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Novel Sources of Resistance to *Stagonospora nodorum* and Role of Effector-Susceptibility Gene Interactions in Wheat of Russian Breeding

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Abstract: Virulence factors of the pathogen *Stagonospora nodorum* Berk. are numerous necrotrophic effectors (NEs) (SnTox), which interact with the products of host susceptibility genes (*Snn*), causing the development of the disease. In this study, 55 accessions of bread spring and winter wheat were screened for sensitivity to NEs SnToxA, SnTox1, and SnTox3 using different isolates of *S. nodorum*. In the studied panel of wheat, 47 accessions were modern commercial cultivars grown in Russia and 8 cultivars were historic wheat accessions from the N. I. Vavilov Institute of Plant Genetic Resources in Russia. In general, our wheat panel differed from other wheat collections with available data in that it was less sensitive to SnToxA and SnTox3, and more sensitive to SnTox1. Six sources of strong SNB resistance were identified in our wheat panel. In addition, during the study, wheat cultivars were identified as appropriate objects in which to study the different effects of SnTox-*Snn* interactions, which is important for marker-assisted selection for SNB resistance. The current study has shown, for the first time, that the expression level of *Snn1* and *Tsn1* susceptibility genes and the disease severity of the different wheat cultivars are interconnected. Future work should focus on the deep characterization of SnTox-*Snn* interactions at the molecular level.

Keywords: breeding; gene-for-gene interactions; fungal pathogens; necrotrophic fungal effectors; SnToxA; SnTox3; SnTox1; susceptibility genes *Tsn1*, *Snn1* and *Snn3*; Septoria nodorum blotch; *Triticum aestivum* L.

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1. Introduction

In the last three to four decades, pathogens of various kinds of blotching have begun to progress on wheat crops around the world. Among the causative agents of blotch, one of the most harmful is the pathogenic fungus *Stagonospora nodorum* Berk. (synonyms: *Septoria*, *Parastagonospora*, *Phaeosphaeria*), which causes Septoria nodorum blotch (SNB) of wheat [1]. *S. nodorum* damages the leaves and glumes of wheat, causing necrotic and chlorotic lesions that later develop into dark brown blotches. Leaf blotch leads to a decrease in the plant surface area, thereby limiting photosynthesis, overall crop growth, and yield, and the glume blotch directly affects the quality of the grain. Due to such damage, SNB is known to cause yield losses of up to 50% [2]. *S. nodorum* is widespread in wheat growing regions, including North America, Australia, Europe, and Asia, where it can cause severe economic losses [3,4]. Disease management of SNB includes cultivar resistance, fungicide treatment, seed cleaning, and stubble management [5]. Although chemical control is an important part of SNB disease management, the use of cultivars with increased genetic resistance

helps to support more environmentally sustainable wheat production. Thus, the study of host genetic resistance in the *S. nodorum*–wheat pathosystem is an actual problem of wheat breeding.

S. nodorum belongs to the order of fungal pathogens Pleosporales, whose representatives synthesize host-specific toxins (HST) or necrotrophic effectors (NE) that facilitate the infection process [6]. Such NEs ensure the virulence of the pathogen strain to the host plant if the plant genome contains the dominant susceptibility gene corresponding to the effector [7]. In the *S. nodorum*–wheat pathosystem, the interaction of the products of the NE pathogen virulence genes (*SnTox*) with the products of the host plant susceptibility genes (*Snn*) mostly follows an inverse gene-for-gene model and causes the development of the disease [6]. This phenomenon is known as dominant susceptibility and has been described for several other pathogens, including species of *Cochliobolus*, *Alternaria*, *Pyrenophora*, and *Bipolaris* [8,9].

Currently, eight such effectors and nine matching host dominant susceptibility genes have been identified and characterized to different extents. The following interactions are described: SnToxA–*Tsn1*, SnTox1–*Snn1*, SnTox2–*Snn2*, SnTox3–*Snn3-B1*, SnTox3–*Snn3-D1*, SnTox4–*Snn4*, SnTox5–*Snn5*, SnTox6–*Snn6*, and SnTox7–*Snn7* [10–20]. Recently, gene cloning experiments have shown that NEs SnTox2, SnTox6, and SnTox7 were the same protein, and therefore, the NE was redesignated SnTox267 [20]. All of these interactions have been identified both at the seedling stage and in adult plants under different environmental conditions. To date, five effectors (SnToxA, SnTox1, SnTox3, SnTox5, and SnTox267) have been cloned and characterized in the *S. nodorum* genome [4,18,20]. However, only *Tsn1* and *Snn1* of the susceptibility genes from *Triticum aestivum* L. have been cloned and characterized [12,17]. Although the *Snn3-B1* homeolog of the *Snn3-D1* gene, found on chromosome 5D in the diploid wild wheat relative *Aegilops tauschii,* has been cloned and its genomic sequence determined, the corresponding locus in the D subgenome of hexaploid wheat has not been reported [4,19].

Effectors SnToxA, SnTox1, and SnTox3 are considered the main effectors in the pathogen *S. nodorum* and are quite widespread among strains and isolates [4]. Compatible interactions SnToxA–*Tsn1*, SnTox1–*Snn1* и SnTox3–*Snn3-B1* play a significant role in the development of SNB [4]. Wheat susceptibility gene *Tsn1*, which determines sensitivity to NE SnToxA, is located on the long arm of chromosome 5B and encodes a serine/threonine protein kinase and a nucleotide binding site leucine rich repeat protein (NB-LRR) [12]. The SnToxA-*Tsn1* interaction explains 25–95% of the phenotypic variation of SNB in both tetraploid and hexaploid wheat [4]. Gene *Tsn1* is expressed in leaves, stems, and immature spikes [12]. The presence of *Tsn1* in different wheat collections varies from 26.5% to 86% of wheat cultivars [4]. The wheat susceptibility gene *Snn1*, which determines sensitivity to NE SnTox1, is located on the short arm of wheat chromosome 1B and encodes a member of the wall-associated kinase (WAK) class of plant receptor kinases [17]. A compatible SnTox1-*Snn1* interaction has explained up to 58% of the SNB variation [21]. The *Snn1* gene is specifically expressed only in wheat leaves [17]. The sensitivity to SnTox1 of various wheat lines ranges from 16 to 76% in various collections of the world [4]. The SnTox3-*Snn3-B1* interaction has been shown to significantly contribute to SNB incidence, accounting for 13 to 51% of disease severity [5]. Sensitivity locus *Snn3-B1* is located on the short arm of chromosome 5B, but gene cloning has not been complete [4,16]. A high frequency of occurrence of wheat genotypes sensitive to SnTox3 was found both in Europe (up to 55% of spring wheat) and in Australia (up to 91.3% of wheat cultivars) [4,22].

In the *S. nodorum*–wheat pathosystem, each SnTox–*Snn* interaction contributes to disease development. Resistance to SNB is inherited quantitatively, and it is complex. The complexity of the situation lies in the fact that multiple NEs expressed by *S. nodorum* isolates provide functional redundancy of the pathogen on wheat cultivars that have multiple dominant susceptibility genes [23]. There are two ways that breeders and geneticists use to discover susceptibility genes. The first way is to use molecular markers linked to susceptibility genes. Gene-based markers have been developed for *Tsn1* and *Snn1* [12,17].

In addition, microsatellite markers have been developed for the *Snn3-B1* locus [11,16]. Another method is to infiltrate plants with various *S. nodorum* isolates or synthesized toxins. The first method shows only the presence of dominant susceptibility gene alleles, while the second method is more accurate in showing which plants are truly insensitive or sensitive to a particular NE [4]. However, the level of sensitivity of different wheat genotypes to NEs can vary from strong to weak. It is believed that this may depend on various factors, in particular, on the expression of wheat susceptibility genes [4,17]. Unfortunately, the expression of host susceptibility genes has been largely unexplored, and such studies are needed to further characterize this pathosystem at the molecular level.

To characterize the *S. nodorum*–wheat pathosystem, the wheat collection is commonly investigated. Screening wheat collections to detect susceptibility genes to *S. nodorum* and to detect wheat sensitivity to NEs can help to expedite research progress on the *S. nodorum*–wheat pathosystem and give breeders new tools to effectively breed against multiple susceptibility genes at once. However, much remains to be explored in this pathosystem.

The aim of this study was to use a combination of wheat diversity in a panel of 55 bread spring and winter wheat cultivars along with *S. nodorum* isolates differing in effector gene profile to identify *Snn* susceptibility genes, search for new sources of SNB resistance, and to further analyze the complexity of SNB in the presence of various combinations of SnToxA-*Tsn1*, SnTox1-*Snn1*, and SnTox3-*Snn3* interactions. Furthermore, one of the main questions of this study was determination of the relationship between the sensitivity of the wheat genotype to NE and the expression of the *Snn* susceptibility gene. The screening of 55 wheat accessions with three pathogen isolates resulted in the discovery of 6 resistant cultivars. Correlation analysis revealed a positive relationship between sensitivity to NE and the level of *Snn* gene expression.

2. Materials and Methods

2.1. Research Objects

The object of the study was a wheat panel consisting of 55 accessions of bread spring and winter wheat (*Triticum aestivum* L.), which included 47 accessions that were modern commercial cultivars grown in Russia and 8 cultivars that were historic wheat accessions from the N.I. Vavilov Institute of Plant Genetic Resources in Russia. Other wheat accessions were obtained from the National Grain Center, named after P.P. Lukyanenko (Krasnodar, Russia); the Institute of Biochemistry and Genetics Ufa Federal Research Center of the Russian Academy of Sciences (Ufa, Russia); and the Federal Research Center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (Novosibirsk, Russia). All wheat accessions were hydroponically grown for seven days on 10% Hoagland–Arnon nutrient medium in a KBW E6 plant growth chamber (Binder GmbH, Tuttlingen, Germany) at 20/24 °C (night/day) at the irradiance 146 W/m^2 PAR (Osram lamps L 36W/77) with a 16 h photoperiod.

The pathogenic objects were three isolates of the fungus *S. nodorum*: SnB, Sn9MN-3A, and Sn1SP (from the collection of Institute of Biochemistry and Genetics, Ufa Federal Research Centre, Russian Academy of Sciences, Ufa, Russia). All *S. nodorum* isolates were maintained on potato-glucose agar (PGA) at 21 °C with a 12 h photoperiod.

2.2. Experimental Design

Genotyping of the accessions from the wheat panel and studying the expression of susceptibility genes were carried out using intact 7-day-old seedlings. The resistance/susceptibility of genotypes was evaluated on the separated first leaves [24].

The 7-day-old seedlings were placed in separate vessels and were sprayed with suspensions of *S. nodorum* isolates SnB, Sn9MN-3A, or Sn1SP with 10^6 spores mL^{-1} in 0.02% Tween-20. Control plants were sprayed with a solution containing only the wetting agent Tween-20 (0.02%) [24]. The volumes of all solutions allowed full moistening of leaves. To study plant-host genes expressions, the shoots of intact wheat seedlings were fixed in

liquid nitrogen 24 and 72 h after inoculation with *S. nodorum* isolates SnB, Sn9MN-3A, or Sn1SP.

Segments of the first wheat leaves of 7-day-old plants were placed in Petri dishes on wet cotton wool containing 0.004% benzamidazole (10–12 leaves/dish). Then 5 µL of a spore suspension of *S. nodorum* isolates SnB, Sn9MN-3A, or Sn1SP with 10^6 spores mL^{-1} in 0.02% Tween 20 were spotted onto the leaf surface, and the Petri dishes with leaves were transferred to the KBW E6 plant growth chamber (Binder GmbH, Tuttlingen, Germany) under controlled conditions [24].

2.3. Plant Resistance/Susceptibility Assay to NEs SnToxA, SnTox1 and SnTox3

The development of SNB symptoms on wheat leaves was registered on the sixth day after infection with *S. nodorum* isolates using an SP-800UZ Image Stabilization camera (Olympus, Bekasi, Indonesia); the damage area was measured using the ImageJ 1.44 computer program (rsbweb.nih.gov/ij/download.html, accessed on 20 February 2023) and expressed as a percentage of the total leaf area. In addition, the degree of lesion was also evaluated according to the international scale based on the percentage of the damage area of plant organs: RR (0–5%)—cultivars with very high and high resistance; R (6–15%)—resistant cultivars; M (16–25%)—slightly susceptible cultivars; S (26–65%)—susceptible cultivars; and SS (66–100%)—cultivars with very high and high susceptibility [24].

2.4. Isolation of DNA in Plants and Fungi and Performing the Polymerase Chain Reaction (PCR)

DNA was isolated from wheat seedlings and 7-day fungus culture by the phenol-detergent method [24]. Identification of NEs genes *SnToxA*, *SnTox1*, and *SnTox3* in *S. nodorum* isolates SnB, Sn9MN-3A, or Sn1SP was performed by PCR with gene-specific primers and in a TP4-PCR-01-"Tertsik" type amplifier (DNA Technology, Moscow, Russia). Primers for the housekeeping gene tubulin were used as an internal control for the presence of fungal DNA [25].

The dominant allele of the *Tsn1* gene was identified in wheat cultivars by PCR with primers for the microsatellite marker *Xfcp623*, located in the internal region of the *Tsn1* gene [12]. The dominant allele of the *Snn1* gene was identified with primers for the intragenic marker *Snn1* [17]. The allelic state of the *Snn3-B1* locus was determined by PCR with primers for the microsatellite markers *Xcfd20* and *Xgwm234* [16,26]. The presence of an amplification product proved the existence of a dominant allele of the gene, and the absence of an amplification product indicated the presence of a null (recessive) allele. The sequences of primers are presented in Table S1 (Supplementary Materials). PCR products were separated in 7% PAGE stained with ethidium bromide using GeneRuler DNA Ladders (Thermo Fisher Scientific, Waltham, MA, USA). The gels were photographed using a documenting system of GelDoc XR (Bio-Rad Laboratories, Hercules, CA, USA).

2.5. Gene Expression Analysis

Total RNA from wheat seedlings and 7-day fungus culture was extracted using TRIzol™ Reagent (Merck KGaA, Sigma-Aldrich, Darmstadt, Germany) according to the manufacturer's instructions. cDNA synthesis was carried out as described previously [24]. Analysis of *SnToxA*, *SnTox1*, and *SnTox3* gene expression in different isolates of *S. nodorum* in vitro was performed with the same primers using semi-quantitative PCR (Table S1, Supplementary Materials). PCR with the cDNA template was performed in a TP4-PCR-01-Tertsik type amplifier (DNA Technology, Moscow, Russia). PCR products were separated in 7% PAGE stained with ethidium bromide using GeneRuler DNA Ladder (Thermo Fisher Scientific, Waltham, MA, USA). The gels were photographed using a documenting system of GelDoc XR (Bio-Rad Laboratories, Hercules, CA, USA). Primers for wheat genes (*TaSnn1* and *TaTsn1*) for qRT-PCR were designed using a web-based primer designing tool from IDT (http://eu.idtdna.com/Scitools/Applications/Primerquest, accessed on 20 February 2023) (USA). The sequences of the primers are presented in Table S2 (Supplementary Materials). Quantitative PCR was performed using a set of predefined reagents EvaGreenI (Synthol, Moscow,

Russia) and CFX Connect realtime PCR Detection System device (BioRad Laboratories, Hercules, CA, USA). To standardize the data, the wheat gene *TaRLI* (RNaseLinhibitor-like) (Table S2, Supplementary Materials) was used as an internal reference [27]. The delta–delta Ct method was used to quantify the relative gene expression [28,29]. Three independent biological and three technical replications were performed for each experiment.

2.6. Statistical Analysis

All experiments were repeated 3 times with a different number of biological repetitions from 3 to 10. Experimental data were expressed as means ± SE, which were calculated in all treatments using MS Excel. The significance of the differences was assessed by ANOVA followed by Duncan's test ($p < 0.05$) with STATISTICA 10.0 software. Calculation of the correlation coefficient and regression analysis were performed using Microsoft Office Excel 2010.

3. Results

3.1. Analysis of the Allelic State of the Tsn1, Snn1 Genes and the Snn3-B1 Locus

In this work, 55 wheat accessions were studied for the presence of alleles of the *Tsn1* and *Snn1* genes and alleles of the *Snn3-B1* locus. (Figures S1–S3, Table S3, Supplementary Materials).

This included 17 cultivars of bread spring wheat and 38 cultivars of bread winter wheat, of which 8 are historic wheat accessions (Saratovskaya 29, Susquehanna, Mironovskaya 808, Amelio, Selkirk, Atlas 66, Salamoni, Chinese Spring), and the other 47 are modern commercial cultivars (Table S3, Supplementary Materials). Based on DNA analysis using intragenic markers, the dominant allele of the *Tsn1* gene was found in 4 spring wheat cultivars and 11 winter wheat cultivars, which amounted to 23.5 and 29%, respectively (Figure 1A and Figure S1, Table S3, Supplementary Materials). The remaining 40 cultivars had a null allele (Figure S1, Table S3, Supplementary Materials). The dominant allele of the *Snn1* gene was absent in one winter cv. Don Mira. Of the remaining 54 cultivars, 17, amounting to 100% of spring wheat panel, and 37, representing 97.4% of the winter wheat panel, contained the dominant allele of the *Snn1* gene (Figure 1A and Figure S2, Table S3, Supplementary Materials).

For PCR diagnostics of the allelic state of the *Snn3-B1* locus, specific primers for two microsatellite markers, *Xcfd20* and *Xgwm234*, were used [16,26]. Amplified fragments of both markers were polymorphic across different wheat accessions (Figure S3, Supplementary Materials). The null allele of the *Snn3-B1* locus for two markers was found only in one cv. Don Mira (Figure S3, Table S3, Supplementary Materials). In addition, a null allele for the *Xcfd20* marker was found in two cultivars—Bashkirskaya 26 and Boevchanka (Figure S3A, Table S3, Supplementary Materials). Among the remaining 52 wheat accessions, the *Xcfd20* marker was detected in one allele in 26 accessions (an allele with sizes of 500 bp was found in 24 accessions, and an allele with sizes of 370 bp was found in 2 accessions) and two or more different alleles in 26 accessions (380 bp, 500 bp, about 700 bp), of which 15 accessions were spring wheat (88%) and 37 accessions were winter wheat (97.4%) (Table S3, Figure S3A, Supplementary Materials, Figure 1A).

The *Xgwm234* marker detected a null allele in one accession (Don Mira), one allele with a size of 244 bp in 5 accessions, and two alleles with sizes of 244 and 255 bp in 49 wheat accessions (Figure S3B, Table S3, Supplementary Materials). Out of 55 samples, we selected 21 accessions in which the *Xcfd20* detected two alleles with sizes of 380 and 500 bp, and in which the *Xgwm234* found two alleles with sizes of 244 and 255 bp (Figure S3B, Table S3, Supplementary Materials).

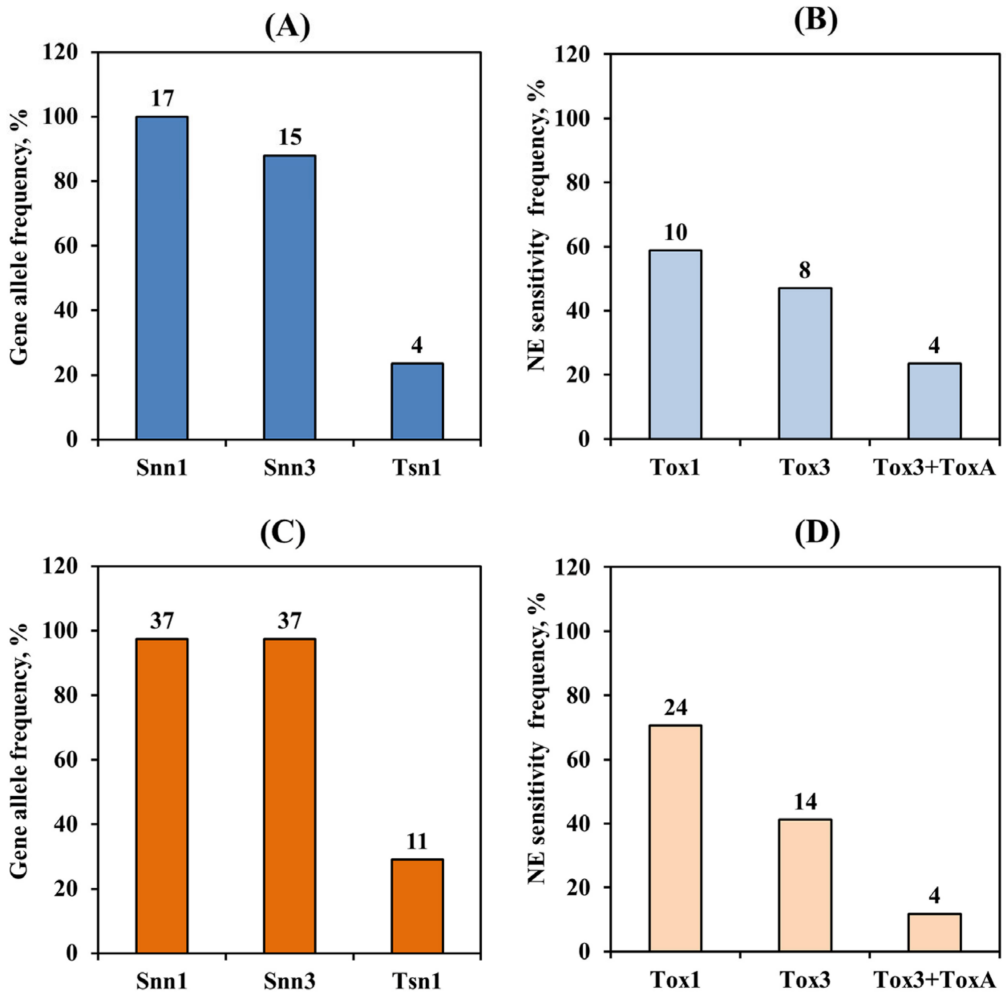

Figure 1. Frequency of alleles of susceptibility genes *Tsn1*, *Snn1*, and *Snn3-B1* in 17 accessions of spring wheat (**A**) and 38 accessions of winter wheat (**C**). Frequency of NE sensitivity against SnTox1, SnTox3, and SnToxA+SnTox3 in 17 accessions of spring wheat (**B**) and 34 accessions of winter wheat (**D**). The frequency is expressed as a percentage; the wheat panel is taken as 100 percent. The numerals on the histograms indicate the amount of wheat accessions that have susceptibility genes or are sensitive to NE.

3.2. Analysis of Resistance/Susceptibility of Wheat Accessions to NEs SnToxA, SnTox1 and SnTox3

The presence of a dominant allele of the susceptibility gene does not always mean the susceptibility of the cultivar; the cultivar may be resistant [4]. The degree of cultivar resistance was tested by the damage area of the leaf when infected with different *S. nodorum* isolates (SnB, Sn9MN-3A, and Sn1SP), which carried a different set of NEs genes (Figure 2A). The isolates SnB and Sn9MN-3A contained the two NEs genes *SnToxA* and *SnTox3* in their genome (Figure 2A). The isolate Sn1SP contained only the one NE gene, SnTox1, in its genome (Figure 2A). In all isolates of *S. nodorum*, SnB, Sn9MN-3A, and Sn1SP, all identified NEs genes were expressed in vitro (Figure 2B).

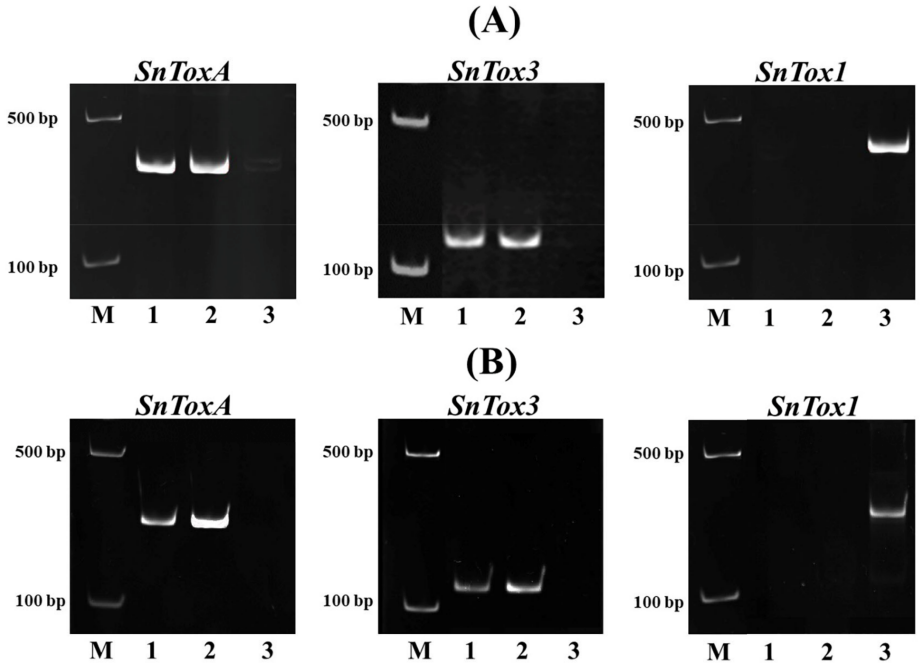

Figure 2. Identification of the *SnToxA*, *SnTox3*, and *SnTox1* genes by PCR (**A**) and analysis of transcriptional activity of the *SnToxA*, *SnTox1*, and *SnTox3* effector genes (in vitro) (**B**) in three isolates of *S. nodorum*: 1-SnB; 2-Sn9MN-3A; 3–Sn1SP; M-DNA molecular weight ladder 100–1000 bp.

The frequency of sensitivity to NE differed from the frequency of susceptibility genes, and was, in almost all cases, less by 17–56% (Figure 1B). Only 10 spring and 24 winter wheat accessions were sensitive to SnTox1, which amounted to 58.8 and 70.6%, respectively, in the studied wheat panel (Figure 1B). Sensitivity to SnTox3 was found in an even smaller number of accessions, with at least one dominant allele for *Xcfd20* and Xgwm234 markers, in 8 spring wheat and 14 winter wheat accessions, which amounted to 47 and 41.2%, respectively (Figure 1B). Since the SnB isolate expressing SnToxA and SnTox3 was used in this study (Figure 2B), we were unable to separate the susceptibility to these two NEs. Susceptibility to SnB (susceptibility to SnToxA + SnTox3) was found in 100% of spring wheat accessions with the dominant allele of the *Tsn1* gene, and only 11.8% of winter wheat accessions with the *Tsn1* gene (Figure 1B).

In this work, one completely resistant cultivar of bread winter wheat Don Mira was identified; it had null alleles of the *Tsn1* and *Snn1* genes, as well as a null allele for the markers *Xcfd20* and *Xgwm234* (Figures S1–S3, Table S3, Supplementary Materials). When this cultivar was infected with three different isolates of *S. nodorum* SnB (SnToxA/SnTox3), Sn9MN-3A (SnToxA/SnTox3), and Sn1SP (SnTox1), damage areas were not found except for a hypersensitivity reaction, which is evidence of the cultivar's resistance (Figure 3C).

As expected, the 21 accessions that had two different alleles for both *Xcfd20* (370/380 and 500 bp) and *Xgwm234* (244 and 255 bp) markers were sensitive to NE SnTox3 (numbers 1, 2, 5–9, 14, 26, 36, 37, 42, 44, 46, 47, 49–54 in Tables S3 and S4, Supplementary Materials). All 20 accessions (no data available for accession 14) were susceptible to isolates of SnB (SnToxA/SnTox3) and Sn9MN-3A (SnToxA/SnTox3) (Table S4, Supplementary Materials). However, these pathogen isolates express SnToxA in addition to SnTox3 (Figure 2B). Among 20 susceptible accessions, the dominant allele of the *Tsn1* gene was found in six accessions (numbers 7, 8, 26, 37, 52, 54); these accessions may be sensitive to SnToxA (Table S4, Supplementary Materials). All 20 accessions had a dominant allele of the *Snn1* gene (Table S3, Supplementary Materials), but only 12 accessions were sensitive to SnTox1 when infected with the Sn1SP isolate (SnTox1) (numbers 1, 2, 5–9, 26, 42, 44, 46, 47 in Tables S3 and S4, Supplementary Materials), and 8 accessions were insensitive to SnTox1 (numbers 36, 37, 49–54) (Table S4, Supplementary Materials). Photo of accessions leaves with numbers

26—cv. Dmitry, 36—cv. Kazakhstanskaya 10, 37—cv. Iren, 42—cv. Tulaykovskaya 108, 50—cv. Mironovskaya 808, 52—cv. Selkirk, and 54—cv. Salamoni are presented in Figure 3. Thus, in this work, 12 wheat cultivars susceptible to three isolates of *S. nodorum* (SnB, Sn9MN-3A, and Sn1SP) were found (Table S4, Supplementary Materials).

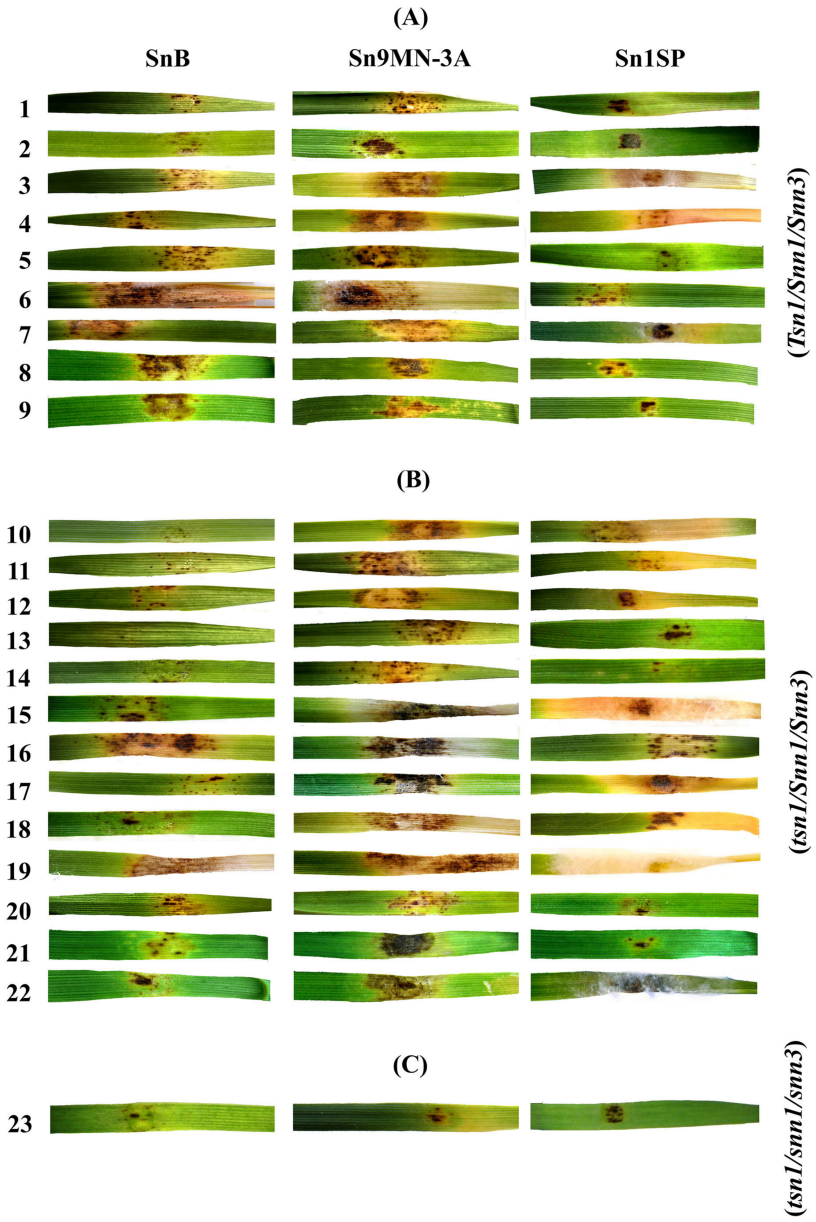

Figure 3. Analysis of phenotypes of gene-for-gene interactions in various compatible and incompatible combinations in the *S. nodorum*–wheat pathosystem. Photographs represent results of a typical variant from a series of experiments. Experiments were carried out on the separated first leaves. The development of symptoms was recorded 7 days after infection of 23 wheat accessions with different allelic states of susceptibility genes (*Tsn1/Snn3/Snn1*) (**A**), (*tsn1/Snn3/Snn1*) (**B**), and (*tsn1/snn3/Snn1*) (**C**) with three isolates of *S. nodorum* SnB (SnToxA/SnTox3), Sn9MN-3A (SnToxA/SnTox3), and Sn1SP (SnTox1) carrying a different set of NEs genes. The 23 cultivars are as follows: 1—Grom, 2—Vassa, 3—Dmitriy, 4—Gratsiya, 5—Kalym, 6—Zhnitsa, 7—Iren, 8—Selkirk, 9—Salamoni, 10—Tabor, 11—Stan, 12—Brigada, 13—Yeremeyavna, 14—Lebed, 15—Bashkirskaya 26, 16—Kazahstanskaya 10, 17—Omskaya 35, 18—Salavat Yulaev, 19—Tulajkovskaya 108, 20—Saratovskaya 29, 21—Mironovskaya 808, 22—Chinese Spring, 23—Don Mira.

Among the remaining 33 accessions (there are no data for 3 cultivars because these accessions were omitted from analysis due to poor growth or lack of seeds during different stages of the experiment), 30 accessions were resistant to varying degrees (Table S4, Supplementary Materials).

Cultivars Bashkirskaya 26 (no. 34) and Boevchanka (no. 45), with null alleles for the *Tsn1* gene and for the *Xcfd20* marker and a dominant allele for the *Snn1* gene (Table S3, Supplementary Materials), were resistant to the SnB isolate, and accession no. 45 was additionally resistant to Sn9MN-3A, but both accessions were susceptible to the Sn1SP isolate (Table S4, Supplementary Materials). These two accessions were insensitive to NEs SnToxA and SnTox3, but sensitive to NE SnTox1 (Table S4, Supplementary Materials). The susceptibility of the cv. Bashkirskaya 26 to the Sn9MN-3A isolate suggests the presence of one or more undetermined NEs in this isolate (Figure 3).

Five accessions (numbers 38, 40, 41, 43, 48 in Tables S3 and S4, Supplementary Materials) with the null allele for the *Tsn1* gene and one allele for the *Xgwm234* marker (244 bp) were resistant to the SnB isolate (insensitive to SnToxA and SnTox3) and were susceptible to the Sn9MN-3A isolate (Table S4, Supplementary Materials), which further confirms the assumption that the Sn9MN-3A isolate has an additional NE. All five accessions had the dominant allele of the *Snn1* gene (Table S3, Supplementary Materials), but only four accessions (38, 40, 41, 43) were sensitive to SnTox1 when infected with the Sn1SP isolate, and the accession no. 48—cultivar Saratovskaya 29—was insensitive to SnTox1 when infected with Sn1SP isolate (Table S4, Supplementary Materials). Photos of accession leaves with numbers 38—cv. Omskaya 35, 40—cv. Salavat Yulaev, and 48—cv. Saratovskaya 29 are shown in Figure 3.

Among the remaining 23 accessions, all had one allele for the *Xcfd20* marker (380 bp) (numbers 3, 4, 10, 11, 16, 17, 19–25, 27–33, 35, 39, 55 in Tables S3 and S4, Supplementary Materials) (Figure S3A, Supplementary Materials). The dominant allele for the *Tsn1* gene was found in 8 accessions (numbers 3, 10, 20, 23, 24, 28, 32, 35 in Tables S3 and S4, Supplementary Materials). Of the 23 accessions, 21 were resistant to the SnB isolate and therefore insensitive to SnTox3. Among these 21 resistant accessions, there were six that had a dominant allele for the *Tsn1* gene; despite this fact, they were insensitive to SnToxA. These were accessions 3—cv. Esaul, 10—cv. Trio, 20—cv. Alekseich, 23—cv. Grom, 24—cv. Vassa, and 28—cv. Gratsiya (Table S4, Supplementary Materials). Only 2 out of 23 accessions were susceptible to the SnB isolate; both accessions had a dominant allele of the *Tsn1* gene, and therefore they could be sensitive to both SnTox3 and SnToxA. These are accessions were 32—cv. Kalym and 35—cv. Zhnitsa (Figure 3, Table S4, Supplementary Materials). All 23 accessions had a dominant allele of the *Snn1* gene; however, seven accessions were insensitive to SnTox1 when infected with Sn1SP isolate, and other accessions were susceptible to this NE (Table S4, Supplementary Materials). Of the seven non-susceptible accessions, two (32 and 35) were resistant to only one *S. nodorum* isolate, Sn1SP (Figure 3, Table S4, Supplementary Materials), and five accessions were resistant to all three *S. nodorum* isolates (SnB, Sn9MN-3A, and Sn1SP) (Table S4, Supplementary Materials). These were accessions 23—cv. Grom, 24—cv. Vassa, 29—cv. Zhiva, 30—cv. Yeremeyavna, and 31—cv. Lebed, which suggests their insensitivity to SnToxA, SnTox3, and SnTox1, as well as to indeterminate NE in isolate Sn9MN-3A. Photos of accession leaves from Grom, Vassa, Yeremeyavna, and Lebed are presented in Figure 3. Excluding these five accessions, the remaining 18 accessions were susceptible to the Sn9MN-3A isolate (Table S4, Supplementary Materials).

Thus, in this work, 51 wheat accessions were analyzed for resistance/susceptibility to different isolates of *S. nodorum* (Figure 4A). The scatterplot analysis of 51 accessions highlighted that this wheat collection contained mostly resistant and slightly susceptible accessions to at least one pathogen isolate, and there were relatively few highly susceptible accessions (Figure 4A). In this study, six wheat accessions that were resistant to three isolates of *S. nodorum* (SnB, Sn9MN-3A, and Sn1SP) and insensitive to three NEs, SnToxA, SnTox3, and SnTox1 (Figure 4A). Two accessions were resistant to two pathogen isolates

at the same time. Cultivar Saratovskaya 29 was resistant to SnB and Sn1SP, and cultivar Boevchanka was resistant to SnB and Sn9MN-3A (Table S4, Supplementary Materials).

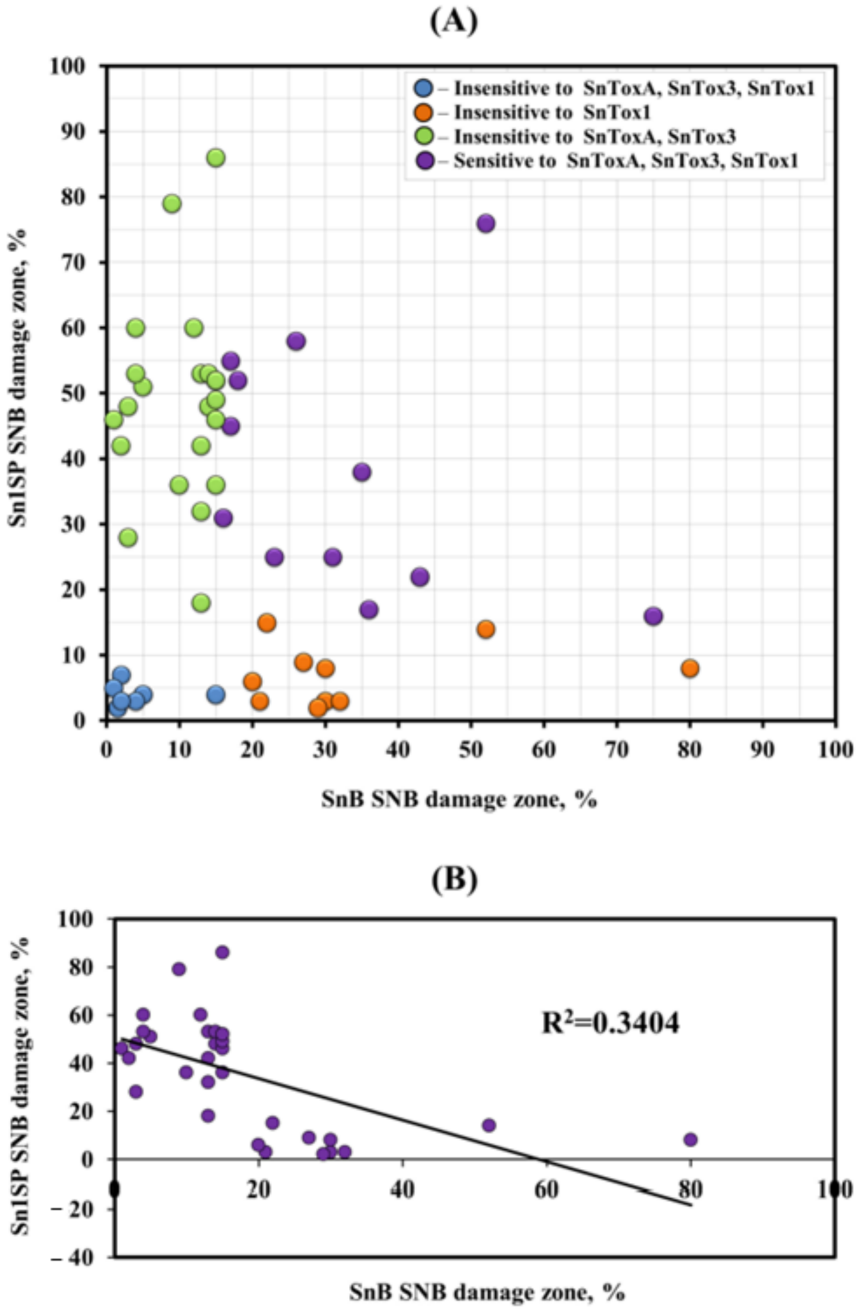

Figure 4. Comparative analysis of the resistance/susceptibility in the wheat panel to *S. nodorum* SnB and Sn1SP isolates. (**A**) Dot plot comparisons of Sn1SP and SnB SNB damage zones in the 51 wheat accessions. (**B**) Regression line for relationship between sensitivity to SnTox3 and SnToxA (wheat accessions susceptible to isolate SnB) and sensitivity to SnTox1 (wheat accessions resistant to isolate Sn1SP) in the 32 wheat accessions. The correlation coefficient of Pearson's was $r = -0.58$, coefficient of determination was $R^2 = 0.34$, p-value = 0.0005.

The SnB-only resistance was found in 21 accessions, and the Sn1SP-only resistance was found in 10 accessions (Figure 4A). Of the 51 wheat accessions tested, only 12 accessions were susceptible to all three *S. nodorum* isolates (Figure 4A).

This work also analyzed the relationship between sensitivity of wheat to one NE and insensitivity to other NEs in 32 accessions that were resistant to one or two pathogen

isolates (Figure 4B). In this work, a negative relationship was found between sensitivity to SnTox3 and SnToxA (wheat accessions susceptible to isolate SnB) and sensitivity to SnTox1 (wheat accessions resistant to isolate Sn1SP) (Figure 4B, Table S5, Supplementary Materials). If the wheat accession was sensitive to SnTox1, then it was insensitive to SnToxA and SnTox3 (Figure 4B). The correlation coefficient of Pearson's was $r = -0.58$, the coefficient of determination was $R^2 = 0.34$, and the p-value = 0.0005 (Figure 4B, Table S5, Supplementary Materials).

3.3. Analysis of the Expression of the Tsn1 and Snn1 Susceptibility Genes

Despite the presence of dominant alleles of the Tsn1 and Snn1 susceptibility genes, some wheat accessions were resistant to SnB and Sn1SP isolates. Therefore, the transcriptional activity of the *Tsn1* and *Snn1* genes was studied in 26 selected accessions that were infected with SnB or Sn1SP isolates, respectively. Photos of leaves with symptoms of the disease of 22 accessions out of 26 selected accessions are presented in Figure 3.

The *Snn1* gene expression was studied in 22 accessions (Figure 5A,B). In ten SnTox1 NE-sensitive accessions—Dmitry, Gratsiya, Tabor, Stan, Brigada, Bashkirskaya 26, Omskaya 35, Salavat Yulaev, Tulajkovskaya 108, and Chinese Spring—a high transcript level of the *Snn1* gene was found both in the control and in the Sn1SP isolate-infected wheat leaves (Figure 5A,B). At the same time, with the development of infection, the transcript level decreased in all susceptible cultivars (Figure 5A,B). In 12 SnTox1-insensitive samples, *Snn1* gene expression was inhibited by 50% or more under normal conditions and was greatly reduced in the Sn1SP isolate-infected wheat leaves compared to susceptible accessions (Figure 5A,B). Some resistant accessions—Grom, Vassa, Lebed, Saratovskaya 29, and Mironovskaya 808—either did not have *Snn1* gene expression or it was minimal (Figure 5A,B).

Among the 26 accessions, the dominant allele of the *Tsn1* gene was found in 13 accessions, and the expression of this susceptibility gene was studied in these accessions observed during infection with the SnB isolate (SnToxA/SnTox3) (Figure 5C). However, the SnB isolate was expressed SnTox3 in addition to SnToxA. Five accessions (Grom, Vassa, Gratsiya, Esaul, and Alekseich) were resistant to SnB isolate; therefore, they were insensitive to SnToxA and SnTox3 (Table S4, Supplementary Materials). Analysis of the *Tsn1* gene expression in four resistant accessions showed a low transcript level of this gene in the norm and during infection (Figure 5C). In the cv. Gratsiya, transcription of the *Tsn1* gene was practically absent (Figure 5C). The remaining eight accessions were susceptible to the SnB isolate; therefore, they could be susceptible to both SnToxA and SnTox3 (Table S4, Supplementary Materials). The transcript level of the *Tsn1* gene in eight susceptible accessions ranged from almost zero to a high level (Figure 5C). In the cv. Kalym and cv. Iren, transcription of the *Tsn1* gene was almost absent (Figure 5C). In several susceptible cultivars (Selkirk, Salamoni, and Yubileynaya 100), the transcript level of the *Tsn1* gene was strongly reduced, as in resistant accessions (Figure 5C). In three susceptible cultivars—Dmitry, Zhnitsa, and Ermak—the transcript level of the *Tsn1* gene increased by 1.4–2.4 times 72 h after infection with the SnB isolate compared to the control (Figure 5C). This was the highest expression level of *Tsn1* during infection among all cultivars studied (Figure 5C).

In accordance with the results obtained, the relationship between the degree of the cultivar sensitivity to NE and the expression level of the corresponding NE host susceptibility gene was studied. Correlation analysis showed a positive relationship between the expression level of the *Snn1* gene 24 h after infection with the Sn1SP isolate and the severity and magnitude of damage in all 22 wheat cultivars ($r = 0.92$, $R^2 = 0.85$, p-value = 9.22709×10^{-10}) (Figure 6A, Table S6, Supplementary Materials).

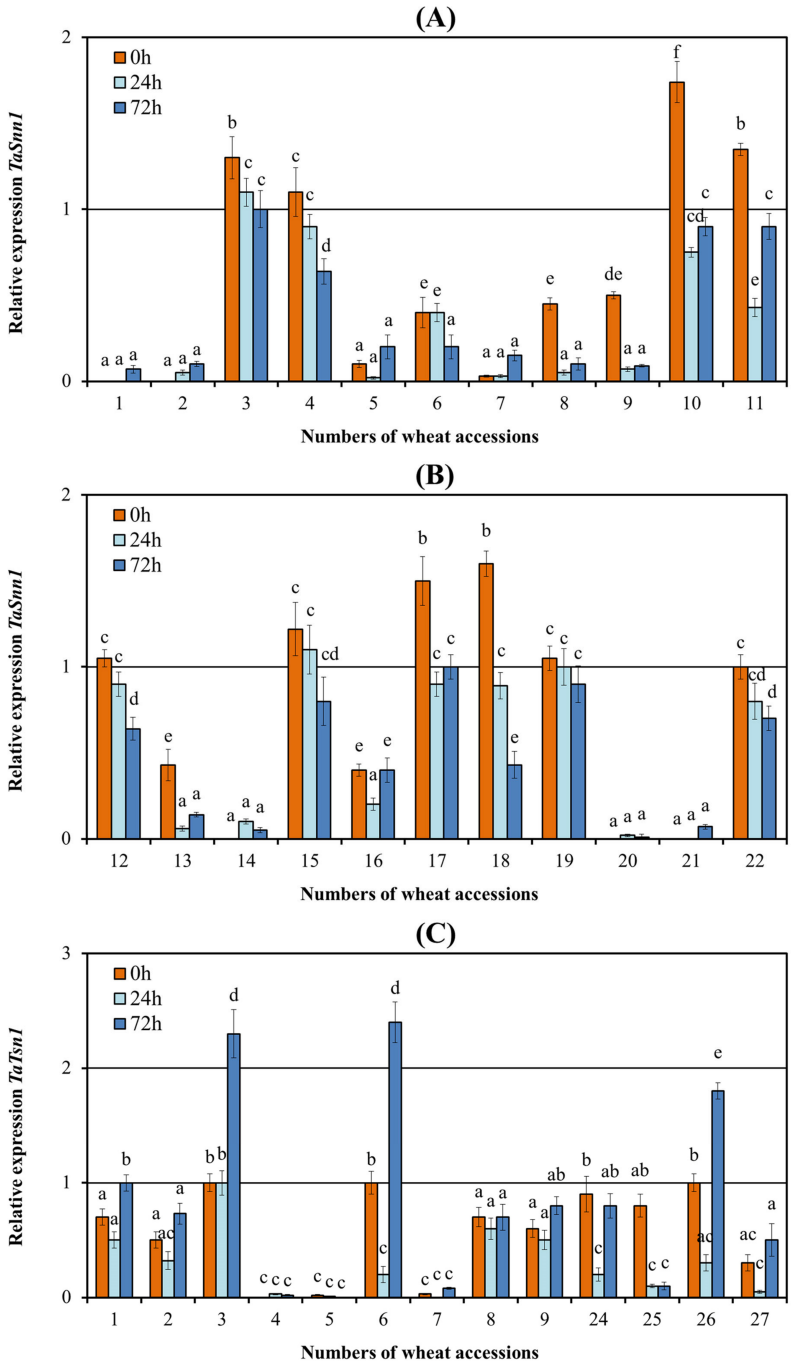

Figure 5. Expression of host susceptibility genes to NEs in the wheat panel during infection with *S. nodorum* isolates. (**A**,**B**) The expression level of the host susceptibility gene *TaSnn1* infected with the Sn1SP isolate in the 22 wheat cultivars. (**C**) The expression level of the host susceptibility gene *TaTsn1* infected with the SnB isolate in the 13 wheat cultivars. The samples are indicated as follows: 0 h—accessions uninfected with *S. nodorum*; 24 h—accessions infected with *S. nodorum* within 24 h; 72 h—accessions infected with *S. nodorum* within 72 h. Wheat cultivar numbers (1–22) are the same as in Figure 3. Other cultivars are as follows: 24—Esaul, 25—Yubilejnaya 100, 26—Ermak, 27—Alekseich. Expression values were normalized to the housekeeping gene *TaRLI* as an internal reference. Figures present means ± SE (n= 3). Columns of each histogram marked with different letters represent the mean values that are statistically different from each other according to the Duncan's test ($p \leq 0.05$).

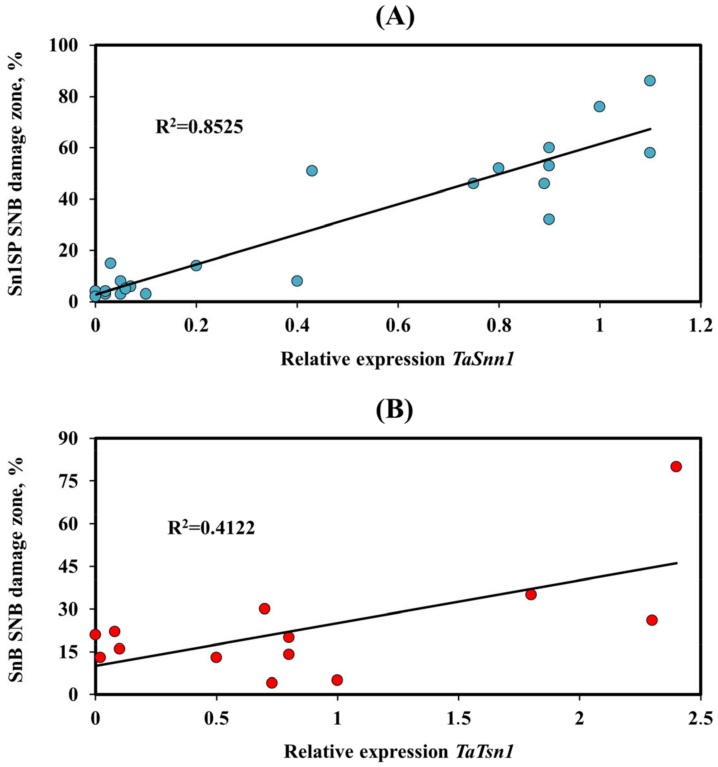

Figure 6. Relationship between the degree of cultivar sensitivity to NE and the expression level of the corresponding NE host susceptibility gene. (**A**) Regression line for relationship between damage zones and expression level of the host susceptibility gene *TaSnn1* 24 h after infection with the Sn1SP isolate in the 22 wheat cultivars; (r = 0.92, R^2 = 0.85, p-value = 9.22709×10^{-10}); (**B**) regression line for relationship between damage zones and expression level of the host susceptibility gene *TaTsn1* 72 h after infection with the SnB isolate in the 13 wheat cultivars; (r = 0.64, R^2 = 0.41, p-value = 0.02).

The direct dependence of the *Tsn1* gene expression 72 h after infection with SnB isolate and the degree of damage on the leaves was also found in 13 wheat cultivars (r = 0.64, R^2 = 0.41, p-value = 0.02) (Figure 6B, Table S7, Supplementary Materials).

4. Discussion

4.1. Effector Sensitivity in Russian Wheat Panel and Its Relevance to SNB

Advances in the understanding of SNB resistance have been used in breeding programs since 2005. For example, in Australia, effector-assisted breeding is used, in which wheat is selected based on NE insensitivity. However, the relationship between effector insensitivity and cultivar resistance in the *S. nodorum*–wheat pathosystem is not always as clear as in the *Pyrenophora tritici-repentis*–wheat pathosystem [5]. However, deletion of effector susceptibility genes has never been shown to reduce SNB resistance or to have any other detrimental effect. This either has no effect or a positive effect on resistance [5]. Unfortunately, natural genetic variants determining resistance to many NEs have not been identified, and so screening collections with toxins or isolates is probably the most objective approach for reliably determining wheat genotype susceptibility to *S. nodorum*.

In this study, 55 cultivars were screened with different isolates of *S. nodorum*. This study was carried out with several goals. First, modern commercial cultivars of bread wheat grown in Russia have been genotyped for the presence of *Snn* susceptibility genes; a search has been made for novel sources of resistance for breeders. Secondly, different wheat genotypes have been selected to help dissect SNB at the molecular level and to study various SnTox-*Snn* interactions. Thirdly, the expression of *Snn* susceptibility genes has been studied.

In the studied panel of wheat, 47 cultivars are modern commercial cultivars grown in Russia that have never been genotyped for susceptibility to *S. nodorum*, and 8 cultivars are historic wheat accessions from the Vavilov wheat collection (Table S3, Supplementary Materials). In this panel, the frequency of SnTox1 sensitive cultivars was 67%, which is comparable to the frequency found for Australian cultivars of 72% [30], but it contrasts markedly with the frequency found for Scandinavian cultivars (12%) [22], British cultivars (28%) [31], commercial bread red winter wheat cultivars from the southeastern United States (0%) [26], and bread spring wheat from global collections (16%) [17]. The frequency of SnTox3 sensitive cultivars in this panel was 43%, which was similar to the frequency found for British (42%) and Scandinavian wheat cultivars (55%) [22,31]. On the contrary, for Australian cultivars, sensitivity to SnTox3 was 91% [30], and it was 67% for cultivars from the southeastern United States [26]. In this work, wheat cultivars sensitive to SnToxA + SnTox3 were found, the frequency of which was 16%. This is much lower than in other wheat collections. For example, 45% in Scandinavian cultivars [22], 65% in Western Australian cultivars [32], and 31% in cultivars from the southeastern United States were sensitive to SnToxA [26]. However, this is comparable to the frequency found for British cultivars (10%) [31]. We assume that, in our panel of wheat, the share of cultivars sensitive only to SnToxA was even less. Based on our results of *Tsn1* gene expression (Figure 5C) and correlation analysis of the relationship between expression and the degree of damage (Figure 6B), we can assume that, among winter wheat cultivars, there were only two cultivars sensitive to SnToxA: Dmitry and Ermak. Among spring wheat cultivars, only one cultivar, Zhnitsa, was sensitive, which, together, amounted to 6% in the studied wheat panel.

Thus, our panel of wheat differs from the European, American, and Australian wheat collections with available data, being less sensitive to SnToxA and SnTox3, and more sensitive to SnTox1 (Figure 1B). A panel of 295 bread wheat accessions from the Vavilov collection comprising landraces, breeding lines, and cultivars collected from 28 countries between 1922 and 1990 was recently screened, and it showed a moderate frequency of sensitivity to SnToxA, SnTox1, and SnTox3 [33]. A large proportion of accessions in the collection consisted of wheats from the former Soviet Union, which generally showed a high frequency of NE insensitivity. Sensitivity to SnToxA was found in 29.3% of accessions, 26.8% of accessions were sensitive to SnTox1, and 51.2% of accessions were sensitive to SnTox3 [33].

Our wheat panel differs greatly from the panel out of Vavilov's collection only in sensitivity to SnTox1; sensitivity to the other two effectors was comparable. The increased frequency of sensitivity to SnTox1 can be explained by the fact that our cultivars are more modern (with the exception of the 8 historical ones, in which the frequency of sensitivity to SnTox1 was 12.5%) and selection in the last 30 years was not aimed at selecting cultivars insensitive to SnTox1. This fact may create big problems in the future because the SnTox1-*Snn1* interaction has been shown to play a key role in the determination of SNB in seedlings and adult wheat plants [34,35].

The low sensitivity to SnToxA in accessions from the Vavilov's collection and our panel of modern wheat cultivars is presumably due to selective breeding that results in indirect selection against *Tsn1*, since the SnToxA–*Tsn1* interaction was well manifested in adult plants during field infection [33,36]. In addition, it was previously found that the tan spot (syn. yellow spot) of the wheat fungus *Pyrenophora tritici-repentis* (*Ptr*) also uses effector ToxA to promote the development of the disease on wheat lines that carry the dominant susceptibility gene *Tsn1* [10]. It was hypothesized that *Ptr* may have acquired ToxA through lateral gene transfer, presumably from *S. nodorum*, and this occurred as early as the first half of the 20th century [10]. Tan spot has become increasingly damaging on wheat since the 1960s and the 1970s [37], and it was hypothesized that the selection was breeding ToxA-insensitive wheat lines [33]. Importantly, ToxA has also been found in some isolates of *Parastagonospora avenae* (causes SNB) [38], and, recently, ToxA has been identified in *Bipolaris sorokiniana*, the causative agent of wheat blotch [39]. Therefore, the

low sensitivity to SnToxA of modern commercial cultivars of bread wheat grown in Russia is important and relevant.

Moderate sensitivity to SnTox3 in accessions from the Vavilov's collection and our panel of modern wheat cultivars is presumably due to the absence of indirect selection for NE insensitivity [33]. It has been suggested that the high prevalence of sensitivity to SnTox3 in International Maize and Wheat Improvement Center (CIMMYT) breeding materials, International Center for Agricultural Research in the Dry Areas (ICARDA) breeding materials [33], Australian cultivars [30], European cultivars, and American cultivars [26,31] can be explained by the fact that *Snn3* may be closely linked to other desirable breeding traits, or its direct removal entails a trait penalty other than yield [40]. The SnTox3-*Snn3* interaction was also one of the major disease determinants using SnB in our wheat panel. Previously, it was found that SnTox3 was present in 60% of the 923 *S. nodorum* isolates worldwide and was an important pathogen virulence factor [34]. This indicates that where there is SnTox3 in the local pathogen population, the presence of *Snn3* in local cultivars may contribute to the severity of SNB disease in that region [4]. It should be taken into account that the SnTox3–*Snn3* interaction plays an important role in the development of the disease not only in seedlings, but also in adult plants in the field [41].

Thus, it is necessary to deepen the study of the role and contribution of both NE and *Snn* susceptibility genes in the development of SNB.

4.2. The Role of Various SnTox-Snn Interactions in the Development of SNB

In this study, six cultivars were found to be insensitive to three NEs (SnToxA, SnTox3, and SnTox1) and to an indeterminate NE produced by isolate Sn9MN-3A. These are the cultivars Don Mira, Yeremeyavna, Lebed, Zhiva, Grom, and Vassa (Table S4, Supplementary Materials). Only one cultivar out of six had null alleles for all susceptibility genes—Don Mira. The other cultivars had alleles of these genes, but were not sensitive to NE. These cultivars are not only sources of SNB resistance, but are also important objects for further study of the *S. nodorum*–wheat pathosystem.

In this study, the frequency of sensitivity to NE was much lower than the frequency of the occurrence of susceptibility genes (Figure 1B). Therefore, some SnTox-*Snn* interactions did not result in susceptibility and disease development. This may be due to various regulatory mechanisms that are activated by either the pathogen or the host. On the part of the pathogen, a diversity of manifestations of one interaction can be caused with epistatic or additive effects between different SnTox-*Snn* interactions, which are partly associated with different levels of NE gene expression during plant infection or partly with the influence of host susceptibility genes [23,35]. On the part of the host plant, mutations contained in the *Snn* susceptibility gene, which lead to plant insensitivity to NE, can contribute to the diversity of responses, and, as recent data have shown, the regulation of transcription of the *Snn* susceptibility gene can provide resistance to the cultivar [17]. However, all of these mechanisms of regulation of SnTox-*Snn* interactions are practically not studied and need further research.

In our wheat panel, 10 winter and 7 spring wheat cultivars had the *Snn1* gene allele but were insensitive to SnTox1 (Table S4, Supplementary Materials). Previously, two single-nucleotide polymorphisms were found in some SnTox1-insensitive accessions that caused nonsynonymous mutations at amino acid positions 347 and 429 *Snn1* alleles [16]. We hypothesized that insensitive cultivars in our wheat panel could also have mutations in the *Snn1* gene. We have yet to verify this assumption. In addition, in the works of other authors, cases of cultivar insensitivity in the absence of mutations in the *Snn1* gene were found, for example, in the durum cultivar Lebsock [17]. Transcriptional analysis revealed that the gene was not expressed, thus providing an explanation for the lack of SnTox1 sensitivity in Lebsock [17]. In our panel of wheat, *Snn1* gene expression was absent or minimal in five cultivars insensitive to SnTox1 (Figure 5A,B). In addition, correlation analysis showed a positive dependence of the level of *Snn1* gene expression on the degree of cultivar sensitivity

to SnTox1 (Figure 6A). These results suggest some regulatory mechanisms in the host plant that may influence resistance. This phenomenon requires further comprehensive study.

Despite the fact that, in our wheat panel, an *Snn3-B1* locus was found in 94.6% of the accessions, insensitivity to SnTox3 was found in 29 cultivars, which amounted to 56.9% (Tables S3 and S4, Supplementary Materials). The *Snn3-B1* gene has not yet been sequenced. The *Snn3-B1* locus has been mapped to the distal end of chromosome arm 5BS [11]. Efforts are underway to develop diagnostic markers for the detection of the *Snn3-B1* gene [16,31]. However, evaluation of markers has indicated that they are poor at diagnosing the presence of the gene, possibly due to the high degree of recombination that has occurred in the *Snn3-B1* region in natural populations [4].

To identify the *Snn3-B1* locus in our wheat panel, the SSR markers *Xcfd20* and *Xgwm234* were used, which detected a null allele, as well as one or more alleles in different wheat accessions (Figure S3, Supplementary Materials). Null alleles for one or both markers (cultivars Bashkirskaya 26, Boevchanka, and Don Mira) most likely indicate a deletion at the *Snn3-B1* locus [16]. The presence of diverse alleles or a different number of alleles may indicate various degrees of sensitivity to SnTox3 [16]. Thus, two wheat lines, BG220 and Sumai3, carrying different alleles of the *Snn3-B1* locus, showed various degrees of sensitivity to SnTox3 [16]. In this work, wheat accessions that did not have the 500 bp fragment for marker *Xcfd20* and 244 bp fragment for the marker *Xgwm234* were insensitive to SnTox3 (Figure S3, Table S4, Supplementary Materials).

In addition, correlation analysis showed that insensitivity to SnTox3 was associated with sensitivity to SnTox1 and vice versa (Figure S3, Supplementary Materials). This suggests an epistatic interaction between the *Snn1* and *Snn3-B1* susceptibility genes. Previously, it was shown that SnTox1-*Snn1* interaction is epistatic to SnTox3-*Snn3-B1*, at least in part by suppressing *SnTox3* expression mediated by *SnTox1* [35]. It was later reported that the SnTox3-*Snn3-B1* interaction is epistatic to the SnTox1-*Snn1* interaction [21]. A possible explanation for the differences between the two studies is likely the presence of different host alleles at the *Snn1* and/or *Snn3-B1* loci [21]. In other words, the role of the *Snn1* and *Snn3-B1* susceptibility genes in the effects of these interactions is assumed. However, this role of susceptibility genes has been poorly understood and requires further in-depth research. Wheat cultivars from our panel are an appropriate object for such studies.

In this work, the dominant allele of the *Tsn1* gene was found in 14 wheat cultivars with effects on resistance/susceptibility to SNB (Table S4, Supplementary Materials). However, six of them were insensitive to SnToxA. These cultivars could presumably have mutations in the *Tsn1* gene. Thus, in the works of other researchers, genotyping of the 386 *Triticum* accessions with the PCR marker *Xfcp623* indicated that 6 ToxA-insensitive lines contained the *Tsn1* gene [12]. Sequence analysis of these six lines indicated that three lines had a nonsense mutation at the same position within the LRR domain, and the other three lines had frameshift mutations at different positions within the gene [12].

We have analyzed the expression of the *Tsn1* gene in both susceptible and resistant cultivars (Figure 5C). The correlation analysis showed a positive relationship between the degree of damage and the level of *Tsn1* gene expression (Figure 6B). The transcriptional regulation of the *Tsn1* susceptibility gene is practically not explored. The study of the *Tsn1* gene expression in different wheat genotypes under pathogen attack has not been carried out, and the expression during infiltration with the SnToxA toxin has been studied in only one cultivar within 48 h, and it did not lead to significant changes in the transcript level [12]. Our data on the *Tsn1* gene expression in different wheat cultivars were obtained for the first time and are of great importance.

Eight cultivars in our wheat panel showed susceptibility to the *S. nodorum* SnB isolate (SnToxA/SnTox3), so they could be sensitive both NEs (Tables S3 and S4, Supplementary Materials). These are cultivars Dmitry, Kalym, Zhnitsa, Iren, Selkirk, Salamoni, Yubileinaya 100, and Ermak (Table S4, Supplementary Materials). However, it has been known that SnToxA-*Tsn1* and SnTox3-*Snn3-B1* interactions play a significant role in SNB, but often their effects were not additive because the presence of both *Snn3-B1* and *Tsn1* did not

make plants significantly more susceptible than plants with one of the two genes [21]. The exceptions were cases where there was no SnTox1-*Snn1* interaction [21]. In our panel, seven out of eight cultivars showed a moderate susceptibility to the SnB isolate (SnToxA/SnTox3), while Zhnitsa was highly susceptible to SnB and showed the highest transcript level of the *Tsn1* gene while being insensitive to SnTox1 (Figure 5C, Table S4, Supplementary Materials). Therefore, an additive effect of two interactions, SnToxA-*Tsn1* and SnTox3-*Snn3-B1*, was observed in this cultivar (Figure 3). Two good examples of the absence of an additive effect were the cultivars Dmitry and Ermak, which also showed a high transcript level of the *Tsn1* gene, but were moderately susceptible to SnB and sensitive to SnTox1 (Figure 5C, Table S4, Supplementary Materials). All eight cultivars provide an appropriate panel for a deeper study of the effects of SnToxA-*Tsn1* and SnTox3-*Snn3-B1* interactions.

5. Conclusions

In the course of this work, sources of strong resistance to SNB were identified; the frequency of susceptibility genes and the frequency of sensitivity to NEs were studied in 47 modern commercial cultivars of winter and spring wheat grown in Russia. These results are important for marker-assisted selection for SNB resistance [4]. The current study has shown, for the first time, that the expression levels of *Snn1* and *Tsn1* susceptibility genes and the disease severity of the different wheat cultivars are interconnected. These results may provide additional information on the resistance/susceptibility of the cultivars. In addition, during the study, wheat cultivars were identified that are appropriate objects with which to study the different effects of SnTox-*Snn* interactions. Now that relative progress has been achieved in cloning host susceptibility genes and their corresponding NEs genes, future work should focus on the deep characterization of SnTox-*Snn* interactions at the molecular level [4]. These host–pathogen interactions are complex and can be affected by multiple factors, and more work related to the interplay between the interactions, pathogen NEs genes, and host sensitivity genes is required.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijpb14020031/s1, Table S1: Primers used to identify pathogen effector genes and plant susceptibility genes from genomic DNA; Table S2: PCR primers used for qRT-PCR analysis; Figure S1: Identification of alleles of *Tsn1* gene in 55 wheat accessions by PCR. Amplified fragments with sizes of 467 bp were separated in a polyacrylamide gel. Numbers from 1 to 55 wheat accessions correspond to the numbers in Table S3. M-DNA markers. Marker sizes are indicated on the right. The presence of the amplification product indicates the existence of a dominant allele of the *Tsn1* gene; the absence of the amplification product indicates a null (recessive) *tsn1* allele; Figure S2: Identification of alleles of *Snn1* gene in 55 wheat accessions by PCR. Amplified fragments with sizes of 80 bp were separated in a polyacrylamide gel. Numbers from 1 to 55 wheat accessions correspond to the numbers in Table S3. M-DNA markers. Marker sizes are indicated on the right. The presence of the amplification product indicates the existence of a dominant allele of the *Snn1* gene; the absence of the amplification product indicates a null (recessive) *snn1* allele; Figure S3: Identification of alleles of the *Snn3-B1* locus in 55 wheat samples using primers to SSR markers *Xcfd20* (A) and *Xgwm234* (B) by PCR. The results show that marker *Xcfd20* amplifies different fragments in wheat (370 bp, 380 bp, 500 bp, about 700 bp), marker *Xgwm234* amplifies two fragments in wheat with sizes of 244 and 255 bp. Numbers from 1 to 55 wheat samples correspond to the numbers in Table S3. M-DNA markers. Marker sizes are indicated on the right. The absence of an amplification product indicates a null (recessive) *snn3-B1* allele; Table S3: The 55 *Triticum* accessions evaluated for the presence of the *Tsn1*, *Snn1*, and *Snn3-B1* DNA sequence; Table S4: The 55 *Triticum* accessions evaluated for reaction to SnTox3, SnToxA, and SnTox1; Table S5: Regression statistics for 32 wheat accessions that were resistant to one or two pathogen isolates; Table S6: Regression statistics for 22 wheat accessions, in which the expression of the *Snn1* gene and damage zones were analyzed during infection with the Sn1SP isolate; Table S7: Regression statistics for 13 wheat accessions, in which the expression of the *Tsn1* gene and damage zones were analyzed during infection with the SnB isolate.

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