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Wheat Transcriptional Corepressor TaTPR1 Suppresses Susceptibility Genes *TaDND1/2* and Potentiates Post-Penetration Resistance against *Blumeria graminis forma specialis tritici*

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Abstract: The obligate biotrophic fungal pathogen *Blumeria graminis forma specialis tritici* (*B.g. tritici*) is the causal agent of wheat powdery mildew disease. The TOPLESS-related 1 (TPR1) corepressor regulates plant immunity, but its role in regulating wheat resistance against powdery mildew remains to be disclosed. Herein, TaTPR1 was identified as a positive regulator of wheat post-penetration resistance against powdery mildew disease. The transient overexpression of *TaTPR1.1* or *TaTPR1.2* confers wheat post-penetration resistance powdery mildew, while the silencing of *TaTPR1.1* and *TaTPR1.2* results in an enhanced wheat susceptibility to *B.g. tritici.* Furthermore, *Defense no Death 1* (*TaDND1*) and *Defense no Death 2* (*TaDND2*) were identified as wheat susceptibility (*S*) genes facilitating a *B.g. tritici* infection. The overexpression of *TaDND1* and *TaDND2* leads to an enhanced wheat susceptibility to *B.g. tritici*, while the silencing of wheat *TaDND1* and *TaDND2* leads to a compromised susceptibility to powdery mildew. In addition, we demonstrated that the expression of *TaDND1* and *TaDND2* is negatively regulated by the wheat transcriptional corepressor TaTPR1. Collectively, these results implicate that TaTPR1 positively regulates wheat post-penetration resistance against powdery mildew probably via suppressing the *S* genes *TaDND1* and *TaDND2*.

Keywords: TOPLESS-related 1; *Defense no Death 1*; *Defense no Death 2*; bread wheat; powdery mildew

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1. Introduction

As the most widely cultivated cereal crop, allohexaploid bread wheat (*Triticum aestivum* L.) provides approximately 20% of the total calories in human food [1]. The world's population is projected to reach 9.7 billion by 2050 and rise further to 11.2 billion in 2100, which drives the global demand for wheat grains [2]. However, the plant growth and global production of bread wheat are challenged by stressful environments, particularly invading pathogens and pests (P and Ps) [3]. Wheat powdery mildew disease caused by the pathogenic fungus *Blumeria graminis forma specialis tritici* (*B.g. tritici*) adversely affects the global wheat production [4,5]. Exploring the molecular mechanism underlying the wheat–*B. g. tritici* interaction and developing wheat varieties with an improved powdery mildew resistance are essential for controlling the powdery mildew epidemic and securing wheat production.

During the long-term coevolution, adapted pathogens and their host plants have acquired sophisticated strategies to facilitate their infection and defense, respectively. *Susceptibility* (*S*) genes from host plants are exploited by adapted pathogens to support the compatibility of the pathogens with plants probably via promoting pathogen (pre)penetration, suppressing plant immunity, and facilitating pathogen sustenance [6,7]. Upon the detection of invading pathogens, plants initiate two intertwined layers of induced defenses, pattern-triggered immunity (PTI) and effector-triggered immunity (ETI), to defend against pathogen infections [8–18]. During PTI and ETI, massive transcriptomic reprogramming is usually initiated, and this defense-related transcriptomic reprogramming is under the tight control of transcriptional regulators [19–21]. Identifying *S* genes and defense-related tran-

scriptional regulators could deepen our understanding of the wheat–*B.g. tritici* interaction and assist wheat breeding for *B.g. tritici* resistance.

TOPLESS (TPL)/TOPLESS-related (TPR) transcriptional corepressors regulate plant development and environmental adaptation. In the model plant *Arabidopsis thaliana* (L.) Heynh, the transcriptional repressor AtAUX/IAA interacts with AtTPL to suppress the expression of auxin response factor (AtARF) target genes in the absence of auxin, whereas transcription factors BRI1-EMS-SUPPRESSOR 1 (AtBES1) and BRASSINAZOLE-RESISTANT 1 (AtBZR1) associate with the AtTPL-AtHDA19 complex to regulate the *Arabidopsis* brassinosteroid (BRs) signaling pathway [22,23]. There is increasing evidence showing that TPR1 plays a vital role in the regulation of plant immunity [24]. Indeed, knocking out *Arabidopsis AtTPR1* and its close homologs compromises the immunity mediated by the toll-like/interleukin-1 receptor (TIR)-NB-LRR R protein, a suppressor of npr1-1, constitutive 1 (AtSNC1), whereas the overexpression of *AtTPR1* constitutively activates AtSNC1-mediated immune responses [25,26]. Similarly, the silencing of *NbTPR1* in *Nicotiana benthamiana* compromised the flg22-triggered PTI defense response [27]. However, the potential function of wheat TPR1 homologs in the regulation of the wheat–*B.g. tritici* interaction is poorly understood.

Arabidopsis S genes *Defense no Death 1* (*AtDND1*) and *Defense no Death 2* (*AtDND2*) encode cyclic nucleotide-gated cation channels (CNGC; also known as AtCNGC2 and AtCNGC4, respectively). *Arabidopsis dnd1* and *dnd2* mutants exhibited a broad-spectrum disease resistance against a wide range of pathogens, including the bacterial pathogen *Pseudomonas syringae* pv. tomato and the oomycete pathogen *Hyaloperonospora parasitica* [28–30]. Similarly, the silencing of *StDND1* and *SlDND1*, *Arabidopsis AtDND1* orthologs, in potato and tomato crops, respectively, leads to an elevated resistance to late blight (*Phytophthora infestans*), powdery mildew (*Oidium neolycopersici* and *Golovinomyces orontii*), and grey mold (*Botrytis cinerea*) [31–33]. However, whether and how the wheat *DND1* and *DND2* homologs regulate the powdery mildew resistance remains unknown.

Herein, *TaTPR1.1* and *TaTPR1.2* are identified as positive regulators of wheat post-penetration resistance against powdery mildew disease. The transient overexpression of *TaTPR1.1* or *TaTPR1.2* confers wheat post-penetration resistance to powdery mildew, while the silencing of *TaTPR1.1* and *TaTPR1.2* results in an enhanced wheat susceptibility to *B.g. tritici.* Furthermore, *TaDND1* and *TaDND2* were identified as wheat *S* genes facilitating a *B.g. tritici* infection. The overexpression of *TaDND1* and *TaDND2* leads to an enhanced wheat susceptibility to *B.g. tritici*, while the silencing of wheat *TaDND1* and *TaDND2* leads to a compromised susceptibility to powdery mildew. In addition, we demonstrated that the expression of *TaDND1* and *TaDND2* is negatively regulated by the wheat transcriptional corepressor TaTPR1. This evidence strongly supports that TaTPR1 corepressors positively regulate wheat post-penetration resistance against powdery mildew by suppressing the expression of the *S* genes *TaDND1* and *TaDND2*. These findings could enhance our understanding of the genetic basis of wheat–*B.g. tritici* interactions and provide a new avenue for breeding wheat varieties with powdery mildew resistance.

2. Results

2.1. Homology-Based Identification of Wheat TaTPR1

In this study, a wheat homolog of *Arabidopsis* AtTPR1 was identified and characterized in the regulation of the wheat–*B.g. tritici* interaction. *TaTPR1.1* and *TaTPR1.2* were obtained from the reference genome of the hexaploid wheat by using the amino acid sequence of AtTPR1 (At1g80490) as a query. Three highly homologous sequences of *TaTPR1.1* genes separately located on chromosomes 4A, 4B, and 4D were obtained from the wheat genome sequence and designated as *TaTPR1.1-4A* (*TraesCS4A02G083300*), *TaTPR1.1-4B* (*TraesCS4B02G220900*), and *TaTPR1.1-4D* (*TraesCS4D02G221200*). Similarly, three highly homologous sequences of *TaTPR1.2* genes separately located on chromosomes 7A, 7B, and 7D were obtained from the wheat genome sequence and designated as

TaTPR1.2-7A (*TraesCS7A02G296100*), *TaTPR1.2-7B* (*TraesCS7B02G189300*), and *TaTPR1.2-7D* (*TraesCS7D02G293500*).

As shown in Figure 1A, these predicted TaTPR1.1-4A, TaTPR1.1-4B, TaTPR1.1-4D, TaTPR1.2-7A, TaTPR1.2-7B, and TaTPR1.2-7D proteins shared over a 66% of their identities with *Arabidopsis* AtTPR1. The TaTPR1.1-4A, TaTPR1.1-4B, TaTPR1.1-4D, TaTPR1.2-7A, TaTPR1.2-7B, and TaTPR1.2-7D proteins all contain two conserved WD domains (WD40) (Figure 1B). The coding regions of these *TaTPR1.1* and *TaTPR1.2* genomic sequences all contain 25 exons and 24 introns (Figure 1C). Further phylogenetic analysis revealed that the TaTPR1.1-4A, TaTPR1.1-4B, TaTPR1.1-4D, TaTPR1.2-7A, TaTPR1.2-7B, and TaTPR1.2-7D proteins share over 70% of their identities with the AtTPR1, AtTPL, and rice OsTPR1 proteins (Figure 2). In contrast, AtTPR2 and AtTPR3 reside in the distinct 'TPR2' clade together with wheat TaTPR2-3A, TaTPR2-3B, TaTPR2-3D, and rice OsTPR2 (Figure 2).

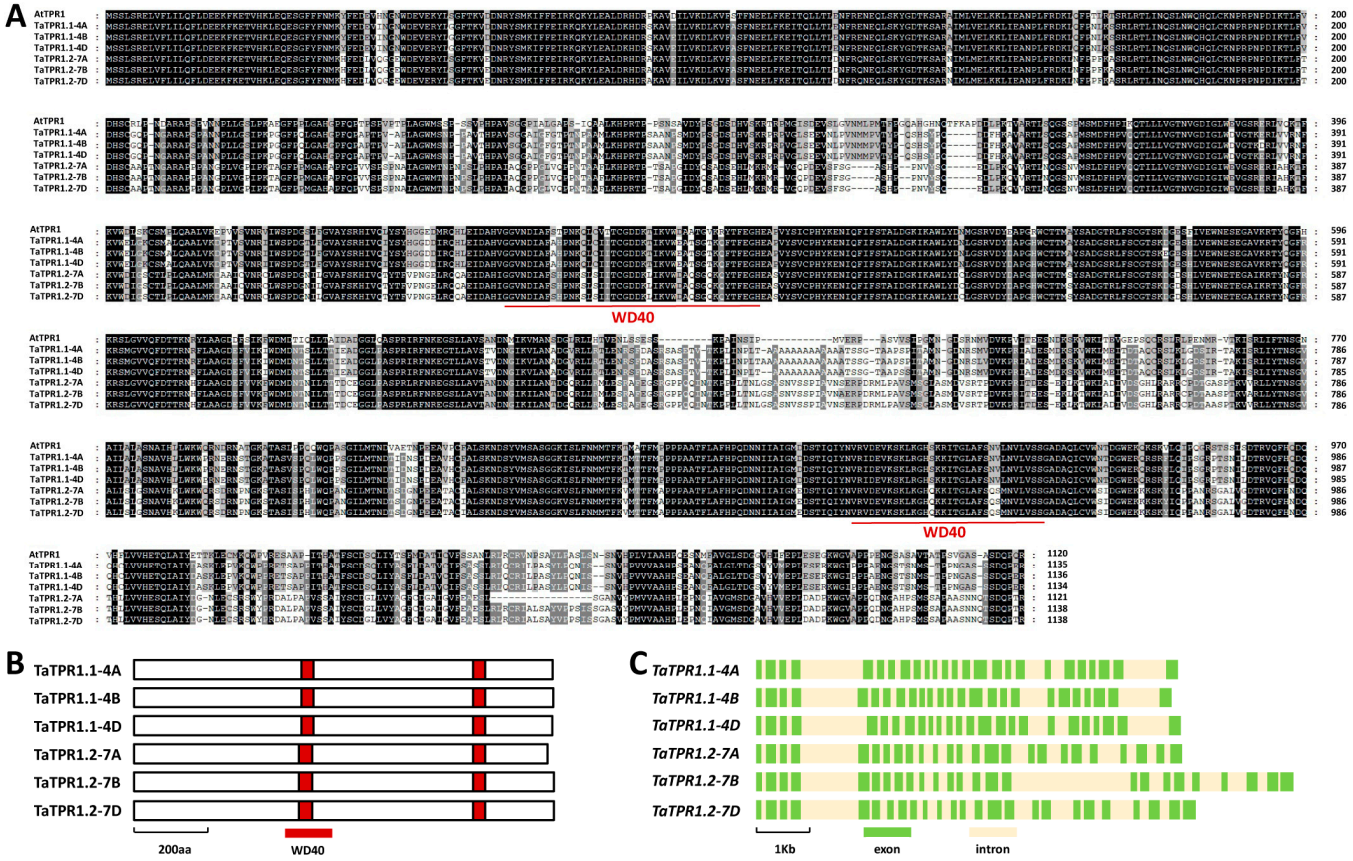

Figure 1. Identification of wheat TaTPR1 based on homology with *Arabidopsis* AtTPR1. (**A**) Protein sequence alignments of wheat TaTPR1.1, TaPRR1.2, and *Arabidopsis* AtTPR1. Identical residues among 7 protein sequences are shaded in dark, while residues conserved in at least 4 of the 7 proteins are shaded in gray. (**B**) Domain structures of wheat TaTPR1.1 and TaTPR1.2 proteins. (**C**) Gene architectures of wheat *TaTPR1.1* and *TaTPR1.2* genes.

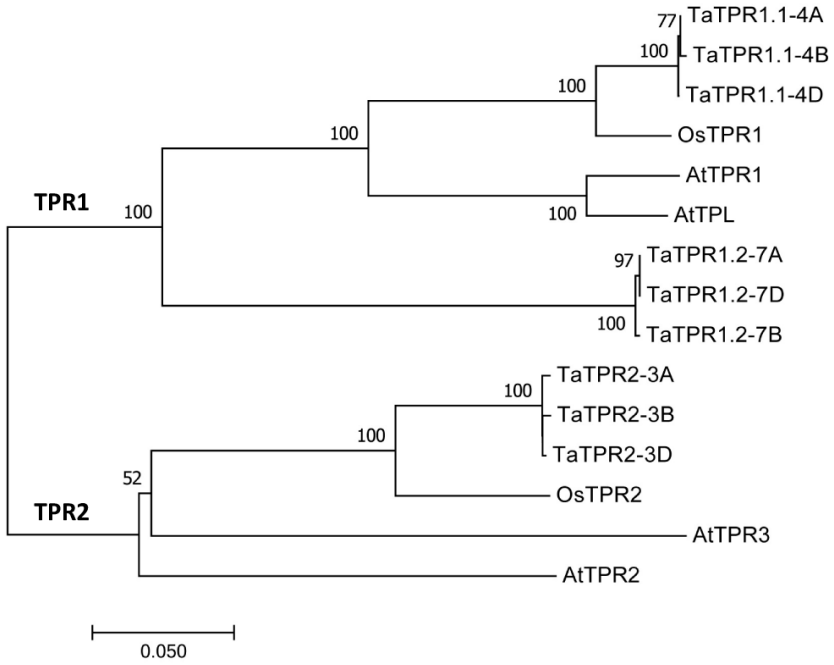

Figure 2. Phylogenetic relationships of the TPR1 and TPR2 homologs in *Arabidopsis*, rice, and bread wheat. The phylogenetic tree was constructed using the neighbor-joining method with 1000 bootstraps.

2.2. TaTPR1 Potentiates Wheat Post-Penetration Resistance against Powdery Mildew

These *TaTPR1.1-4A*, *TaTPR1.1-4B*, *TaTPR1.1-4D*, *TaTPR1.2-7A*, *TaTPR1.2-7B*, or *TaTPR1.2-7D* genes were overexpressed in the leaf epidermal cells of the powdery mildew-susceptible wheat cultivar Yannong 999 using transient gene expression assays. After the inoculation of conidia from the virulent *B.g. tritici* isolate E09, the formation of *B.g. tritici* haustoria was statistically analyzed to evaluate the wheat post-penetration susceptibility to powdery mildew. As shown in Figure 3A, the *B.g. tritici* haustorium index (HI%) decreased from 58% for the empty vector (OE-EV) control to below 37% on wheat cells overexpressing *TaTPR1.1* or *TaTPR1.2* genes. These results suggested that the overexpression of *TaTPR1* could enhance the formation of *Bgt* haustoria and attenuate the wheat post-penetration susceptibility to the fungal pathogen *B.g. tritici*.

Thereafter, transiently induced gene silencing (TIGS) assays were performed to separately silence all endogenous *TaTPR1.1* or *TaTPR1.2* genes in the wheat epidermal cells. As shown in Figure 3B, the single silencing of *TaTPR1.1* or *TaTPR1.2* genes failed to cause a significant change in the HI%, compared to 38% for the empty vector (OE-EV) controls. In contrast, the simultaneous silencing of *TaTPR1.1* and *TaTPR1.2* could lead to a significant increase in the HI% to approximately 50%, suggesting that *TaTPR1.1* and *TaTPR1.2* might redundantly attenuate the formation of *Bgt* haustoria and contribute to the post-penetration resistance of wheat to *B.g. tritici* (Figure 3B).

To further verify the function of *TaTPR1* genes in the regulation of the wheat–*B.g. tritici* interaction, we employed barley stripe mosaic virus (BSMV)-induced gene silencing (BSMV-VIGS) to silence all endogenous *TaTPR1.1* or *TaTPR1.2* genes in the wheat leaves. A qRT-PCR assay demonstrated that the expression levels of *TaTPR1.1* or *TaTPR1.2* declined in the indicated VIGS plants (Figure 3C). After the inoculation of *B.g. tritici* conidia, the formation of microcolonies was statistically analyzed to evaluate the wheat post-penetration susceptibility to powdery mildew. As shown in Figure 3D, the microcolony index (MI%) increased to approximately 64% on *BSMV-TaTPR1.1as* + *BSMV-TaTPR1.2as* plants, compared with 55% for the *BSMV-γ* plants, 57% for the *BSMV-TaTPR1.1as* plants, and 54% for the *BSMV-TaTPR1.2as* plants (Figure 3D). These data confirm that *TaTPR1.1* and *TaTPR1.2* redundantly contribute to the post-penetration resistance of wheat to *B.g. tritici.*

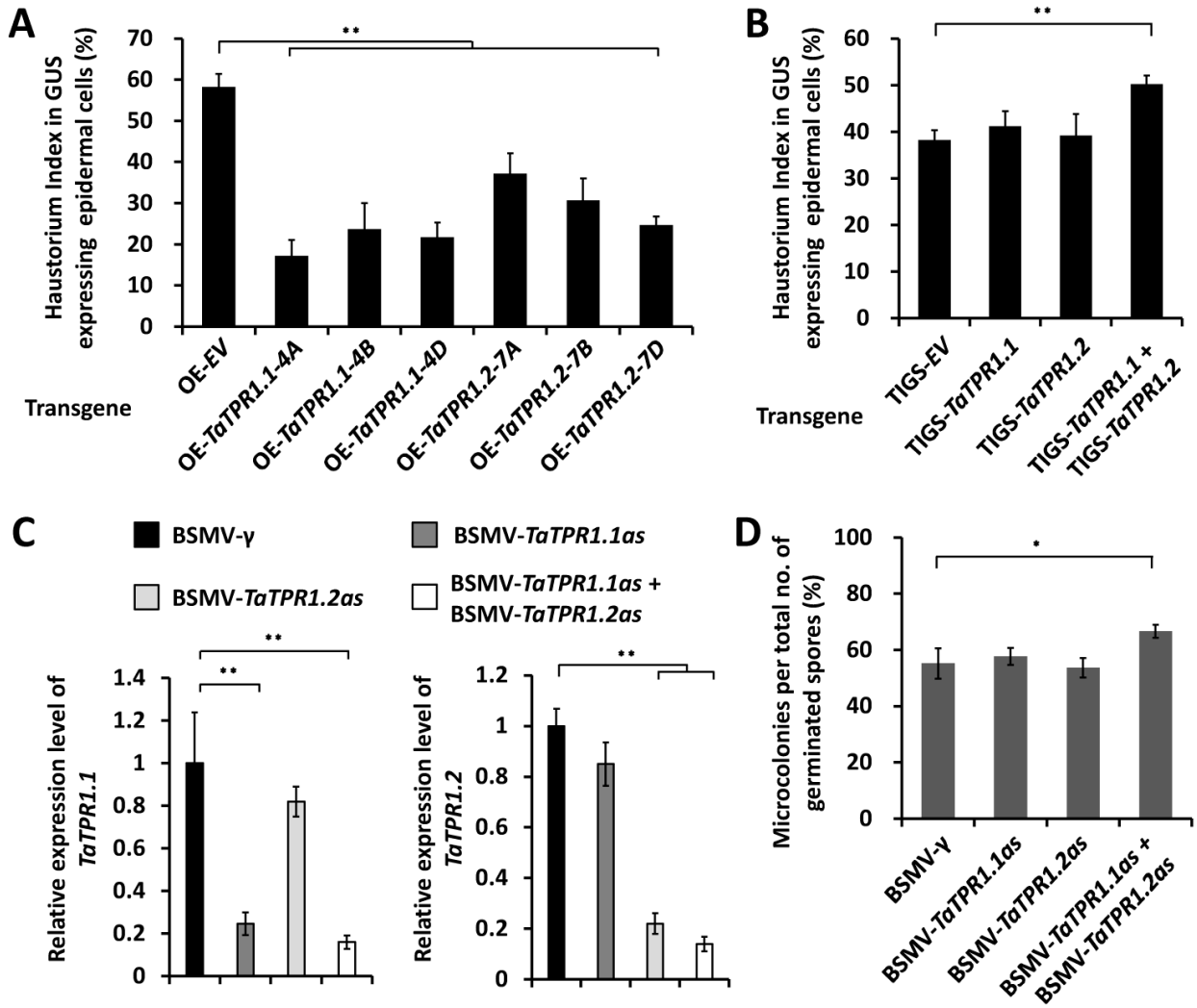

Figure 3. Functional analyses of *TaTPR1* genes in wheat–*Bgt* interaction. (**A**) Haustorial formation analysis in wheat epidermal cells transiently overexpressing *TaTPR1.1* (*OE-TaTPR1.1*) and *TaTPR1.2* (*OE-TaTPR1.2*). Haustorium index (HI%) on wheat epidermal cells bombarded with empty vector (*OE-EV*) was statistically analyzed as a control. More than 50 wheat cells were analyzed for each experiment. (**B**) Haustorial formation analysis in wheat epidermal cells transiently silencing *TaTPR1.1* (*TIGS-TaTPR1.1*) and *TaTPR1.2* (*TIGS-TaTPR1.2*) or cosilencing *TaTPR1.1* and *TaCAMTA3* (*TIGS-TaCAMTA2* + *TIGS-TaCAMTA3*). (**C**) qRT-PCR analysis of *TaTPR1.1* and *TaTPR1.2* expression in the wheat leaves infected with indicated BSMV vectors. BSMV-*γ* empty vector was employed as the negative control. (**D**) *Bgt* microcolony index analysis on wheat leaves silencing *TaTPR1.1* (*BSMV-TaTPR1.1as*) and *TaTPR1.2* (*BSMV-TaTPR1.2as*) or cosilencing *TaTPR1.1* and *TaTPR1.2* (*BSMV-TaTPR1.1as* + *BSMV-TaTPR1.2as*). For (**A**–**D**), three independent biological replicates were statistically analyzed for each treatment (*t*-test; * $p < 0.05$, ** $p < 0.01$).

2.3. Homology-Based Identification of TaDND1 and TaDND2 in Bread Wheat

Previous studies have revealed that the *Arabidopsis* transcriptional corepressor AtTPR1 targets the *S* genes *AtDND1* and *AtDND2* [1–3,12,13]. In this study, wheat homologs of Arabidopsis *AtDND1* and *AtDND2* were identified and characterized in the regulation of the wheat–*B.g. tritici* interaction. *TaDND1*, *TaDND2.1*, and *TaDND2.2* were obtained from the reference genome of the hexaploid wheat by using the amino acid sequences of *Arabidopsis* AtDND1 (At5g15410) and AtDND2 (AT5G54250) as queries. Three highly homologous sequences of *TaDND1* genes separately located on wheat chromosomes 5A, 5B, and 5D were obtained and designated as *TaDND1-5A* (*TraesCS5A02G395300*), *TaDND1-5B*

(*TraesCS5B02G400100*), and *TaDND1-5D* (*TraesCS5D02G404600*). Three highly homologous sequences of *TaDND2.1* genes separately located on wheat chromosomes 3A, 3B, and 3D were obtained and designated as *TaDND2.1-3A* (*TraesCS3A02G316300*), *TaDND2.1-3B* (*TraesCS3B02G350500*), and *TaDND2.1-3D* (*TraesCS3D02G315000*). Similarly, three highly homologous sequences of *TaDND2.2* genes separately located on wheat chromosomes 1A, 1B, and 1D were obtained and designated as *TaDND2.2-1A* (*TraesCS1A02G321700*), *TaDND2.2-1B* (*TraesCS1B02G334100*), and *TaDND2.2-1D* (*TraesCS1D02G322000*).

As shown in Figure 4A, these predicted TaDND1-5A, TaDND1-5B, and TaDND1-5D proteins shared about 67% of their identities with *Arabidopsis* AtDND1. The TaDND1-5A, TaDND1-5B, and TaDND1-5D proteins all contain an ion transport (Ion_trans) domain (Figure 4B). The coding regions of these allelic *TaDND1* genomic sequences all contain five exons and four introns (Figure 4D). As shown in Figure 4E, these predicted TaDND2.1-3A, TaDND2.1-3B, TaDND2.1-3D, TaDND2.2-1A, TaDND2.2-1A, and TaDND2.2-1D proteins shared over 59% of their identities with *Arabidopsis* AtDND2. The TaDND2.1-3A, TaDND2.1-3B, TaDND2.1-3D, TaDND2.2-1A, TaDND2.2-1A, and TaDND2.2-1D proteins all contain an Ion_trans domain and a cyclic nucleotide-binding (cNMP binding) domain (Figure 4F). The coding regions of these allelic *TaDND2.1* genomic sequences all contain five exons and four introns, whereas the coding regions of allelic *TaDND2.2* genomic sequences all contained four exons and three introns (Figure 4F).

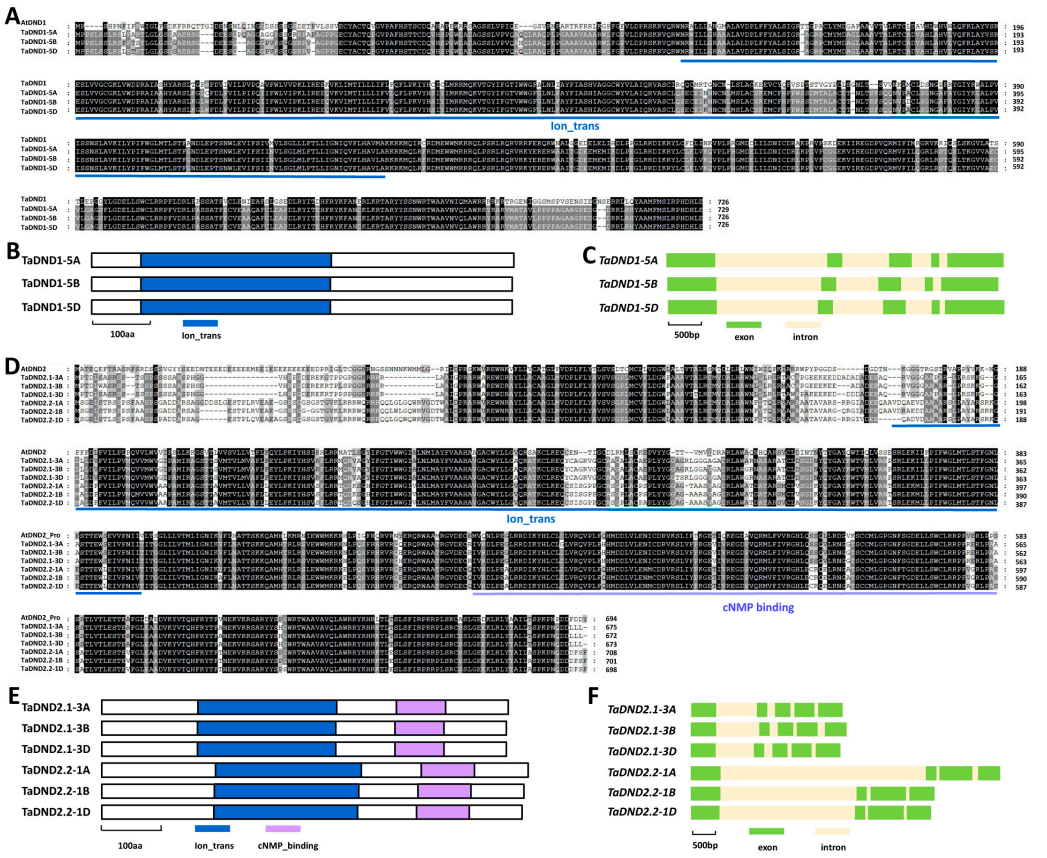

Figure 4. Identification of wheat TaDND1 and TaDND2 based on homology with *Arabidopsis* AtDND1 and AtDND2. (**A**) Sequence alignments of wheat TaDND1 and *Arabidopsis* AtDND1 proteins. Residues conserved in at least 2 of the 4 proteins are shaded in gray, while identical residues among 4 protein sequences are shaded in dark. (**B**) Domain structures of wheat TaDND1 proteins. (**C**) Gene architectures of wheat *TaDND1* genes. (**D**) Sequence alignments of wheat TaDND2.1, TaDND2.2, and *Arabidopsis* AtDND2 proteins. Residues conserved in at least 3 of the 6 proteins are shaded in gray, while identical residues among 6 protein sequences are shaded in dark. (**E**) Domain structures of wheat TaDND2.1 and TaDND2.2 proteins. (**F**) Gene architectures of wheat *TaDND2.1* and *TaDND2.2* genes.

2.4. TaDND1 and TaDND2 Positively Contribute to the Wheat Susceptibility to B.g. tritici

To characterize the functions of *TaDND1* and *TaDND2* in the regulation of the wheat–*B.g. tritici* interaction, we first employed transient gene expression assays to overexpress *TaDND1-5A*, *TaDND1-5B*, *TaDND1-5D*, *TaDND2.1-3A*, *TaDND2.1-3B*, *TaDND2.1-3D*, *TaDND2.2-1A*, *TaDND2.2-1A*, or *TaDND2.2-1D* genes in the wheat leaf epidermal cell. As shown in Figure 5A, the HI% increased from 55% for the empty vector control (OE-EV) to over 67% on wheat cells overexpressing *TaDND1* or *TaDND2* genes. These results suggest that the overexpression of *TaDND1* or *TaDND2* significantly attenuates the formation of *Bgt* haustoria and potentiates the wheat post-penetration susceptibility to *B.g. tritici*.

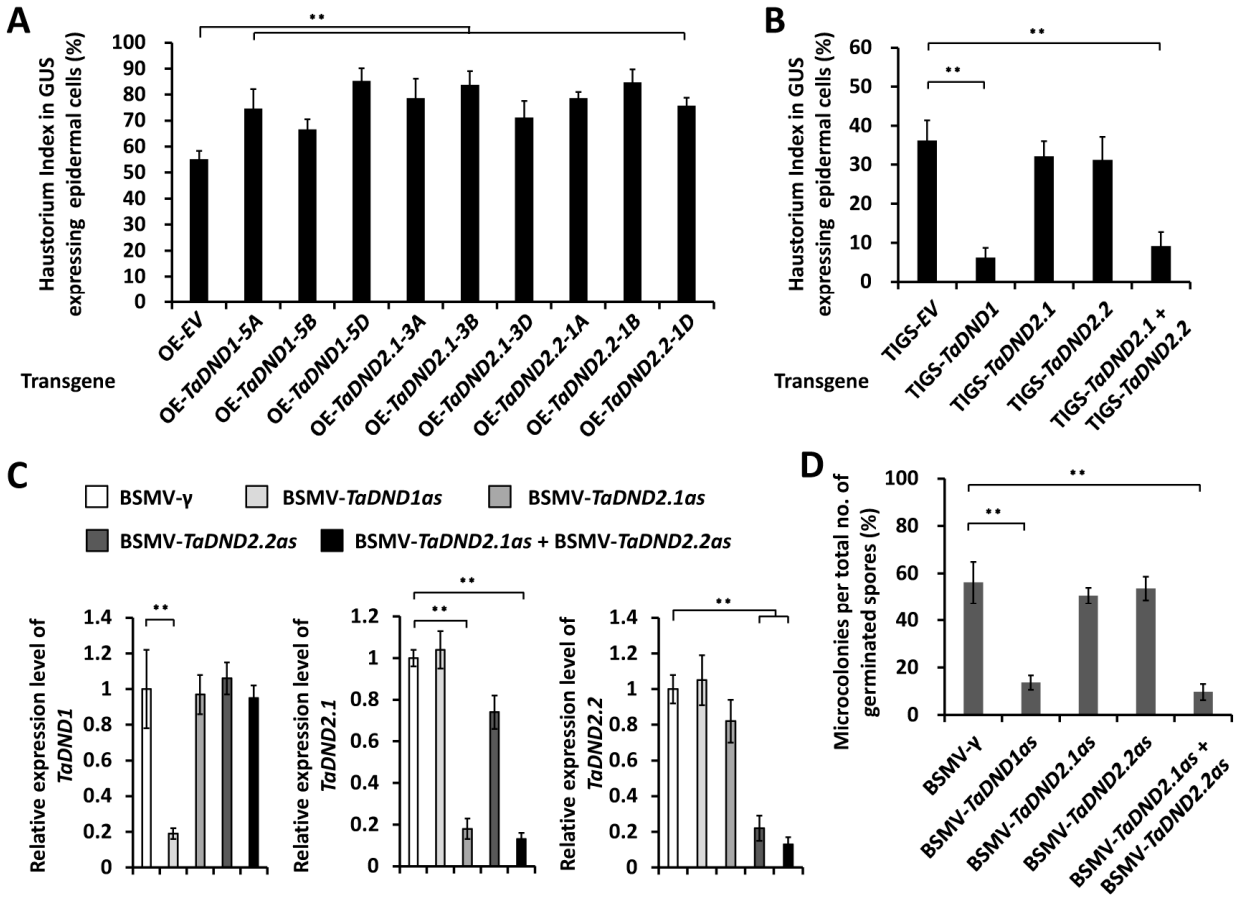

Figure 5. Functional analyses of *TaDND1* and *TaDND2* genes in wheat–*Bgt* interaction. (**A**) Haustorial formation analysis in wheat epidermal cells transiently overexpressing *TaDND1* (*OE-TaDND1*), *TaDND2.1* (*OE-TaDND2.1*), and *TaDND2.2* (*OE-TaDND2.2*). (**B**) Haustorium index analysis in wheat epidermal cells transiently silencing *TaDND1* (*TIGS-TaDND1*), *TaDND2.1* (*TIGS-TaDND2.1*), and *TaDND2.2* (*TIGS-TaDND2.2*) or cosilencing *TaDND2.1* and *TaDND2.2* (*TIGS-TaDND2.1*+ *TIGS-TaDND2.2*). (**C**) qRT-PCR analysis of *TaDND1*, *TaDND2.1*, and *TaDND2.2* expressions in the wheat leaves infected with indicated BSMV vectors. (**D**) *Bgt* microcolony index analysis on wheat leaves silencing *TaDND1* (*BSMV-TaDND1as*), *TaDND2.1* (*BSMV-TaDND2.1as*), and *TaDND2.2* (*BSMV-TaDND2.2as*) or cosilencing *TaDND2.1* and *TaDND2.2* (*BSMV-TaDND2.1as* + *BSMV-TaDND2.2as*). For (**A**–**D**), three independent biological replicates were statistically analyzed for each treatment (*t*-test; ** $p < 0.01$).

Thereafter, we employed the TIGS assays to silence all endogenous *TaDND1* or *TaDND2* genes in the leaf epidermal cell of the *B.g. tritici*-susceptible wheat cultivar Yannong 999. As shown in Figure 5B, the silencing of *TaDND1* genes resulted in a notable HI% reduction to about 6%, compared to 36% for the empty vector controls. Although the silencing of the *TaDND2.1* or *TaDND2.2* genes failed to cause a significant change in the

HI%, the simultaneous silencing of *TaDND2.1* and *TaDND2.2* could lead to a remarkable decrease in the HI% to approximately 9% (Figure 5B). These results suggest that the redundant *TaDND2.1* and *TaDND2.2* attenuate the formation of *Bgt* haustoria and contribute to the wheat post-penetration susceptibility to *B.g. tritici*.

In addition, we employed BSMV-VIGS to silence all endogenous *TaDND1*, *TaDND2.1*, or *TaDND2.2* genes in the leaves of the *B.g. tritici*-susceptible wheat cultivar Yannong 999 (Figure 5C). As shown in Figure 5D, the *B.g. tritici* MI% decreased to about 14% on the BSMV-TaDND1as plants, compared with 56% for the BSMV-γ plants. Although the silencing of the *TaDND2.1* or *TaDND2.2* genes failed to cause an obvious change in the MI%, the simultaneous silencing of *TaDND2.1* and *TaDND2.2* could lead to a significant decrease in the MI% to about 10% (Figure 5D). Collectively, these results support that *TaDND2.1* and *TaDND2.2* contribute to the wheat post-penetration susceptibility to the adapted fungal pathogen *B.g. tritici*.

2.5. TaTPR1 Is a Transcriptional Corepressor and Suppresses the Expression of TaDND1 and TaDND2

It has been demonstrated that *Arabidopsis* TPR1 functions as a transcriptional corepressor [13]. To quantify the transcriptional regulatory activities of TPR1 proteins, we performed the *Arabidopsis* leaf protoplast transfection assay. As shown in Figure 6A, the LucA ratio has decreased from 1 for the Gal4 DNA binding domain (DBD) control to less than 0.45 under the presence of DBD-TaTPR1.1-4A, DBD-TaTPR1.1-4B, DBD-TaTPR1.1-4D, DBD-TaTPR1.2-7A, DBD-TaTPR1.2-7B, or DBD-TaTPR1.2-7D, indicating that TaTPR1.1 and TaTPR1.2 proteins exhibit a transcriptional repressing activity.

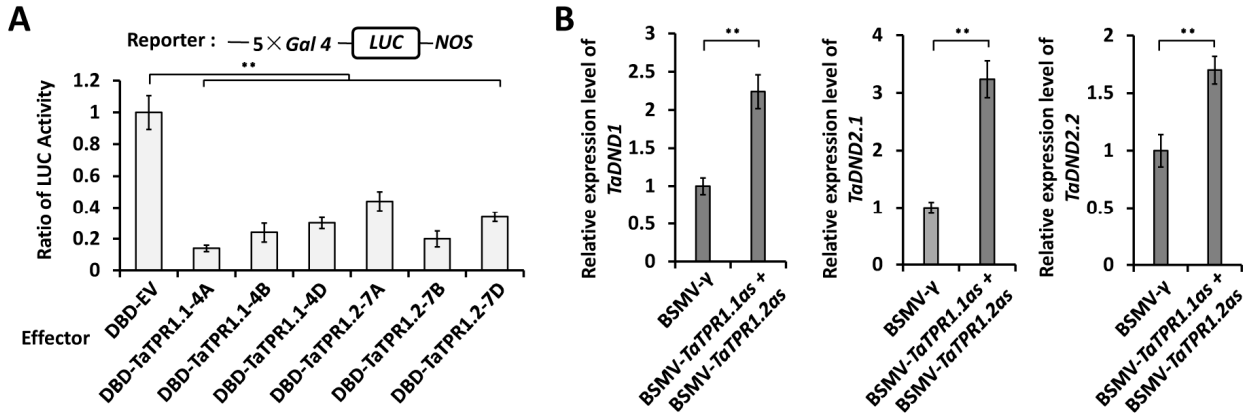

Figure 6. Analysis of the transcriptional suppression of *TaDND1* and *TaDND2* genes by TaTPR1. (**A**) Transcriptional repression activity analysis of TaTPR1.1 and TaTPR1.2 in *Arabidopsis* protoplast cells. (**B**) qRT-PCR analysis of *TaDND1* and *TaDND2* expression levels in *TaTPR1*-silenced wheat leaves. For (**A**) and (**B**), three independent biological replicates were statistically analyzed for each treatment (*t*-test; ** $p < 0.01$).

To further confirm the regulation of *TaTPR1* on the expression of wheat *TaDND1* and *TaDND2* genes, we employed BSMV-VIGS to silence all endogenous *TaTPR1* genes, including *TaTPR1.1* and *TaTPR1.2* genes, in the leaves of the wheat cultivar Yannong 999. As shown in Figure 6B, the silencing of the *TaTPR1.1* and *TaTPR1.2* genes could lead to a significant increase in the expression levels of *TaDND1* and *TaDND2*, indicating that the transcriptional corepressor TaTPR1 negatively regulates the expression of *TaDND1* and *TaDND2*. Collectively, these results support the idea that the transcriptional corepressor TaTPR1 directly suppresses the expression of *TaDND1* and *TaDND2*.

3. Discussion

3.1. TaTPR1 Positively Regulates Wheat Powdery Mildew Immunity

In this study, six *AtTPR1* homologs (*TaTPR1.1-4A*, *TaTPR1.1-4B*, *TaTPR1.1-4D*, *TaTPR1.2-7A*, *TaTPR1.2-7B*, and *TaTPR1.2-7D*) were identified from bread wheat. The overexpression of *TaTPR1.1* or *TaTPR1.2* could confer wheat post-penetration resistance against *B.g. tritici*. Although the single silencing of *TaTPR1.1* or *TaTPR1.2* genes failed to pose a significant effect on haustorium development and microcolony formation of *B.g. tritici*, the simultaneous silencing of *TaTPR1.1* and *TaTPR1.2* led to a significantly compromised resistance against *B.g. tritici*, implicating that *TaTPR1.1* and *TaTPR1.2* redundantly contribute to the post-penetration resistance of wheat to *B.g. tritici*. Similarly, knocking out *AtTPR1* and its close homologs in *Arabidopsis* or the silencing of *NbTPR1* in *N. benthamiana* compromised the plant ETI and PTI [24–26]. It was recently demonstrated that the *Arabidopsis* TPR1 protein could reduce the detrimental effects associated with an activated transcriptional immunity [14]. It is therefore intriguing to examine the potential contribution of wheat TaTPR1 to mitigate the deleterious effects of induced immunity in future research. In addition, *Arabidopsis* transcription factors AtAUX/IAA, AtBES1, and AtBZR1 could interact with AtTPL, the homolog of TaTPR1, to regulate plant responses to auxin and BRs [22,23]. The potential effects of TaTPR1 overexpression on wheat plant development and yields need to be characterized in future research.

3.2. TaDND1 and TaDND2 Contribute to Wheat Powdery Mildew Susceptibility

Herein, three *AtDND1* homologs (*TaDND1-5A*, *TaDND1-5B*, and *TaDND1-5D*) and six *AtDND2* homologs (*TaDND2.1-3A*, *TaDND2.1-3B*, *TaDND2.1-3D*, *TaDND2.2-1A*, *TaDND2.2-1A*, and *TaDND2.2-1D*) were identified from bread wheat. Overexpressing *TaDND1* leads to an enhanced wheat susceptibility to powdery mildew, while the silencing of *TaDND1* confers wheat post-penetration resistance against powdery mildew, suggesting that TaDND1, resembling its homolog *AtDND1* in *Arabidopsis*, positively contribute to the wheat powdery mildew susceptibility. Similarly, the overexpression of *TaDND2.1* or *TaDND2.2* significantly potentiates a wheat powdery mildew susceptibility. Although the single knockdown of *TaDND2.1* or *TaDND2.2* failed to pose a significant effect on haustorium development and microcolony formation of *B.g. tritici*, the simultaneous silencing of *TaDND2.1* and *TaDND2.2* resulted in the significantly elevated resistance against *B.g. tritici*, implicating that *TaDND2.1* and *TaDND2.2* redundantly contribute to the wheat powdery mildew susceptibility. It was previously demonstrated that the knockout of *Arabidopsis AtDND1* and *AtDND2* or silencing the homologs of *AtDND1* in potatoes and tomatoes resulted in an elevated plant resistance against bacterial, fungal, and oomycete pathogens [28–33]. This study further confirmed the contribution of the wheat *S* genes *TaDND1* and *TaDND2* in facilitating the wheat–*B.g. tritici* interaction.

Previous studies have identified *S* genes governing multiple processes in the wheat–*B.g. tritici* interaction [7]. For instance, the S factors TaMLO, TaEDR1, TaPOD70, TaHDA6, TaHOS15, TaHDT701, and TaCAMTA2/3 negatively regulate wheat defense-related gene expression and suppress the wheat post-penetration resistance to *B.g. tritici* [34–43]. In *Arabidopsis*, mutations that attenuated SA biosynthesis or signaling (*sid2*, *npr1*, and *ndr1*) abolished the enhanced resistance of *dnd* mutants against the bacterial pathogen *P. syringae* and the oomycete pathogen *H. parasitica*, but not the fungal pathogen *B. cinerea* [44]. In contrast, the disruption of *Arabidopsis* ethylene signaling (*ein2*) partially attenuated the enhanced resistance to *B. cinerea* but not to *P. syringae* or *H. parasitica* [44]. Therefore, more experiments are needed to elucidate the molecular mechanisms underlying the resistance to *B.g. tritici* in *TaDND1*- or *TaDND2*-silenced wheat plants. In addition, the activation of plant defense usually results in a fitness cost. The yield penalty associated with *TaDND1* or *TaDND2* silencing needs to be characterized in future research.

There is increasing evidence demonstrating that the inactivation of *S* genes could reduce the compatibility of host plants with adapted pathogens and confer plant disease resistance [7,39,45–50]. For instance, the knockout of wheat *S* genes *TaMLO* and *TaEDR1* by

genome editing system transcription activator-like effector nucleases (TALENs) enhances powdery mildew resistance, whereas the targeted knockout of *TaMLO* using clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 (CRISPR–associated 9) systems confers wheat powdery mildew resistance without a yield penalty [41,42,51]. Similarly, wheat *tamlo* mutant lines identified in the screen using targeting-induced local lesions in genomes (TILLING) techniques exhibited an enhanced resistance against *B.g. tritici* [39]. Therefore, it is intriguing to examine the potential of inactivating the *S* genes *TaDND1* and *TaDND2* via genome editing and TILLING techniques in the future when breeding for wheat powdery mildew resistance.

3.3. Transcriptional Corepressor TaTPR1 Suppresses Expression of TaDND1 and TaDND2

As demonstrated in the *Arabidopsis* protoplast transrepression assay, TaTPR1.1 and TaTPR1.2 proteins exhibit a transcriptional repressing activity. In addition, we showed that the silencing of *TaTPR1.1* and *TaTPR1.2* genes by BSMV-VIGS led to the potentiated expression of *TaDND1* and *TaDND2* in wheat leaves. These experiments indicate that the wheat transcriptional corepressor TaTPR1 suppresses the expression of *TaDND1* and *TaDND2*. Previous studies have demonstrated that *Arabidopsis* AtTPR1 is associated with the promoters of *AtDND1* and *AtDND2* genes and represses the expression of *AtDND1* and *AtDND2* [25]. Collectively, these studies strongly support that the suppression of *DND1* and *DND2* genes by TPR1 might be conserved among dicots and monocots. *Arabidopsis* AtTPR1 is demonstrated to associate with histone deacetylase (HDAC) 19 [25]. Although whether the wheat TaTPR1 protein interacts with HDACs remains unknown, there is increasing evidence demonstrating that wheat HDACs are involved in the regulation of wheat powdery mildew resistance [52,53]. For instance, the RPD3 (reduced potassium dependency protein 3)-type HDAC TaHDA6 and the HD2 (histone deacetylase 2)-type HDAC TaHDT701 negatively regulate wheat defense to *B.g. tritici* by mediating histone deacetylation at the promoter regions of defense-related genes [52,53]. Identifying wheat HDACs associated with TaTPR1 might shed light on the molecular mechanism underlying TaTPR1's function in the wheat–*B.g. tritici* interaction in future research.

4. Materials and Methods

4.1. Plant and Pathogen Materials

One wheat genotype, *B.g. tritici*-susceptible wheat cultivar Yannong 999, was employed in this study. Wheat seeds were surface sterilized and kept in pots containing soil in the greenhouse under a 16 h/8 h, 20 °C/18 °C day/night cycle with a 70% relative humidity. *A. thaliana* ecotype Columbia (Col-0) was used in this study. *A. thaliana* seeds were surface sterilized and kept in pots containing soil in a growth chamber under a 16 h/8 h light period at 23 °C with a 70% relative humidity. One *B.g. tritici* genotype, virulent *B.g. tritici* isolate E09, was used in this study. The *B.g. tritici* was maintained on the leaves of Yannong 999 wheat plants and kept at a 70% relative humidity and a 20 °C day/18 °C night cycle. The *B.g. tritici* inoculation and maintenance were performed as described previously [34].

4.2. Quantitative Reverse-Transcription PCR (qRT-PCR)

Total RNA was extracted using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). The RNA quality was examined according to previous studies [54,55]. Two µg of total RNA was used to generate the cDNA template under the TransScript one-step gDNA removal and cDNA synthesis supermix according to the manufacturer's instructions. The real-time PCR assay was performed using a qPCR master mix (Invitrogen). The *TaGADPH* gene was employed as the internal control, and the expressions of *TaTPR1.1*, *TaTPR1.2*, *TaDND1*, *TaDND2.1*, and *TaDND2.2* were analyzed using the primers 5′-ATCATTAAAACTAGGTGAT-3′/5′-GGCCTCATCAGGACTATTG-3′, 5′-GCATTTTCTCAATCAATG A-3′/5′-GCAGTGC ATCTCTTGGGTA-3′, 5′-ATGCCTCCATCGCTCTCCT-3′/5′-GGCTGCGTGCACGCGTAAC-3′, 5′-TCCTCGCCTTCTTCCTCGT-3′/5′-CTTGGACCTCGGCAGCCGA-3′, and 5′-CGGCC ACGGCGGTTGC GCG-3′/5′-CGGATCATCGCCGGCGCCG-3′, respectively. For the qRT-

PCR, three independent biological replicates were statistically analyzed (*t*-test; * $p < 0.05$, ** $p < 0.01$) for each treatment. The qRT-PCR analysis experiments were repeated three times with similar results.

4.3. BSMV-Mediated Gene Silencing and Microcolony Index Analysis

For the BSMV-mediated gene silencing assay, antisense fragments of *TaTPR1.1*, *TaTPR1.2*, *TaDND1*, *TaDND2.1*, and *TaDND2.2* were cloned into the pCa-γbLIC vector using the primers 5′-AAGGAAGTTTAGCGGGTAGCTATGGCTCTGC-3′/5′-AACCACCACCACCG TTGGACCCTTTCAACCTGCAC-3′, 5′-AAGGAAGTTTAGTGCGAACAACTTGTTTGG-3′/5′-AACCACCACCACCGTTGGTTGGATGACAAATCCCA-3′, 5′-AAGGAAGTTTACAT AAGCAAAGGCGCCATTG-3′/5′-AACCACCACCACGTTCATTGCCTCTCATATTGCA-3′, 5′-AAGGAAGTTTAGCCCGATCGCCGCCAGCCG-3′/5′-AACCACCACCACCGTGAC CGACCTCTCGGCGTCG-3′, and 5′-AAGGAAGTTTAGCCCCAGCCCCAGCTGCTG-3′/ 5′-AACCACCACCACCGTGCTCTCCACGCGCTCGTCG-3′. The BSMV-mediated gene silencing assay and microcolony index (MI) analysis were performed as described previously [56]. At least 2000 wheat-*Bgt* interaction sites were counted in one experiment for each treatment, and three independent biological replicates were statistically analyzed (*t*-test; * $p < 0.05$, ** $p < 0.01$) for each treatment. The MI analysis experiments were repeated three times with similar results.

4.4. Single-Cell Transient Gene Silencing/Overexpression Assays and Haustorium Index Analysis

For the single-cell transient gene silencing assay, antisense fragments of *TaTPR1.1*, *TaTPR1.2*, *TaDND1*, *TaDND2.1*, and *TaDND2.2* were cloned into the pIPKb007 vector using the primers 5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGCGGGTAGCTATGGCT CTGC-3′/5′-GGGGACCACTTTGTACAAGAAAGCTGGGTCTGACCCTTTCAACCTGC AC-3′, 5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGTGCGAACAACTTGTTTG G-3′/5′-GGGGACCACTTTGTACAAGAAAGCTGGGTCTGGTTGGATGACAAATCCCA-3′, 5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCATAAGCAAAGGCGCCATTG-3′/5′-GGGGACCACTTTGTACAAAAAGCTGGGTCTCATTGCCTCTCATATTGCA-3′, 5′-GGGGACAAGTTTGTACAAAAAA GCAGGCTTCGCCCGATCGCCGCCAGCCG-3′/5′-GGGGACCACTTTGTACAAGAAAGCTGGGTCGACCGACCTCTCGGCGTCG-3′, and 5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGCCCCAGCCCCAGCTGCTG-3′/ 5′-GGGGACCACTTTGTACAAGAAAGCTGGGTCGCTCTCCACGCGCTCGTCG-3′, respectively. For the single-cell transient gene overexpression assay, coding regions of *TaTPR1.1-4A*, *TaTPR1.1-4B*, *TaTPR1.1-4D*, *TaTPR1.2-7A*, *TaTPR1.2-7B*, *TaTPR1.2-7D*, *TaDND1-5A*, *TaDND1-5B*, *TaDND1-5D*, *TaDND2.1-3A*, *TaDND2.1-3B*, *TaDND2.1-3D*, *TaDND2.2-1A*, *TaDND2.2-1A*, and *TaDND2.2-1D* were cloned into the pIPKb001 vector using the primers 5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTTCTCTCAGCCGGGA-3′/ 5′-GGGGACCACTTTGTACAAGAAAGCTGGGTCTTATCTTTCTGGTTGATCAGA-3′ (for amplifying coding regions of *TaTPR1.1-4A*, *TaTPR1.1-4B*, and *TaTPR1.1-4D*), 5′-GGGGA CAAGTTTGTACAAAAAAGCAGGCTTCATGTCGTCGCTCAGCAGGGA-3′/5′-GGGGA CCACTTTGTACAAGAAAGCTGGGTCTCATCTCGTTGGCTGATCAGA-3′ (for amplifying coding regions of *TaTPR1.2-7A*, *TaTPR1.2-7B*, and *TaTPR1.2-7D*), 5′-GGGGACAAGTTTG TACAAAAAAGCAGGCTTCATGCCTCCATCGCTCTCCTC-3′/5′-GGGGACCACTTTGTA CAAGAAAGCTGGGTCCTACTCGAGGTGGTCGTGCG-3′ (for amplifying coding regions of *TaDND1-5A*, *TaDND1-5B*, and *TaDND1-5D*), 5′-GGGGACAAGTTTGTACAAAAAAGCA GGCTTCATGCCGACCGACCTCTCGGCGT-3′/5′-GGGGACCACTTTGTACAAGAAAGC TGGGTCTCAGAGCAGGAGGTCGTCCTG-3′ (for amplifying coding regions of *TaDND2.1-3A*, *TaDND2.1-3B*, and *TaDND2.1-3D*), and 5′-GGGGACAAGTTTGTACAAAAAAGCAGG CTTCATGTCCGGCGAGCTCTCCAC-3′/5′-GGGGACCACTTTGTACAAGAAAGCTGGG TCCTAGAAGGAGAAGTCGTCGTC-3′ (for amplifying coding regions of *TaDND2. 2-1A*, *TaDND2.2-1A*, and *TaDND2.2-1D*), respectively. The single-cell transient gene silencing/overexpression assays and haustorium index (HI) analysis were performed as described [34]. At least 100 cells were analyzed in one experiment, and three independent

biological replicates were statistically analyzed (*t*-test; * $p < 0.05$, ** $p < 0.01$) for each treatment. The HI analysis experiments were repeated three times with similar results.

5. Conclusions

In this study, we characterized the function of wheat TaTPR1 in the regulation of the wheat–*B.g. tritici* interaction and demonstrated that *TaTPR1.1* and *TaTPR1.2* positively contribute to the wheat post-penetration resistance against *B.g. tritici.* The overexpression of *TaTPR1.1* or *TaTPR1.2* confers wheat post-penetration resistance against *B.g. tritici*, while the silencing of *TaTPR1.1* and *TaTPR1.2* results in a compromised wheat resistance against *B.g. tritici.* Furthermore, we found that *TaDND1* and *TaDND2* function as wheat *S* genes contributing to the wheat powdery mildew susceptibility. The knockdown of *TaDND1* or *TaDND2* expression using transient- or virus-induced gene-silencing attenuates the post-penetration susceptibility to *B.g. tritici*. In addition, we demonstrated that the expression of *TaDND1* and *TaDND2* is negatively regulated by the wheat transcriptional corepressor TaTPR1. These results collectively suggest that TaTPR1 positively regulates the wheat post-penetration resistance against *B.g. tritici* probably via suppressing the *S* genes *TaDND1* and *TaDND2*. These findings could enhance our understanding of the genetic basis of wheat–*B.g. tritici* interactions and promote breeding programs for future wheat varieties with an enhanced powdery mildew resistance.

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