Genetic and Physical Mapping of a Gene Conditioning Sensitivity in Wheat to a Partially Purified Host-Selective Toxin Produced by *Stagonospora nodorum*

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ABSTRACT

Liu, Z. H., Faris, J. D., Meinhardt, S. W., Ali, S., Rasmussen, J. B., and Friesen, T. L. 2004. 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.

A toxin, designated SnTox1, was partially purified from culture filtrates of isolate Sn2000 of *Stagonospora nodorum*, the causal agent of wheat leaf and glume blotch. The toxin showed selective action on several different wheat genotypes, indicating that it is a host-selective toxin (HST). The toxic activity was reduced when incubated at 50°C and activity was eliminated when treated with proteinase K, suggesting that the HST is a protein. The synthetic hexaploid wheat W-7984 and hard red spring wheat Opata 85, the parents of the International Triticeae Mapping Initiative (ITMI) mapping population, were found to be sensitive and in-

sensitive, respectively, to SnTox1. The ITMI mapping population was evaluated for toxin reaction and used to map the gene conditioning sensitivity. This gene, designated *Snn1*, mapped to the distal end of the short arm of chromosome 1B. The wheat cv. Chinese Spring (CS) and all CS nullisomic-tetrasomic lines were sensitive to the toxin, with the exception of N1BT1D. Insensitivity also was observed when the 1B chromosome of CS was substituted with the 1B chromosome of an insensitive accession of *Triticum dicoccoides*. In addition, a series of 1BS chromosome deletion lines were used to physically localize the sensitivity gene. Physical mapping indicated that *Snn1* lies within a major gene-rich region on 1BS. This is the first report identifying a putative proteinaceous HST from *S. nodorum* and the chromosomal location of a host gene conferring sensitivity.

Additional keywords: *Leptosphaeria*, *Phaeosphaeria*, *Septoria*.

Stagonospora nodorum Berk. (syn. *Septoria nodorum* (Berk.), teleomorph *Phaeosphaeria nodorum* (syn. *Leptosphaeria nodorum* E. Müller) is the causal agent of *Stagonospora nodorum* blotch (SNB) of common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD genomes) and durum wheat (*T. turgidum* L., $2n = 4x = 28$, AABB genomes). SNB is an economically important disease in many wheat-growing areas throughout the world, not only because it causes yield losses up to 50% (13,17,44), but also because it can affect grain quality (7,24). Typical SNB symptoms include lens-shaped necrotic and chlorotic lesions, whereas only small restricted lesions develop on resistant genotypes.

Host-selective toxins (HSTs) are important factors in many host–pathogen interactions, and have been reported in many pathosystems (35). Most fungal-produced HSTs are nonribosomally synthesized peptides or polyketide-derived compounds, but some are small proteins (32). Proteinaceous HSTs are primary gene products, and therefore are well suited for molecular biological investigations involving the interaction between fungal pathogen and host plant. The wheat tan spot pathogen *Pyrenophora tritici-repentis* currently is the most well-characterized fungus producing proteinaceous HSTs. The best characterized of these toxins is Ptr ToxA, which is a 13.2-kDa protein that induces necrosis when infiltrated into sensitive wheat genotypes (41). The

gene responsible for the production of the toxin has been cloned and characterized from the fungus (2,4). In the host, sensitivity to the toxin is associated with susceptibility to the fungus (18) and is controlled by a single dominant gene (19). This gene was mapped on the long arm of chromosome 5B using restriction fragment length polymorphisms in common wheat (8) as well as durum wheat (1).

In the case of *S. nodorum*, several chemical compounds, including cationic acid, ochracine, and septorin, have been isolated from culture filtrates. These compounds have been shown to induce symptom-like reactions on wheat leaves. However, they proved to be nonspecific and not significant determinants of disease (15). Keller et al. (16) demonstrated that crude extract of *S. nodorum* had selective action on wheat embryos originating from cultivars with different disease resistance levels. This in vitro screening method was used successfully for genetic analyses and breeding (43). However, proteinaceous HSTs have not yet been identified and isolated from *S. nodorum*.

A large, coordinated effort to develop molecular marker-based genetic linkage maps of all the wheat chromosomes began in the early 1990s with the establishment of the International Triticeae Mapping Initiative (ITMI) and utilized a population of recombinant inbred (RI) lines. Dense genetic maps of all 21 chromosomes now exist and include hundreds of marker loci (23,29–31,33,42). Many major genes and quantitative trait loci (QTL) for disease resistance and agronomically important characters have been mapped using this well-characterized population (3,9,12,21,27,28,38,39), including the gene conferring sensitivity to Ptr ToxC, an HST produced by *P. tritici-repentis* (5).

The polyploid nature of common wheat has allowed researchers to develop a vast array of aneuploid stocks. Stocks such as

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monosomic, nullisomic-tetrasomic (NT), ditelosomic, and inter-varietal disomic substitution lines are powerful tools for investigations of genetics and genomics, and they allow the physical location of genes and markers to specific chromosomes or chromosome arms (26,37). Recently, more than 400 chromosome deletion lines have been developed (6). These lines allow genes and DNA markers to be located to subchromosomal regions or bins (10,11,14,34,40).

The objective of this study was to physically and genetically map the gene conferring sensitivity to a partially purified HST from *S. nodorum*. Symbols for designating the toxin and the host sensitivity gene are proposed.

MATERIALS AND METHODS

Plant material. The genetic mapping population consisted of 106 RI lines (partial ITMI mapping population) derived from a cross between the synthetic hexaploid wheat W-7984 and the hard red spring wheat cv. Opata 85 (PI 591776), and was provided by M. E. Sorrells, Cornell University, Ithaca, NY. Development of this population was described in Nelson et al. (30). Wheat-cytogenetic stocks, including NT lines (37) (N2AT2D not included), chromosome 1BS deletion lines (6), Chinese Spring-*T. dicoccoides* (CS-DIC) disomic chromosome substitution lines (CS-DIC 3A and CS-DIC 6B not included), and *T. dicoccoides* ($2n = 4x = 28$, AABB genomes) used to create the substitution lines were provided by the Wheat Genetics Resource Center at Kansas State University. All these lines were subjected to infiltration with partially purified toxin for genetic analysis and physical mapping. Wheat genotypes ND495, BR-34, Erik, Grandin, Kulm, and CS were used to check the activity of partially purified toxin and determine host selective properties.

Culture filtrate production and toxin partial purification. *S. nodorum* isolate Sn2000 was collected from a wheat field in North Dakota in 1980. Toxin-containing Sn2000 culture filtrates were generated by growing the fungus on V8-potato dextrose agar medium until pycnidia began to release spores. Each plate then was washed with 50 ml of sterile distilled water, and 200 μ l of the spore suspension was added to 50 ml of liquid Fries medium (5 g of ammonium tartrate, 1.0 g of ammonium nitrate, 0.5 g of magnesium sulfate, 1.3 g of potassium phosphate [dibasic], 2.6 g of potassium phosphate [monobasic], 30.0 g of sucrose, 1.0 g of yeast extract, dissolved in 1,000 ml of water) and placed on an orbital shaker at 80 rpm for 48 h followed by 3 weeks of stationary growth. Both growth steps took place in the dark at room temperature. Culture filtrates were filtered through two layers of cheese cloth followed by vacuum filtration through a Whatman No. 1 filter and a 0.45- μ m Whatman cellulose nitrate filter. Culture filtrate (100 ml) was concentrated 16-fold using an Amicon YM10 membrane, diluted 9-fold with 20 mM glycyl-glycine, pH 8.0, and concentrated to 3 ml. The dilution with glycyl-glycine was repeated once and the concentrated 3-ml sample passed through a DEAE Sepharose Fast Flow column, 1.6 by 12 cm. The flow-through was collected, concentrated to 3 ml, and separated on a Superdex HR75 column (Pharmacia), 1.6 by 60 cm, equilibrated with 20 mM sodium acetate and 150 mM NaCl, pH 4.8. Active fractions were combined and used for assays.

Infiltration of partially purified toxin and toxin partial characterization. Plants were grown in plastic cones containing SB100 professional grow mix (Sungrow Horticulture, Dellevue,

WA) in the greenhouse at an average temperature of 21°C with a 12-h photoperiod. Two replicates of at least three plants of each parent, the RI lines, and cytogenetic stocks were infiltrated with the partially purified toxin. When the secondary leaf was fully expanded, it was assayed by infiltrating ≈ 25 μ l of partially purified toxin using a 1-ml syringe with the needle removed. The boundaries of the infiltration sites were marked with a nontoxic felt pen before water-soaking disappeared. After infiltration, all plants were moved to a growth chamber at 21°C with a 12-h photoperiod. Leaves were evaluated 3 days after infiltration and scored as insensitive or sensitive. Sensitive reactions were characterized by necrosis, whereas insensitivity showed no reaction within the infiltrated area.

To identify the putative proteinaceous nature of this toxin, the partially purified toxin samples along with samples treated with proteinase K at 1 mg ml⁻¹ were incubated at both room temperature and 50°C for 4 h. Proteinase K at 1 mg ml⁻¹ alone was used as a control (Table 1). The untreated and treated samples along with the control were infiltrated into the leaves of the toxin-sensitive cv. Grandin. The toxin activity was assayed by scoring plant leaves as sensitive or insensitive as mentioned previously.

The size of the toxin in culture filtrates was identified using size-based ultrafiltration. Filtrates and concentrates were tested using cutoff filters of 5, 10, and 30 kDa (Millipore, Billerica, MA). Filtrates and concentrates were infiltrated as described previously using toxin-sensitive cv. Grandin.

Linkage analysis. The mapping data for the ITMI population was obtained from the Graingenes database. The phenotypic data of the toxin reaction was treated as a marker locus and tested for linkage with the data set of 541 markers spanning the entire genome. The computer program MAPMAKER (version 2.0 for Macintosh) (20) was used to calculate linkage of the toxin-sensitivity gene with molecular markers using a minimum log likelihood ratio (LOD) of 3.0.

RESULTS

In vitro reaction of partially purified toxin. The reaction of several common wheat genotypes to the partially purified toxin was either sensitive or insensitive. Among them, ND495, Grandin, Kulm, and CS showed sensitivity (necrosis) within the infiltrated area, but lines BR34 and Erik both were insensitive to the toxin. The two parents used to develop the ITMI population also showed a differential reaction to this toxin, with W-7984 being sensitive and Opata 85 being insensitive (Fig. 1, top). The selective action of this partially purified toxin preparation on different genotypes suggested that it contains a host-selective toxin.

Activity assay of partially purified toxin after temperature, proteinase K treatment, and size-based filtration. Partially purified toxin incubated at room temperature and at 50°C showed necrosis 3 days postinfiltration (Table 1; Fig. 1, bottom). However, neither partially purified toxin samples treated with proteinase K (1 mg ml⁻¹) nor proteinase K (1 mg ml⁻¹) alone showed necrosis when infiltrated into sensitive wheat leaves (Table 1; Fig. 1, bottom).

Concentrates of all size-based filtration experiments induced necrosis when infiltrated into sensitive cv. Grandin. Filtrates of the 5- and 10-kDa filter did not induce necrosis, whereas filtrates of the 30-kDa filter did induce necrosis, indicating that the toxin is between 10 and 30 kDa.

TABLE 1. Effect of partially purified SnTox1 on sensitive wheat leaves after temperature and proteinase K treatment

Treatment	Reaction temperature (4 h)	
	Room temperature	50°C
Partially purified toxin alone	Sensitive	Sensitive
Partially purified toxin treated with proteinase K (1 mg ml ⁻¹)	Insensitive	Insensitive
Proteinase K (1 mg ml ⁻¹) alone	Insensitive	Insensitive

Fig. 1. Reaction of leaves 3 days after infiltration with the partially purified toxin produced by *Stagonospora nodorum* isolate Sn2000 and the partially purified toxin treated with proteinase K. Top panel: Leaves from the parents of International Triticeae Mapping Initiative population: **A**, Opata 85, **B**, W-7984; middle panel: **A**, Chinese Spring, **B**, *Triticum dicoccoides*, **C**, N1AT1D, **D**, N1BT1D, **E**, N1DT1B, **F**, CS-DIC1A, **G**, CS-DIC1B, **H**, CS1BS-4, **I**, CS1BS-18; bottom panel: leaves of Grandin treated with **A**, proteinase K only at 1 mg/ml, **B**, partially purified toxin treated with proteinase K at 1 mg/ml, and **C**, partially purified toxin only.

Genetic mapping of toxin insensitivity. The RI population segregated in a ratio of 48:58 sensitive/insensitive and were consistent with the expected 1:1 ratio after χ^2 analysis ($\chi^2 = 0.33$). This suggests that a single nuclear gene is responsible for conferring reaction to the partially purified toxin from the *S. nodorum* isolate Sn2000. Linkage analysis revealed that the toxin-sensitivity locus is located at the distal end of the short arm of chromosome 1B at a distance of 4.7 centimorgans (cM) from the marker *XksuD14* (Fig. 2).

Physical mapping of toxin sensitivity. CS was found to be sensitive to the Sn2000 HST, and an accession of *T. dicoccoides* used to create disomic chromosome substitution lines in CS was found to be insensitive (Fig. 1, middle). Among the set of CS-DIC substitution lines and CS NT lines, only CS-DIC1B and N1BT1D showed insensitive reactions (Fig. 1, middle). Therefore, this result correlated with the genetic linkage mapping result in that the toxin sensitivity gene resides on chromosome 1B.

To further refine the physical location of the Sn2000 HST sensitivity gene, we screened six CS chromosome 1B short arm deletion lines. Infiltration analysis revealed that all 1BS deletion lines were insensitive to the toxin, with the exception of 1BS-4 (Fig. 1, middle), which is the most terminal chromosome deletion. Therefore, the physical location of the toxin sensitivity gene lies between the breakpoints of 1BS-4 and 1BS-18 (Fig. 2).

DISCUSSION

Sn2000 is an aggressive isolate of *S. nodorum* that was collected in North Dakota. Using filtration, ion-exchange, and gel filtration chromatography, we partially purified a toxin from culture filtrates of this isolate. Some reports of chemical toxic compounds purified from *S. nodorum* exist, but most of these toxic compounds do not appear to be HSTs and are likely secondary metabolites or general cell wall-degrading enzymes (15). Culture filtrates that did show selective action in previous studies (16,43) were tested only on embryos and never were partially purified or characterized. In our study, the partially purified toxin from Sn2000 was shown to pass through a 30-kDa cutoff filter but not through a 5- or 10-kDa filter, indicating a size of between 10 and 30 kDa. Toxin action also was found to be labile to the treatment of proteinase K. Size and sensitivity to proteinase K both suggest that the toxin is proteinaceous in nature. Even though multiple proteins are present with this partially purified toxin, it is hypothesized that only one protein is host selectively active as a toxin due to the 1:1 segregation of host sensitivity. The isolation and characterization of this putative protein is in progress. There is no precedent for naming toxins from *S. nodorum*; therefore, we propose SnTox1 to designate this toxin, which is the first proteinaceous HST identified from *S. nodorum*.

In general, host-selective toxins are recognized as primary determinants (pathogenicity factors) of disease, and they delimit the host range of the fungal pathogen. Victorin is an HST produced by *Cochliobolus victoriae* Nelson that infects only oat (*Avena sativa* L.) with the genetically dominant *Vb* gene (25). Another known HST is HC toxin, produced by *C. carbonum* race 1, and is necessary to cause disease only in toxin-sensitive maize lines (36). ND495, Kulm, Grandin, and CS are sensitive to SnTox1 and susceptible to isolate Sn2000. In contrast, Erik and BR34 are insensitive to SnTox1 and highly resistant to Sn2000. In related work, we evaluated the ITMI population for reaction to fungal inoculation of Sn2000. A major QTL was identified at the toxin-sensitivity locus, and it accounted for a large portion of the phenotypic variation (22). It appears that SnTox1 plays an important role in disease development and appears to be a major virulence factor.

In the wheat-*P. tritici-repentis* interaction, Ptr ToxA also is considered an important factor in the development of tan spot. Insensitivity to the toxin often is correlated with resistance to the

Fig. 2. Comparison between the physical and genetic maps of wheat chromosome 1BS. The toxin sensitivity locus is designated *Snn1*. On the physical map, deletion bins are indicated by the boxes to the right of the chromosome. On the genetic map, markers are listed at the right. The centimorgan (cM) distance between *Snn1* and *XksuD14* is 4.7.

fungus. In addition, sensitivity to the toxin is controlled by a dominant gene. It was suggested that Ptr ToxA requires interaction with the product of the host sensitivity gene to manifest necrosis (1). In this study, we found that CS N1BT1D and the CS-DIC1B substitution line are insensitive to SnTox1. This insensitivity can be explained by the fact that the sensitivity gene was absent because of the absence of CS 1B. The toxin-sensitivity locus on chromosome 1B may be a sensitivity gene that produces a protein that directly or indirectly interacts with SnTox1 and gives rise to a sensitive reaction. The genetics of the SnTox1 and Ptr ToxA wheat interactions are very similar despite the fact they are produced by two different pathogens. It may be that such mechanisms were evolutionarily conserved by different pathogens.

Comparison of the physical and genetic map (Fig. 2) indicates that the toxin-sensitivity gene and DNA markers *XksuD14*, *Xmwg938*, *XGli*, and *XksuF43* all map on 1BS. The deletion bin harboring these markers accounts for $\approx 7\%$ of the physical size of the 1B short arm (34). The corresponding segment of the genetic linkage map accounts for ≈ 27 cM and is one of two gene-rich regions along the short arm of chromosome 1B. This region and the homologous regions of 1AS and 1DS also harbor many disease resistance genes (*Lr21*, *Sr33*, *Pm3*, and *Qndsu.Tsc*), agronomically important genes (*Rf3*, *Qphs.cnl*, and seed storage proteins), and more than 50 DNA markers (14,34). Here, we propose the symbol *Snn1* to designate the gene on 1BS that confers the sensitivity to the toxin produced by isolate Sn2000 of *S. nodorum*. The marker *XksuD14* has been converted to a polymerase chain reaction-based STS marker and would be suitable for marker-assisted selection.

SNB is one of the most important fungal diseases in wheat; however, to date, little research has been done regarding the host-pathogen interaction. We are the first to identify a putative proteinaceous HST of *S. nodorum* and provide the genetic and physical map locations of the toxin-sensitivity gene. These discoveries will greatly facilitate the understanding of the mechanism of pathogenicity and resistance in the *S. nodorum*-wheat pathogen system.

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