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**1–3. 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 cytoplasm4–6. This process often triggers a localized hypersensitive response, which halts further disease development5. 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 effector6–8 and confers pathogen resistance without a hypersensitive response7. 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) disease9–12.

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. 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 over, 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 microbes14–16. 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)17–19. The causal agent of STB is the fungus *Z. tritici*, which has recently been described as a latent necrotroph20 with a strictly extracellular mode of plant pathogenesis21. The emergence and dispersal of fungicide resistance in fungal populations18–20 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 genetically22. 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 wheat23, and targeted stacking of isolate-specific Stb resistance genes are also being considered24,25.

*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 evaluation24,25. It is inherited and manifests as a semidominant trait26 (Supplementary Fig. 1a), controlling a gene-for-gene type resistance effective against *Z. tritici* isolates, such as IPO323, which carry a matching *AvrStb6* gene encoding a small cysteine-rich effector protein7,8. *Stb6* is particularly interesting because it confers pathogen resistance in the absence of a hypersensitive response7. This gene has been suggested to exist in wheat since the mid-Neolithic period24, and it contributes to field resistance26. *Stb6* resides in the subtelomeric portion on 3AS in the wheat varieties Flame27, Chinese Spring (CS) and Cadenza (Cad) (Fig. 1 and Supplementary Fig. 1b,c). To better understand the

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

Using published wheat genetic maps27,28 and exploiting the synteny between wheat genomes and model grass genomes29, 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, including two markers that 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).

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* IFO323 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* 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 resequencing34,35. Mutant families with predicted nonsense and missense mutations in *TaWAKL3* and *TaWAKL4* were tested for resistance to *Z. tritici* IFO323, 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). *T₄* plants generated for each construct were self-fertilized. Analysis of the T₁ generation identified families segregating for resistance to *Z. tritici* IFO323 and, with the predicted full-length coding sequence of this gene driven by its native promoter or the maize *Ubi1* promoter showed specific gene-for-gene resistance to *Z. tritici* IFO323 (Supplementary Fig. 8).
*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-aspartate36 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...
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 in the corresponding amino acid changes). 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).

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, thus affirming the absence of a strict separation between PRRs and resistance proteins. 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) and the two recently cloned maize WAKs implicated in broad-spectrum, but partial, resistance proteins. Studying 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 oligagalacturonides, respectively. There is evidence that some WAKs may also bind proteinaceous ligands. A recent study has identified wheat susceptibility/sensitivity protein Sn1 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.
We hypothesized that Stb6 might bind to its matching recently cloned 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 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. 1). 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/](http://www.wheat-tilling.com/); WebLogo, [http://weblogo.berkeley.edu/](http://weblogo.berkeley.edu/); GrainGenes, [http://wheat.pw.usda.gov/GG3/](http://wheat.pw.usda.gov/GG3/); The Wheat Portal, [https://wheat-urgi.versailles.inra.fr/](https://wheat-urgi.versailles.inra.fr/); Ensembl Fungi, [http://fungi.ensembl.org/index.html/](http://fungi.ensembl.org/index.html/); INRA-CNRS Plant Genomic Resources Center, [https://cnrgv.toulouse.inra.fr/en/](https://cnrgv.toulouse.inra.fr/en/); Hisat2, [https://ccb.jhu.edu/software/hsat2/index.shtml](https://ccb.jhu.edu/software/hsat2/index.shtml); Hierarchical Genome Assembly Process (HGAP), [https://github.](https://github.)
23. McDonald, B. A. & Mundt, C. C. How knowledge of pathogen population resistance and escape to the control of Septoria tritici blotch of wheat. Plant Pathol. 58, 910–922 (2009).

24. 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).

25. Saintenac, C., Jiang, D., Wanga, S. & Akhunov, E. Sequence-based mapping of the polyloid wheat genome. G3 (Bethesda) 3, 1105–1114 (2013).

26. Bolot, S. et al. The ‘inner circle’ of the cereal genomes. Curr. Opin. Plant Biol. 13, 119–125 (2009).

27. Antolin-Llovera, M., Ried, M. K., Binder, A. & Parniske, M. Receptor kinase signaling pathways in plant-microbe interactions. Annu. Rev. Phytopathol. 50, 451–473 (2012).

28. 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).

29. 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).

30. 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).

31. King, R. et al. Mutation scanning in wheat by exon capture and next-generation sequencing. PLoS One 10, e0137549 (2015).

32. Krasileva, K. V. et al. Uncovering hidden variation in polyloid wheat. Proc. Natl. Acad. Sci. USA 114, E913–E921 (2017).

33. 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).

34. Stutz, H. U., Mitroussia, 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).

35. De Wit, P. J. G. M. Apolastic fungal effectors in historic perspective; a personal view. New Phytol. 212, 805–813 (2016).

36. Diener, A. C. & Ausubel, F. M. RESISTANCE TO FUSARIUM OXYSPORUM f.sp. TRITICI, a dominant Arabidopsis disease-resistance gene, is not race specific. Genetics 171, 305–321 (2005).

37. 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 102, 8780–8785 (2015).

38. Zuo, W. et al. A maize wall-associated kinase confers quantitative resistance to head smut. Nat. Genet. 47, 151–157 (2015).

39. 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).

40. Ferrari, S. et al. Oligogalacturonoidases: plant damage-associated molecular patterns and regulators of growth and development. Front. Plant Sci. 4, 49 (2013).

41. Kohorn, B. D. & Kohorn, S. L. The cell wall-associated kinases, WAKs, as pectin receptors. Front. Plant Sci. 3, 88 (2012).

42. Kohorn, B. D. Cell wall-associated kinases and pectin perception. J. Exp. Bot. 67, 489–494 (2016).

43. Park, A. R. et al. Interaction of the Arabidopsis receptor protein kinase WAK1 with a glycinine-rich protein. AGRIP3, J. Biol. Chem. 286, 26688–26693 (2001).

44. 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.

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Critical recombinant F2 plants were selfed, and their progeny (at least 20 F3 plants) described above as well as subjected to a fungal pathoassay. Forty experiments with CS and Cad wheat, respectively. For each experiment, and data were pooled from a minimum of three and two independent samples from each virus treatment were counted in each experiment, for silencing candidate Stb6 genes was carried out essentially as previously described. 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 F2 CS × CT progeny was first genotyped with gwm369 and cfa3010, which flank Stb6, and individuals with recombination events between these markers were then genotyped with new genetic markers (Supplementary Tables 9 and 10) described above as well as subjected to a fungal pathoassay. Forty critical recombinant F3 plants were selfed, and their progeny (at least 20 F4 plants per family) were tested in pathoassays.

For the construction of 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 TACGACv1 (Supplementary Note). Three large-scale contiguous sequences combined contained genetic markers csi8311 and cfn80023, which cosegregated with Stb6, as well as markers csi8311F12/csi8311R12, which flank 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. Gene-silencing constructs were created by cloning fragments of wheat gene sequences into the BSMV RNA(A)-derived binary vector pCa-yPlac33 (Agilent Technologies), which is part of a joint project between the University of California, Davis and the University of Arizona. The integrity of the cloned genomic sequence was confirmed by Sanger sequencing of PCR fragments produced with a set of primer pairs distributed along the Stb6 gene sequence (Supplementary Table 9). In addition, all these individual susceptible M5 plants were self-fertilized, and their progeny (at least 24 M6 plants per family) were tested in pathoassays to confirm the susceptibility phenotype.

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, (1/NSK) qRT–PCR data were analyzed as previously described, where NRI is the normalized relative quantity (2-ΔCt/2-ΔCt 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.

To determine the expression of silencing candidate Stb6 genes in wheat through VIGS on the VIGS of the Z. tritici infection, GenStat 18 (https://www.vsni.co.uk/software/genstat/) was used as described previously. 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 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 on 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 with a t test for between-family and 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 the two-tailed t test. 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 M1, mutant families of Cad wheat 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 genome for geis 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 (TILLING database), which is part of a joint project between the University of California, Davis and the University of Arizona. The integrity of the cloned genomic sequence was confirmed by Sanger sequencing of PCR fragments produced with a set of primer pairs distributed along the Stb6 gene sequence (Supplementary Table 9). In addition, all these individual susceptible M5 plants were self-fertilized, and their progeny (at least 24 M6, 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 831F12/831R12 (Supplementary Table 9) and cloned into the pCR8/GW/TOPO vector (Thermo Fisher Scientific). The integrity of the cloned genomic sequence was confirmed 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 gene silencing experiments. The corresponding TOPO vector with a double digestion with EcoRV and PstIOML and the cloned wheat genomic DNA fragment was purified after agarose gel electrophoresis and dephosphorylated as previously described. 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 with a PIDS 1000 He device (Bio-Rad). Regeneration of plants and bar virus selection were performed essentially as previously reported. Detection of the Stb6 gene in T0 plants was performed by PCR amplification using plant genomic DNA as the template and primers pCR8/GW_Stb6F1 and pCR8/GW_Stb6R1.
Triticum monococcum
Stb6
incubated with 500
pellet). The final clarified supernatant containing the soluble proteins was then
(4 ml per bacterial
construct 3 transformants were analyzed with primers UbiPro4 and R-gene-rev.
′
both analyzed with the primer pair R-gene-fwd and Nos5
rev. Additionally,
DNA Purification kit (Promega). PCR analysis was carried out for the gene of
well-known susceptible wheat varieties Obelisk, Riband and Longbow6,24,59,60; recent
accessions comprising varieties previously reported as potential carriers of
Z. tritici
isolates IPO323 at the seedling stage.

Aat
EcoRI
CDS digested with
Not
I, and then cloned upstream of the
Nos
terminator into the pRRES14, RR.001_65 vector codigested with Smal and NotI to create the plasmid p658-Rpromo-CDS (construct 2). After digestion with NotI and Stbl, the Stb6 CDS was cloned between the maize Ubi promoter and the Nos terminator into the pRRES14, RR.1m021_125 vector codigested with AafII and NorI to create the plasmid p125CDS-R (construct 3). Each of these plasmids was mixed with pBAD plasmid pBDM2 containing the constitutive bar gene for resistance to herbicide12 and transformed into immature embryos of Bobwhite wheat after a particle-bombardment procedure, essentially as previously described11. 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). Transgenic and bar+ plants were turned out in T1

and current widely cultivated UK and French varieties with good field resistance

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 and then cloned upstream of the
Nos
terminator into the Gateway-compatible vector pDONR221 with BP Clonase II and Gateway 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 Sb6 bait constructs and the corresponding fungal effector prey constructs (including a negative-control vector Z10), and vice versa. The same yeast strain was transformed with the A. thaliana GAL (bait) and ARR1 (prey) protein constructs was used as a positive control for protein–protein interaction44. 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 were scored by growth of single 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, KY485194, KY485195, KY485196, KY485197, 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 to subvert disease control by 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, e24668 (2011).
54. Rieu, I. & Powers, S. J. Real-time quantitative RT-PCR: design, calculations, and statistics. Plant Cell 19, 1029–1039 (2007).
55. Lee, W.-S., Rudd, J. J., Hammond-Kosack, K. E. & Kanyuka, Y. Mycosphaerella graminicola 1xM effector-mediated stealth pathogenesis subverts recognition through both CERK1 and CEBP homologues in wheat. Mol. Plant Microbe Interact. 27, 236–243 (2014).
56. Sass, C., Partier, A., Becker, M., Foulle, C. & Barret, P. Biolistic transformation of wheat: increased production of plants with simple insertions and inheritable 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 triticil 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. Marin-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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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.

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   | □ X The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
   | □ X A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly.
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   | □ X The test results (e.g. p values) given as exact values whenever possible and with confidence intervals noted.
   | □ X A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range).
   | □ X 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/hsat2/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.

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No eukaryotic cell lines were used in this study

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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

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