LETTERS
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Wheat receptor-kinase-like protein Stb6 controls gene-for-gene resistance to fungal pathogen *Zymoseptoria tritici*

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Deployment of fast-evolving disease-resistance genes is one of the most successful strategies used by plants to fend off pathogens[1,2]. In gene-for-gene relationships, most cloned disease-resistance genes encode intracellular nucleotide-binding leucine-rich-repeat proteins (NLRs) recognizing pathogen-secreted isolate-specific avirulence (Avr) effectors delivered to the host cytoplasm[3,4]. This process often triggers a localized hypersensitive response, which halts further disease development[5]. Here we report the map-based cloning of the wheat *Stb6* gene and demonstrate that it encodes a conserved wall-associated receptor kinase (WAK)-like protein, which detects the presence of a matching apoplastic effector[6–8] and confers pathogen resistance without a hypersensitive response[9]. This report demonstrates gene-for-gene disease resistance controlled by this class of proteins in plants. Moreover, *Stb6* is, to our knowledge, the first cloned gene specifying resistance to *Zymoseptoria tritici*, an important foliar fungal pathogen affecting wheat and causing economically damaging septoria tritici blotch (STB) disease[10–12].

More than a decade ago, the concept of plant innate immunity as a two-layer defense system comprising broad-spectrum pattern-triggered immunity (PTI) and isolate-specific effector-triggered immunity (ETI) was first proposed[3]. PTI is thought to be orchestrated by conserved cell-surface pattern-recognition receptors (PRRs)[13], such as receptor-like proteins and receptor-like kinases (RLKs) including WAKs, after perception of conserved pathogen-associated or plant-derived damage-associated molecular patterns, such as chitin or pectic oligogalacturonide derivatives of fungal or plant cell walls, respectively. After this first level of defense is overcome, plants may deploy ETI with highly variable, often dispensable resistance proteins (cytoplasmic NLRs or extracellular-receptor-like proteins) that detect matching Avr effectors, which are also highly variable. ETI frequently culminates in a hypersensitive response and is often described as faster and stronger than PTI. The above concepts are being challenged by the accumulation of new data suggesting that plant immunity is likely to be a continuous surveillance system that evolves to detect invading microbes[14,15]. In particular, there may be no strict dichotomy between PTI and ETI, or between PRRs and resistance proteins.

Wheat, one of the most important staple food crops, provides 20% of the total daily calories consumed by humans worldwide and in that regard is second only to rice. STB is a devastating disease in most wheat-growing areas of the world. It is the primary foliar disease of wheat in Europe and is responsible for annual wheat losses of 5–10%, with a value of more than $800 (€720) million, despite the use of fungicide treatments estimated to cost farmers additional $1.2 billion (€1 billion)[10,11]. The causal agent of STB is the fungus *Z. tritici*, which has recently been described as a latent necrotroph[16] with a strictly extracellular mode of plant pathogenesis[17]. The emergence and dispersal of fungicide resistance in fungal populations[11,18–20] severely threatens wheat production and compromises food security; therefore, STB-resistance breeding is considered a high priority. To date, 21 major genes for resistance to STB (*Stb* resistance genes), most of which have different specificities based on reactions to pathogen isolates, and numerous minor-effect resistance quantitative trait loci (QTLs) have been mapped genetically[21]. However, none of these genes have been cloned, and the mechanisms of resistance remain poorly understood. Owing to a lack of well-defined QTLs with additive effects and the near absence of diagnostic markers, current STB-resistance breeding strategies rely primarily on phenotypic evaluation of breeding materials rather than targeted-genotyping-based selection, although deployment of broad-spectrum resistance genes, such as *Stb16q* identified in synthetic wheat[22], and targeted stacking of isolate-specific *Stb* resistance genes are also being considered[22,23].

Stb6, the best-characterized gene for resistance to STB, has been reported to be present in wheat used in breeding programs worldwide, on the basis of phenotypic evaluation[24,25]. It is inherited and manifests as a semidominant trait[24] (Supplementary Fig. 1a), controlling a gene-for-gene type resistance[6] effective against *Z. tritici* isolates, such as IPO323, which carry a matching *AvrStb6* gene encoding a small cysteine-rich effector protein[7,8]. *Stb6* is particularly interesting because it confers pathogen resistance in the absence of a hypersensitive response[9]. This gene has been suggested to exist in wheat since the mid-Neolithic period[24], and it contributes to field resistance[26]. *Stb6* resides in the subtelomeric portion on 3AS in the wheat varieties Flame[6], Chinese Spring (CS) and Cadenza (Cad) (Fig. 1 and Supplementary Fig. 1b,c). To better understand the

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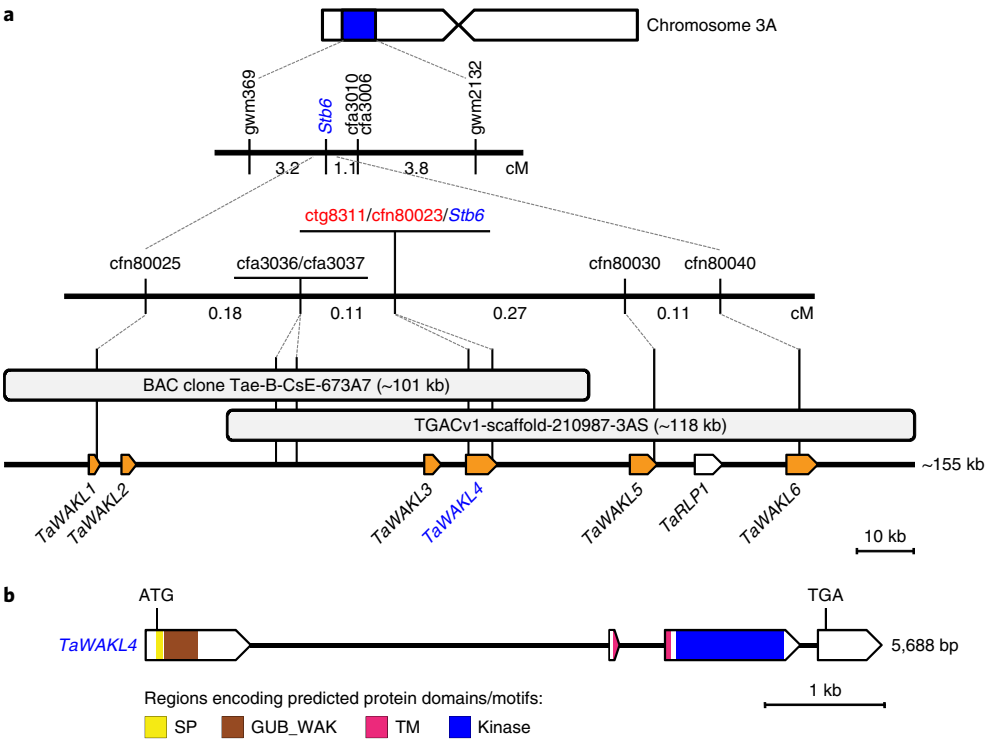

Fig. 1 | Map-based cloning of the *Stb6* resistance gene. a, Sequential fine genetic and physical mapping of the *Stb6* locus. Initial genetic mapping positioned *Stb6* within an 8.1-cM interval flanked by the simple-sequence-repeat markers gwm369 and gpw2132 on the short arm of chromosome 3 A. Further fine-resolution genetic mapping delimited *Stb6* to the 0.56-cM interval between the SNP markers cfn80025 and cfn80030, derived from RLK genes. Markers ctg8311 and cfn80023, derived from the coding sequence of *TaWAKL4*, cosegregated with *Stb6*. Using these SNP markers as probes, we then defined *Stb6* to an ~155-kb physical interval comprising one BAC clone and one long genomic-DNA scaffold (indicated as overlapping unfilled rectangles) and containing five *WAKL* genes (yellow pentagons). **b**, Gene structure and protein-domain prediction for *TaWAKL4* (putative *Stb6*). Pentagons and solid black lines represent exons and introns, respectively. Positions of start and stop codons are indicated with thin vertical lines. Regions encoding predicted protein domains and motifs are shown in color: SP, signal peptide; GUB_WAK, wall-associated receptor-kinase galacturonan-binding domain; TM, transmembrane region; S/T kinase, serine/threonine kinase domain.

mechanism of resistance against *Z. tritici*, we isolated the *Stb6* gene from CS by using a map-based cloning approach.

Using published wheat genetic maps[27,28] and exploiting the synteny between wheat genomes and model grass genomes[29], we identified a physical region in the *Brachypodium distachyon* genome syntenic to the *Stb6* locus in wheat. Close to the center of this 769-kb region lies a cluster of 19 genes (Supplementary Fig. 2) annotated as RLKs, a class of genes that includes well-known regulators of plant innate immunity[30]. Homologous genes were identified in the wheat CS chromosome-arm 3AS assembly and used for developing new genetic markers (Supplementary Fig. 2). Five of these markers were mapped by using a large F_2 population developed from a cross between CS and the susceptible wheat variety Courtot (Ct) within an ~0.67-cM interval, including two markers that cosegregated with *Stb6* (Fig. 1). Using a BAC library and an available draft CS whole-genome assembly, we delimited the *Stb6* locus to either of two candidate genes: *TaWAKL3* and *TaWAKL4* (Fig. 1 and Supplementary Fig. 3).

Five complementary approaches were then used for functional validation of candidate genes. First, gene expression analysis at six different time points after mock or *Z. tritici* IPO323 inoculation of CS wheat showed that *TaWAKL3* was only minimally expressed under these conditions, whereas *TaWAKL4* showed a moderate level of expression and was upregulated approximately twofold during attempted infection (Supplementary Fig. 4). Second, exon resequencing identified no polymorphisms in *TaWAKL3* between resistant (CS) and susceptible (Ct) wheat, whereas the coding sequence of *TaWAKL4* in Ct contained a missense mutation causing a p.Ile447Asp amino acid change (Supplementary Fig. 5). Third, knockdown of expression of *TaWAKL4* but not *TaWAKL3* through virus-induced gene silencing (VIGS)[31,32] compromised *Stb6*-mediated resistance in CS and Cad wheat (Fig. 2, Supplementary Figs. 6 and 7, and Supplementary Tables 1 and 2). Fourth, we took advantage of the Targeting Induced Local Lesions In Genome (TILLING)[33] population of Cad and the corresponding database cataloging mutations identified in 1,200 mutant families through exome resequencing[34,35]. Mutant families with predicted nonsense and missense mutations in *TaWAKL3* and *TaWAKL4* were tested for resistance to *Z. tritici* IPO323, and mutations were verified by targeted sequencing (Supplementary Tables 3 and 4). All ten families with mutations in *TaWAKL3* remained resistant to this fungal isolate (Fig. 3a,b), whereas susceptible individuals from eight families homozygous for critical mutations in *TaWAKL4* were identified (Fig. 3c,d). Finally, we stably transformed the susceptible wheat varieties Ct and Bobwhite with *TaWAKL4* or with the predicted full-length coding sequence of this gene driven by its native promoter or the maize polyubiquitin promoter (*Ubi1*) (Fig. 4a). T_0 plants generated for each construct were self-fertilized. Analysis of the T_1 generation identified families segregating for resistance to *Z. tritici* IPO323, and all resistant individuals tested positive for the corresponding transgene (Fig. 4b–d). These results verified the *Stb6* gene identity and the accuracy of the inferred gene structure. Importantly, Bobwhite transgenic plants expressing *Stb6* from the native promoter or the maize *Ubi1* promoter showed specific gene-for-gene resistance to *Z. tritici* isolate IPO323 (Supplementary Fig. 8).

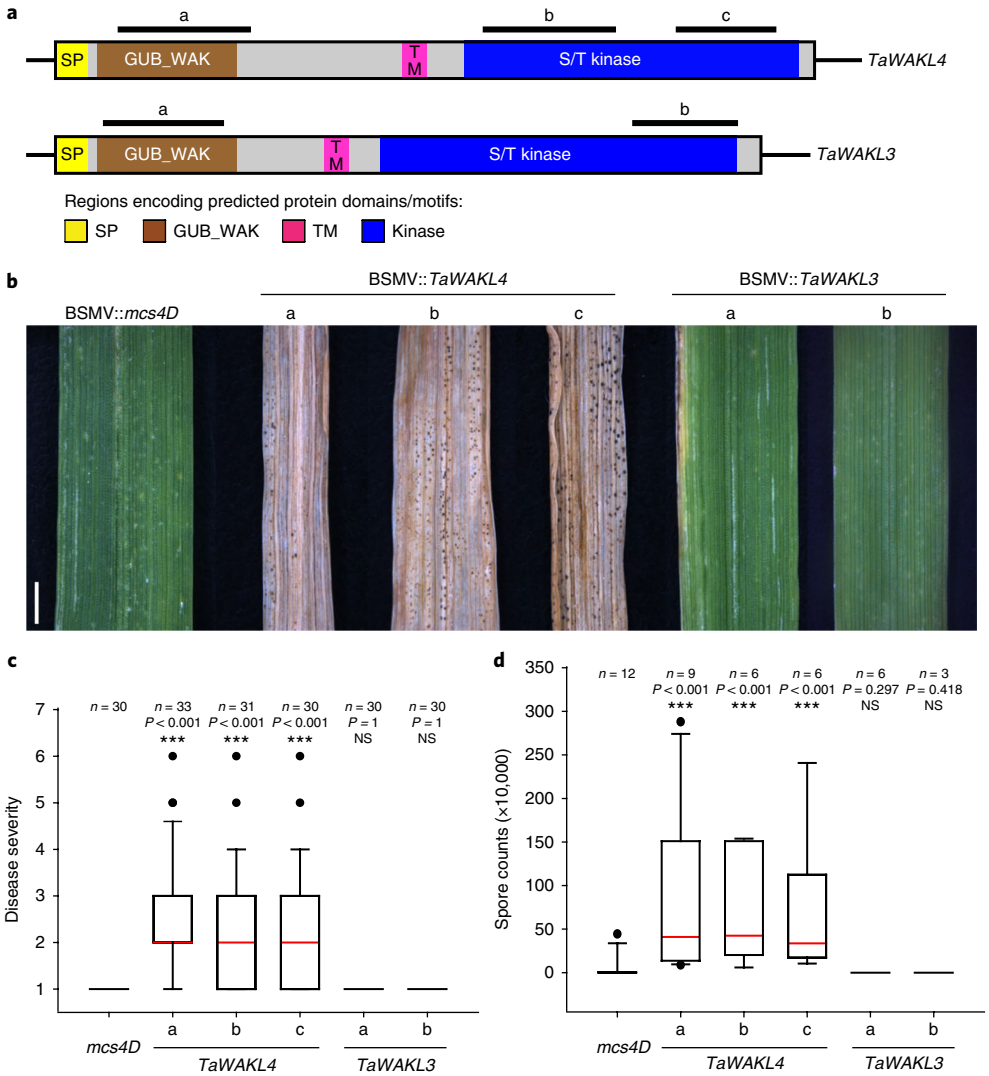

Fig. 2 | Functional analysis of candidate genes through barley stripe mosaic virus (BSMV)-mediated VIGS. a, Schematic representation of inferred *TaWAKL4* and *TaWAKL3* mRNAs. Coding sequences are shown as rectangles. Black bars above mRNA models indicate regions targeted in specific VIGS constructs. **b**–**d**, Assessment of *Z. tritici* IPO323-induced disease in CS wheat plants pretreated with either different gene-specific VIGS constructs or the negative control BSMV::*mcs4D* at 21 d after fungal inoculation. **b**, Symptoms on leaves of representative plants (from a total of 42, 21, 29, 20, 29, and 25 plants treated with BSMV:mcs4d, BSMV:TaWAKL4a, BSMV:TaWAKL4b, BSMV:TaWAKL4c, BSMV:TaWAKL3a, and BSMV:TaWAKL3b, respectively). Scale bar, 25 mm. **c**, A highly significant (***) effect of silencing specific candidate genes on disease severity, assessed on a scale from 1 (no symptoms) to 6 (80–100% leaf area covered by pycnidia-bearing necrotic lesions). The number of leaves (*n*) and the *P* values for the approximate two-tailed *t* test for comparison of silenced plants to those treated with a negative control are shown. **d**, A highly significant (***) effect of silencing specific candidate genes on fungal sporulation in the infected leaves. The number of replicate samples (*n*) of spores washed off the infected leaves and the *P* values for the post hoc two-tailed *t* test for comparison of VIGS-treated and negative-control-treated plants are shown. In each box-and-whisker plot, the center lines (red) indicate the medians; the bottom and top edges of the boxes indicate the twenty-fifth and seventy-fifth percentiles; whiskers mark the range of the data from the tenth to ninetieth percentiles, and black dots indicate data points that lie outside of this interval. NS, not significant.

TaWAKL4/*Stb6* contains four exons and three introns, and the first intron and the second exon are particularly long (2.8 kb) and short (36 bp), respectively (Fig. 1). A transcript originating from this gene mapped with RACE PCR (data not shown) showed leaf-specific developmentally regulated expression peaking in flag leaves after anthesis (Supplementary Fig. 9). The predicted Stb6 resistance protein consists of 647 amino acids and contains an extracellular galacturonan-binding domain (GUB_WAK), an intracellular non-arginine-aspartate[36] protein kinase, and a complex-topology concanavalin A–like domain (Supplementary Fig. 10). In contrast, all other known WAKs implicated in pathogen defense contain additional extracellular domains located downstream of GUB_WAK, such as wall-associated receptor-kinase C-terminal or EGF-like calcium-binding domains (Supplementary Fig. 11).

Exon resequencing identified a notable sequence conservation of *Stb6* in the hexaploid bread wheat *Triticum aestivum*. Only eight haplotypes were identified among 98 accessions (Supplementary Tables 5 and 6), and a single resistance haplotype predominated, including in 15 of 25 of the most highly resistant and in 10 of 19 of the most commonly grown recent and current UK varieties (Supplementary Tables 7 and 8). This result indicates that defense pathways activated by *Stb6* may have no or minimal associated fitness cost. Remarkably, *Stb6* haplotypes were also identified in several A-genome-containing domesticated and wild tetraploid and diploid

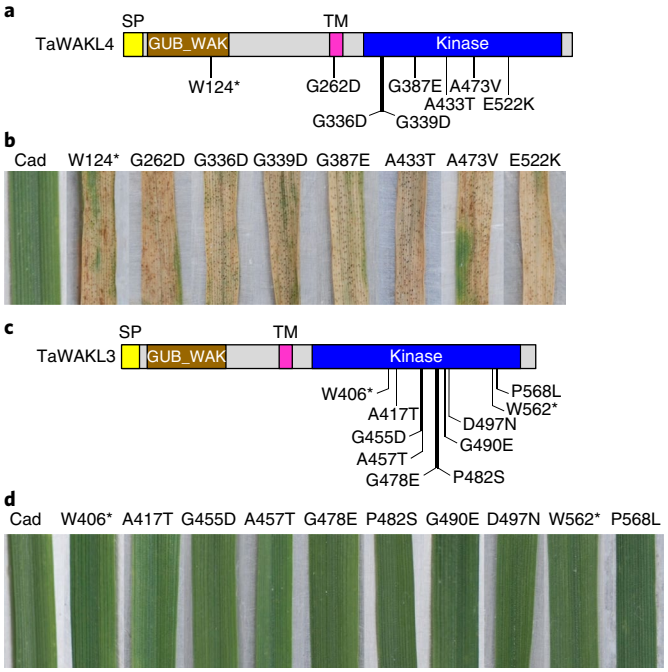

Fig. 3 | Mutational analysis of *TaWAKL4* and *TaWAKL3*. An ethyl methanesulfonate-mutagenized (TILLING) population of *Stb6*-containing Cad wheat was used. Mutant lines carrying single nonsense or missense mutations in the coding *TaWAKL4* and *TaWAKL3* gene sequences were identified by reference to exon capture data[31] and tested along with the wild-type Cad parent for resistance to *Z. tritici* IPO323 in a glass-house-based attached-seedling-leaf bioassay. **a**,**c**, Schematic representation of TaWAKL4 (**a**) and TaWAKL3 (**c**) proteins with predicted domains and motifs (colored rectangles). Thin vertical lines indicate positions of the corresponding amino acid changes. **b**,**d**, Disease resistance assays. Representative images (from a total of 4 or 5 individual plants) taken at 21 d after-fungal inoculation. Eight TILLING lines carrying mutations in *TaWAKL4* developed typical disease symptoms and therefore were susceptible to *Z. tritici* IPO323 (**b**), whereas the parental Cad wheat and 11 TILLING lines with mutations in *TaWAKL3* remained completely resistant (**d**).

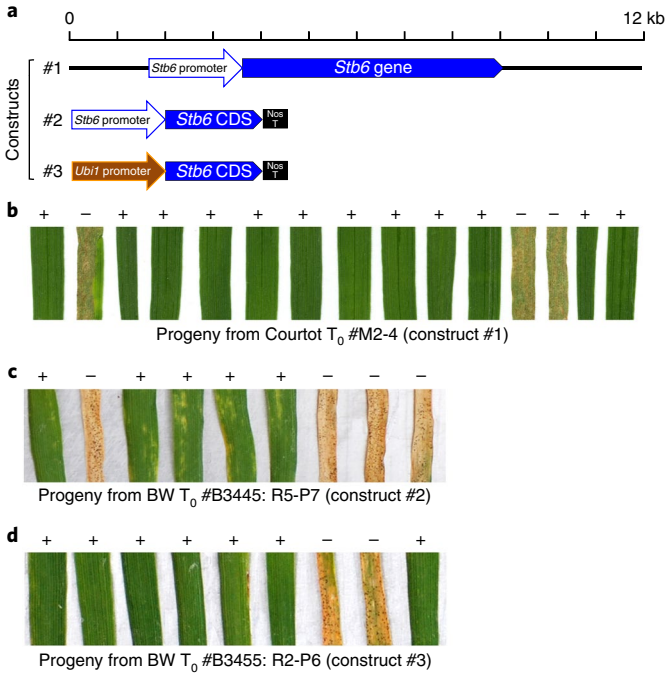

Fig. 4 | *TaWAKL4* confers resistance to *Z. tritici* IPO323 when stably transferred into a susceptible wheat background. a, Schematic diagram of one genomic and two gene coding sequence (CDS) constructs used for transformation of susceptible Ct and Bobwhite (BW) wheat. The putative *Stb6* gene promoter comprised 2 kb of sequence upstream of the predicted start (ATG) codon. *Ubi1*, promoter and the first intron of the maize polyubiquitin gene; Nos T, termination sequence of the *Agrobacterium tumefaciens* nopaline synthase gene. **b**–**d**, Examples of screening of T_1 progeny from the indicated transgene-containing T_0 plants for resistance to *Z. tritici* IPO323. Representative images taken at 21–23 d after fungal inoculation are shown (in **b**, all 15 individuals of this transgenic line are shown; in **c** and **d**, 12 selected plants for each line are shown). For each *Stb6* construct, the presence (+) or absence (–) of the corresponding transgene corresponded well to the resistance/susceptibility phenotype observed for the individual tested T_1 plants.

wheat species (Fig. 5a and Supplementary Tables 5 and 6). The prevalence of a resistance haplotype in *Triticum dicoccum*, one of the earliest cultivated forms of wheat, suggests that *Stb6* might have been introduced into agriculture during early wheat domestication, thus potentially explaining its widespread occurrence in bread wheat.

Ct wheat contains an expressed susceptible haplotype of *Stb6*, which differs from the resistance haplotype by a single nonsynonymous SNP (Supplementary Fig. 5) causing a change from a conserved isoleucine residue to an aspartate at position 447 in the catalytic site of the protein kinase domain (Fig. 5b). All mutations associated with susceptibility to *Z. tritici* IPO323 identified in the Cad TILLING population (except for one nonsense mutation and one potential splice-site mutation) also led to changes at conserved amino acid residues in the kinase domain of Stb6 (Fig. 3a). Biochemical assays suggested that disease susceptibility associated with these mutations probably results from a loss of kinase catalytic activity and thus abrogated immunological signaling (Fig. 5c).

Z. tritici belongs to a group filamentous ascomycete fungal pathogens that do not penetrate host cells or form specialized feeding structures (haustoria) but instead colonize and extract nutrients from the plant extracellular space[17]. Many of these pathogens are also of utmost agronomic importance[37,38], for example, *Mycosphaerella fijiensis*, *Leptosphaeria maculans*, and *Rhynchosporium commune*, which cause major diseases affecting banana, canola, and barley, respectively. Host resistance to these pathogens is typically governed by the PRR-like receptor-like proteins or RLKs (rather than cytoplasmic NLRs), which recognize fungal secreted effectors in the plant apoplastic space and transduce defense signals through interaction with the accessory RLKs[37,38], thus affirming the absence of a strict separation between PRRs and resistance proteins[14,15]. Our study provides additional evidence supporting this concept, confirming that WAK receptor proteins are new players in plant innate immunity against extracellular pathogens and adding another twist by demonstrating that PRR-like proteins of this class, such as wheat Stb6, can control qualitative pathogen resistance in a gene-for-gene manner through recognition of apoplastic Avr effectors. This functionality contrasts with that of *Arabidopsis thaliana RFO1* (WAK-like 22)[39] and the two recently cloned maize WAKs implicated in broad-spectrum, but partial, quantitative resistance[40,41]. WAKs monitor and respond to changes in the cell wall during plant development or pathogen attack through binding to cross-linked cell-wall pectin or oligogalacturonides, respectively[42–45]. There is evidence that some WAKs may also bind proteinaceous ligands[46]. A recent study[47] has identified wheat susceptibility/sensitivity protein Snn1 as a WAK that interacts with the secreted protein Tox1 from the necrotrophic fungus *Parastagonospora nodorum*, thereby inducing extensive tissue necrosis and consequently providing nutrients for pathogen growth and reproduction in a process termed necrotrophic-effector-triggered susceptibility.

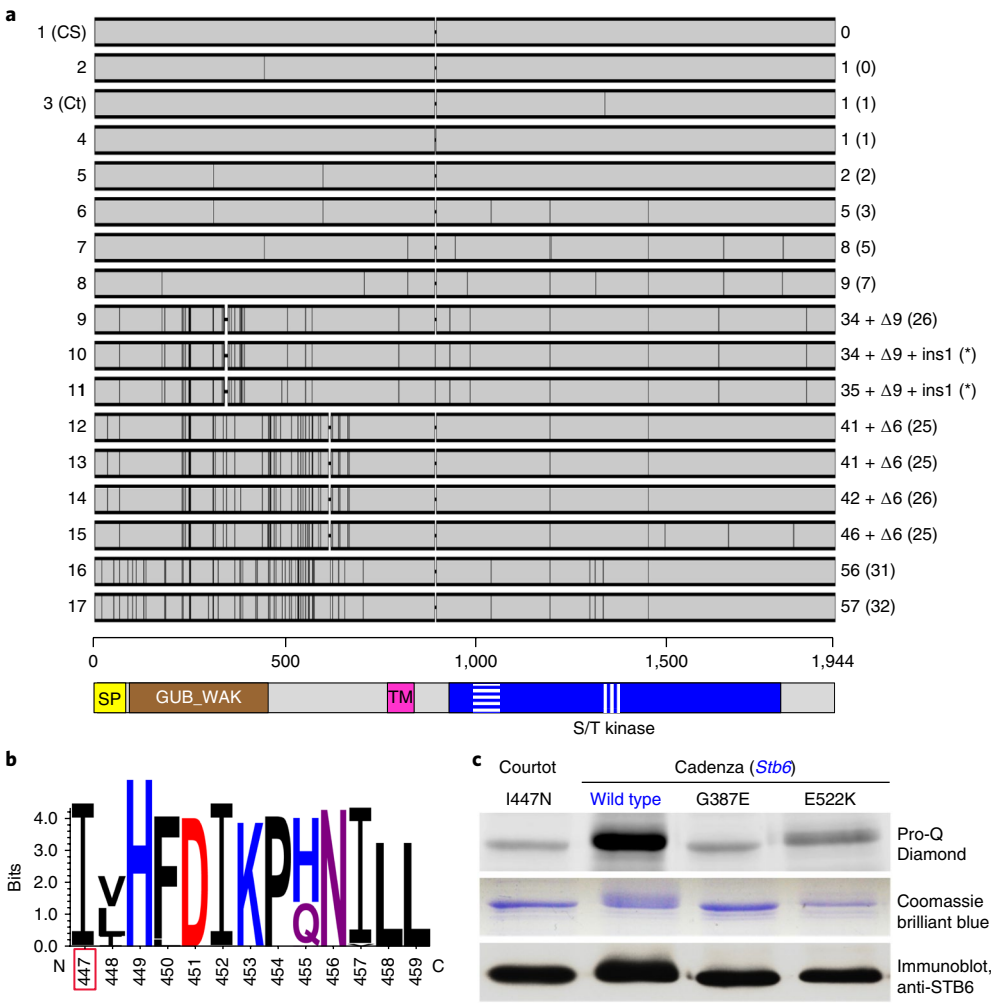

Fig. 5 | Sequence and biochemical characterization of natural and induced *Stb6* haplotypes. a, Alignment of 17 haplotypes (gray rectangles) identified in wheat germplasm through resequencing *Stb6* exons. Haplotypes 1 and 3 were obtained from the resistant wheat CS and the susceptible wheat Ct. Vertical black lines and empty areas inside the rectangles indicate positions of SNPs and short insertions and deletions (indels), respectively, as compared with haplotype 1. Numbers on the right indicate SNPs and indels identified for each haplotype, and numbers in parentheses indicate numbers of resulting polymorphic amino acid residues in the predicted encoded proteins, as compared with haplotype 1. Asterisks indicate haplotypes containing a 1-nt insertion at position 904 that results in a frame shift and a premature stop codon at nucleotide positions 1033–1035. **b**, Graphical representation, produced in WebLogo, of a multiple sequence alignment for predicted protein kinase active sites from Stb6 and 218 protein kinase sequences from diverse plant species. The highly conserved isoleucine residue at position 447 (I447; boxed, and outlined in red) in Stb6 is replaced by aspartate (N) in the protein encoded by haplotype 3 found in susceptible wheats such as Ct. **c**, Biochemical analysis of four variants of the Stb6 protein kinase domain expressed in *Escherichia coli* from resistant and natural or induced susceptible mutants. Pro-Q Diamond phosphoprotein stain shows substantial autophosphorylation of Stb6 from wild-type Cad and near-complete absence of autophosphorylation of Stb6 variants from the three susceptible wheat genotypes.

We hypothesized that Stb6 might bind to its matching recently cloned[7,8] effector AvrStb6 from the avirulent *Z. tritici* isolate IPO323. Because this effector has been reported to show a high level of polymorphism but no presence/absence variation in field populations of *Z. tritici*, we therefore also anticipated that its alternative alleles[7,8] from virulent fungal isolates cannot be recognized by Stb6. We used yeast two-hybrid (Y2H) assays to test this hypothesis, but no direct interaction between Stb6 and any of the three different AvrStb6 sequence variants was detected (Supplementary Fig. 12). Y2H may be suboptimal for assaying interactions between apoplastic proteins, and further tests, including in planta assays, will be required to confirm this initial result. If, however, the lack of direct Stb6–AvrStb6 interaction is genuine, at least two alternative scenarios may be possible: (i) initiation of the immune response may involve additional interactions possibly involving pectin, oligogalacturonides or other plant cell-wall-derived signals, and/or (ii) AvrStb6 may interact with another protein that is 'guarded' (monitored) by Stb6.

Cloning of *Stb6*, together with the recent discovery that the matching *Z. tritici* effector is maintained in fungal populations because avirulent isolates can mate with virulent isolates even on resistant host plants, emphasizes the value of *Stb6* for controlling STB disease while also providing new fundamental insights into the molecular control of plant–pathogen interactions.

URLs. Wheat TILLING, http://www.wheat-tilling.com/; WebLogo, http://weblogo.berkeley.edu/; GrainGenes, http://wheat.pw.usda.gov/GG3/; The Wheat Portal, https://wheat-urgi.versailles.inra.fr/; Ensembl Fungi, http://fungi.ensembl.org/index.html/; INRA-CNRGV Plant Genomic Resources Center, https://cnrgv.toulouse.inra.fr/en/; Hisat2, https://ccb.jhu.edu/software/hisat2/index.shtml; Hierarchical Genome Assembly Process (HGAP), https://github.

com/PacificBiosciences/Bioinformatics-Training/wiki/HGAP/; Ensembl Plants, http://plants.ensembl.org/index.html/; HMMER, http://hmmer.org/; Picard Tools suite, https://broadinstitute.github.io/picard/.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41588-018-0051-x.

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References

1. Michelmore, R. W., Christopoulou, M. & Caldwell, K. S. Impacts of resistance gene genetics, function, and evolution on a durable future. *Annu. Rev. Phytopathol.* **51**, 291–319 (2013).
2. Krattinger, S. G. & Keller, B. Molecular genetics and evolution of disease resistance in cereals. *New Phytol.* **212**, 320–332 (2016).
3. Jones, J. D. & Dangl, J. L. The plant immune system. *Nature* **444**, 323–329 (2006).
4. Dodds, P. N. & Rathjen, J. P. Plant immunity: towards an integrated view of plant-pathogen interactions. *Nat. Rev. Genet.* **11**, 539–548 (2010).
5. Dickman, M. B. & Fluhr, R. Centrality of host cell death in plant-microbe interactions. *Annu. Rev. Phytopathol.* **51**, 543–570 (2013).
6. Brading, P. A., Verstappen, E. C. P., Kema, G. H. J. & Brown, J. K. M. A gene-for gene relationship between wheat and *Mycosphaerella graminicola*, the Septoria tritici blotch pathogen. *Phytopathology* **92**, 439–445 (2002).
7. Zhong, Z. et al. A small secreted protein in *Zymoseptoria tritici* is responsible for avirulence on wheat cultivars carrying the *Stb6* resistance gene. *New Phytol.* **214**, 619–631 (2017).
8. Kema, G.H.J. et al. Stress and sexual reproduction affect the dynamics of the wheat pathogen effector AvrStb6 and strobilurin resistance. *Nat. Genet.* https://doi.org/10.1038/s41588-018-0052-9 (2018).
9. Rudd, J. J., Keon, J. & Hammond-Kosack, K. E. The wheat mitogen-activated protein kinases TaMPK3 and TaMPK6 are differentially regulated at multiple levels during compatible disease interactions with *Mycosphaerella graminicola*. *Plant Physiol.* **147**, 802–815 (2008).
10. Fones, H. & Gurr, S. The impact of Septoria tritici Blotch disease on wheat: An EU perspective. *Fungal Genet. Biol.* **79**, 3–7 (2015).
11. Torriani, S. F. et al. *Zymoseptoria tritici*: a major threat to wheat production, integrated approaches to control. *Fungal Genet. Biol.* **79**, 8–12 (2015).
12. Kettles, G. J. & Kanyuka, K. Dissecting the molecular interactions between wheat and the fungal pathogen *Zymoseptoria tritici*. *Front. Plant Sci.* **7**, 508 (2016).
13. Zipfel, C. Plant pattern-recognition receptors. *Trends Immunol.* **35**, 345–351 (2014).
14. Thomma, B. P., Nürnberger, T. & Joosten, M. H. Of PAMPs and effectors: the blurred PTI-ETI dichotomy. *Plant Cell* **23**, 4–15 (2011).
15. Cook, D. E., Mesarich, C. H. & Thomma, B. P. H. J. Understanding plant immunity as a surveillance system to detect invasion. *Annu. Rev. Phytopathol.* **53**, 541–563 (2015).
16. Sánchez-Vallet, A., McDonald, M. C., Solomon, P. S. & McDonald, B. A. Is *Zymoseptoria tritici* a hemibiotroph? *Fungal Genet. Biol.* **79**, 29–32 (2015).
17. Kema, G. H. J., Yu, D. Z. & Rijkenberg, F. H. J. Histology of the pathogenesis of *Mycosphaerella graminicola* in wheat. *Phytopathology* **86**, 777–786 (1996).
18. Torriani, S. F. F., Brunner, P. C., McDonald, B. A. & Sierotzki, H. QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola*. *Pest Manag. Sci.* **65**, 155–162 (2009).
19. Cools, H. J. & Fraaije, B. A. Update on mechanisms of azole resistance in *Mycosphaerella graminicola* and implications for future control. *Pest Manag. Sci.* **69**, 150–155 (2013).
20. Estep, L. K. et al. Emergence and early evolution of fungicide resistance in North American populations of *Zymoseptoria tritici*. *Plant Pathol.* **64**, 961–971 (2015).
21. Brown, J. K., Chartrain, L., Lasserre-Zuber, P. & Saintenac, C. Genetics of resistance to *Zymoseptoria tritici* and applications to wheat breeding. *Fungal Genet. Biol.* **79**, 33–41 (2015).
22. Tabib Ghaffary, S. M. et al. New broad-spectrum resistance to septoria tritici blotch derived from synthetic hexaploid wheat. *Theor. Appl. Genet.* **124**, 125–142 (2012).
23. McDonald, B. A. & Mundt, C. C. How knowledge of pathogen population biology informs management of Septoria tritici blotch. *Phytopathology* **106**, 948–955 (2016).
24. Chartrain, L., Brading, P. A. & Brown, J. K. M. Presence of the *Stb6* gene for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in cultivars used in wheat-breeding programmes worldwide. *Plant Pathol.* **54**, 134–143 (2005).
25. Arraiano, L. S. & Brown, J. K. M. Identification of isolate-specific and partial resistance to Septoria tritici blotch in 238 European wheat cultivars and breeding lines. *Plant Pathol.* **55**, 726–738 (2006).
26. Arraiano, L. S. et al. Contributions of disease resistance and escape to the control of Septoria tritici blotch of wheat. *Plant Pathol.* **58**, 910–922 (2009).
27. Ghaffary, S. M. et al. Genetic analysis of resistance to septoria tritici blotch in the French winter wheat cultivars Balance and Apache. *Theor. Appl. Genet.* **123**, 741–754 (2011).
28. Saintenac, C., Jiang, D., Wang, S. & Akhunov, E. Sequence-based mapping of the polyploid wheat genome. *G3 (Bethesda)* **3**, 1105–1114 (2013).
29. Bolot, S. et al. The 'inner circle' of the cereal genomes. *Curr. Opin. Plant Biol.* **12**, 119–125 (2009).
30. Antolín-Llovera, M., Ried, M. K., Binder, A. & Parniske, M. Receptor kinase signaling pathways in plant-microbe interactions. *Annu. Rev. Phytopathol.* **50**, 451–473 (2012).
31. Lee, W. S., Hammond-Kosack, K. E. & Kanyuka, K. Barley stripe mosaic virus-mediated tools for investigating gene function in cereal plants and their pathogens: virus-induced gene silencing, host-mediated gene silencing, and virus-mediated overexpression of heterologous protein. *Plant Physiol.* **160**, 582–590 (2012).
32. Lee, W. S., Rudd, J. J. & Kanyuka, K. Virus induced gene silencing (VIGS) for functional analysis of wheat genes involved in *Zymoseptoria tritici* susceptibility and resistance. *Fungal Genet. Biol.* **79**, 84–88 (2015).
33. McCallum, C. M., Comai, L., Greene, E. A. & Henikoff, S. Targeting induced local lesions in genomes (TILLING) for plant functional genomics. *Plant Physiol.* **123**, 439–442 (2000).
34. King, R. et al. Mutation scanning in wheat by exon capture and next-generation sequencing. *PLoS One* **10**, e0137549 (2015).
35. Krasileva, K. V. et al. Uncovering hidden variation in polyploid wheat. *Proc. Natl. Acad. Sci. USA* **114**, E913–E921 (2017).
36. Dardick, C., Schwessinger, B. & Ronald, P. Non-arginine-aspartate (non-RD) kinases are associated with innate immune receptors that recognize conserved microbial signatures. *Curr. Opin. Plant Biol.* **15**, 358–366 (2012).
37. Stotz, H. U., Mitrousia, G. K., de Wit, P. J. G. M. & Fitt, B. D. L. Effector-triggered defence against apoplastic fungal pathogens. *Trends Plant Sci.* **19**, 491–500 (2014).
38. De Wit, P. J. G. M. Apoplastic fungal effectors in historic perspective; a personal view. *New Phytol.* **212**, 805–813 (2016).
39. Diener, A. C. & Ausubel, F. M. *RESISTANCE TO FUSARIUM OXYSPORUM 1*, a dominant *Arabidopsis* disease-resistance gene, is not race specific. *Genetics* **171**, 305–321 (2005).
40. Hurni, S. et al. The maize disease resistance gene *Htn1* against northern corn leaf blight encodes a wall-associated receptor-like kinase. *Proc. Natl. Acad. Sci. USA* **112**, 8780–8785 (2015).
41. Zuo, W. et al. A maize wall-associated kinase confers quantitative resistance to head smut. *Nat. Genet.* **47**, 151–157 (2015).
42. Brutus, A., Sicilia, F., Macone, A., Cervone, F. & De Lorenzo, G. A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. *Proc. Natl. Acad. Sci. USA* **107**, 9452–9457 (2010).
43. Ferrari, S. et al. Oligogalacturonides: plant damage-associated molecular patterns and regulators of growth and development. *Front. Plant Sci.* **4**, 49 (2013).
44. Kohorn, B. D. & Kohorn, S. L. The cell wall-associated kinases, WAKs, as pectin receptors. *Front. Plant Sci.* **3**, 88 (2012).
45. Kohorn, B. D. Cell wall-associated kinases and pectin perception. *J. Exp. Bot.* **67**, 489–494 (2016).
46. Park, A. R. et al. Interaction of the *Arabidopsis* receptor protein kinase Wak1 with a glycine-rich protein, AtGRP-3. *J. Biol. Chem.* **276**, 26688–26693 (2001).
47. Shi, G. et al. The hijacking of a receptor kinase-driven pathway by a wheat fungal pathogen leads to disease. *Sci. Adv.* **2**, e1600822 (2016).

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Author contributions

K.K., K.E.H.-K., C.S., and T.L. conceived the project. W.M. and H.B. screened the wheat BAC library. A.L.P., R.C.K., and C.U. provided the TILLING data. R.C.K. performed bioinformatics analyses and analyzed RNA-seq data. W.-S.L. performed VIGS. J.J.R. performed biochemical assays. S.J.P. performed statistical analysis. F.C., C.S., and K.K. carried out all other experiments and analyzed the data. K.K. and C.S. wrote the manuscript, and all authors revised the manuscript.

Competing interests

Rothamsted Research filed an International Patent Application (no. PCT/GB2016/053929 entitled 'Plant Fungal Resistance Gene') related to the content of this manuscript, on behalf of K.K., C.S., F.C., T.L., W.-S.L., and K.E.H.-K.

Additional information

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Methods

Plant materials and *Zymoseptoria tritici* resistance assays. Parents of published or available in-house wheat mapping populations were used in initial pathoassays to verify the data from early reports[24,25] suggesting that *Stb6* or an allelic gene is present in CS and Cad wheat but absent in Avalon (Av) and Ct wheat. A selection from these and/or the well-known highly susceptible wheat Riband[9] were used as controls in other pathoassays in the study. The wild-type CS along with the lines 3AS4-0.45, 3AS2-0.23, and 3AS1-0.13, carrying induced deletions[48] of chromosome arm 3AS were used for locating *Stb6* to the 0.45–1.00 deletion bin in the subtelomeric region on 3AS. Twenty-seven F_2 progeny of a Cs×Ct cross were used in pathoassays for reconfirming an earlier observation[6] that *Stb6* is a semidominant gene.

Ninety-six doubled-haploid lines derived from a CS×Ct cross[49] and 40 doubled-haploid lines derived from an Av×Cad cross[50,51] were used in pathoassays in conjunction with genotyping assays using publicly available simple-sequence-repeat markers (selected from the GrainGenes database) for production of a low-resolution genetic map around the *Stb6* locus. A biparental mapping population derived from a CS×Ct cross comprising 1,962 F_2 individuals was established for fine mapping *Stb6*.

All wheat phenotyping for resistance to the *Z. tritici* isolate IPO323, which carries AvrStb6 (refs [6–8]), were done with the attached-seedling-leaf bioassay[52].

Genetic mapping, physical map construction, sequencing, and annotation. New genetic markers used for construction of a high-resolution map at the *Stb6* locus in wheat were developed as described in the Supplementary Note. To expedite fine mapping, the F_2 CS×Ct population was first genotyped with gwm369 and cfa3010, which flank *Stb6*, and individuals with recombination events between these marker loci were then genotyped with all other markers (Supplementary Tables 9 and 10) described above as well as subjected to a fungal pathoassay. Forty critical recombinant F_2 plants were selfed, and their progeny (at least 20 F_3 plants per family) were tested in pathoassays.

To construct the physical map spanning the *Stb6* interval, we initially (i) identified and sequenced an ~100-kb BAC clone after screening the wheat CS Tae-B-CsE BAC library and (ii) identified an overlapping ~118-kb genomic contig through BLASTn analysis against the wheat whole-genome assembly TGACv1 (Supplementary Note). These two large continuous sequences combined contained genetic markers ctg8311 and cfn80023, which cosegregated with *Stb6*, as well as markers cfn80025 and cfn80030/cfn80040, which flanked *Stb6*. Subsequently, when the substantially improved IWGSC wheat CS whole genome assembly v1.0 became available, we confirmed the above data by identifying a continuous 400-kb genomic sequence containing *Stb6* and spanning the interval between markers cfn80025 and cfa3010. The gene models were annotated and manually curated as described in the Supplementary Note.

Virus-induced gene silencing (VIGS). VIGS for functional analysis of candidate wheat genes was carried out essentially as previously described[32]. Gene-silencing constructs were created by cloning fragments of wheat gene sequences into the BSMV RNAγ-derived binary vector pCa-γbLIC[53] in antisense orientation. Three nonoverlapping fragments of *TaWAKL4*, one from the GUB_WAK domain and two from the kinase domain, were cloned separately into pCa-γbLIC to generate three independent VIGS constructs designed to target this gene for silencing (BSMV::as*TaWAKL4a*, BSMV::as*TaWAKL4b*, and BSMV::*TaWAKL4c*). Two constructs designed to target the neighboring gene *TaWAKL3* (BSMV::*TaWAKL3a* and BSMV::*TaWAKL3b*) were also generated. The target gene fragments for cloning were generated by standard RT–PCR with primers described in Supplementary Table 9, and total RNA was extracted from CS leaf tissue as a template for reverse transcription. The BSMV::*mcs4D* control construct contained a 275-nt noncoding DNA sequence amplified from the multiple cloning site of the pBluescript II SK vector (Agilent Technologies).

Samples for qRT–PCR analysis to determine target-gene silencing success were harvested from the tips of the third leaves of wheat plants at 11–14 d post virus inoculation (dpi) and immediately before *Z. tritici* inoculation. A minimum of three independent samples per virus treatment, each sample harvested from an individual plant, were analyzed. Quantification of gene expression with primers described in Supplementary Table 9 was carried out with SYBR Green Jumpstart Ready Mix (Sigma Aldrich), with an annealing temperature of 60 °C, in an ABI 7500 Real-Time PCR system (Applied Biosystems).

Attached-wheat-leaf infection assays with the *Z. tritici* isolate IPO323 on virus-infected plants were carried out as described previously[32]. Disease was assessed at 21 dpi by scoring the area of *Z. tritici*–inoculated leaf tissue that was both necrotic and evenly covered by fungal asexual fruiting bodies (pycnidia). The disease severity was scored on a scale from 1 to 6 corresponding to 0, 1–20, 21–40, 41–60, 61–80, and 80–100 percent leaf coverage by fungal pycnidia. After visual assessment, pycnidiospores were washed from *Z. tritici*–inoculated leaf segments and counted as previously described[32]. Each sample comprised three 6-cm-long leaf segments, each from an individual wheat plant. Pycnidiospores from a minimum of three replicate samples from each virus treatment were counted in each experiment, and data were pooled from a minimum of three and two independent experiments with CS and Cad wheat, respectively.

Statistical analyses. To determine whether treatments of wheat plants with specific BSMV VIGS constructs resulted in decreased expression of target genes but not nontarget genes, we applied statistical analysis through analysis of variance (ANOVA) (*F* test) followed by comparison of means with post hoc two-tailed *t* test. For this analysis, the $\log_2$ (1/NRQ) qRT–PCR data were analyzed as previously described[54], where NRQ is the normalized relative quantity ($2^{-Ct\ target}/2^{-Ct\ reference}$), for the target genes *TaWAKL4* and *TaWAKL3* and the reference gene *CDC48*, which has previously been determined to be a suitable reference gene in BSMV-infected leaf tissue[55].

To determine the effect of silencing candidate *Stb6* genes in wheat through VIGS on the outcome of *Z. tritici* infection, GenStat 18 (https://www.vsni.co.uk/software/genstat/) was used as described previously[55]. A generalized linear model was fitted to the disease-severity data (scores from 1 to 6), by assuming a Poisson distribution and using a log-link function, to test (*F* test) for the overall significance of difference between genotypes. Comparison of mean disease scores of *TaWAKL4*- and *TaWAKL3*-silenced plants with those of plants treated with the negative control BSMV::*mcs4D* was made with approximate *t* tests. Separate modeling exercises were done for data derived from CS and Cad wheat backgrounds. ANOVA was applied to the fungal-spore-count data on the natural log scale with an adjustment of +1 to account for observations of zero counts. The transformation ensured an approximate normal distribution and homogeneous variance over the genotypes, on the basis of checked residuals from the analysis. After a significant ($P<0.05$) *F*-test result, means for spore counts from *TaWAKL4*- and *TaWAKL3*-silenced plants were compared with those of plants treated with the negative control BSMV::*mcs4D* by using post hoc two-tailed *t* tests based on the residual variance and degrees of freedom from the ANOVA.

Two-way ANOVA was applied to the CS wheat RNA-seq data (an infection time course) for the two genes, *TaWAKL4* and *TaWAKL3*, testing the main effects and interactions between the factor of treatment (mock inoculated and *Z. tritici* inoculated) and time (2, 5, 8, 11, 14, and 17 dpi). Comparison of means was done with post hoc two-tailed *t* tests.

One-way ANOVA was applied to natural-log (FPKM + 0.5) data for the *TaWAKL4* gene expression in the leaf tissue only (because no nonzero FPKM data were obtained from other tissues to contribute variation for the analysis). FPKM means for the three growth stages (Z10, Z23, and Z71) were compared with post hoc two-tailed *t* tests.

Analysis of EMS-derived mutants. An ethyl methanesulfonate (EMS)-mutagenized population of 1,200 M_5 mutant families of Cad wheat[34,35] containing *Stb6* was used in this study. An 84-Mb exome capture assay comprising overlapping probes covering 82,511 nonredundant wheat genes was used to capture (through Roche NimbleGen array technology) and sequence (through Illumina GA II 110-bp paired-end-read technology) the coding gene regions from all mutant families and to identify induced mutations, as detailed in ref. [35]. Potential mutations in *TaWAKL3* and *TaWAKL4* were identified with BLASTn analysis of these gene sequences against the database of mutations induced in Cad wheat (wheat TILLING database), which is part of a joint project[35] between the University of California Davis in the US, and Rothamsted Research, Earlham Institute, and the John Innes Centre in the UK. All 28 and 11 randomly selected M_5 families (five or six individuals per family) with predicted mutations in the coding *TaWAKL3* and *TaWAKL4* gene sequence, respectively, identified through in silico analysis (Supplementary Tables 3 and 4), were tested for fungal resistance in a glass-house-based bioassay[52]. The presence of predicted mutations and mutation zygosity in each of the M_5 individuals identified as susceptible to *Z. tritici* IPO323 was verified through exon resequencing in the target genes by using primers described in Supplementary Table 9. In addition, all these individual susceptible M_5 plants were self-fertilized, and their progeny (at least 24 M_6 plants per family) were tested in pathoassays to confirm the susceptibility phenotype.

Wheat transformation and analysis of transgenic plants. A genomic sequence of approximately 12 kb containing the full-length *TaWAKL4*/*Stb6* gene (construct 1) was PCR amplified from the BAC clone Tae-B-CsE-673A7 with Phusion High-Fidelity PCR Master Mix (Thermo Fisher Scientific) and primers 8311F12/8311R12 (Supplementary Table 9) and cloned into the pCR8/GW/TOPO vector (Thermo Fisher Scientific). The integrity of the cloned genomic sequence was verified by Sanger sequencing of PCR fragments produced with a set of primer pairs distributed along the *Stb6* gene sequence (Supplementary Table 9). A deletion toward the 3′ end of the sequence was identified; however, because it was located downstream of the *Stb6* transcriptional termination site inferred from the 3′-RACE analysis, the cloned *Stb6* gene sequence was full length and therefore suitable for genetic complementation. The pCR8/GW/TOPO vector carrying *Stb6* was double digested with *Eco*RV and *Psp*OMI, and the cloned wheat genomic DNA fragment was purified after agarose gel electrophoresis and dephosphorylated as previously described[56]. This fragment was then mixed with the *bar* dephosphorylated cassette at a 2:1 ratio and used for transformation of immature embryos of Ct wheat by particle bombardment[56] with a PDS 1000 He device (Bio-Rad). Regeneration of plants and *bar* selection were performed essentially as previously reported[56]. Detection of the *Stb6* gene in T_0 plants was performed by PCR amplification using plant genomic DNA as the template and primers pCR8/GW_Stb6F1 and

Stb6_pCR8GW_R1 (Supplementary Table 9). Six independent transgenic lines were identified. T_0 plants were allowed to self-pollinate, and the resulting progeny (at least 12 T_1 individuals originating from each T_0 parent plant) were assessed for resistance to *Z. tritici* IPO323 at the seedling stage.

The inferred full-length *Stb6* gene CDS and a 2-kb putative *Stb6* promoter from wheat CS, flanked by *Aat*II and *Not*I or *Eco*RV and *Aat*II restriction sites, respectively, were synthesized commercially (Life Technologies). The *Stb6* promoter sequence digested with *Eco*RV and *Aat*II was combined with the *Stb6* CDS digested with *Aat*II and *Not*I, and then cloned upstream of the *Nos* terminator into the pRRes14_RR.001_65 vector codigested with SmaI and *Not*I to create the plasmid p65:R-promo-CDS (construct 2). After digestion with *Aat*II and *Not*I, the *Stb6* CDS was cloned between the maize *Ubi1* promoter and the *Nos* terminator into the pRRes14_RR.1m201_125 vector codigested with *Aat*II and *Not*I to create the plasmid p125:CDS-R (construct 3). Each of these plasmids was mixed with the plasmid pAHC20 containing the selectable *bar* gene for resistance to herbicide[57] and transformed into immature embryos of Bobwhite wheat after a particle-bombardment procedure, essentially as previously described[58]. Regenerated plantlets in soil were analyzed by PCR to identify transformants. Genomic DNA was extracted from young leaf material with a Wizard Genomic DNA Purification kit (Promega). PCR analysis was carried out for the gene of interest and the selectable marker gene (*bar*) as follows. Constructs 2 and 3 were both analyzed with the primer pair R-gene-fwd and Nos5′rev. Additionally, construct 3 transformants were analyzed with primers UbiPro4 and R-gene-rev. The *bar* gene was detected with primers bar1 and bar2 (Supplementary Table 9). Transgene and *bar* gene copy number analyses were carried out in T_0 and T_1 generations by iDNA Genetics (Norwich, UK). Identified T_0 plants were selfed, and the resulting progeny, at least ten T_1 individuals originating from each T_0 parent plant, were assessed for resistance to *Z. tritici* IPO323 at the seedling stage. Selected T_1 individuals, mostly those predicted to be homozygous for the corresponding transgene, were selfed. The resulting progeny, at least ten T_2 individuals originating from each T_1 parent plant, were tested for resistance to *Z. tritici* isolates IPO323 (avirulent on *Stb6* wheat genotypes), and IPO88004 and RRes116 (both virulent on *Stb6*-containing wheat) with the attached-seedling-leaf bioassay[52].

Haplotype analysis. We assembled a collection of 98 bread wheat (*T. aestivum*) accessions comprising varieties previously reported as potential carriers of *Stb6*; the well-known susceptible wheat varieties Obelisk, Riband and Longbow[6,24,59,60]; recent and current widely cultivated UK and French varieties with good field resistance to STB, obtained from different seed companies; and 48 highly genetically diverse genotypes selected from a worldwide bread-wheat core collection[61] with MSTRAT software[62] (Supplementary Tables 5–8). This collection was used for resequencing *Stb6* exons with the primers listed in Supplementary Table 9. Similarly, six diploid (one *Triticum monococcum*, two *Triticum boeticum*, and three *Triticum urartu*) and 31 tetraploid (five *Triticum dicoccoides*, five *T. dicoccum*, two *Triticum polonicum*, two *Triticum turgidum*, and 12 *Triticum durum*) wheat accessions were used for resequencing *Stb6* exons to evaluate the evolutionary origin of *Stb6*. Comparison of the different identified haplotypes was performed with Molecular Evolutionary Genetics Analysis (MEGA) v5.10 software[63].

Kinase assay. Coding *Stb6* sequence corresponding to the cytoplasmic S/T kinase domain containing a region of the encoded WAK protein was PCR amplified with primers Kin1186-attB1-F1 and Kin1186-attB1-R1 (Supplementary Table 9) with Phusion High-Fidelity DNA polymerase (Finnzymes) and either the plasmid p125:CDS-R containing the full-length *Stb6* CDS from CS wheat or the first-strand cDNA derived from total RNA extracted from *Z. tritici* IPO323–infected Av wheat (containing the same susceptibility *Stb6* allele as that in Ct) as a template. Two additional PCR fragments, each containing one SNP (preceding the amino acid change p.Gly387Glu or p.Glu522Lys as in Cad TILLING mutants #1495 and #0449, respectively) were generated by overlap-extension PCR[64] with primers Kin1186-attB1-F1 and Kin1186-attB1-R1 in combination with primers G387E-F1 and G387E-R1, or E522K-F1 and E522K-R1, containing the corresponding point mutation (Supplementary Table 9). These PCR fragments were recombined into the pDONR221 vector (Thermo Fisher Scientific) with Gateway BP Clonase II enzyme mix (Thermo Fisher Scientific), and the resulting entry clones were verified through Sanger sequencing at Eurofins Genomics (Ebersberg, Germany). Entry clones were then recombined with Gateway LR Clonase II enzyme mix (Thermo Fisher Scientific) into the pDEST-HisMBP vector[65] for expression of recombinant proteins with an N-terminal hexahistidine–maltose-binding protein dual tag and transformed into the *E. coli* strain Rosetta (DE3) (Novagen).

For protein expression, PCR-verified bacterial colonies were first inoculated into 5 ml LB and grown at 37 °C overnight (16 h) with shaking at 220 r.p.m. One milliliter of cell suspension was then inoculated into 100 ml of fresh LB and grown for approximately 2–3 h at 18 °C with shaking at 220 r.p.m. until the OD_{600} reached 0.5. At that point, IPTG was added to a final concentration of 0.5 mM. Protein expression then continued for a further 20 h at 18 °C with shaking at 220 r.p.m. Cells were collected by centrifugation and lysed with CelLytic B Cell Lysis Reagent (Sigma Aldrich) according to the manufacturer's protocol (4 ml per bacterial pellet). The final clarified supernatant containing the soluble proteins was then incubated with 500 µL of prewashed HIS-Select HF Nickel Affinity Gel (Sigma Aldrich), and affinity binding, washing, and elution were performed according to the supplier's protocol.

To directly determine the phosphorylation levels of each eluted protein, we followed a previously described protocol[66] for recombinant expression of plant receptor protein kinases. Briefly, equal protein amounts were separated on SDS–PAGE gels (Laemmli). One gel was subjected to the ProQ Diamond (Thermo Fischer Scientific) phosphoprotein staining protocol[66] and imaged in an Odyssey Fc Imaging System (LI-COR Biosciences) with image capture with Image-Studio V5.2. Those gels were subsequently counterstained with Coomassie brilliant blue to verify equal protein loading. A second series of gels were equally loaded and blotted onto nitrocellulose membranes, then subjected to western blot analysis with an anti–Stb6 kinase domain peptide (sequence DVQSGSSTRSEETSL) antiserum produced at Eurogentec (Seraing, Belgium) with standard protocols.

Yeast two-hybrid (Y2H) protein–protein interaction assay. Nucleotide sequences corresponding to the predicted extracellular and intracellular regions of Stb6 (amino acids 25–257 and 281–647, respectively); mature (lacking signal peptide) AvrStb6 from isolates IPO323 (avirulent), IPO88004 (virulent), and RRes16 (virulent); and mature (lacking signal peptide) *Z. tritici* effector Zt10 (Ensembl Fungi database accession Mycgr3P111505) not known to be recognized by Stb6 were PCR amplified from the plasmid p125:CDS-R containing the full-length *Stb6*; from the first-strand cDNAs derived from total RNA extracted from the corresponding 6-d-old fungal cultures grown on solid YPD agar medium; and from a cDNA clone[67], respectively. PCR amplifications were performed with Phusion High-Fidelity DNA Polymerase (Finnzymes). The obtained attB-flanked PCR products were first cloned into the Gateway-compatible vector pDONR221 with BP clonase II enzyme mix and were then recombined into the ProQuest Two-Hybrid System yeast expression vectors pDEST32 and pDEST22 with LR clonase II enzyme mix, according to the manufacturer's (Thermo Fisher Scientific) instructions. All generated Y2H prey and bait constructs were verified by sequencing. Primers for PCR and sequencing are detailed in Supplementary Table 9.

A yeast (*Saccharomyces cerevisiae*) strain MaV203 was then cotransformed with specific Stb6 bait constructs and the corresponding fungal effector prey constructs (including a negative-control effector Zt10), and vice versa. The same yeast strain cotransformed with the *A. thaliana* GAI (bait) and ARR1 (prey) protein constructs was used as a positive control for protein–protein interaction[68]. Four representative yeast transformants selected on SC/–Leu/–Trp agar plates from each transformation were picked for assessing induction of *HIS3* and *URA3* genes reporting positive protein–protein interactions. Histidine and uracil auxotrophy was tested by spotting yeast cells diluted in sterile saline onto SC/–Leu/–Trp/–His agar plates containing 0, 10, 25, 50, or 100 mM *HIS3* inhibitor 3-amino-1,2,4-triazole (3AT) and onto SC/–Leu/–Trp/–Ura agar plates, respectively.

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Data availability. Sequence data that support the findings of this study have been deposited in NCBI GenBank under accession numbers KY485188, KY485190, KY485191, KY485192, KY485193, KY485194, KY485195, KY485196, KY485197, KY485198, KY485199, KY485200, KY485201, KY485202, KY485203, and KY485204, and in the European Nucleotide Archive under accession numbers LT727683 and LT727684.

References

48. Endo, T. R. & Gill, B. S. The deletion stocks of common wheat. *J. Hered.* **87**, 295–307 (1996).
49. Sourdille, P. et al. An update of the Courtot x Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. *Theor. Appl. Genet.* **106**, 530–538 (2003).
50. Allen, A. M. et al. Transcript-specific, single-nucleotide polymorphism discovery and linkage analysis in hexaploid bread wheat (*Triticum aestivum* L.). *Plant Biotechnol. J.* **9**, 1086–1099 (2011).
51. Wang, S. et al. Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. *Plant Biotechnol. J.* **12**, 787–796 (2014).
52. Keon, J. et al. Transcriptional adaptation of *Mycosphaerella graminicola* to programmed cell death (PCD) of its susceptible wheat host. *Mol. Plant Microbe Interact.* **20**, 178–193 (2007).
53. Yuan, C. et al. A high throughput *barley stripe mosaic virus* vector for virus induced gene silencing in monocots and dicots. *PLoS One* **6**, e26468 (2011).
54. Rieu, I. & Powers, S. J. Real-time quantitative RT-PCR: design, calculations, and statistics. *Plant Cell* **21**, 1031–1033 (2009).
55. Lee, W.-S., Rudd, J. J., Hammond-Kosack, K. E. & Kanyuka, K. *Mycosphaerella graminicola* LysM effector-mediated stealth pathogenesis subverts recognition through both CERK1 and CEBiP homologues in wheat. *Mol. Plant Microbe Interact.* **27**, 236–243 (2014).
56. Tassy, C., Partier, A., Beckert, M., Feuillet, C. & Barret, P. Biolistic transformation of wheat: increased production of plants with simple insertions and heritable transgene expression. *Plant Cell Tissue Organ Cult.* **119**, 171–181 (2014).

57. Christensen, A. H. & Quail, P. H. Ubiquitin promoter-based vectors for high-level expression of selectable and/or screenable marker genes in monocotyledonous plants. *Transgenic Res.* **5**, 213–218 (1996).
58. Sparks, C. A. & Jones, H. D. Genetic transformation of wheat via particle bombardment. *Methods Mol. Biol.* **1099**, 201–218 (2014).
59. Eriksen, L., Borum, F. & Jahoor, A. Inheritance and localisation of resistance to *Mycosphaerella graminicola* causing septoria tritici blotch and plant height in the wheat (*Triticum aestivum* L.) genome with DNA markers. *Theor. Appl. Genet.* **107**, 515–527 (2003).
60. Chartrain, L., Brading, P. A., Widdowson, J. P. & Brown, J. K. Partial resistance to Septoria tritici blotch (*Mycosphaerella graminicola*) in wheat cultivars Arina and Riband. *Phytopathology* **94**, 497–504 (2004).
61. Balfourier, F. et al. A worldwide bread wheat core collection arrayed in a 384-well plate. *Theor. Appl. Genet.* **114**, 1265–1275 (2007).
62. Gouesnard, B. et al. MSTRAT: an algorithm for building germ plasm core collections by maximizing allelic or phenotypic richness. *J. Hered.* **92**, 93–94 (2001).
63. Tamura, K. et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739 (2011).
64. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K. & Pease, L. R. Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene* **77**, 51–59 (1989).
65. Nallamsetty, S., Austin, B. P., Penrose, K. J. & Waugh, D. S. Gateway vectors for the production of combinatorially-tagged His6-MBP fusion proteins in the cytoplasm and periplasm of *Escherichia coli*. *Protein Sci.* **14**, 2964–2971 (2005).
66. Taylor, I., Seitz, K., Bennewitz, S. & Walker, J. C. A simple in vitro method to measure autophosphorylation of protein kinases. *Plant Methods* **9**, 22 (2013).
67. Kettles, G. J., Bayon, C., Canning, G., Rudd, J. J. & Kanyuka, K. Apoplastic recognition of multiple candidate effectors from the wheat pathogen *Zymoseptoria tritici* in the nonhost plant *Nicotiana benthamiana*. *New Phytol.* **213**, 338–350 (2017).
68. Marín-de la Rosa, N. et al. Genome wide binding site analysis reveals transcriptional coactivation of cytokinin-responsive genes by DELLA proteins. *PLoS Genet.* **11**, e1005337 (2015).

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Experimental design

1. Sample size

   Describe how sample size was determined.

   More than 5 samples, and generally more than 10 samples, were used in each experiment. Each experiment was replicated at least twice. Please see the figure legends and online methods. Minimal (or in some cases exact) sample sizes used are indicated in specific sections of online methods.

2. Data exclusions

   Describe any data exclusions.

   No data were excluded from the analyses

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   For each experiment all attempts at replication were successful

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   Replicated samples were allocated in groups on a random basis

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly.
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☒	☐	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *p* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Assembly of the NGS sequence reads was performed using the HGAP workflow (https://github.com/PacificBiosciences/Bioinformatics-Training/wiki/HGAP) of the SMRT® Analysis v2.2.0 software (Pacific Biosciences). RNA-seq data was mapped to the wheat genomic sequence using Hisat2 v2.0.4 (https://ccb.jhu.edu/software/hisat2/index.shtml). The BAM file was imported into Geneious v8.1.5 (Biomatters Ltd.) and the gene models curated by producing gene coding sequence (CDS) annotations that matched the mapped RNA-seq data. To identify and construct pseudogene annotations, the curated exons and TGACv1 WGA WAK-like gene exon annotations extracted from BioMart on Ensembl Plants (http://plants.ensembl.org/index.html) were aligned to the reference using Lastz v7.0 in Geneious. The genomic sequence was then translated on all 6 frames and the resulting amino acid sequences subjected to a scan for Pfam domains using HMMER v3.1 (http://hmmer.org/) to assist in curation.
The NGS (Illumina) paired-end reads from RNA-seq experiments were mapped to wheat genomic DNA contigs using Tophat2 v2.0.13 with a mate inner distance set to 300 (-r), a mate standard deviation to 300, no mismatches are allowed (-m 0 -N 0) and qualities are set to --solexa1.3-quals. Mapping results were processed using the Picard Tools suite v1.124 (https://broadinstitute.github.io/picard/), to accept only reads with a mapping quality above 30. The duplicates were removed with MarkDuplicates in Picard. The transcript assembly was performed using Cufflinks v2.2.1 using filtered mapped reads. All the assemblies were then merged using Cuffmerge in Cufflinks, and FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values were calculated using default parameters.
Comparison of the different identified haplotypes was performed using Molecular Evolutionary Genetics Analysis (MEGA) v5.10 software.
Statistical analyses were done using GenStat (2016, 18th edition, VSN International, Hemel Hempstead, UK).

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The *Nature Methods* guidance for providing algorithms and software for publication may be useful for any submission.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All unique materials used are readily available from the authors

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

An anti-Stb6 kinase domain peptide (sequence – DVQSGSSTRSEETSL) antiserum was custom produced at Eurogentec (Seraing, Belgium) and its specificity tested using a dot blot at 1:1000 dilution vs 200 ng peptide.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used in this study

b. Describe the method of cell line authentication used.

n/a

c. Report whether the cell lines were tested for mycoplasma contamination.

n/a

d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

n/a

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	No animals were used in this study

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	This study did not involve human research participants