Serine/threonine kinase gene *Stpk-V*, a key member of powdery mildew resistance gene *Pm21*, confers powdery mildew resistance in wheat

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Powdery mildew resistance gene *Pm21*, located on the chromosome 6V short arm of *Haynaldia villosa* and transferred to wheat as a 6VS·6AL translocation (T6VS·6AL), confers durable and broad-spectrum resistance to wheat powdery mildew. *Pm21* has become a key gene resource for powdery mildew resistance breeding all over the world. In China, 12 wheat varieties containing *Pm21* have been planted on more than 3.4 million hectares since 2002. *Pm21* has been intractable to molecular genetic mapping because the 6VS does not pair and recombine with the 6AS. Moreover, all known accessions of *H. villosa* are immune to powdery mildew fungus. *Pm21* is still defined by cytogenetics as a locus. In the present study, a putative serine and threonine protein kinase gene *Stpk-V* was cloned and characterized with an integrative strategy of molecular and cytogenetic techniques. *Stpk-V* is located on the *Pm21* locus. The results of a single cell transient expression assay showed that *Stpk-V* could decrease the haustorium index dramatically. After the *Stpk-V* was transformed into a susceptible wheat variety Yangmai158, the characterized transgenic plants showed high and broad-spectrum powdery mildew resistance similar to T6VS·6AL. Silencing of the *Stpk-V* by virus-induced gene silencing in both T6VS·6AL and *H. villosa* resulted in their increased susceptibility. *Stpk-V* could be induced by *Bgt* and exogenous H_2O_2, but it also mediated the increase of endogenous H_2O_2, leading to cell death and plant resistance when the plant was attacked by *Bgt*.

gene cloning | microarray | transgenic wheat | disease resistance gene | hypersensitive response

Wheat is the most widely grown food crop in the world and ranks first in world crop production. It is the national staple food of 43 countries and feeds at least one third of the world's population (1). Currently, China is the biggest wheat producer and consumer in the world (2). Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is one of the most destructive diseases of wheat worldwide (3). Heavily infected seedlings gradually become yellow and dry, significantly affecting the efficiency of photosynthesis. The disease can usually lead to a 13% to 34% loss of yield, but loss can be as severe as 50% when the flag leaf becomes severely diseased during the heading and filling stages (4). To date, there have been 59 powdery mildew resistance alleles identified and designated at 43 loci (5), among which only the *Pm3b* and its alleles have been cloned (6, 7). Cloning of more genes which are highly resistant to the available powdery mildew pathogens is necessary for wheat breeding through biotechnological methods.

Race-specific resistance and broad-spectrum resistance (BSR) are two major types of disease resistance in plants. BSR refers to resistance against two or more types of pathogen species or the majority of races of the same pathogen species (8). Several BSR genes have been cloned, such as powdery mildew resistance gene *mlo* in barley (9) and leaf rust resistance gene *Lr34* and stripe rust resistance gene *Yr36* in wheat (10, 11). The mechanisms of action of these BSR genes are not characterized simply in a "gene-to-gene" model, and BSR, which cannot easily be overcome by newly developed pathogens, is usually correlated with durability. The broad spectrum and durability properties make BSR genes highly valuable in breeding programs.

Haynaldia villosa (*Dasypyrum villosum*, 2n = 2x = 14, VV) is a wild relative species of wheat. *Pm21* from *H. villosa* has been identified to confer durable and BSR to *Bgt* worldwide since the late 1970s. The *Pm21* gene was located to the short arm of chromosome 6V by the development of the wheat-*H. villosa* translocation line T6VS·6AL (12), and was then further localized to the 6VS bin [fraction length (FL) 0.45–0.58] with the construction of the resistant alien deletion line del6VS-1 (FL 0.58) (13) and susceptible alien deletion line del6VS-2 (FL 0.45) (14). It was reported in many other cases that the disease resistance was the integrated effect of a cluster of genes located tightly in one chromosome region or separately in different chromosome regions. It is still unclear whether *Pm21* is a single gene functioning independently or if it is a cluster of genes working simultaneously; therefore, *Pm21* is still defined as a locus by cytogenetics. T6VS·6AL has been released to 71 scientific research institutes in China and 23 other countries since 1995, and feedback from these researchers has revealed that *Pm21* shows high resistance in all these regions of the world and still confers immunity to all of the analyzed *Bgt* strains. T6VS·6AL has been used widely as a parent in breeding programs, and 12 new resistant varieties have been developed and released since 2002. The newly developed varieties have been cultivated on more than 3.4 million hectares by farmers in China, and they have been rapidly expanding since 2007. Our previous study showed that the introduction of T6VS·6AL in the newly developed varieties had no obvious adverse effect on the other agronomic traits (15). With other *Pm* genes losing their resistance in China, it is expected that *Pm21* will be widely used as a main resistance gene resource in future breeding programs.

Although *Pm21* is a pivotal gene in wheat breeding for powdery mildew resistance, little is known about the nature of the gene and its mechanism of BSR. However, as a result of the low

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See Commentary on page 7657.

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frequency of pairing and suppressed recombination between the 6VS of *H. villosa* and 6AS of wheat, it is extremely difficult to characterize the *Pm21* locus through a map-based cloning strategy. In this study, an integrated strategy was conducted to clone a resistance gene from the *Pm21* locus by using a GeneChip microarray combined with genetic mapping using a series of alien deletion and translocation lines. *Stpk-V*, a putative serine and threonine kinase gene cloned from the *Pm21* locus of *H. villosa*, will potentially play an important role in wheat resistance breeding via genetic engineering. This study also provides a step toward understanding the nature of the high and BSR to powdery mildew of *Pm21*.

Results

A Serine/Threonine Protein Kinase Gene Is Induced by *Bgt* in *H. villosa*. Cloning of the resistance gene in the *Pm21* locus by map-based cloning failed because no recombination was found between 6VS and 6AS in our previous study. Microarray is a feasible method for cloning a target gene that is expressed differentially between different samples. In this study, a GeneChip microarray was used to identify *Bgt*-induced genes in *H. villosa*. The 196 genes, whose signal intensities in the *Bgt*-inoculated *H. villosa* were twofold higher than in the uninoculated *H. villosa*, were selected as differentially expressed genes. Among the 131 genes with putative functions, there were pathogenesis-related genes, defense response genes, signal transduction and transcription factor genes, and resistance gene analogues (RGAs). To select the candidate genes located in the *Pm21* locus, we focused first on the four RGAs, including Contig17515 (serine and threonine protein kinase), Contig16386 (putative disease resistance protein), Contig7534 (serine and threonine protein kinase), and Contig13968 (*Mlo*-like seven-transmembrane protein). However, only *Stpk-V*, the homologue of Contig17515 in *H. villosa*, was chosen as the candidate gene based on its cytogenetic localization to 6VS and its expression pattern (as detailed later). A pair of primers, *CINAU15*-F and *CINAU15*-R, were designed based on Contig17515 to clone its homologue from *H. villosa*. *Stpk-V* (a putative serine and threonine protein kinase gene from *H. villosa*), a 448-bp fragment isolated from *Bgt*-inoculated *H. villosa* by RT-PCR, shared 96% similarity with Contig17515 (16). RT-PCR confirmed that *Stpk-V* was significantly up-regulated by *Bgt* (16). In the resistant T6VS·6AL, *Stpk-V* was induced by *Bgt* (Fig. S1*A*, 1), but not by *Fusarium graminearum* (Fig. S1*A*, 2) or abiotic stresses such as heat and salt treatments (Fig. S1*A*, 3 and 4).

The full-length cDNA of *Stpk-V* was obtained by RACE, and the ORF contains 401 amino acids (HM241655). To obtain the genomic sequence of *Stpk-V*, the transformation-competent artificial chromosome (TAC) library of T6VS·6AL was screened by pooled PCR using the *CINAU15*-F and *CINAU15*-R. A 30-kb TAC clone and a 5,160-bp subclone containing the whole genomic sequence of *Stpk-V* were obtained (HQ864471). Sequence comparisons indicated that the cDNA of *Stpk-V* matches the TAC subclone completely. *Stpk-V* contains six exons and five introns (Fig. S2*A*), and a putative serine/threonine protein kinase domain was found within Stpk-V. The HRDIKASNIL sequence is the putative catalytic loop, and DFGLAKLLPP, ISTRV, and GTLGYLAPE form the putative activation loop. The amino acid residues Y, N, L, and SDF form the putative ATP binding pocket, and K, TR, G, and LG form the putative substrate binding pocket (Fig. S2*B*). The comparisons of the protein (Figs. S3 and S4) and putative promoter sequence (Fig. S5) of the *Stpk-V* with its homologues from other species are described in *SI Results*.

***Stpk-V* Is Located in the 6VS Bin FL0.45–0.58.** The location of the *Stpk-V* gene was determined by PCR using *CINAU15*-F and *CINAU15*-R. The partial sequence of *Stpk-V* (902 bp in length) was amplified from materials containing 6VS, including *H. villosa*, *T. durum–H. villosa* amphiploid, T6VS·6AL, and wheat–*H. villosa* disomic addition line of chromosome 6V (DA6V), but not from Yangmai158 and other addition lines. These data localized *Stpk-V* to 6VS of *H. villosa*. Further amplification in the resistant alien deletion del6VS-1 (FL 0.58) and susceptible alien deletion del6VS-2 (FL 0.45) mapped *Stpk-V* to the bin FL 0.45–0.58 (Fig. S6*A*).

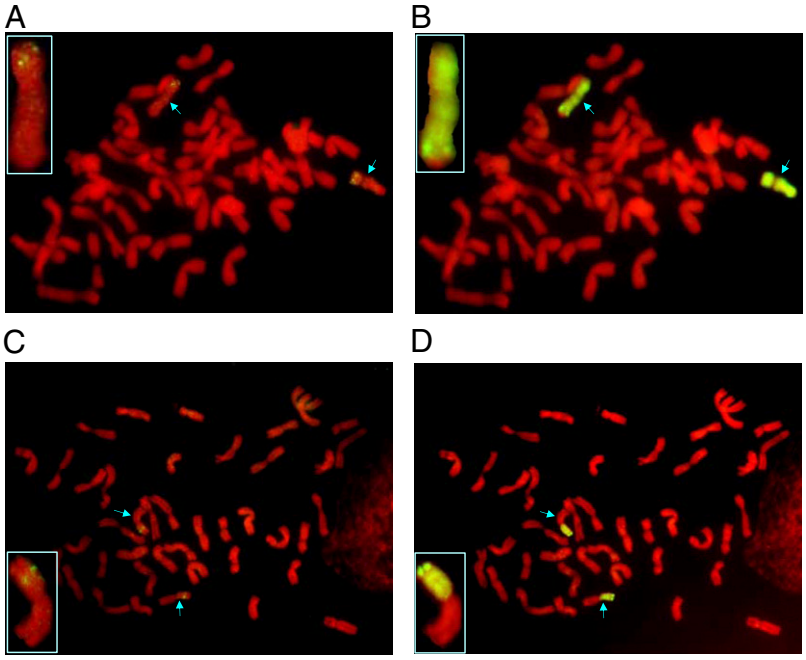

Fig. 1. Mapping of *Stpk-V* by FISH and sequential GISH on mitotic metaphase chromosomes of T6VS·6AL and del6VS-1. (*A* and *C*) FISH with 30-kb TAC clone as the probe, and the green signal shows the *Stpk-V* position. (*B* and *D*) Sequential GISH with the genomic DNA of *H. villosa* as the probe. The arrows in *A* and *B* show the deletion 6V chromosomes, and in *C* and *D* they indicate the translocation chromosomes.

To precisely map *Stpk-V*, FISH using the 30-kb TAC clone with *Stpk-V* as the probe and the following sequential genomic in situ hybridization (GISH) using gDNA of *H. villosa* as the probe were conducted. A TAC signal was detected near the telomeres of 6VS in del6VS-1, and the signal was located from FL 0.45–0.58 of 6VS in T6VS·6AL (Fig. 1). Both the PCR and FISH results indicated that *Stpk-V* is located in the same region as *Pm21*, i.e., from FL 0.45–0.58 of 6VS.

Stpk-V Is Localized to the Membrane, Cytoplasm, and Nucleus of Epidermal Cells and Decreases the Haustorium Index of *Bgt* in Susceptible Yangmai 158. A single-cell transient expression assay of *Stpk-V-YFP* in the epidermal cells of *H. villosa* localized Stpk-V to the membrane, cytoplasm, and nucleus (Fig. S6*B*).

Haustorium is a key structure for nutrient extraction during *Bgt* development. If the haustorium cannot form successfully, the host–*Bgt* interaction is considered to be incompatible. The haustorium index is usually used as a criterion to estimate the compatibility of interactions between the host and *Bgt*. The haustorium index was 54.8% in epidermal cells of susceptible Yangmai158 transformed with a single GUS gene, but it decreased to 13.6% in epidermal cells cotransformed with *GUS* and *Stpk-V*. In epidermal cells of T6VS·6AL transformed with *GUS*, the haustorium index was 14.1% (Fig. S7). The results indicate that *Stpk-V* acts by preventing the formation of the haustoria.

***Stpk-V* Shows High Resistance and BSR to *Bgt*.** The function of *Stpk-V* was further studied in transgenic wheat plants stably expressing *Stpk-V* from the T_0 to T_3 generation. *Stpk-V* transgenic plants were produced by transforming pAHC:*Stpk-V* into the susceptible variety Yangmai158 by particle bombardment. Southern blotting (T_0 generation), BASTA resistance evaluation (T_1 generation), *Stpk-V* expression analysis (T_1 generation), and detection of the transformed genes (T_3 generation) on the transgenic plants verified the stable incorporation and expression of *Stpk-V* (Fig. S8 *A*–*D*). RT-PCR analysis showed that *Stpk-V* was induced by *Bgt* in T6VS·6AL, and its expression was undetectable in the susceptible Yangmai158, but *Stpk-V* was constitutively expressed even in the uninoculated seedlings in the transgenic plants (Fig. S8*E*).

Leaves detached from the T_0 plants were inoculated with mixed *Bgt* collected from eastern China, and HRs were observed (Fig. 2*A*). In the T_1 generation, only plants with *Stpk-V* showed high resistance similar to that of T6VS·6AL (Fig. 2*B*). By the T_3 generation, the resistance conferred by *Stpk-V* has been stably transmitted (Fig. 2*C*). The homozygous resistant lines of the T_3 generation were used to evaluate the function of *Stpk-V* using eight different strains isolated from a population of *Bgt* from northern China. The transgenic plants showed the highest level of resistance—grade 0 or 0;—to all eight strains; however, the receptor Yangmai158 showed the highest level of susceptibility—grade 3 or 4—to all eight strains (Table S1). These results suggested that *Stpk-V* may be a BSR gene.

Resistance to *Bgt* Is Compromised by *Stpk-V* Silencing in *H. villosa* and T6VS·6AL. A virus-induced gene silencing (VIGS) system (17) established in *H. villosa* (18) was used to evaluate the function of *Stpk-V*. The expression levels of *Stpk-V* decreased by 2.5- to 10-fold in the fifth leaves of both resistant materials challenged with barley stripe mosaic virus (BSMV):*Stpk-V* (Fig. 3*A*).

The fifth fully expanded leaves inoculated with BSMV:*Stpk-V* were detached from the plants for inoculation with fresh *Bgt* conidia, using plants infected with BSMV:*GFP* as a control. In T6VS·6AL and *H. villosa* inoculated with BSMV:*GFP*, most of the germinated spores formed only the appressorium penetration peg (App), and approximately 15% of the spores could form haustorium with no more than four branches of hyphae (Fig. 3*B*, 1 and 3). However, approximately 40% of the germinated spores could produce more branches of hyphae in BSMV:*Stpk-V*–

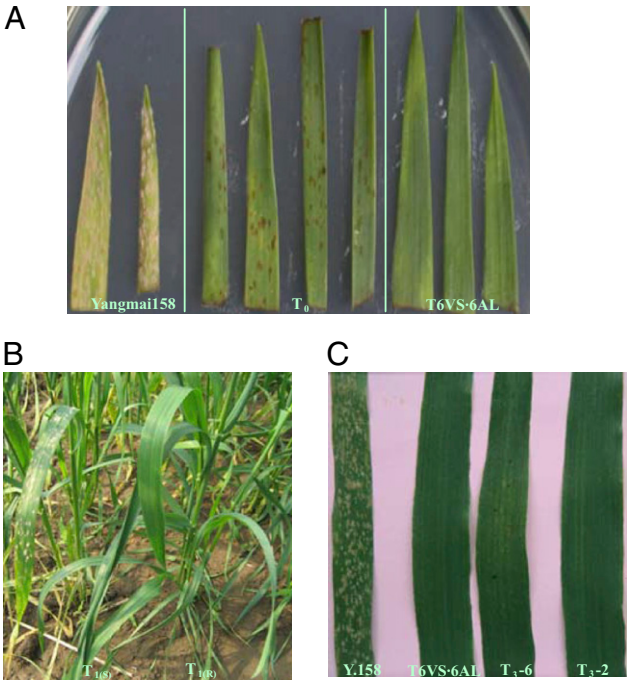

Fig. 2. Functional analysis of *Stpk-V* by stable transformation into the susceptible wheat variety Yangmai158. (*A*) Detached leaves of the T_0 plants showed HRs 7 d after inoculation with *Bgt*, whereas the leaves of Yangmai158 were covered with colonies. (*B*) T_1 plant without *Stpk-V* ($T_{1(S)}$) showed susceptibility, whereas the T_1 plant with *Stpk-V* ($T_{1(R)}$) showed high resistance in the greenhouse. (*C*) T_3 lines showed a similar high level of resistance in the field as T6VS·6AL. T_3-6, resistant line with hypersensitive mosaics, T_3-2, resistant line without hypersensitive mosaics.

treated leaves of *H. villosa* and T6VS·6AL, some of which even formed conidiophore (Fig. 3*B*, 2 and 4). Although gene silencing was not complete, the decreased expression of *Stpk-V* in the BSMV:*Stpk-V*–treated plants compromised the resistance of *H. villosa* and T6VS·6AL to *Bgt*.

The sequence homology between *Stpk-V* and the three orthologous genes (*Stpk-A*, *Stpk-B*, and *Stpk-D*) of wheat was greater than 95%. Therefore, in the homozygous wheat–*H. villosa* translocation line T6VS·6AL, besides the *Stpk-V* in the V genome, the other two copies in the B and D genomes (*Stpk-B* and *Stpk-D*) may also be silenced if they are expressed. As *Stpk-B* and *Stpk-D* made no contribution to the resistance in common wheat, we proposed that the phenotypic change of T6VS·6AL was most likely a result of the silencing of *Stpk-V*.

Resistance of *Stpk-V* Is Correlated with Hypersensitive Cell Death and H_2O_2 Accumulation. Obvious hypersensitive cell death was observed in the leaves of T_0 (Fig. 2*A*) and T_3 plants (Fig. 2*C* and Table S1), indicating that the hypersensitive response (HR) was a crucial mechanism of resistance mediated by the over-expression of *Stpk-V*. HR is usually associated with a burst of reactive oxygen species, especially H_2O_2. To study whether the production of H_2O_2 is responsible for HR, the subcellular localization of H_2O_2 at the sites of interaction and development of *Bgt* were investigated.

For the susceptible Yangmai158, a small area of brown staining around the App, indicative of H_2O_2 production, was observed at 6 h (Fig. 4, S6). At 24 h, the haustorium beneath the App and several branches of primary hyphae were formed (Fig. 4, S24). At 48 h, a small area of brown staining was again detected under the secondary penetration peg (Fig. 4, S48). At 120 h, the hyphae and conidiophores covered the leaf surface (Fig. 4, S120). In the whole process, no HR was observed.

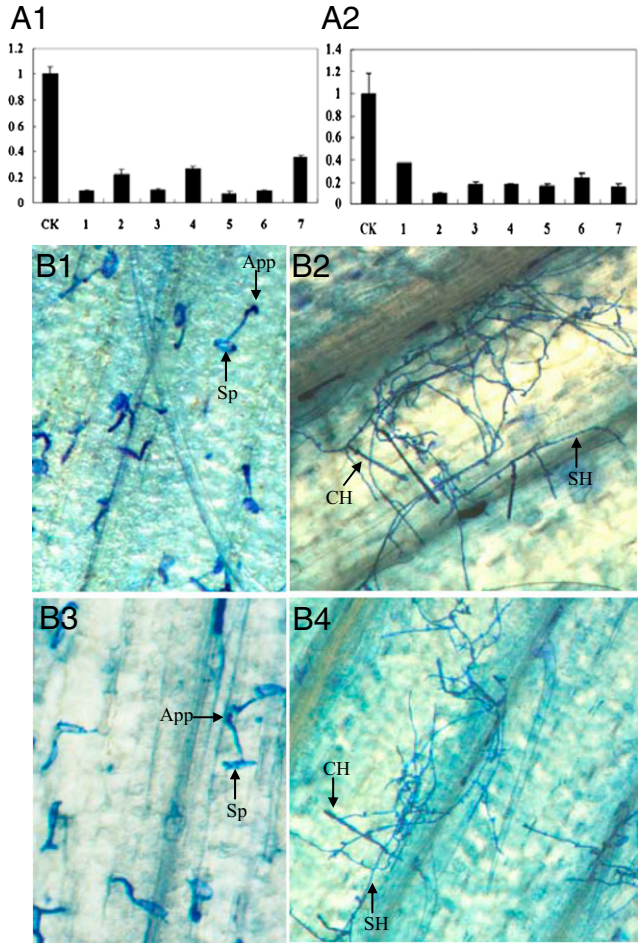

Fig. 3. Functional analysis of *Stpk-V* by VIGS. (*A*) Quantitative RT-PCR analysis of the expression level of *Stpk-V* in the plants inoculated with BSMV:*Stpk-V* using the plants inoculated with BSMV:*GFP* as the control (CK). In seven BSMV:*Stpk-V*–inoculated *H. villosa* plants (*A1*) and seven BSMV:*Stpk-V*–inoculated T6VS·6AL plants (*A2*), the expression level of *Stpk-V* was decreased. (*B*) Observations of the development of *Bgt* in the BSMV:*Stpk-V*–inoculated plants using the BSMV:*GFP* inoculated plants as the control. *H. villosa* (*B1*) and T6VS·6AL (*B3*) were infected with BSMV:*GFP* and then inoculated with *Bgt*. Infection with BSMV:*GFP* did not alter the resistance to *Bgt*. *H. villosa* (*B2*) and T6VS·6AL (*B4*) infected with BSMV:*Stpk-V* and then inoculated with *Bgt*. *Stpk-V* silencing resulted in increased susceptibility. Arrows indicate spore (Sp), appressorium (App), secondary hyphae (SH), and conidiophore (CH).

For the resistant T6VS·6AL, approximately 44% of the germinated spores induced a brown staining response throughout the whole cell at 6 h (Fig. 4, R6) and HR were observed at 24 h (Fig. 4, R24). Fifteen percent of the spores developed haustoria at 24 h (Fig. 4, R24′), but hyphae developed slowly and no conidiophore formed at 120 h (Fig. 4, R120′).

In the transgenic plants, 76% of the germinated spores induced brown staining in the whole infected cells at 6 h (Fig. 4, T6), and HR but no haustoria was observed at 24 h in the *Bgt*-infected cells (Fig. 4, T24). However, the haustorium index was 13.6% in the single-cell transient experiments, indicating that there was a low percentage of GUS expressing cells without *Stpk-V* expression.

H_2O_2, salicylic acid (SA), or jasmonic acid (JA) has proven to be closely correlated with disease resistance by inducing the pathogenesis related genes or acting as early signaling components. In this study, *Stpk-V* could be induced by H_2O_2 (Fig. S1*B*, 1), but not by SA (Fig. S1*B*, 2) and JA (Fig. S1*B*, 3). H_2O_2 can induce cell death (19), promote cross-linking of cell wall structural proteins (20), or act directly as the defense substance (21). Observations of the T6VS·6AL and transgenic plants revealed that H_2O_2 production was tightly correlated with the HR and the suppression of fungus growth, and the *Stpk-V* gene has a crucial role in mediating the accumulation of H_2O_2.

Discussion

In this study, the combined evidence from gene mapping, stable transformation, transient expression assay, and gene silencing strongly suggests that *Stpk-V*, a putative serine/threonine protein kinase gene, is a key gene conferring the durable BSR in the *Pm21* locus. Serine/threonine protein kinases (EC 2.7.11.1) phosphorylate the OH group of serine or threonine residues leading to a functional change of the target protein, and protein phosphorylation has been identified as one of the most important events in the disease resistance pathway. The tomato bacterial speck disease resistance gene *Pto* encodes serine/threonine protein kinases (22); bacterial blight disease resistance gene *Xa21* (23) in rice, flagellin perception gene *FLS2* (24) and AvrPphB recognition gene *PBS1* (25) in *Arabidopsis*, stem rust resistance gene *Rpg5* (26) in barley, and stripe rust resistance gene *Yr36* (11) in wheat also contain a serine/threonine protein kinase domain. However, the homology of Stpk-V with these genes was lower than 40%, and the homologous region was limited to 39 to 201 aa.

HR, involving the rapid development of cell death immediately surrounding infection sites, is a common weapon used by plants to limit development of the pathogen. Oxidative bursts occur at early stages after pathogen infection and induce HR later (27). There are two distinct phases of reactive oxygen species production during plant–pathogen interactions (19, 21). In this study, the production of H_2O_2 in resistant and susceptible plants was observed. The first H_2O_2 burst in the basal resistance stage in susceptible Yangmai158 made no contribution to resistance; however, the first H_2O_2 burst in the basal resistance stage in the resistant plant T6VS·6AL could induce the expression of *Stpk-V*, which then mediated the second round of H_2O_2 burst, leading to HR. The H_2O_2-induced expression of *Stpk-V*, which was revealed by RT-PCR, supported this hypothesis.

The formation of mature haustoria and the development of secondary hyphae are prerequisites for the establishment of a compatible interaction between the host and the parasite (28). In our study, some primary haustoria were formed in T6VS·6AL, but the hyphae developed slowly and no colony was observed. In the transgenic plants, no haustorium was observed. The difference in the development pattern of *Bgt* in both resistant plants may be a result of the different expression patterns of *Stpk-V*. In the transgenic plants, the constitutive overexpression of *Stpk-V* allowed the leaves to respond to *Bgt* very quickly, and the fungus could not produce even primary haustoria. However, in the T6VS·6AL plants, the *Stpk-V* was induced by *Bgt*, and the defense response mediated by *Stpk-V* was slower, thus leading to the formation of primary haustoria.

Wild species have been widely used as genetic resources for introgression of useful traits into cultivated species by wide hybridization (2). The cloning of genes from wild relatives and the use of these genes in transgenic studies is an efficient way for modern genetic improvement. However, because of the low frequency of pairing and recombination between the chromosomes from the wild and cultivated species, map-based cloning of these elite genes from wild species is a challenge. Our research is a successful example of cloning useful wild genes by integrative cytogenetic and molecular methods.

Stpk-V, a gene from a wild species conferring BSR against *Bgt*, can now be used in wheat breeding for powdery mildew resistance by genetic engineering. The gene cloning strategies used in this study will benefit other research aimed at cloning genes from wild species. In the follow-up research, we will evaluate the

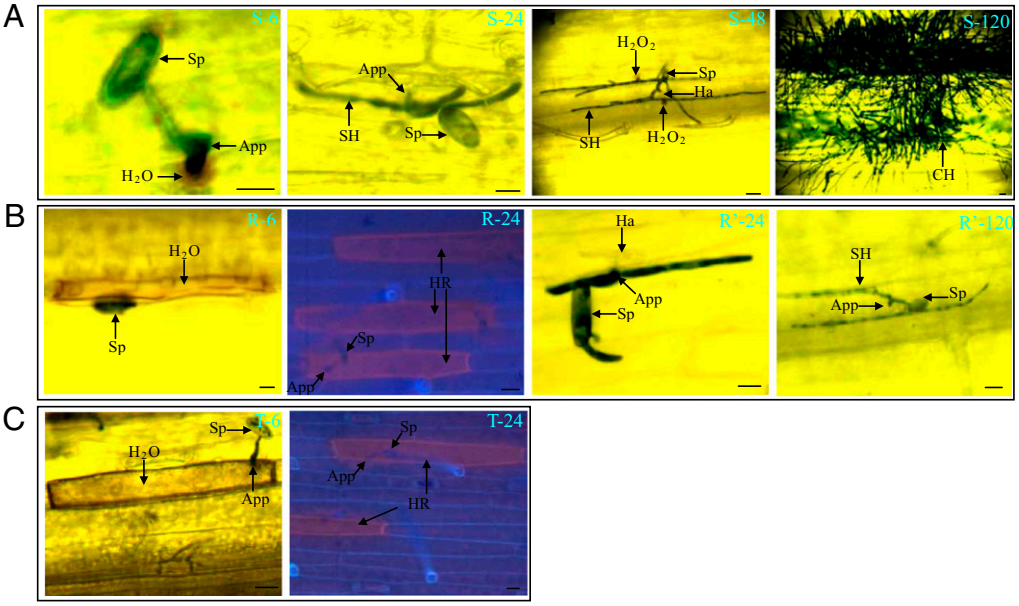

Fig. 4. Characterization of the resistance mechanism of *Stpk-V* by observing of H_2O_2 and HRs after *Bgt* inoculation in leaves of Yangmai158 (S), T6VS·6AL (R), and the transgenic plants (T). (*A*) In Yangmai158, *Bgt* developed normally. H_2O_2 was localized around the primary penetration peg at 6 h (S-6) and then appeared under the newly developed penetration pegs at 48 h (S-48). At 120 h, the leaves were covered with hyphae and conidiophores (S-120). (*B*) In the T6VS·6AL, there were two types of *Bgt* development patterns. For the first type, the development of *Bgt* stopped after App formation. H_2O_2 was detected in the interaction cell (R-6), and the HR was then observed at 24 h (R-24). For the second one, the haustoria could form (R′-24), and the hyphae elongated, but very slowly (R′-120). (*C*) In the transgenic plants, *Bgt* could not develop after App formation. H_2O_2 was detected in the interaction cell (T-6), and the HR was observed at 24 h (T-24). Arrows indicate spore (Sp), appressorium (App), haustoria (Ha), secondary hyphae (SH), HR cell (HR), and conidiophore (CH). Numbers indicate the hours of *Bgt* inoculation. (Scale bar, 25 μm.)

function of *Stpk-V* by using its endogenous promoter, and focus on the polymorphisms between *Stpk-V* with its orthologous genes in the susceptible wheat varieties. It will be interesting to identify the protein(s) interacting with Stpk-V to investigate the molecular mechanism of BSR mediated by *Stpk-V*. We will also create more cytogenetic stocks and mutants involving the 6VS to help us determine how many genes are involved in the *Pm21* locus, and whether *Stpk-V* is the only one necessary to confer the resistance phenotype.

Materials and Methods

Plant Materials. *H. villosa* (introduced from Cambridge Botanical Garden, United Kingdom), *T. durum–H. villosa* amphiploid [developed by the Cytogenetics Institute of Nanjing Agricultural University (CINAU); accession no. NAU201], wheat–*H. villosa* translocation line T6VS·6AL (developed by CINAU; accession no. NAU405), DA1V-DA7V (seven *T. aestivum–H. villosa* alien addition lines, each containing one pair of chromosomes of *H. villosa* from 1V to 7V in the *T. aestivum* cv. Chinese Spring background, developed by CINAU; accession nos. NAU307–NAU313), del.6VS-1 and del.6VS-2 (deletion addition lines that lack the distal 42% and 55% part of the 6VS chromosome respectively, developed by CINAU; seed accession nos. NAU453 and NAU454) were used in this study. All the NAU accession numbers were given by Cytogenetics Institute of Nanjing Agricultural University, China.

GeneChip Microarray. GeneChip microarray was conducted only to screen genes for independent analysis in the later study. Leaves of 3-wk-old seedlings of resistant *H. villosa* were collected at 24, 48, and 72 h after inoculation with a mixture of *Bgt*, and total RNA isolated from three samples was pooled as the test. The uninoculated leaves were collected at the same three time points, and the total RNAs were pooled as the control. More information about data analysis is supplied in *SI Materials and Methods*.

Semiquantitative RT-PCR. *Stpk-V* expression level was analyzed by semiquantitative RT-PCR under different abiotic and biotic stresses in *H. villosa*, T6VS·6AL, and transgenic plants. Details are provided in *SI Materials and Methods*.

TAC Library Screening and RACE. To isolate the genomic sequence of *Stpk-V*, *CINAU15*-F and *CINAU15*-R (*SI Materials and Methods*) were used to screen a genomic TAC library of T6VS·6AL (29) by a pooled PCR screening procedure. The 30-kb positive TAC clone was obtained and digested with *Eco*RI, and then a 5,160-bp subclone containing the whole *Stpk-V* was further obtained. Details of RACE are provided in *SI Materials and Methods*.

FISH and Sequential GISH. FISH using 30-kb TAC as probe in del.6VS-1 and T6VS·6AL was conducted as described (30). After TAC-FISH, the hybridization signals were removed for the following GISH, which was performed using DNA of *H. villosa* as probe. Details are provided in *SI Materials and Methods*.

Vector Construction and Wheat Transformation. The vector pAHC:*Stpk-V* was constructed by replacing the *GUS* gene with ORF of *Stpk-V* in the plant expression vector pAHC 25, and the pAHC:*Stpk-V* transgenic wheat plants were produced by particle bombardment of calli cultured from immature embryos of susceptible variety Yangmai158. Details are provided in *SI Materials and Methods*.

Evaluation of Powdery Mildew Resistance of Transgenic Plants. Powdery mildew resistance of the transgenic plants was evaluated via inoculation with *Bgt* using Yangmai158 and T6VS·6AL as the control. The detached leaves from T_0 transgenic plants were inoculated with a mixture of *Bgt* collected from Eastern China. The adult T_1- and T_3-generation transgenic plants were inoculated with a mixture of *Bgt* in the natural field of Eastern China. Two lines (T_3-6 and T_3-2) of homozygous resistant T_3 transgenic plants were also inoculated with eight different individual strains (*Bgt11*, *Bgt18*, *Bgt311*, *Bgt411*, *Bgt401*, *Bgt413*, *Bgt611*, and *Bgt711*) collected from Northern China to evaluate the BSR. The level of resistance was classified as grades 0 to 4 (0, 0;, 1, 2, 3, and 4) according to the standard of Sheng et al. (31). Grade 0 indicates the highest resistance level to *Bgt* at which leaves are immune, grade "0;" represents high resistance at which only hypersensitive mosaics are observed, and grade 4 is the highest susceptible level at which all leaves were covered with vast stretches of hyphae producing large amounts of spores.

Single-Cell Transient Expression Assay. The single-cell transient expression assay was performed according to Shirasu et al. (32). Reporter plasmids

containing β-glucuronidase (*GUS*) genes and the plasmids pAHC:*Stpk-V* were mixed before coating of the particles (molar ratio of 1:1; 1 μg of total DNA). The bombarded leaves were transferred to 1% agar plates supplemented with 85 μM benzimidazole and incubated at 18 °C for 8 h before high-density inoculation with single *Bgt* spores. Leaves were stained for GUS to identify *Stpk-V* transformed cells at 48 h after spore inoculation. The haustorium index (percentage of GUS-staining cells with haustoria in the total GUS-staining cells attacked by *Bgt*) is indicated by the mean of three independent experiments, each contributing at least 40 interactions.

Functional Analysis of *Stpk-V* by VIGS. VIGS was mediated by BSMV (17). Vector construction, plasmid linearization, in vitro transcription, and virus infection were conducted as described by Wang et al. (18) and Zhou et al. (33). Details are provided in *SI Materials and Methods*.

Subcellular Localization of H_2O_2 and Cell Death at Interaction Sites in Leaves After Inoculation with *Bgt*. The first leaves were cut from Yangmai158, T6VS·6AL, and pAHC:*Stpk-V* transgenic plants at 6, 24, 48, 72, 96, and 120 h after *Bgt*18 inoculation. Detection of H_2O_2 was performed by in situ histochemical staining using DAB as described by Thordal-Christensen et al. (34). The development of fungus and H_2O_2 production were observed under an Olympus microscope in bright field. For dead cells localization, the inoculated leaves were observed in the UV light after bleaching in 90% ethanol.

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