-659	PI 471709	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-660	PI 471710	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-668	PI 471716	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-682	PI 471727	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-684	PI 471729	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-685	PI 471730	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-686	PI 471731	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-687	PI 471732	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-688	PI 471733	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-689	PI 471734	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-691	PI 471735	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

G-692	PI 471736	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-719	PI 471761	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-720	PI 471762	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-280-1BM	PI 471768	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-28-2BM	PI 471776	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-29-1M-8	PI 471777	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-90-1-1BM	PI 471779	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-117-1-1-1-2M	PI 471780	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-288-3-5M	PI 471788	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-303-1M	PI 471789	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-316-2-5M	PI 471795	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-332-1-3-5M	PI 471798	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-342-2-2M	PI 471799	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-348-4M	PI 471801	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-395-7M	PI 471803	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-416-4M	PI 471804	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-457-1	PI 471805	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-503M	PI 471813	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-275-1M	PI 471817	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-765	PI 478686	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-759M	PI 478716	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-529M	PI 478730	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-620	PI 478735	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-672	PI 478741	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-TD28	PI 478742	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
G-23-1M	PI 478744	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-510M	PI 478748	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
B-7	PI 479777	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
H-5	PI 479778	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
I-2	PI 479779	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-583-1B	PI 481478	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-632-M	PI 481479	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-729	PI 481489	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-731	PI 481491	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-732	PI 481492	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-733	PI 481493	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-734	PI 481494	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-735	PI 481495	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-736	PI 481496	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-737	PI 481497	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-738	PI 481498	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-739	PI 481499	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-740	PI 481500	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-741	PI 481501	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-742	PI 481502	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-743	PI 481503	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-745	PI 481504	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-746	PI 481505	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-748	PI 481507	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-750	PI 481508	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-751	PI 481509	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-752	PI 481510	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

G-753	PI 481511	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-755	PI 481513	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-757	PI 481515	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-758	PI 481516	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-760	PI 481517	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-761	PI 481518	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-762	PI 481519	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-764	PI 481520	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-TD29	PI 481521	4X	*Triticum*	*turgidum*	*dicoccoides*	-	+
G-769	PI 481522	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G-771	PI 481523	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G2996	PI 538669	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G2997	PI 538670	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G2998	PI 538671	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G2999	PI 538672	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3000	PI 538673	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3001	PI 538674	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3003	PI 538676	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3004	PI 538677	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3006	PI 538678	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3007	PI 538679	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3012	PI 538680	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3013	PI 538681	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3014	PI 538682	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3017	PI 538684	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3035	PI 538686	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3038	PI 538688	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3050	PI 538690	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3051	PI 538691	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3054	PI 538694	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3063	PI 538695	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3064	PI 538696	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3065	PI 538697	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
G3066	PI 538698	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 14-2	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 14-13	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 14-25	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 14-34	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 14-35	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 14-36	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 14-40	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 14-45	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 14-47	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 14-49	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 15-2	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 15-17	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 15-18	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 15-19	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 15-26	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
Td 15-32	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 15-37	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 15-43	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 15-52	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

Td 16-1	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
Td 16-29	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
Td 17-1	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 17-2	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 17-22	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 18-1	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
Td 18-10	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
Td 18-15	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 18-16	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 18-20	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
Td 18-24	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 18-37	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 18-48	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 18-49	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 18-56	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	+
Td 19-6	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 19-7	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 19-20	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 19-23	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 19-24	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 19-25	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 19-29	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 19-36	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 19-45	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 19-50	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 19-51	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 20-8	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 20-9	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 20-11	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 20-20	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 20-22	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 20-31	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 20-34	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 20-35	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 20-37	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 20-38	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 20-42	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 30	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
Td 32-14	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 32-25	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 32-29	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 32- 30	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 32-44	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 32-45	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 32-46	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 36-12	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
Td 36-17	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 36-21	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 36-22	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 36-25	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 36-26	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 36-33	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 36-37	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

Td 36-38	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 36-39	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 36-40	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD A-33	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
TD A-35	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
TD A-51	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD A-52	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD A-56	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD A-57	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD A-61	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD A-69	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD B-6*	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	+
TD B-8	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD B-13	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD B-16	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	+
TD B-19	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD B-31	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD B-32	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD B-34	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD B-35	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD B-37	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD B-40	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD C-7	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD C-13	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD C-19	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
TD C-21	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD C-27	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD C-30	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD C-36	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
TD C-52	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD C-55	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD F-3	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD F-7	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD F-18	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD F-25	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD F-28	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD F-37	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD F-38	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD F-43	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD F-54	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD F-65	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD F-81	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD G-11*	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	+
TD G-15	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD G-22	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD G-42	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD G-47	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD G-50	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD G-52	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD G-56	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD G-58	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD G-59	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD G-61	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

TD I-4	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD I-15	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD I-17	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD I-18	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD I-20	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD I-29	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD I-39	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD I-40	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD I-44	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD I-45	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD I-50	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	+
TD K-19	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD K-32	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD K-36	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD K-45	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD K-46	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD K-50	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD L-1	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
TD L-10	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
TD L-25	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD L-28	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD L-29	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD L-33	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
TD L-34	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD L-40	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
TD L-43	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD L-56	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
TD L-61	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td T-7-1	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td T-7-5	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td T-7-7	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td T-7-8	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td T-7-15	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td T-7-18	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td T-7-21	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td T-7-26	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td T-7-30	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
Td T-7-35	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td T-7-40	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td T-7-45	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	-
Td 15-1	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 15-55	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	NA
Td 328	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	+
Td 582	NA	4X	*Triticum*	*turgidum*	*dicoccoides*	-	+
Ae 1	CIae 45	2X	*Aegilops*	*speltoides*		-	-
Ae 2	CIae 57	2X	*Aegilops*	*speltoides*		-	+
Ae 3	CIae 61	2X	*Aegilops*	*speltoides*		-	-
Ae 4	PI 170203	2X	*Aegilops*	*speltoides*		-	-
Ae 5	PI 170204	2X	*Aegilops*	*speltoides*		-	-
Ae 6	PI 172685	2X	*Aegilops*	*speltoides*		-	-
Ae 7	PI 173614	2X	*Aegilops*	*speltoides*		-	-
Ae 8	PI 174010	2X	*Aegilops*	*speltoides*		-	-
Ae 9	PI 219867	2X	*Aegilops*	*speltoides*		-	-

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

Ae 10	PI 254865	2X	*Aegilops*	*speltoides*	-	-
Ae 11	PI 266817	2X	*Aegilops*	*speltoides*	-	-
Ae 12	PI 315853	2X	*Aegilops*	*speltoides*	-	-
Ae 13	PI 330488	2X	*Aegilops*	*speltoides*	-	-
Ae 14	PI 369581	2X	*Aegilops*	*speltoides*	-	-
Ae 15	PI 369582	2X	*Aegilops*	*speltoides*	-	-
Ae 16	PI 369583	2X	*Aegilops*	*speltoides*	-	-
Ae 17	PI 369584	2X	*Aegilops*	*speltoides*	-	-
Ae 18	PI 369585	2X	*Aegilops*	*speltoides*	-	-
Ae 19	PI 369586	2X	*Aegilops*	*speltoides*	-	-
Ae 20	PI 369587	2X	*Aegilops*	*speltoides*	-	-
Ae 21	PI 369588	2X	*Aegilops*	*speltoides*	-	-
Ae 22	PI 369589	2X	*Aegilops*	*speltoides*	-	-
Ae 23	PI 369591	2X	*Aegilops*	*speltoides*	-	-
Ae 24	PI 369592	2X	*Aegilops*	*speltoides*	-	-
Ae 25	PI 369593	2X	*Aegilops*	*speltoides*	-	-
Ae 26	PI 369594	2X	*Aegilops*	*speltoides*	-	-
Ae 27	PI 369595	2X	*Aegilops*	*speltoides*	-	-
Ae 28	PI 369596	2X	*Aegilops*	*speltoides*	-	-
Ae 29	PI 369597	2X	*Aegilops*	*speltoides*	-	-
Ae 30	PI 369598	2X	*Aegilops*	*speltoides*	-	-
Ae 31	PI 369599	2X	*Aegilops*	*speltoides*	-	-
Ae 32	PI 369600	2X	*Aegilops*	*speltoides*	-	-
Ae 33	PI 369601	2X	*Aegilops*	*speltoides*	-	-
Ae 34	PI 369602	2X	*Aegilops*	*speltoides*	-	-
Ae 35	PI 369603	2X	*Aegilops*	*speltoides*	-	-
Ae 36	PI 369604	2X	*Aegilops*	*speltoides*	-	-
Ae 37	PI 369605	2X	*Aegilops*	*speltoides*	-	-
Ae 38	PI 369606	2X	*Aegilops*	*speltoides*	-	-
Ae 39	PI 369607	2X	*Aegilops*	*speltoides*	-	-
Ae 40	PI 369608	2X	*Aegilops*	*speltoides*	-	-
Ae 41	PI 369609	2X	*Aegilops*	*speltoides*	-	-
Ae 42	PI 369610	2X	*Aegilops*	*speltoides*	-	-
Ae 43	PI 369611	2X	*Aegilops*	*speltoides*	-	-
Ae 44	PI 369613	2X	*Aegilops*	*speltoides*	-	-
Ae 45	PI 369614	2X	*Aegilops*	*speltoides*	-	-
Ae 46	PI 369615	2X	*Aegilops*	*speltoides*	-	-
Ae 47	PI 369616	2X	*Aegilops*	*speltoides*	-	-
Ae 48	PI 369617	2X	*Aegilops*	*speltoides*	-	-
Ae 49	PI 369618	2X	*Aegilops*	*speltoides*	-	-
Ae 50	PI 369620	2X	*Aegilops*	*speltoides*	-	-
Ae 51	PI 369621	2X	*Aegilops*	*speltoides*	-	-
Ae 52	PI 369622	2X	*Aegilops*	*speltoides*	-	-
Ae 53	PI 369623	2X	*Aegilops*	*speltoides*	-	-
Ae 54	PI 369624	2X	*Aegilops*	*speltoides*	-	-
Ae 55	PI 369625	2X	*Aegilops*	*speltoides*	-	-
Ae 56	PI 369626	2X	*Aegilops*	*speltoides*	-	-
Ae 57	PI 369660	2X	*Aegilops*	*speltoides*	-	-
Ae 58	PI 369661	2X	*Aegilops*	*speltoides*	-	-
Ae 59	PI 369662	2X	*Aegilops*	*speltoides*	-	-
Ae 60	PI 369663	2X	*Aegilops*	*speltoides*	-	-
Ae 61	PI 369664	2X	*Aegilops*	*speltoides*	-	-

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

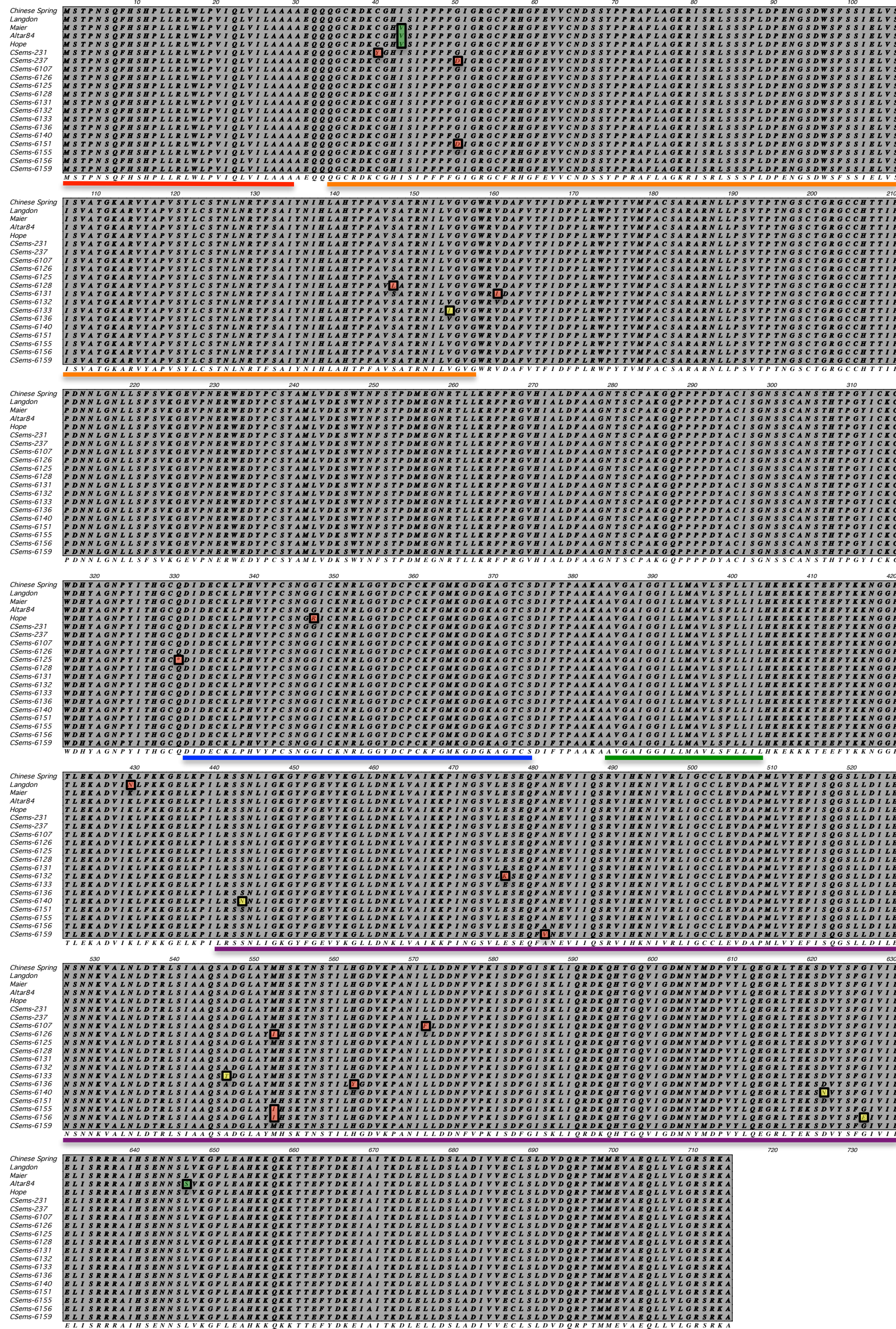
Ae 62	PI 369665	2X	*Aegilops*	*speltoides*		-	-
Ae 63	PI 369666	2X	*Aegilops*	*speltoides*		-	-
Ae 64	PI 393492	2X	*Aegilops*	*speltoides*		-	-
Ae 65	PI 393494	2X	*Aegilops*	*speltoides*		-	-
Ae 66	PI 393495	2X	*Aegilops*	*speltoides*		-	-
Ae 67	PI 422448	2X	*Aegilops*	*speltoides*		-	-
Ae 68	PI 449338	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 69	PI 449339	2X	*Aegilops*	*speltoides*		-	-
Ae 70	PI 449340	2X	*Aegilops*	*speltoides*		-	-
Ae 71	PI 449341	2X	*Aegilops*	*speltoides*		-	-
Ae 72	PI 487231	2X	*Aegilops*	*speltoides*		-	-
Ae 73	PI 487232	2X	*Aegilops*	*speltoides*		-	-
Ae 74	PI 487235	2X	*Aegilops*	*speltoides*		-	-
Ae 75	PI 487238	2X	*Aegilops*	*speltoides*		-	-
Ae 76	PI 542238	2X	*Aegilops*	*speltoides*		-	-
Ae 77	PI 542239	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 78	PI 542240	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 79	PI 542241	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 80	PI 542242	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 81	PI 542243	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 82	PI 542244	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 83	PI 542245	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 84	PI 542246	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 85	PI 542247	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 86	PI 542248	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 87	PI 542249	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 88	PI 542250	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 89	PI 542252	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 90	PI 542253	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 91	PI 542255	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 92*	PI 542256	2X	*Aegilops*	*speltoides*	*ligustica*	-	+
Ae 93	PI 542261	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 94	PI 542262	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 95	PI 542265	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 96	PI 542266	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 97	PI 542267	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 98	PI 542269	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 99	PI 542271	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 100	PI 542272	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 101	PI 542273	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 102	PI 542274	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 103	PI 542276	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 104	PI 554291	2X	*Aegilops*	*speltoides*		-	-
Ae 105	PI 554292	2X	*Aegilops*	*speltoides*		-	-
Ae 106	PI 554296	2X	*Aegilops*	*speltoides*		-	-
Ae 107	PI 554297	2X	*Aegilops*	*speltoides*		-	-
Ae 108	PI 554298	2X	*Aegilops*	*speltoides*		-	-
Ae 109	PI 554299	2X	*Aegilops*	*speltoides*		-	-
Ae 110	PI 554300	2X	*Aegilops*	*speltoides*		-	-
Ae 111	PI 554303	2X	*Aegilops*	*speltoides*		-	-
Ae 112	PI 554304	2X	*Aegilops*	*speltoides*		-	-
Ae 113	PI 554305	2X	*Aegilops*	*speltoides*	*ligustica*	-	-
Ae 114	PI 560527	2X	*Aegilops*	*speltoides*	*ligustica*	-	-

ACCESSIONS OF *TRITICUM* AND *AEGILOPS SPELTOIDES* DEPLOYED IN THE SNTOX1-*SNN1* INTERACTION STUDY (continued)

Ae 115	PI 560529	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 116	PI 560530	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 117	PI 560747	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 118	PI 560749	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 119	PI 560750	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 120	PI 560752	2X	*Aegilops*	*speltoides*	*speltoides*	-	-
Ae 121	PI 573449	2X	*Aegilops*	*speltoides*		-	-
Ae 122	PI 573450	2X	*Aegilops*	*speltoides*		-	-
Ae 123	PI 573452	2X	*Aegilops*	*speltoides*		-	-

[a]Accessions in bold with asterisks were sequenced for phylogenetic analysis; [b]Plus (+) and minus (-) indicate sensitive and insensitive to SnTox1; [c]Plus (+) and minus (-) indicate presence and absence of *Snn1* alleles based on PCR assay with marker *Xfcp667*

APPENDIX C. DEDUCED AMINO ACID SEQUENCE ALIGNMENT OF MUTANTS AND INFORMATIVE LINES

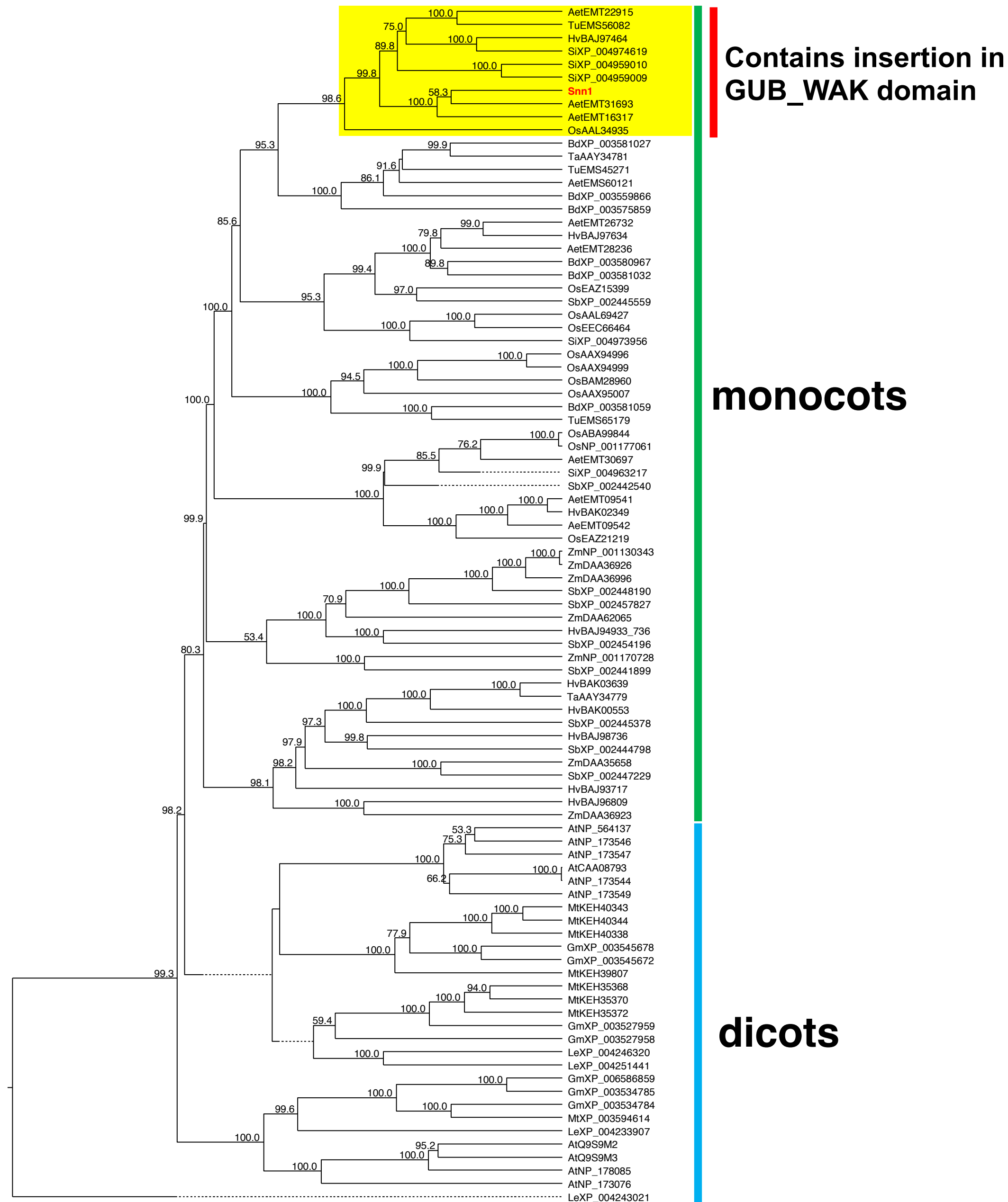


Residues highlighted in green are known to not alter Snn1 function. Residues highlighted in red are known to alter Snn1 function. Residues highlighted in yellow are unknown if they alter function because they occur in mutants that have had two EMS-induced mutations. The predicted signal sequence, GUB_WAK binding, EGF_CA binding, transmembrane, and PKc domains are underlined in red, orange, blue, green, and purple, respectively.

APPENDIX D. DNA ALIGNMENT OF *SNN1-1B* AND PUTATIVE *SNN1- 1D*

Identical nucleotides are shaded in grey. The *Snn1* start codon and the FGENESH-predicted start codon for the 1D copy are indicated in red boxes. The orange underline indicates *Snn1* exons, and the blue overline indicates the predicted exons for the 1D copy. The predicted signal sequence and GUB_WAK binding, EGF_CA binding, Transmembrane and PKc domains are highlighted in red, orange, blue, green, and purple, respectively.

APPENDIX E. A PHYLOGENETIC TREE OF SNN1 WITH OTHER PLANT WAK PROTEINS



Bootstrapping values (>50%, from 1,000 replicates) are indicated on the branches. Note that Snn1 and another nine WAKs all having an insertion within the galacturonan-binding (GUB_WAK) domain form a subgroup (shaded in yellow at the top) in monocot WAKs. Species abbreviations (followed by Genbank accession numbers): Aet, *Aegilops tauschii*; At, *Arabidopsis thaliana*; Bd, *Brachypodium distachyon*; Gm, *Glycine max*; Hv, *Hordeum vulgare*; Mt, *Medicago truncatula;* Os*, Oryza sativa*; Sb, *Sorghum bicolor*; Si, *Setaria italica* (foxtail millet); Le, *Lycopersicon esculentum*; Ta, *Triticum aestivum;* Tu, *Triticum urartu*; Zm, *Zea mays*.

APPENDIX F. GENBANK ACCESSION NUMBERS OF *SNN1* SEQUENCES USED IN PHYLOGENETIC ANALYSIS

Source	GenBank No.	Source	GenBank No.
Triticum aestivum Chinese Spring	KP085710	*Triticum turgidum* ssp. *dicoccum* PI168673	KP085730
Triticum aestivum Hope	KP085711	*Triticum turgidum* ssp. *dicoccum* PI94682	KP085731
Triticum aestivum DN2378	KP085712	*Triticum turgidum* ssp. *dicoccum* PI164582	KP085732
Triticum aestivum G532	KP085713	*Triticum turgidum* ssp. *dicoccum* PI217640	KP085733
Triticum aestivum Grandin	KP085714	*Triticum aestivum* CSems231	KP085734
Triticum aestivum Amery	KP085715	*Triticum aestivum* CSems237	KP085735
Triticum aestivum Timstein	KP085716	*Triticum aestivum* CSems6107	KP085736
Triticum aestivum Mocho de Espiga Quadrada	KP085717	*Triticum aestivum* CSems6126	KP085737
Triticum turgidum ssp. *durum* Altar84	KP085718	*Triticum aestivum* CSems6125	KP085738
Triticum turgidum ssp. *durum* Laidley	KP085719	*Triticum aestivum* CSems6128	KP085739
Triticum turgidum ssp. *durum* Lebsock	KP085720	*Triticum aestivum* CSems6131	KP085740
Triticum turgidum ssp. *durum* Maier	KP085721	*Triticum aestivum* CSems6132	KP085741
Triticum turgidum ssp. *durum* Mountrail	KP085722	*Triticum aestivum* CSems6133	KP085742
Triticum turgidum ssp. *durum* Souri	KP085723	*Triticum aestivum* CSems6136	KP085743
Ae. speltoides PI542256	KP085724	*Triticum aestivum* CSems6141	KP085744
Triticum turgidum ssp. *dicoccoides*TdG11	KP085725	*Triticum aestivum* CSems6140	KP085745
Triticum turgidum ssp. *dicoccoides* Tdb6	KP085726	*Triticum aestivum* CSems6151	KP085746
Triticum turgidum ssp. *dicoccoides* td 582	KP085727	*Triticum aestivum* CSems6107	KP085747
Triticum turgidum ssp. *dicoccum* CItr12214	KP085728	*Triticum aestivum* CSems6156	KP085748
Triticum turgidum ssp. *dicoccum* CItr14919	KP085729	*Triticum aestivum* CSems6159	KP085749
Triticum aestivum Chinese Spring cDNA sequence	KP091701		