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

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1 SUPPLEMENTARY NOTE

2
3 **Genetic mapping.** A synteny^{70,71} between genomes of wheat and *Brachypodium distachyon* was exploited
4 for identification of a region in the fully sequenced⁷² genome of *B. distachyon* v1.2 syntenic to the wheat
5 *Stb6* locus as follows. With the help of genetic maps available for the Apache × Balance and ITMI
6 (International Triticeae Mapping Initiative) wheat mapping populations^{73,74} and their shared markers, an
7 interval corresponding to *Stb6* was located between the single nucleotide polymorphism (SNP) markers,
8 SyOpL2500 (wsnp_Ra_c9738_16173810) and SyOpL304 (wsnp_CAP12_c2692_1286812), present on the
9 ITMI map. Nucleotide sequences surrounding these SNPs were subjected to similarity searches using
10 BLASTn against *B. distachyon* genome and this allowed identification of a syntenic genome region on the *B.*
11 *distachyon* chromosome 2 spanning approximately 769-kb interval containing 96 predicted gene models
12 between *Bd2g02010.1* and *Bd2g02900.1* as shown in **Supplementary Fig. 2**. These *B. distachyon* genes
13 were then subjected to similarity searches using BLASTn against the different wheat databases^{75,76}. Identified
14 wheat genomic sequences were analyzed for the presence of microsatellite sequences using SSR Locator⁷⁷
15 and the repeat sequences were used for development of 35 new SSR markers (data not shown). Two of these,
16 cfa3006 and cfa3010 (**Supplementary Table 9**), were polymorphic between the parents of wheat mapping
17 populations used in this study and were assigned to the deletion bin 0.45-1.00 on 3AS. SSR genotyping was
18 performed essentially as described⁷⁸. The nineteen predicted RLK-like genes, *Bd2g02426.1* to *Bd2g02537.1*,
19 residing close to the center of the 769-kb interval were subsequently subjected to similarity searches using
20 BLASTn against the International Wheat Genome Sequence Consortium Chromosomes Survey Sequence
21 (IWGSC CSS) assembly v1 (**ref. 79**). This identified 11 wheat chromosome 3A-specific genomic DNA
22 contigs each carrying RLK-like sequences, which we partially re-sequenced from wheat Courtot, Avalon and
23 Cadenza to identify potential polymorphic sites as follows. Fragments of these predicted genes were PCR-
24 amplified using primers listed in **Supplementary Table 9** and AmpliTaq Gold® Master Mix (Applied
25 Biosystems), purified using Agencourt AMPure XP (Beckman Coulter), and Sanger sequenced at GATC
26 Biotech SARL (Konstanz, Germany). Identified single nucleotide polymorphisms (SNPs) were used for
27 developing SNP markers (**Supplementary Table 10**). SNP genotyping was performed using the
28 Kompetitive Allele Specific PCR (KASP™) genotyping assays (LGC Genomics) that were analyzed on the
29 Light Cycler 480 Real-Time PCR System (Roche Applied Science). Feeding an additional available wheat
30 genome sequencing data^{71,76} identified an INDEL (INsertion/DELETion) that was used for developing a co-
31 dominant PCR-based marker, named ctg8311. PCR using the primer pair 8311F3 / 8311R4 (**Supplementary**
32 **Table 9**) produces ~550-bp and ~800-bp DNA fragments from Chinese Spring and Courtot, respectively,
33 that are easily differentiated by size using agarose gel electrophoresis.

34
35 **Physical map construction, sequencing, and annotation.** Markers ctg8311 and cfn80023 co-segregating
36 with *Stb6* as well as one of the closest flanking markers, cfn80025, were used for screening the wheat CS
37 Tae-B-CsE BAC library available at the INRA-CNRGV Plant Genomic Resources Center, Toulouse, France
38 using a set of 6 high-density filters each containing 55,296 BAC clones spotted in duplicates following a
39 previously described⁸⁰ hybridization protocol. Nine positive BAC clones were detected and the largest ~100-
40 kb BAC clone Tae-B-CsE-673A07 containing all three markers was sequenced using the PacBio RS II
41 system (Pacific Biosciences). For this 2 µg of BAC DNA were pooled with DNA from 11 other BAC clones
42 and one 8-12-kb DNA library was generated using the standard PacBio library preparation protocol. This
43 library was sequenced in one Single Molecule, Real-Time (SMRT) Cell using the P6 polymerase in
44 combination with the C4 chemistry at IGM Genomic Center (University of California, San Diego, USA).
45 Assembly of the sequence reads was performed using the HGAP workflow of the SMRT® Analysis v2.2.0
46 software (Pacific Biosciences). Reads were first aligned using the BLASR (Basic Local Alignment with
47 Successive Refinement) tool⁸¹ against complete genome of *E. coli* strain DH10B. Identified *E. coli* reads as
48 well as short (< 500-bp) and low quality (< 0.80) reads were excluded from sequence assembly. Vector
49 sequences were trimmed along the assembly process and each BAC assembly was individualized by

50 matching their BAC end sequences using BLASTn. The BAC Tae-B-CsE-673A07 sequence assembly was
51 then annotated automatically using the TriAnnot pipeline⁸² and then manually curated. Sequences of 4
52 WAK-like genes (*TaWAKL1* – *TaWAKL4*) predicted to reside in this BAC clone were subjected to BLASTn
53 against the TGACv1 wheat Whole Genome Assembly (WGA) available from The Wheat Portal. One
54 117,845-bp scaffold *Triticum_aestivum_CS42_TGACv1_scaffold_210987_3AS*, overlapping with the BAC
55 clone Tae-B-CsE-673A07 over 64-kb and containing additional markers cfn80030 and cfn80040 that flank
56 *Stb6* was identified. Sequences of this genomic scaffold and the BAC clone Tae-B-CsE-673A07 were
57 merged to complete the *Stb6* physical region of 155,870-bp, which was annotated using the TriAnnot
58 pipeline⁸² and then manually curated. Microsatellites identified in the long intergenic region between
59 *TaWAKL2* and *TaWAKL3* were used for developing additional SSRs, cfa3036 and cfa3037 (**Supplementary**
60 **Table 9**), for genotyping.

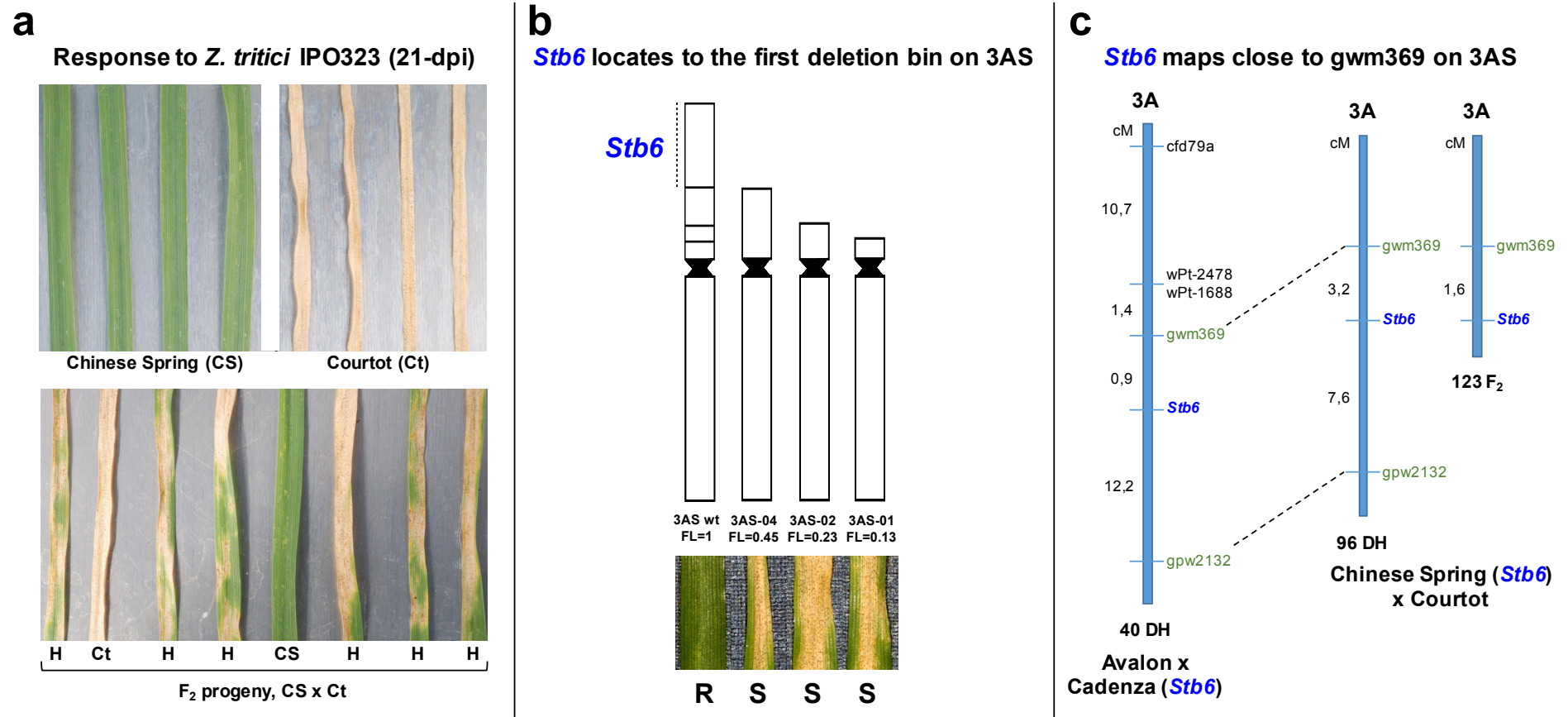
61 Very recently a new substantially improved IWGSC wheat WGA v1.0, comprising of Illumina short
62 sequence reads produced with the DeNovoMAGIC assembly pipeline (NRGene) and further refined by the
63 IWGSC genome assembly team, become available (Kellye Eversole and colleagues at IWGSC,
64 unpublished). A continuous 400-kb genomic sequence containing *Stb6* and spanning the genetic interval
65 between markers cfn80025 and cfa3010, which likely define the borders of the complete WAK-like genes
66 cluster on 3AS, was identified and annotated (**Supplementary Fig. 3**) as follows. RNA-seq data of
67 differently staged wheat tissues available from The Wheat Portal, wheat leaves under drought and/or heat
68 stress from PRJNA257938, root and shoot +/- phosphorous starvation from PRJDB2496, as well as
69 *Zymoseptoria tritici* infection time course on wheat CS (Kanyuka *et al.*, unpublished) and Riband⁸³ was
70 mapped to the 400-kb genomic sequence using Hisat2 v2.0.4. The BAM file was imported into Geneious
71 v8.1.5 (Biomatters Ltd.) and the gene models curated by producing gene coding sequence (CDS) annotations
72 that matched the mapped RNA-seq data. To identify and construct pseudogene annotations, the curated
73 exons and TGACv1 WGA WAK-like gene exon annotations extracted using BioMart tool in Ensembl Plants
74 were aligned to the reference using Lastz v7.0 in Geneious. The 400-kb genomic sequence was then
75 translated on all 6 frames and the resulting amino acid sequences subjected to a scan for Pfam domains using
76 HMMER v3.1 to assist in curation.

77
78 **Gene expression analyses, and Rapid Amplification of cDNA Ends (RACE).** Total RNA extracted in
79 duplicates from root, leaf, stem, spike, and grain at three developmental stages each from healthy wheat
80 Chinese Spring was used for library preparation followed by sequencing by Illumina HiSeq2000 platform as
81 reported⁸⁴. The resulting 100-bp paired-end reads were mapped to the 4,431-bp contig
82 IWGSC_chr3AS_ab_k71_contigs_longerthan_200_3371186 (**Supplementary Fig. 3**) containing exons 3
83 and 4 of *Stb6* using Tophat2 v2.0.13 (**ref. 85**) with a mate inner distance set to 300 (-r), a mate standard
84 deviation to 300, no mismatches are allowed (-m 0 -N 0) and qualities are set to --solexa1.3-quals. Mapping
85 results were processed using the Picard Tools suite v1.124 to accept only reads with a mapping quality above
86 30. The duplicates were removed with MarkDuplicates in Picard. The transcript assembly was performed
87 using Cufflinks v2.2.1 (**ref. 86**) using filtered mapped reads. All the assemblies were then merged using
88 Cuffmerge in Cufflinks, and FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values
89 were calculated using default parameters.

90 To determine the 5'- and 3'-ends of *Stb6* mRNA, RACE were performed using the SMARTer RACE
91 5'/3' kit (Clontech) following the manufacturer's instructions. Leaves from three different wheat Chinese
92 Spring plants were collected at 1, 2, 5, 10, 14 and 17 days post inoculation with *Z. tritici* IPO323 or post
93 mock inoculation in duplicates. Total RNA was extracted with TRIzol® (Life technologies) and treated with
94 the TURBO DNase (Applied Biosystems). First strand cDNA obtained from 1 µg of a pool of 12 DNase-
95 treated RNA samples mixed in equimolar ratio were used as templates for the 5' RACE and 3' RACE using
96 primer 8311R6 and 8311F11, respectively (**Supplementary Table 9**). Resulting PCR products were cloned
97 into the pRACE vector (Clontech) and 17 and 18 plasmids containing the 5' RACE and 3' RACE products,
98 respectively, were Sanger sequenced using M13F and M13R primers at GATC Biotech SARL (Konstanz,

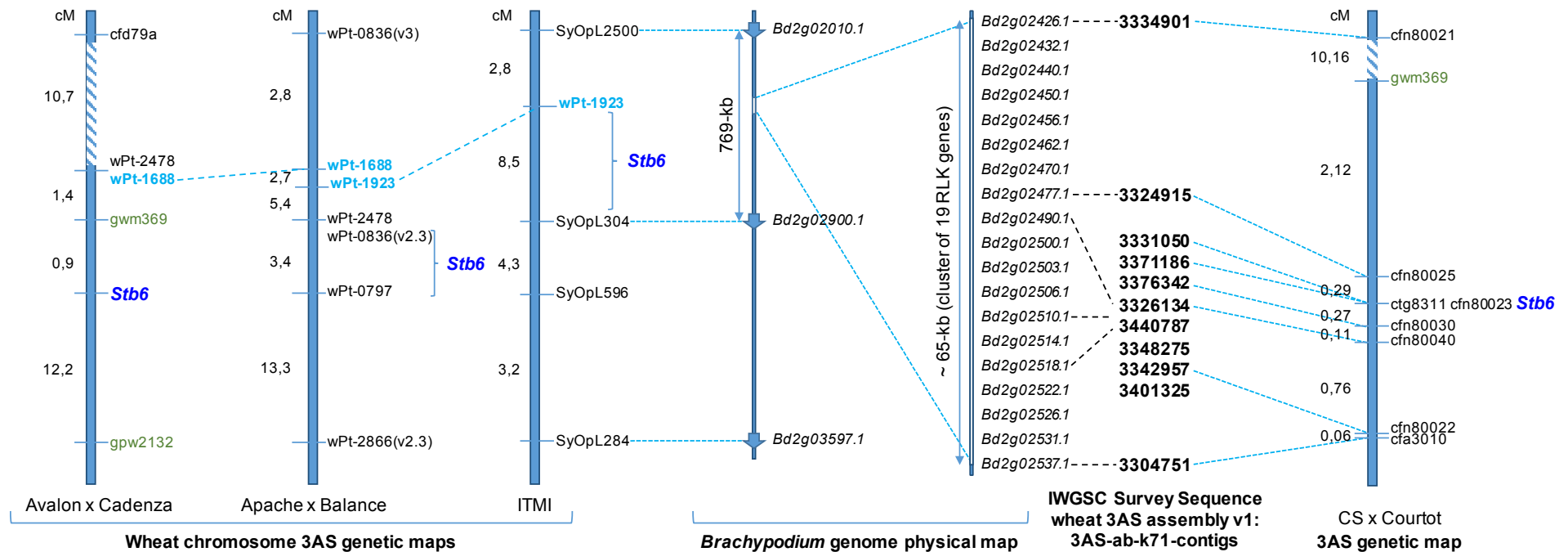
- 99 Germany). These sequences were aligned to the sequence of the BAC clone Tae-B-CsE-673A7 to determine
100 the transcriptional initiation and termination sites of *Stb6* mRNA.
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102
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104 and wheat. *Plant Mol. Biol.* **70**, 47–61 (2009).
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106 comparative whole genome shotgun sequencing. *Nature* **491**, 705–710 (2012).
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126 Successive Refinement (BLASR): theory and application. *BMC Bioinformatics* **13**, 238 (2012).
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136 gene fusions. *Genome Biol.* **14**, R36 (2013).
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138 and isoform switching during cell differentiation. *Nature Biotechnol.* **28**, 511–515 (2010).

Supplementary Figure 1. *Stb6* is inherited as a semi-dominant trait and locates to a sub-telomeric region on chromosome arm 3AS in wheat Chinese Spring and Cadenza.



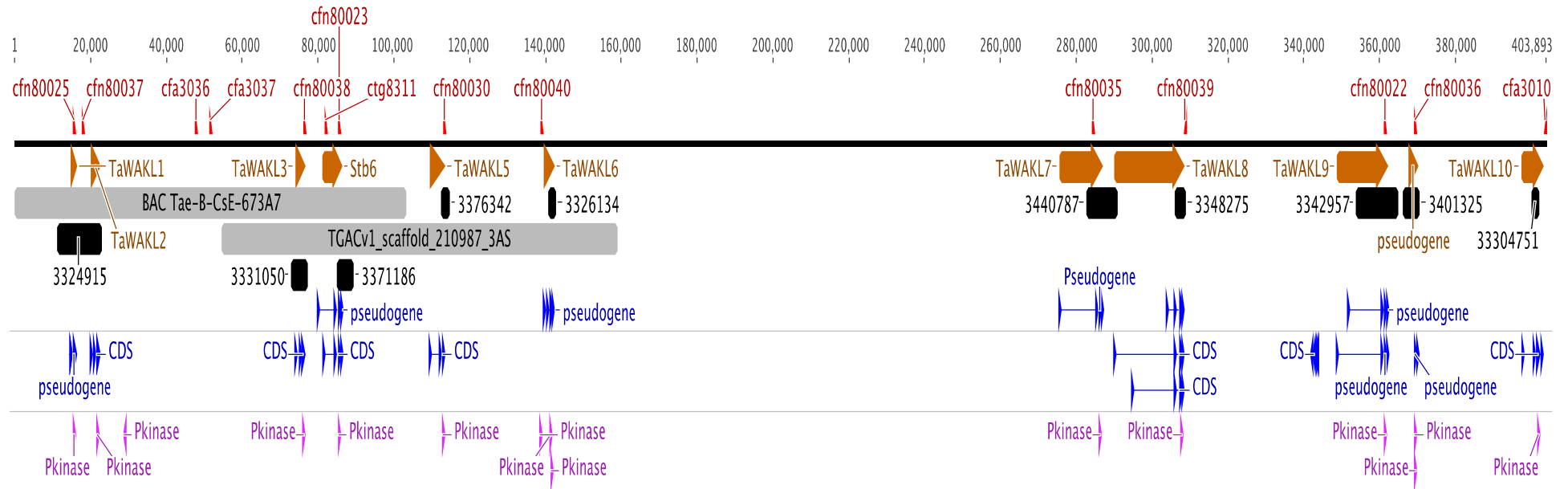
(a) Response to *Z. tritici* IPO323 of wheat cultivars Chinese Spring (CS) and Courtot (Ct), and in the F₂ progeny of a CS × Ct cross. The individuals heterozygous (H) for *Stb6* display a phenotype that is an intermediate between the phenotypes of parental cultivars, confirming that *Stb6* is a semi-dominant gene. **(b)** *Stb6* locates to the 0.45-1.00 marker bin on 3AS in wheat CS. The lines of CS (3AS4-0.45; 3AS2-0.23; 3AS1-0.13) carrying induced deletions of 3AS are fully susceptible to *Z. tritici* IPO323. **(c)** *Stb6* maps close to gwm369 on 3AS in wheat Cadenza and CS. A total of 40 doubled haploid (DH) lines derived from an Avalon × Cadenza cross, 96 DH lines and 123 F₂ plants derived from a CS × Ct cross were tested for resistance to *Z. tritici* IPO323 in conjunction with genotyping assays using publicly available SSR markers. This located *Stb6* at 0.9 cM and 3.2 cM from gwm369 (in Cadenza and CS, respectively), and more precisely between gwm369 and gpw2132.

Supplementary Figure 2. A physical region in the *Brachypodium distachyon* genome syntenic to the *Stb6* genetic interval in wheat.



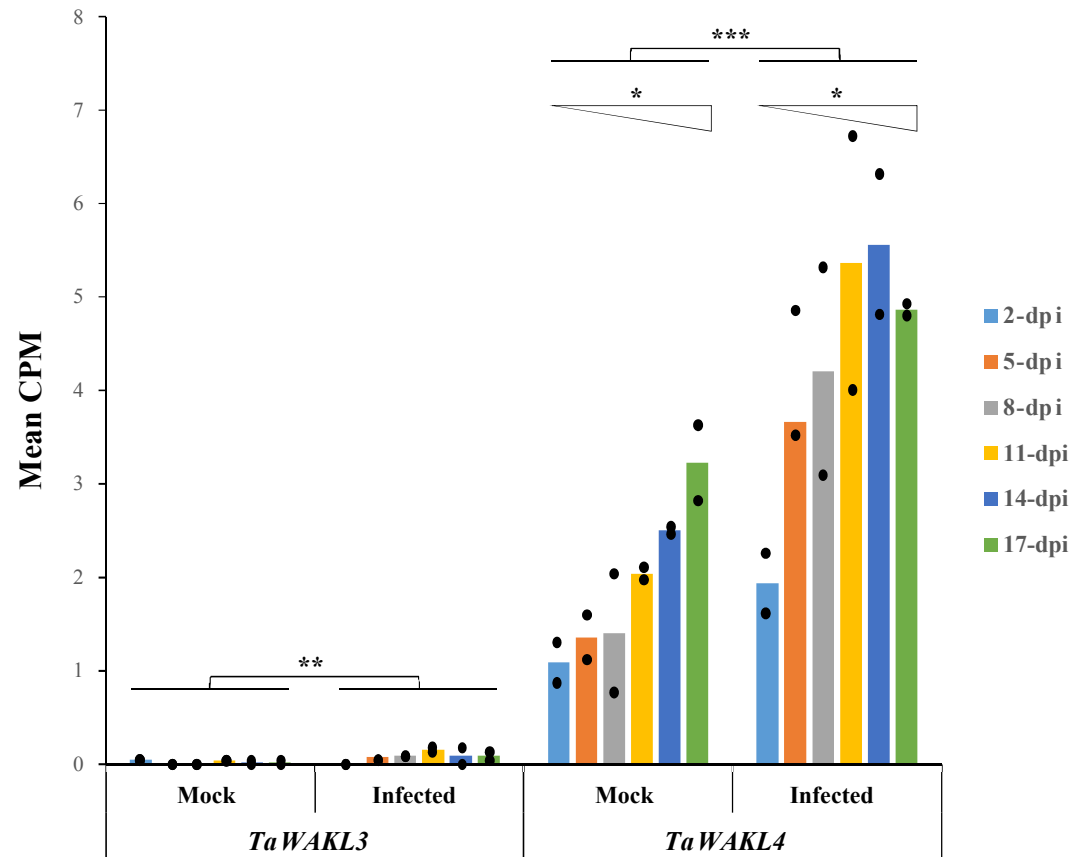
With the help of genetic maps available for the wheat mapping populations Apache × Balance and ITMI (International Triticeae Mapping Initiative) and their shared markers a genetic interval corresponding to *Stb6* was located between the sequence-characterized SyOPL markers present on the ITMI map. Homologous sequences were identified on chromosome 2 of *Brachypodium distachyon* and a physical interval between these sequences, which spanned 769-kb from the predicted gene *Brady2g02010.1* to *Brady2g02900.1*, defined a region syntenic to the wheat *Stb6* locus. This region contains a gene cluster comprising 19 putative RLK genes from *Brady2g02426.1* to *Brady2g02537.1*. Homology search using these RLKs identified eleven wheat chromosome arm 3AS specific DNA contigs, which were used for developing new markers for genotyping in the wheat Chinese Spring × Courtot mapping population. Eight of these new markers were mapped, with two - *ctg8311* and *cfn80023* - co-segregating with *Stb6*.

Supplementary Figure 3. Schematic diagram / annotation of the continuous ~400-kb genomic region, which spans the *Stb6* locus and contains a cluster of WAK-like genes, bordered by markers *cfn80025* and *cfa3010* on 3AS in wheat Chinese Spring (CS).



Genomic dsDNA is indicated by the thick black line. The ten out of eleven wheat CS chromosome 3A-specific IWGSC CSS assembly v1 genomic DNA contigs, which contain sequences homologous to the *Brachypodium distachyon* RLK-like genes clustered in the syntenic region on chromosome 2 (**Supplementary Figure 2**) are shown as black rectangles. The *Stb6* gene-containing BAC Tae-B-CsE-673A07 and TGACv1 WGA scaffold *Triticum aestivum_CS42_TGACv1_scaffold_210987_3AS* are shown as gray rectangles. Molecular markers derived from these sequences and used for fine mapping of *Stb6* are shown in red immediately above the genomic DNA. The WAK-like genes and pseudogenes annotated in this genomic region are indicated in orange as triangles and pentagons immediately below the genomic DNA. Transcribed gene coding sequences (CDS) and pseudogenes imputed from mapping of published and own private RNA-seq data to this ~400-kb genomic DNA sequence (that was extracted from the IWGSC wheat WGA v1.0) are shown in blue. Protein kinase domains identified through scanning the amino acid sequences produced following translation of the 400-kb genomic DNA fragment on all 6 frames are shown in purple.

Supplementary Figure 4. Expression analysis of *TaWAKL3* and *TaWAKL4* at six different time points post mock-inoculation or *Z. tritici* isolate IPO323 inoculation onto leaves of wheat Chinese Spring.



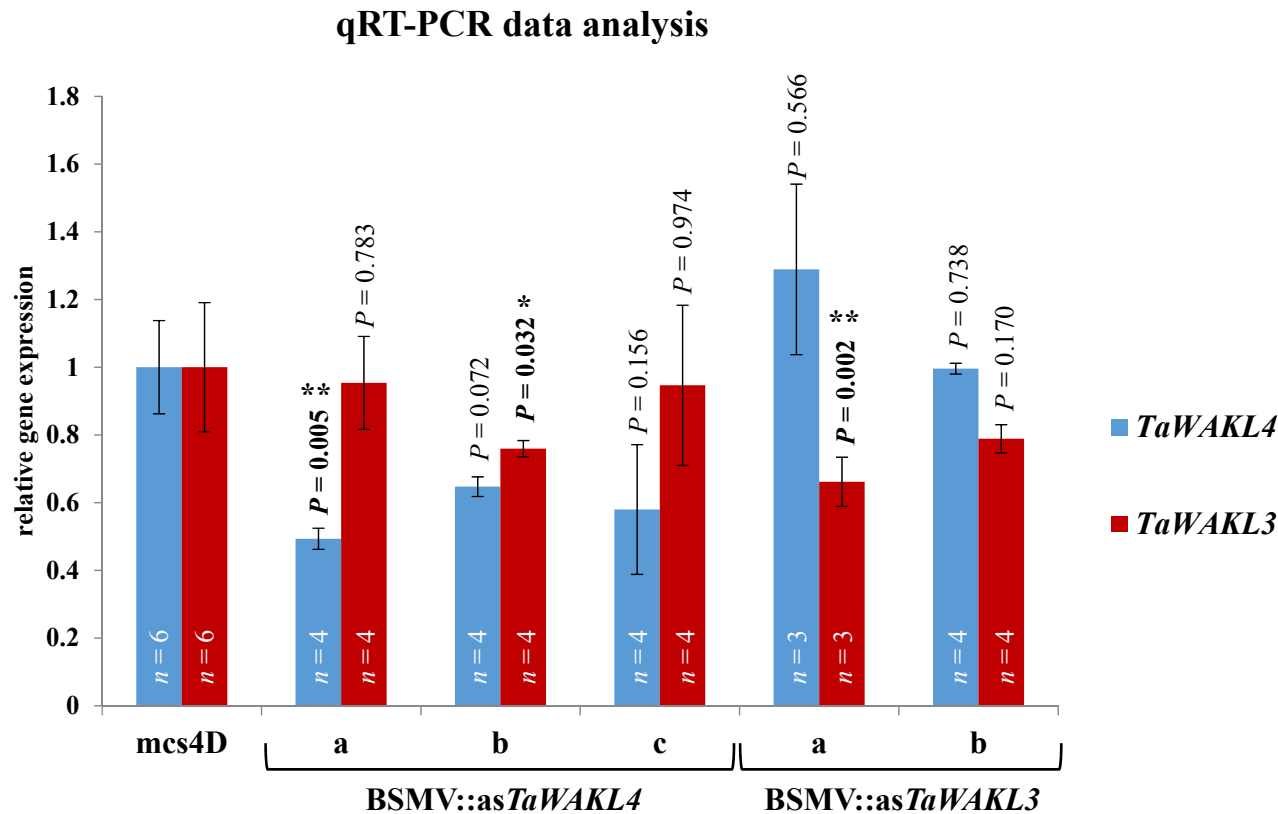
Mean counts per million (CPM) obtained from RNA-sequencing (Kanyuka *et al.*, unpublished) for transcripts of *TaWAKL3* and *TaWAKL4* genes are shown on the Y-axis. Black dots are values for each sample from the two replicated experiments. Comparison of CPM mean values using post-hoc *t*-test for *TaWAKL3* showed that this gene was upregulated during infection ($F = 9.83$ on 1 and 12 df (degrees of freedom), $P = 0.009$) and the means were 0.0215 CPM (mock) and 0.0774 CPM (*Z. tritici* infected); SED (standard error of a difference between 2 means) = 0.01786 on 12 df. For *TaWAKL4* there were additive, independent main effects of treatment ($F = 43.22$ on 1 and 12 df; $P < 0.001$) and time ($F = 4.76$ on 1 and 12 df; $P = 0.013$) indicating expression of this gene increases with time (mean CPMs equal 1.94 (mock) and 4.35 (*Z. tritici* infected); SED = 0.368 on 12 df), and also in response to the pathogen (mean CPMs were 1.51, 2.77, 2.80, 3.70, 4.03 and 4.04 for 2-, 5-, 8-, 11-, 14- and 17 days post treatment; SED = 0.637 on 12 df).

Supplementary Figure 5. Alignment of full-length coding sequences of *TaWAKL4* from the resistant wheat Chinese Spring and the susceptible wheat Courtot.



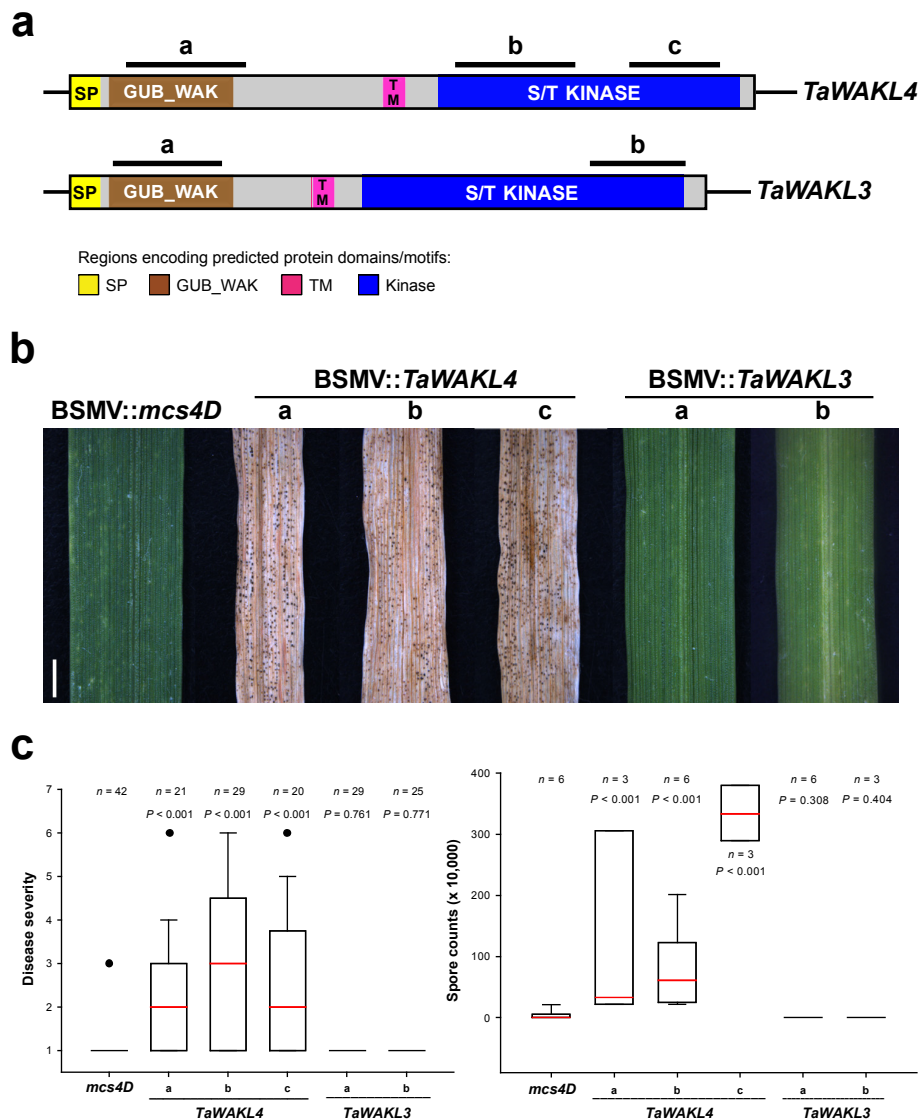
Sequences coding for predicted protein motifs and domains, such as a signal peptide (SP), a galacturonan-binding domain (GUB_WAK), a transmembrane region (TM), and a protein kinase domain, are indicated by differently colored rectangles. Only one single nucleotide polymorphism, T1340A, between the sequences in Chinese Spring (CS) and Courtot (Ct) leading to an amino-acid change Ile447Asp was identified (highlighted).

Supplementary Figure 6. Specific suppression of *TaWAKL4* and *TaWAKL3* expression using BSMV-mediated gene silencing in wheat Chinese Spring.



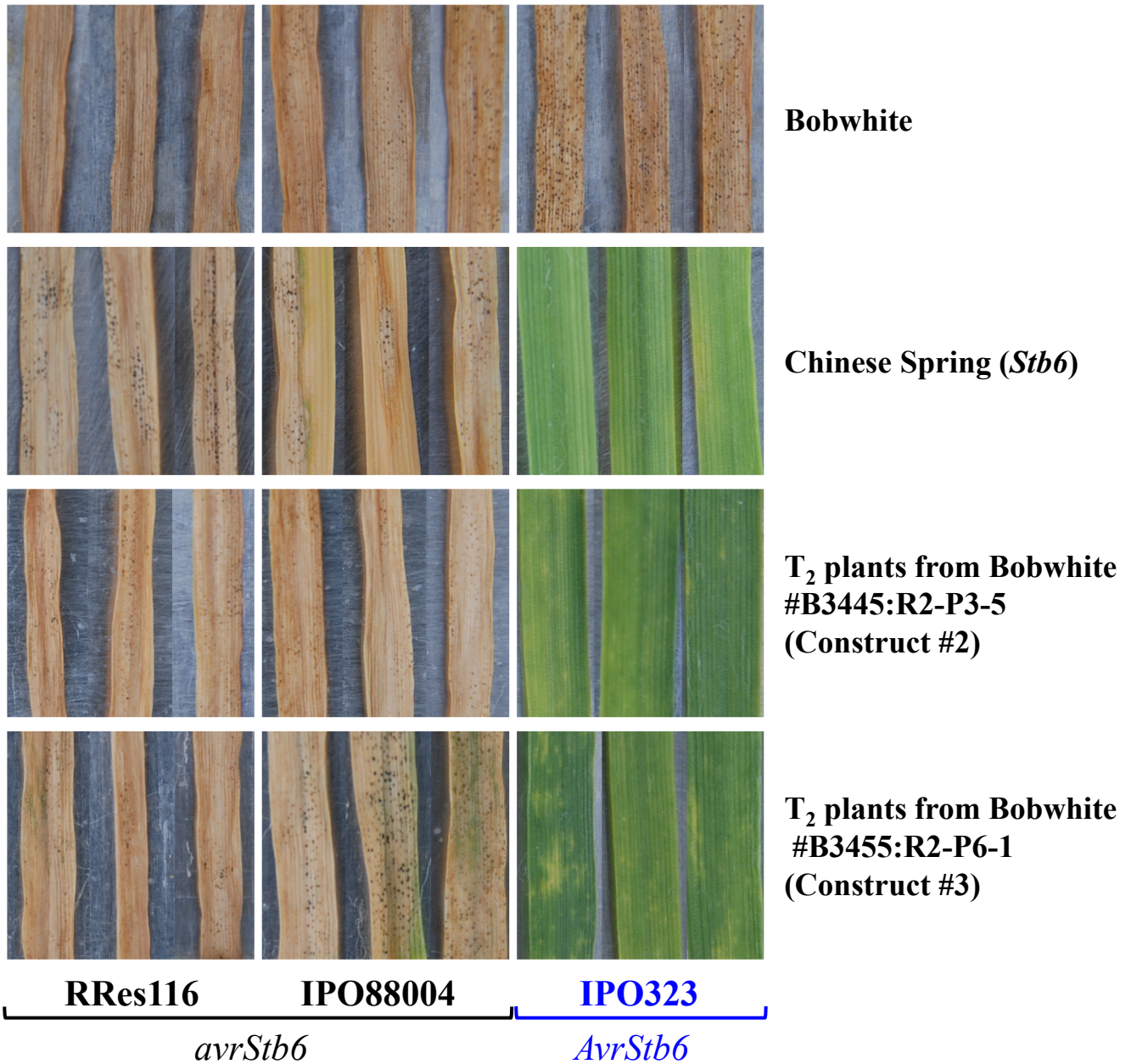
TaWAKL4 and *TaWAKL3* transcripts levels in the third leaves of wheat Chinese Spring plants infected with BSMV-VIGS constructs targeting *TaWAKL4*, *TaWAKL3* or the negative control construct BSMV::*mcs4D* as determined by quantitative RT-PCR. Error bars are mean \pm standard error of the mean of $n = 3-6$ biological replicates (plants), from one experiment only. Three and two different constructs were used for silencing *TaWAKL4* and *TaWAKL3*, respectively. For *TaWAKL4* and *TaWAKL3*, there were overall significant differences between effects of different VIGS construct treatments ($F = 3.29$ on 5 and 18 df (degrees of freedom), $P = 0.028$ / $F = 6.34$ on 5 and 18 df, $P = 0.001$). The post-hoc two-tailed t -test showed that *TaWAKL4* was statistically significantly downregulated only in plants inoculated with BSMV::*TaWAKL4a* (** $P = 0.005$) compared to those inoculated with BSMV::*mcs4D*. *TaWAKL3* was statistically significantly downregulated in plants inoculated with BSMV::*TaWAKL3a* (** $P = 0.002$). There was also a smaller but significant off-target effect on the expression of *TaWAKL3* noted for BSMV::*TaWAKL4b* (* $P = 0.032$). Exact P values are shown above bars.

Supplementary Figure 7. Virus-induced gene silencing of *TaWAKL4* but not *TaWAKL3* compromises resistance to *Z. tritici* isolate IPO323 in the *Stb6*-containing wheat Cadenza.



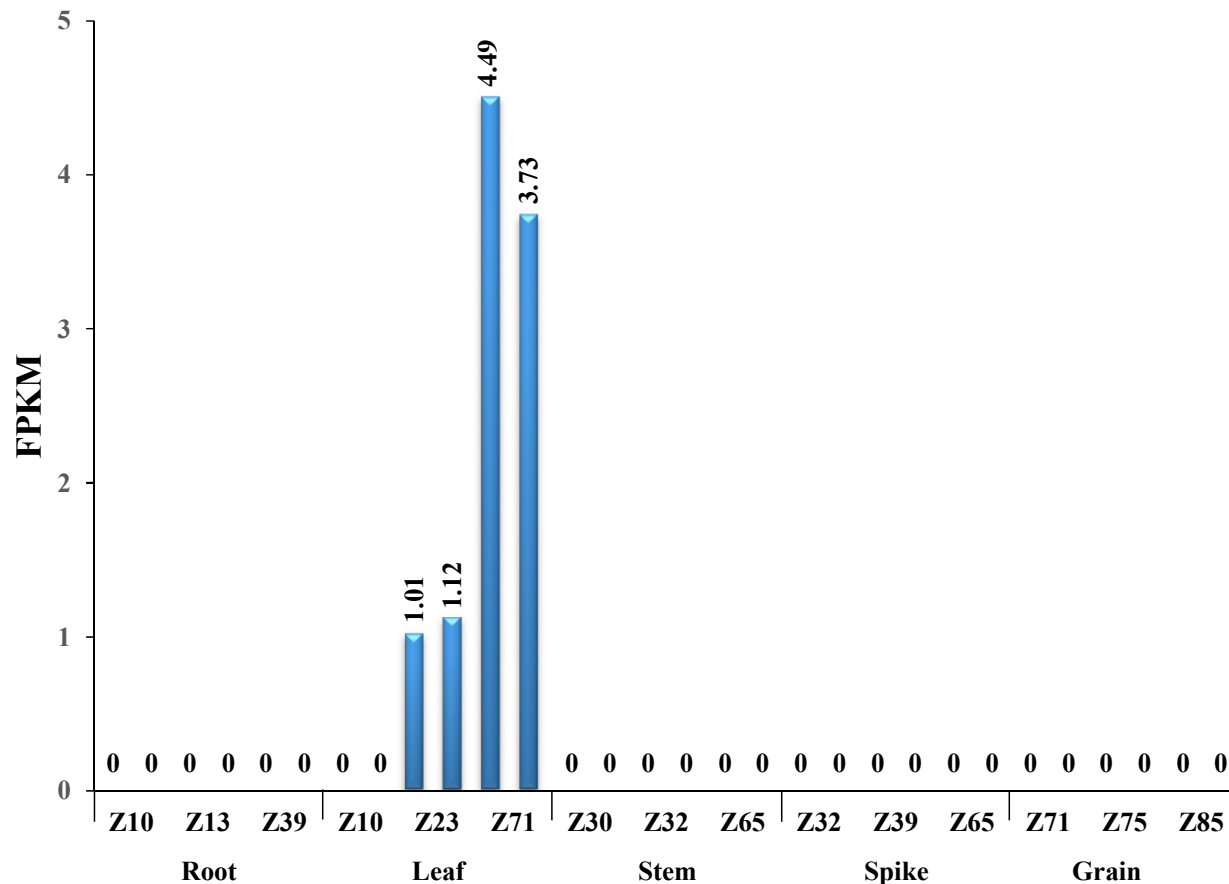
(a) Schematic representation of inferred *TaWAKL4* and *TaWAKL3* mRNAs. Coding and 5'- and 3'-non-coding sequences are shown as rectangles and solid black lines, respectively. Regions encoding predicted protein domains and motifs are labelled and shown as colored blocks: SP, signal peptide; GUB_WAK, galacturonan-binding domain; TM, transmembrane region; S/T Kinase, Serine/Threonine kinase domain. Solid black bars above each mRNA model indicate regions amplified to generate BSMV-VIGS constructs targeting these genes. **(b-d)** Silencing of *TaWAKL4* compromised resistance to *Z. tritici* isolate IPO323 in wheat Cadenza. **(b)** Leaves inoculated with fungal spores developed tan-colored necrotic lesions containing scatterings of black pycnidia (fungal fruiting bodies) when pre-inoculated with one of three independent BSMV-VIGS constructs targeting *TaWAKL4* for silencing, but not when pre-inoculated with the virus control (BSMV::*mcs4D*) or either of the two independent VIGS constructs targeting *TaWAKL3*. Representative images taken at 21 days post-fungal inoculation (dpi). Scale bar = 25 mm. **(c)** Disease severity on a scale from 1 (no fungal-induced necrosis and pycnidia) to 6 (80-10% leaf area covered by pycnidia-bearing necrotic lesions) assessed at 21-dpi. There was a highly significant effect of silencing specific candidate genes ($F = 31.66$ on 5 and 160 df (degrees of freedom), $P < 0.001$). The number of leaves (*n*) and the *P*-value for the approximate *t*-test (160 df) for comparison of *TaWAKL4*- and *TaWAKL3*- silenced plants to those treated with a negative control BSMV::*mcs4D* are shown. **(d)** Counts of pycnidiospores washed from detached leaves. There was a highly significant effect of silencing specific candidate genes ($F = 35.38$ on 5 and 21 df, $P < 0.001$). The number of replicate spore samples (*n*) and the *P*-value for the post-hoc two-tailed *t*-test for comparison of *TaWAKL4*- and *TaWAKL3*- silenced plants to those treated with a negative control BSMV::*mcs4D* are shown. The means on the natural $\log(\text{Count} + 1)$ scale for statistical comparisons were 0.52 (BSMV::*mcs4D*), 4.13, 4.06 and 5.81 (BSMV::*TaWAKL4* a, b, and c), and 0.00 (BSMV::*TaWAKL3* a and b) with residual variance 0.7433 on 21 df for calculation of standard errors of the difference between means. In each box-and-whisker plot, the center values (in red) are the medians. The bottom and top edges of the boxes indicate the 25th and 75th percentiles. Whiskers mark the range of the data from 10th to 90th percentiles, and black dots indicate data points that lie outside of this interval. *** $P < 0.001$; NS, not significant.

Supplementary Figure 8. Transgenic wheat plants stably expressing *TaWAKL4/Stb6* show specific resistance to *Z. tritici* isolate IPO323 carrying *AvrStb6*.



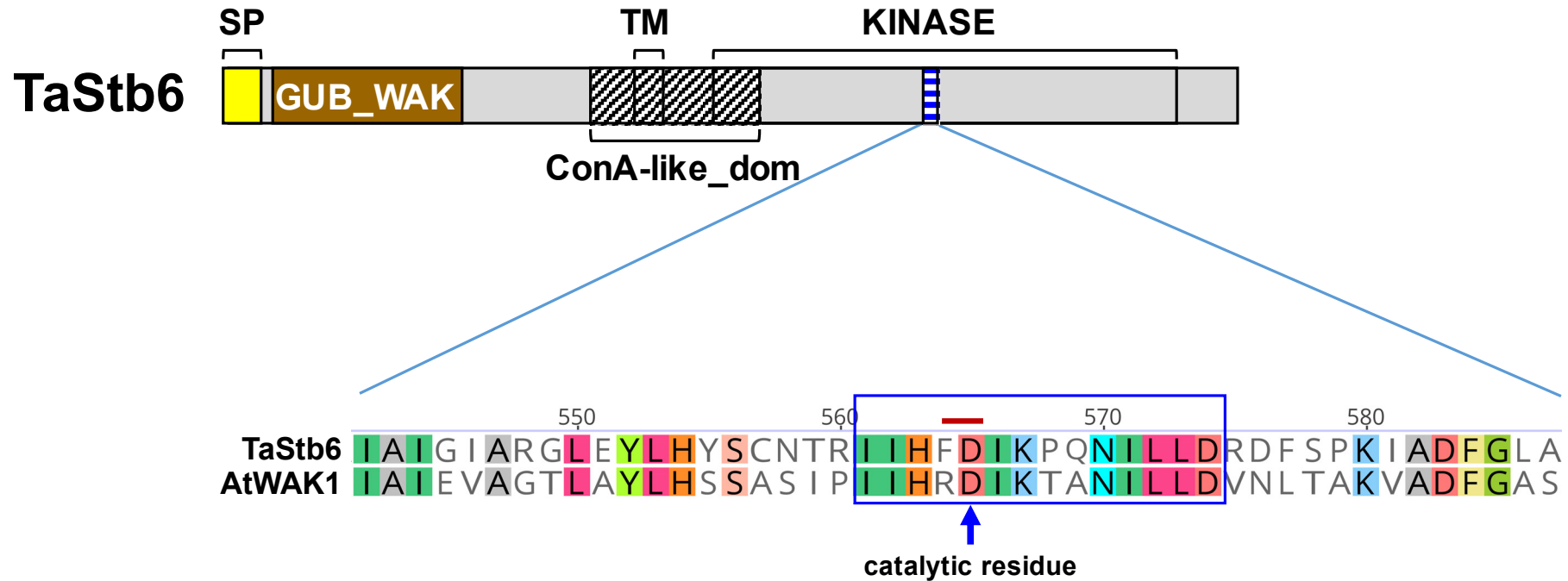
Progeny from Bobwhite wheat T₁ plants carrying *TaWAKL4* expression constructs #2 or #3 (see Fig. 4a for detail of constructs) were tested for resistance to *Z. tritici* isolates that are either avirulent (IPO323) or virulent (RRes116, IPO88004) on *Stb6*-containing wheat varieties. Images taken at 22 days post-fungal inoculation. Note: Bobwhite wheat is fully susceptible to all three *Z. tritici* isolates, whereas Chinese Spring (*Stb6*) and *TaWAKL4* expressing Bobwhite transgenic plants are susceptible only to *Z. tritici* isolates RRes116 and IPO88004 carrying alternative alleles of the matching effector *AvrStb6*.

Supplementary Figure 9. Expression analysis of *TaWAKL4 / Stb6* in 5 different tissues and at different growth stages* in healthy wheat Chinese Spring.



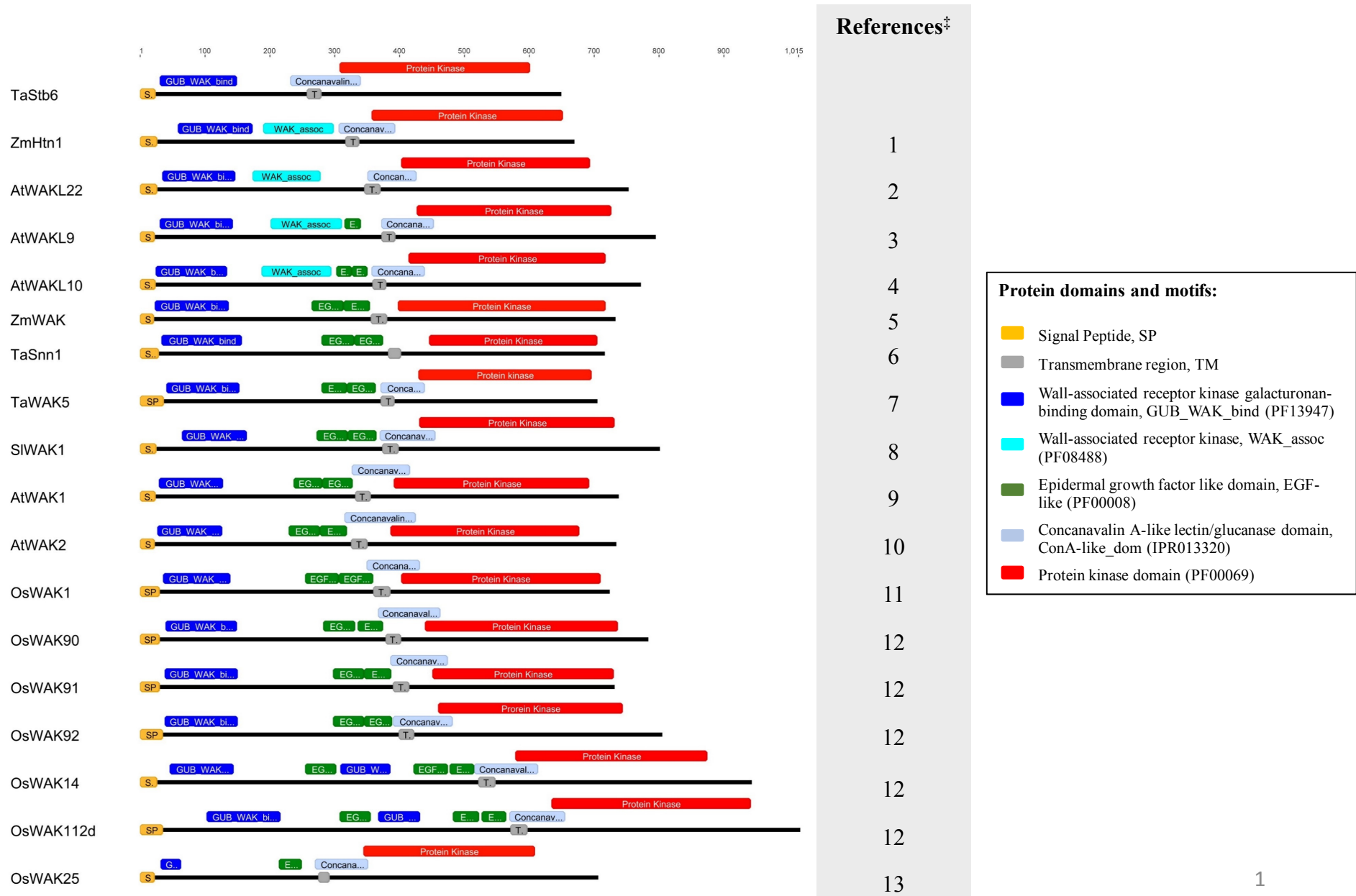
Fragments per kilobase of exon per million fragments mapped (FPKM) values obtained from the RNA-sequencing of healthy (uninfected) wheat tissues at different growth stages [Pingault, L. *et al.* Deep transcriptome sequencing provides new insights into the structural and functional organization of the wheat genome. *Genome Biol.* **16**, 29 (2015)] for the *TaWAKL4 / Stb6* transcript are shown on the Y-axis. FPKM values for the two biological replicates (experiments) of each sample are shown. Statistical analyses using ANOVA indicated overall significant differences in *TaWAKL4* expression in the leaves between different growth stages ($F = 459.11$ on 2 and 3 df (degrees of freedom), $P < 0.001$). The \log_e transformed means for the growth stages were -0.693 (Z10), 0.449 (Z23) and 1.526 (Z71); SED (the standard error of the difference between means) = 0.0732. Two-tailed post-hoc *t*-tests indicate a significant increase in *TaWAKL4* expression ($P < 0.001$) between growth stages Z10 and Z23, and between Z23 and Z71 ($P < 0.001$). Growth stages (Z10, Z13, Z23, Z30, Z32, Z39, Z65, Z71, Z75 and Z85) are defined as in Zadoks *et al.* [Zadoks, J.C., Chang, T.T. & Konzak, C.F. A decimal code for the growth stages of cereals. *Weed Res.* **14**, 415-421 (1974)].

Supplementary Figure 10. *Stb6* encodes a wall associated kinase (WAK)-like receptor protein.



Stb6 is a 647 amino acid long protein with the domain architecture similar to that of WAK proteins. Position of a signal peptide (SP), a galacturonan-binding domain (GUB_WAK), a transmembrane region (TM), a concanavalin A-like domain (ConA-like_dom), and a protein kinase domain are shown in the upper part of the figure. Similarly to most of the plant PRRs characterized to date, *Stb6* falls into the non-arginine-aspartate (non-RD) class of kinases and contains a phenylalanine (F) instead of an arginine (R) next to the catalytic asparagine (D) in the protein's active site (boxed blue in the partial alignment between *Stb6* and the typical RD-kinase WAK1 (UniProtKB accession Q39191) from *Arabidopsis thaliana* shown in the lower part of the figure).

Supplementary Figure 11. Deduced domains architecture of Stb6 compared to that of other WAK-like proteins* implicated in pathogen defense.



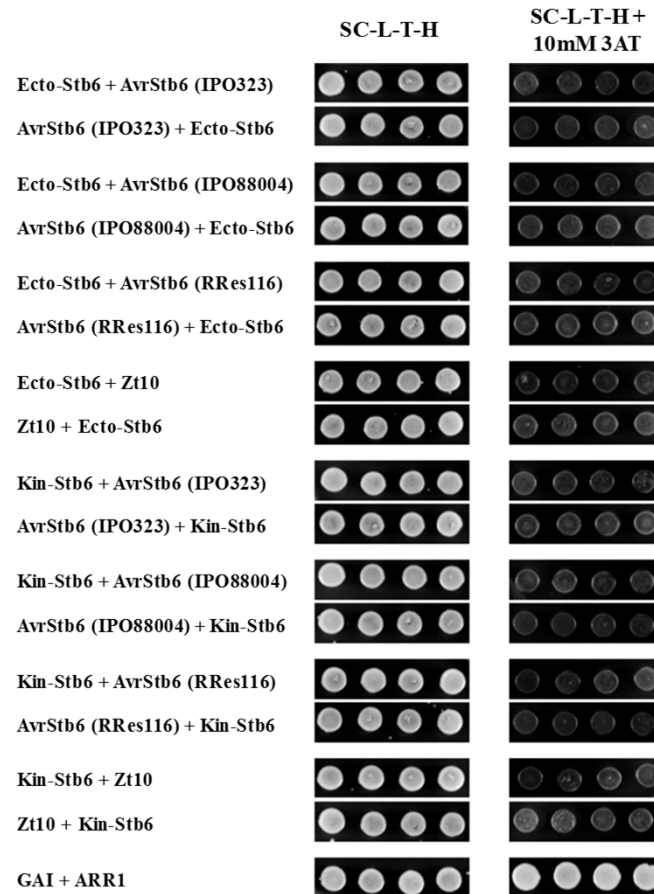
***UniProtKB or NCBI accession numbers for the WAK-like proteins analyzed:**

AtWAK1 (Q39191), AtWAK2 (Q9LMP1), AtWAKL9 (Q9C9L5), AtWAKL10 (Q8VYA3), AtWAKL22 (Q8RY17), SIWAK1 (K4CRU8), TaSnn1 (W5AB81), TaWAK5 (AIK66959), OsWAK1 (Q9AXH6), OsWAK14 (XP_015625923), OsWAK25 (Q8H7R6), OsWAK90 (Q653D0), OsWAK91 (Q653C9), OsWAK92 (Q0IZL6), OsWAK112d (Q33AH2), ZmHtn1/ZmWAK-RLK1 (A0A0H4NTK8), and ZmWAK/qHSR1 (A0A0A7EQ13).

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Supplementary Figure 12. No specific interaction between Stb6 and AvrStb6 proteins can be detected with the yeast two-hybrid system.



Ectodomain and kinase domains of Stb6 (Ecto-Stb6 and Kin-Stb6, respectively) were cloned as bait or prey constructs to allow the forward two-hybrid assay two-ways (minimising identification of false interactions) with AvrStb6 from an avirulent *Z. tritici* isolate IPO323 or from virulent isolates IPO88004 or RRes116. Protein-protein interactions were assessed by spotting four independent transformants, containing the corresponding bait and prey construct pair, onto a synthetic complete dropout media lacking leucine, tryptophan and histidine (SC-L-T-H) and onto a similar media supplemented with 10 to 100 mM 3-Amino-1,2,4-Triazole (3AT; the *HIS3* reporter gene inhibitor). Absence of yeast growth even on the lowest concentration (10 mM) of 3AT indicates the absence of interaction between the two tested proteins. Zt10 (EnsemblFungi accession number Mycgr3P111505) effector protein¹ of *Z. tritici* was used as a negative control. Yeast transformants containing *Arabidopsis thaliana* GAI (bait) and ARR1 (prey) proteins² were used as a positive protein-protein interaction control.

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Supplementary Table 1. Calculated means and standard errors of *Z. tritici* isolate IPO323 pycnidial coverage scores for leaves of silenced and non-silenced wheat Chinese Spring plants.

Virus construct	Pycnidial coverage classes											
	0 %		1-20 %		21-40 %		41-60 %		61-80 %		81-100 %	
	Mean ^a	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
BSMV: <i>mcs4D</i>	9.235	1.0372	0.504	0.2334	0.168	0.1346	0.000	0.0010	0.000	0.0010	0.000	0.0010
BSMV: <i>asTaWAKL4a</i>	2.503	0.6103	2.730	0.6380	1.138	0.4092	0.910	0.3657	0.455	0.2581	0.455	0.2581
BSMV: <i>asTaWAKL4b</i>	3.086	0.8282	1.714	0.6158	1.029	0.4764	1.029	0.4764	0.686	0.3888	1.029	0.4764
BSMV: <i>asTaWAKL4c</i>	2.381	0.6529	2.911	0.726	1.852	0.5725	1.058	0.4289	0.794	0.3703	0.529	0.3015
BSMV: <i>asTaWAKL3a</i>	9.012	1.4398	0.000	0.0013	0.000	0.0013	0.000	0.0013	0.000	0.0013	0.000	0.0013
BSMV: <i>asTaWAKL3b</i>	10.00	1.3093	0.000	0.0012	0.000	0.0012	0.000	0.0012	0.000	0.0012	0.000	0.0012

^aMean values represent the mean number of leaves within each pycnidial coverage class calculated using data from three independent experiments. Between eight and ten leaves per BSMV VIGS construct were scored in each experiment.

Supplementary Table 2. Calculated means and standard errors of *Z. tritici* isolate IPO323 pycnidial coverage scores for leaves of silenced and non-silenced wheat Cadenza plants.

Virus construct	Pycnidial coverage classes											
	0 %		1-20 %		21-40 %		41-60 %		61-80 %		81-100 %	
	Mean ^a	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
BSMV: <i>mcs4D</i>	9.704	1.2243	0.000	0.0007	0.249	0.1938	0.000	0.0007	0.000	0.0007	0.000	0.0007
BSMV: <i>asTaWAKL4a</i>	3.948	1.0885	3.455	1.0180	1.481	0.6659	0.987	0.5436	0.000	0.0010	0.494	0.3843
BSMV: <i>asTaWAKL4b</i>	2.994	0.7779	1.331	0.5183	1.663	0.5795	1.331	0.5183	0.998	0.4488	1.331	0.5183
BSMV: <i>asTaWAKL4c</i>	2.931	0.9393	2.442	0.8563	1.954	0.7648	0.977	0.5393	0.977	0.5393	0.488	0.3808
BSMV: <i>asTaWAKL3a</i>	9.806	1.4265	0.000	0.0008	0.000	0.0008	0.000	0.0008	0.000	0.0008	0.000	0.0008
BSMV: <i>asTaWAKL3b</i>	8.538	1.3879	0.000	0.0008	0.000	0.0008	0.000	0.0008	0.000	0.0008	0.000	0.0008

^aMean values represent the mean number of leaves within each pycnidial coverage class calculated using data from three independent experiments. Between eight and ten leaves per BSMV VIGS construct were scored in each experiment.

Supplementary Table 3. *TaWAKL3* TILLING mutants.

Line	Library	Mutation	Type	AA change	Confirmed[‡]	Phenotype
Cadenza1339	LIB16585	G1218A	nonsense	W406*	yes	R
Cadenza0798	LIB15427	G1249A	missense	A417T	yes	R
Cadenza1247	LIB16255	G1364A	missense	G455D	yes	R
Cadenza1972	LIB17351	G1369A	missense	A457T	yes	R
Cadenza0540	LIB16590	G1433A	missense	G478E	yes	R
Cadenza0773	LIB15417	C1444T	missense	P482S	yes	R
Cadenza0641	LIB11431	G1447A	missense	E483K	no	R
Cadenza0327	LIB10623	G1469A	missense	G490E	yes	R
Cadenza0256	LIB09953	G1489A	missense	D497N	yes	R
Cadenza1692	LIB11270	G1685A	nonsense	W562*	yes	R
Cadenza1068	LIB15968	C1703T	missense	P568L	yes	R

[‡]mutation confirmed by targeted sequencing; R – line shows resistance to *Z. tritici* IPO323

Supplementary Table 4. *TaWAKL4* TILLING mutants.

Line	Library	Mutation	Type	AA change	Confirmed [‡]	Phenotype
Cadenza0178	LIB10954	C184T	missense	P62S	nd	R
Cadenza1519	LIB10271	C263T	missense	A88V	nd	R
Cadenza0188	LIB10919	G299A	missense	R100H	nd	R
Cadenza1051	LIB16127	G371A	nonsense	W124*	yes	S
Cadenza1478	LIB10228	C458T	missense	A153V	nd	R
Cadenza1711	LIB11232	G460A	missense	A154T	nd	R
Cadenza0281	LIB9963	G521A	missense	S174N	nd	R
Cadenza1757	LIB010433	G785A	missense	G262D[#]	yes	S
Cadenza0662	LIB11409	C811T	missense	L271F	nd	R
Cadenza1070	LIB15889	G1007A	missense	G336D	yes	S
Cadenza0861	LIB15456	G1016A	missense	G339D	yes	S
Cadenza1495	LIB10269	G1160A	missense	G387E	yes	S
Cadenza1466	LIB10227	G1186A	missense	A396T	nd	R
Cadenza0784	LIB15410	G1297A	missense	A433T	yes	S
Cadenza1028	LIB16133	C1418T	missense	A473V	yes	S
Cadenza1664	LIB11236	C1433T	missense	T478I	nd	R
Cadenza0352	LIB10633	G1442A	missense	S481N	nd	R
Cadenza0642	LIB11439	C1463T	missense	A488V	nd	R
Cadenza0449	LIB10948	G1564A	missense	E522K	yes	S
Cadenza1358	LIB8413	G1577A	missense	G526E	nd	R
Cadenza1434	LIB8422	G1669A	missense	E557K	nd	R
Cadenza1805	LIB10437	G1709A	missense	R570K	nd	R
Cadenza0376	LIB10635	G1858A	missense	A620T	nd	R
Cadenza0105	LIB10920	G1895A	missense	R632K	nd	R
Cadenza1749	LIB10464	G1915A	missense	A639T	nd	R
Cadenza1488	LIB10308	C1916T	missense	N640V	nd	R
Cadenza1738	LIB10471	C1933T	nonsense	Q645*	nd	R
Cadenza1411	LIB8426	A1943G	missense	Q646R	nd	R

[#]potential splice site mutation

[‡]mutation confirmed by targeted sequencing

R / S – line shows resistance /susceptibility to *Z. tritici* IPO323

nd – no data; mutations in lines resistant to *Z. tritici* IPO323 were not subjected to targeted sequencing

Supplementary Table 5. Wheat accessions used for exons resequencing and identified *Stb6* haplotypes.

Species	Accession name	Notes	Origin	Provider / Acc. Numbers	<i>Stb6</i> haplotype
<i>T. aestivum</i>	Folklor	Cultivar	France	Agri-Obtentions	1
<i>T. aestivum</i>	Koreli	Cultivar	UK	Agri-Obtentions	1
<i>T. aestivum</i>	Armada	Cultivar	UK	BRC / 1008	1
<i>T. aestivum</i>	Atlas 66	Cultivar	USA	BRC / 1072	1
<i>T. aestivum</i>	Aurore	Old cultivar	Australia	BRC / 1110	1
<i>T. aestivum</i>	Balkan	Cultivar	former Yugoslavia	BRC / 1192	1
<i>T. aestivum</i>	Barbu du Finistere	Old cultivar	France	BRC / 1232	1
<i>T. aestivum</i>	Belliei 590	Old cultivar	Hungary	BRC / 1288	1
<i>T. aestivum</i>	Gene	Cultivar	USA	BRC / 13147	1
<i>T. aestivum</i>	Fruh-Weizen	Old cultivar	Germany	BRC / 13310	1
<i>T. aestivum</i>	Opata 85	Breeding line	Mexico	BRC / 13811	1
<i>T. aestivum</i>	Ble Seigle	Old cultivar	France	BRC / 1515	1
<i>T. aestivum</i>	Chinese Spring	Landrace	China	BRC / 2135	1
<i>T. aestivum</i>	Chyamtang	Old cultivar	Nepal	BRC / 2171	1
<i>T. aestivum</i>	RE 99006	Breeding line	France	BRC / 22563	1
<i>T. aestivum</i>	Senat	Cultivar	Denmark	BRC / 22974	1
<i>T. aestivum</i>	Cotipora	Cultivar	Brazil	BRC / 2353	1
<i>T. aestivum</i>	Poros	Cultivar	Germany	BRC / 24237	1
<i>T. aestivum</i>	Fielder	Cultivar	USA	BRC / 3026	1
<i>T. aestivum</i>	USU Apogee	Cultivar	USA	BRC / 31948	1
<i>T. aestivum</i>	Glenlea-Can	Cultivar	Canada	BRC / 3358	1
<i>T. aestivum</i>	Godolloi 15	Old cultivar	Hungary	BRC / 3366	1
<i>T. aestivum</i>	Flame	Cultivar	UK	BRC / 34231	1
<i>T. aestivum</i>	Heines-Kolben	Cultivar	Germany	BRC / 3562	1
<i>T. aestivum</i>	Hereward	Cultivar	UK	BRC / 3572	1
<i>T. aestivum</i>	Bulgaria 88	Cultivar	Bulgaria	BRC / 37164	1
<i>T. aestivum</i>	Israel 493	Cultivar	Israel	BRC / 37166	1
<i>T. aestivum</i>	Tadinia	Cultivar	USA	BRC / 37167	1
<i>T. aestivum</i>	Shafir	Cultivar	Israel	BRC / 37169	1
<i>T. aestivum</i>	Kavlaz K4500 L.6.A.4	Breeding line	Mexico	BRC / 37173	1
<i>T. aestivum</i>	TE 9111	Breeding line	Portugal	BRC / 37174	1
<i>T. aestivum</i>	Arina	Cultivar	UK	BRC / 37176	1
<i>T. aestivum</i>	Mars de Suède rouge barbu	Old cultivar	Sweden	BRC / 4645	1
<i>T. aestivum</i>	Miskaagani	Cultivar	Lebanon	BRC / 4874	1
<i>T. aestivum</i>	Mocho de Espiga Branca	Old cultivar	Portugal	BRC / 4901	1
<i>T. aestivum</i>	Nepal 84	Old cultivar	Nepal	BRC / 5166	1
<i>T. aestivum</i>	Opal	Old cultivar	Germany	BRC / 5486	1
<i>T. aestivum</i>	Pitic 62	Cultivar	Mexico	BRC / 5748	1
<i>T. aestivum</i>	Renan	Cultivar	France	BRC / 6086	1
<i>T. aestivum</i>	Vivant	Cultivar	UK	BRC / 7399	1
<i>T. aestivum</i>	Zanda	Old cultivar	Belgium	BRC / 8058	1
<i>T. aestivum</i>	Cadenza	Cultivar	UK	JIC / W9368	1
<i>T. aestivum</i>	Raffles	Cultivar	UK	JIC / W9908	1
<i>T. aestivum</i>	KWS Crispin	Cultivar	UK	KWS UK	1
<i>T. aestivum</i>	KWS Lili	Cultivar	UK	KWS UK	1
<i>T. aestivum</i>	KWS Santiago	Cultivar	UK	KWS UK	1
<i>T. aestivum</i>	KWS Siskin	Cultivar	UK	KWS UK	1
<i>T. aestivum</i>	Oakley	Cultivar	UK	KWS UK	1
<i>T. aestivum</i>	Robigus	Cultivar	UK	KWS UK	1
<i>T. aestivum</i>	Viscount	Cultivar	UK	KWS UK	1
<i>T. aestivum</i>	Qplus	Cultivar	UK	Limagrain	1
<i>T. aestivum</i>	Claire	Cultivar	UK	Limagrain	1
<i>T. aestivum</i>	Evolution	Cultivar	UK	Limagrain	1
<i>T. aestivum</i>	Panacea	Cultivar	UK	Limagrain	1
<i>T. aestivum</i>	Smuggler	Cultivar	UK	Limagrain	1
<i>T. aestivum</i>	Solstice	Cultivar	UK	Limagrain	1
<i>T. aestivum</i>	Boregar	Cultivar	France	RAGT Seeds	1
<i>T. aestivum</i>	RGT Illustrious	Cultivar	UK	RAGT Seeds	1
<i>T. aestivum</i>	Cougar	Cultivar	UK	RAGT Seeds	1
<i>T. aestivum</i>	Tuxedo	Cultivar	UK	RAGT Seeds	1

<i>T. aestivum</i>	Warrior	Cultivar	UK	RAGT Seeds	1
<i>T. aestivum</i>	Trapez	Cultivar	France	Saatzucht Josef Breun	1
<i>T. aestivum</i>	Duxford	Cultivar	UK	Syngenta	1
<i>T. dicoccum</i>	45383	Wild	Bulgaria	BRC / 33758	1
<i>T. dicoccum</i>	45239	Wild	Italy	BRC / 33760	1
<i>T. dicoccum</i>	45280	Wild	Slovakia	BRC / 33762	1
<i>T. dicoccum</i>	45309	Wild	Slovakia	BRC / 33763	1
<i>T. dicoccum</i>	355484	Landrace	Spain	BRC / 33765	1
<i>T. durum</i>	Primadur	Cultivar	France	BRC / 14063	1
<i>T. durum</i>	82726	Landrace	Turkey	BRC / 33799	1
<i>T. durum</i>	B6Rtchir	Landrace	Bulgaria	BRC / 33803	1
<i>T. aestivum</i>	Skyfall	Cultivar	UK	RAGT Seeds	2
<i>T. aestivum</i>	Balance	Cultivar	France	Syngenta	2
<i>T. aestivum</i>	Ornicar	Cultivar	France	BRC / 13471	3
<i>T. aestivum</i>	Camp Remy	Cultivar	France	BRC / 1743	3
<i>T. aestivum</i>	Courtot	Cultivar	France	BRC / 37172	3
<i>T. aestivum</i>	Recital	Cultivar	France	BRC / 6027	3
<i>T. aestivum</i>	Soissons	Cultivar	France	BRC / 6607	3
<i>T. aestivum</i>	Aifeng No. 4	Cultivar	China	BRC / 822	3
<i>T. aestivum</i>	Avalon	Cultivar	UK	JIC / W2564	3
<i>T. aestivum</i>	Apache	Cultivar	France	Limagrain	3
<i>T. aestivum</i>	Stigg	Cultivar	UK	Limagrain	3
<i>T. aestivum</i>	Coppadra	Cultivar	Turkey	BRC / 2330	4
<i>T. aestivum</i>	Veranopolis	Cultivar	Brazil	BRC / 37165	5
<i>T. dicoccoides</i>	DD.Pseudo-Jordanic.61V	Wild	Czech Republic	BRC / 26676	5
<i>T. dicoccoides</i>	DD.100V	Wild	Hungary	BRC / 26677	5
<i>T. polonicum</i>	330554	Landrace	Cyprus	BRC / 33812	5
<i>T. polonicum</i>	14140	Landrace	NA	BRC / 33817	5
<i>T. boeoticum</i>	BO.D542 Khorramabad 1040V	Wild	Iran	BRC / 26605	6
<i>T. aestivum</i>	Arche	Cultivar	France	BRC / 964	7
<i>T. aestivum</i>	Isengrain	Cultivar	UK	BRC / 13433	7
<i>T. aestivum</i>	Valoris	Cultivar	France	BRC / 13871	7
<i>T. aestivum</i>	Ble de Redon Blanc 1/2 Lache 1 1	Old cultivar	France	BRC / 15658	7
<i>T. aestivum</i>	CF 99007	Breeding line	France	BRC / 32316	7
<i>T. aestivum</i>	Obelisk	cultivar	Netherlands	BRC / 37162	7
<i>T. aestivum</i>	Riband	Cultivar	UK	BRC / 37177	7
<i>T. aestivum</i>	Longbow	Cultivar	UK	BRC / 4340	7
<i>T. aestivum</i>	Cordiale	Cultivar	UK	KWS UK	7
<i>T. aestivum</i>	Grafton	Cultivar	UK	KWS UK	7
<i>T. aestivum</i>	Alchemy	Cultivar	UK	Limagrain	7
<i>T. aestivum</i>	Revelation	Cultivar	UK	Limagrain	7
<i>T. aestivum</i>	Crusoe	Cultivar	UK	Limagrain	7
<i>T. aestivum</i>	Gatsby	Cultivar	UK	Limagrain	7
<i>T. aestivum</i>	Gravitas	Cultivar	UK	Limagrain	7
<i>T. aestivum</i>	Belgrade	Cultivar	UK	Saaten Union UK	7
<i>T. aestivum</i>	Timber	Cultivar	UK	Saaten Union UK	7
<i>T. aestivum</i>	JB Diego	Cultivar	UK	Senova	7
<i>T. aestivum</i>	Spyder	Cultivar	UK	Senova	7
<i>T. aestivum</i>	Reflection	Cultivar	UK	Syngenta	7
<i>T. durum</i>	Karim 80	Cultivar	Tunisia	BRC / 31589	7
<i>T. durum</i>	Kronos	Cultivar	USA	Cristobal Uauy, JIC	7
<i>T. durum</i>	Ofanto	Cultivar	Italy	Huw Jones, RRes	7
<i>T. durum</i>	Svevo	Cultivar	Italy	Huw Jones, RRes	7
<i>T. durum</i>	Luminur	Cultivar	France	RAGT Seeds	7
<i>T. dicoccoides</i>	45963	Wild	Jordan	BRC / 33770	8
<i>T. urartu</i>	UR.G3143	Landrace	Lebanon	BRC / 27037	9
<i>T. dicoccoides</i>	117887	Wild	Syria	BRC / 33772	10
<i>T. urartu</i>	G1812	Wild	Lebanon	NPGS / PI428198	10
<i>T. urartu</i>	UR.G1939	Landrace	Turkey	BRC / 27035	11
<i>T. aestivum</i>	M708//G25/N163	Breeding line	Israel	BRC / 4482	12
<i>T. durum</i>	82715	Landrace	Turkey	BRC / 33800	12
<i>T. durum</i>	84866	Landrace	Syria	BRC / 33796	13
<i>T. durum</i>	Brumaire	Old cultivar	France	BRC / 33801	13

<i>T. durum</i>	Durental	Cultivar	France	BRC / 33802	13
<i>T. turgidum</i>	341300	Landrace	Turkey	BRC / 33819	13
<i>T. turgidum</i>	Canoco	Landrace	Portugal	BRC / 33820	13
<i>T. aestivum</i>	Ralet	Old cultivar	France	BRC / 8048	14
<i>T. dicoccoides</i>	487255	Wild	Syria	BRC / 33779	15
<i>T. monococcum</i>	DV92	Cultivar	USA	BRC / 23861	16
<i>T. boeoticum</i>	<i>Triticum boeoticum</i> d'Arménie	Wild	Armenia	BRC / 23733	17

BRC = Biological resource centre on Small Grain Cereals (INRA, France)

JIC = SeedStor, The Germplasm Resources Unit, John Innes Centre (Norwich, UK)

NPGS = National Plant Germplasm System (USDA, USA)

Supplementary Table 6. *Stb6* haplotypes identified in A genome-containing wheat species.

Haplotype number	Species [‡]	Total Samples	Phenotype
1	Ta (63), Tdc (5), Td (3)	71	R
2	Ta (2)	2	R
3	Ta (9)	9	S
4	Ta (1)	1	unknown
5	Ta (1), Tdd (2), Tp (2)	5	R
6	Tb (1)	1	unknown
7	Ta (20), Td (5)	25	S
8	Tdd (1)	1	unknown
9	Tu (1)	1	unknown
10	Tdd (1), Tu (1)	2	unknown
11	Tu (1)	1	unknown
12	Ta (1), Td (1)	2	unknown
13	Td (3), Tt (2)	5	unknown
14	Ta (1)	1	unknown
15	Tdd (1)	1	unknown
16	Tm (1)	1	unknown
17	Tb (1)	1	unknown

[‡]Numbers of accessions containing the same haplotype are shown in brackets following the abbreviated species names

Abbreviations of species names:

Ta - *Triticum aestivum* (A^uBD)

Tb - *Triticum boeoticum* (A^b)

Td - *Triticum durum* (A^uB)

Tdc - *Triticum dicoccum* (A^uB)

Tdd - *Triticum dicoccoides* (A^uB)

Tm - *Triticum monococcum* (A^b)

Tp - *Triticum polonicum* (A^uB)

Tt - *Triticum turgidum* (A^uB)

Tu - *Triticum urartu* (A^u)

Supplementary Table 7. *Stb6* haplotypes identified in the current UK winter wheat varieties showing average STB disease resistance rating >5.5*.

Variety	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	AVERAGE	<i>Stb6</i> haplotype [‡]
Stigg	-	-	-	-	-	-	-	7.7	7	-	-	-	-	7.4	3 (S)
Cougar	-	-	-	-	-	-	-	-	-	7	7	7	-	7.0	1 (R)
Graham	-	-	-	-	-	-	-	-	-	-	-	-	7	7.0	1 (R)
KWS Siskin	-	-	-	-	-	-	-	-	-	-	-	-	7	7.0	1 (R)
Smuggler	7	7	-	-	-	-	-	-	-	-	-	-	-	7.0	1 (R)
Timber	-	-	-	7	6.9	7	-	-	-	-	-	-	-	7.0	7 (S)
Gatsby	-	-	7	7	6.9	6.9	-	-	-	-	-	-	-	7.0	7 (S)
Warrior	-	-	-	-	-	-	7.1	7.2	6.5	-	-	-	-	6.9	1 (R)
Robigus	7	7	7	6	6.5	6.2	6.1	6	-	-	-	-	-	6.5	1 (R)
Alchemy	-	-	7	7	6.4	6.7	6.7	6.8	5.9	5.9	5.8	5.9	-	6.4	7 (S)
Crusoe	-	-	-	-	-	-	-	-	6.5	6.7	6.3	6.2	6	6.3	7 (S)
Gravitas	-	-	-	-	-	-	-	6.9	6.1	5.9	-	-	-	6.3	7 (S)
Panacea	-	-	-	-	-	-	-	-	-	-	6.4	6	-	6.2	1 (R)
Revelation	-	-	-	-	-	-	-	-	-	6.2	6.2	6.4	6	6.2	7 (S)
Qplus	-	-	-	-	-	6	6.1	-	-	-	-	-	-	6.1	1 (R)
Belgrade	-	-	-	-	-	-	-	-	-	-	-	-	6	6.0	7 (S)
KWS Crispin	-	-	-	-	-	-	-	-	-	-	-	-	6	6.0	1 (R)
RGT Illustrious	-	-	-	-	-	-	-	-	-	-	-	-	6	6.0	1 (R)
Skyfall	-	-	-	-	-	-	-	-	-	-	6	6	6	6.0	7 (S)
Spyder	-	-	-	-	-	-	-	-	-	-	-	-	6	6.0	2 (R)
Tuxedo	-	-	-	-	-	-	-	6.5	5.9	5.9	5.8	5.8	-	6.0	1 (R)
KWS Lili	-	-	-	-	-	-	-	-	-	-	-	5.9	6	6.0	1 (R)
Evolution	-	-	-	-	-	-	-	-	-	-	6	5.5	6	5.8	1 (R)
Claire	6	6	6	6	5.7	5.9	5.9	6	5.2	5.5	5.2	5.3	5	5.7	1 (R)
Hereward	6	6	5	6	5.3	5.4	5.4	-	-	-	-	-	-	5.6	1 (R)

* On the 1-9 scales, high figures indicate that a variety shows higher resistance. Data from the AHDB web site: <https://cereals.ahdb.org.uk/varieties/ahdb-recommended-lists.aspx>.

[‡]Text in brackets indicates whether this is a resistance (R) or a susceptibility (S) haplotype (with respect to *Z. tritici* IPO323)

Supplementary Table 8. *Stb6* haplotype identified in the most popular* current UK wheat varieties.

variety	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	max in one year	average per year	<i>Stb6</i> haplotype [‡]
Jb Diego	-	-	1.2	3.7	8.6	12.0	14.4	16.6	17.9	14.8	17.9	11.2	7 (S)
Solstice	9.2	10.6	9.9	10.4	9.1	7.0	6.0	3.3	1.5	1.6	10.6	6.9	1 (R)
KWS Santiago	-	-	-	0.0	0.3	10.0	14.4	10.8	7.5	4.2	14.4	6.7	1 (R)
Alchemy	17.5	15.9	11.1	9.2	5.9	4.0	0.0	1.8	1.1	0.5	17.5	6.7	7 (S)
Oakley	0.2	6.6	14.1	15.0	13.2	10.0	3.5	0.6	0.1	0.1	15	6.3	1 (R)
Einstein	15.8	12.5	10.3	6.2	3.2	1.4	0.3	0.2	0.0	-	15.8	5.5	unknown
Cordiale	3.9	4.3	5.7	7.5	6.0	5.0	5.0	4.0	3.9	2.9	7.5	4.8	7 (S)
Skyfall	-	-	-	-	-	-	0.0	0.2	7.2	10.7	10.7	4.5	2 (R)
Crusoe	-	-	-	-	-	0.5	1.5	4.4	7.3	8.2	8.2	4.4	7 (S)
Robigus	13.0	7.8	3.6	2.0	1.2	0.5	0.1	-	-	-	13	4.0	1 (R)
Grafton	-	-	0.1	3.1	6.1	5.0	5.9	4.8	3.6	2.8	6.1	3.9	7 (S)
Revelation	-	-	-	-	-	-	0.0	1.9	6.6	7.0	7	3.9	7 (S)
Reflection	-	-	-	-	-	-	-	0.0	0.4	10.4	10.4	3.6	7 (S)
Claire	6.5	5.0	5.3	4.7	3.5	3.0	2.4	2.3	1.7	1.2	6.5	3.6	1 (R)
Viscount	-	0.1	5.7	8.1	6.4	3.0	2.6	3.0	1.9	0.5	8.1	3.5	1 (R)
Gladiator	7.5	6.9	3.8	1.6	0.4	0.1	-	-	-	-	7.5	3.4	unknown
Duxford	-	0.3	3.8	5.8	6.9	6.0	3.4	0.6	0.1	-	6.9	3.4	1 (R)
Evolution	-	-	-	-	-	-	0.0	0.3	5.9	6.1	6.1	3.1	1 (R)
Humber	0.2	6.0	7.1	3.1	1.5	0.1	-	-	-	-	7.1	3.0	unknown
Gallant	-	0.0	0.1	3.3	3.9	6.0	5.9	3.7	2.3	1.2	6	2.9	unknown
Invicta	-	-	-	0.3	3.8	6.0	4.6	3.2	1.2	1.0	6	2.9	unknown
Leeds	-	-	-	-	-	-	0.1	3.5	4.4	3.3	4.4	2.8	unknown
Relay	-	-	-	-	0.0	0.5	3.1	5.4	4.2	3.1	5.4	2.7	unknown
Scout	-	0.0	0.1	2.8	5.3	5.0	3.9	2.7	1.9	1.9	5.3	2.6	unknown
KWS Lili	-	-	-	-	-	-	-	-	0.3	4.8	4.8	2.5	1 (R)
KWS Kielder	-	-	-	-	-	0.0	0.3	5.5	1.7	1.8	5.5	1.9	unknown
Conqueror	-	-	-	0.1	1.6	3.0	3.4	2.4	0.8	0.1	3.4	1.6	unknown
Timber	0.2	2.7	1.2	-	-	-	-	-	-	-	2.7	1.4	7 (S)
Horatio	-	-	-	-	0.0	0.1	1.1	3.7	1.7	0.9	3.7	1.3	unknown
Glasgow	2.8	2.3	1.5	0.9	0.7	0.2	0.1	-	-	-	2.8	1.2	unknown
Hereward	2.7	2.0	1.5	1.4	1.2	0.7	0.4	0.1	0.1	0.1	2.7	1.0	1 (R)
Zebedee	1.8	2.3	1.2	0.6	0.3	0.1	0.0	-	-	-	2.3	0.9	unknown

* Based on the market share figures over the 10 years from 2006 to 2015

[‡]Text in brackets indicates whether this is a resistance (R) or a susceptibility (S) haplotype (with respect to *Z. tritici* IPO323)

Supplementary Table 9. Primers used in this study.

Primer Name	Primer Sequence	Purpose
01864F1	ATTTTCGGAAGCACCCAGATT	WAK resequencing
01864R1	CGCCCTGTTGATCGGATGAG	WAK resequencing
04034F1	ATGGGAGAGGCTTACGGGAT	WAK resequencing
04034R1	CGCCTCCGCTCCATGATT	WAK resequencing
13609F1	CTGAAAAAATAACGAGGCCATGA	WAK resequencing/ <i>Stb6</i> re-sequencing
13609R1	GGCTTGTCTCCTCCGATCTTGT	WAK resequencing
13699F1	CCCGTTGCATGGTTCAGACTT	WAK resequencing
13699R1	TCGGCCAGACTTCTCATGTAAAC	WAK resequencing
32351_F1	TGGGGTCGATCACAAAATAA	WAK resequencing
32351_R1	TGGCCTCGTAATTCTTCTCAGT	WAK resequencing
6342_F1	AAGCGGTAGTCAAGTATTAT	WAK resequencing
6342_R1	TTGCAGAAAGAGATATGTAAACTA	WAK resequencing
32351F3	TGGCTAAACCGAAGATTGTGTG	WAK resequencing
32351R3	TTCCTTCTGCTTCTCATGTGATT	WAK resequencing
06777F2	AATGACGGGAGCTAGAGGAACA	WAK resequencing
06777R2	GCGTATCTCCTCGTATCCAGCA	WAK resequencing
13699F2	CGAGGTACGGTGAGCCACG	WAK resequencing
13699R2	CGGCTGCTGACAATGACTACA	WAK resequencing
20822F7	GATCGCCTCTGTGCGCTGA	WAK resequencing
20822F4	AAGCGCAGTAAAATTCGGCAT	WAK resequencing
03359F7	AGGATGTGCTCGGCGATGT	WAK resequencing
03359R7	TCTGCAGGGTGATGAATTGA	WAK resequencing
6342_F2	GGTTTGGTGCAGTGAGGAT	<i>TaWAKL5</i> resequencing
6342_R2	CACATGGCCTATCTGCTCAA	<i>TaWAKL5</i> resequencing
cfp5026F	CAAACCTTGGCAAGCCCTCAT	BAC library screening
cfp5026R	CAAATACAGGGAGGACGGAA	BAC library screening
27963F1	GCTCATTAGGATTTGGCTTGTTA	BAC library screening
27963F5	CAGCTGACTCAITTTATTTGCCTTA	<i>Stb6</i> re-sequencing
1186F1	GGAGTGGCAACGGGGTGAG	BAC library screening / <i>Stb6</i> re-sequencing
1186R1	GGGAATTGGGGCACTACC	BAC library screening / <i>Stb6</i> re-sequencing
1186R2	GAGCAAGCTTCAATTACAGGAG	<i>Stb6</i> re-sequencing
8311F3	CCGTTTAGCTCGTGTGTGC	<i>Stb6</i> re-sequencing
8311F13	TGGCCCATGATGCTGTAGAG	<i>Stb6</i> re-sequencing
8311F16	GCGACATGGTAGTCAATCAAA	<i>Stb6</i> re-sequencing
8311F19	CGCGGTTCCAGTCACATCAC	<i>Stb6</i> re-sequencing
8311R5	CTCGAAGTCCAGCGGTCCAG	<i>Stb6</i> re-sequencing
8311R9	CCAGTTCCTTTCCCGGTGT	<i>Stb6</i> re-sequencing
8311R16	TTCCTTCCATGGTCCGTAACCTT	<i>Stb6</i> re-sequencing
M13F	GTAAAACGACGGCCAGT	Plasmid inserts sequencing
M13R	CAGGAAACAGCTATGAC	Plasmid inserts sequencing
8311F12	GCGCGACCTTGAAAAACTAT	Amplification of <i>Stb6</i> genomic region
8311R12	CTGGACGGATGTGGCGAGT	Amplification of <i>Stb6</i> genomic region
8311F11	GATTACGCCAAGCTTTCGGCAGCCTGGATAAAGAA	3' RACE
8311R6	GATTACGCCAAGCTTTCGGCAGCCTGGATAAAGAA	5' RACE
ctg8311F3	CCGTTTAGCTCGTGTGTGC	Genotyping, co-dominant marker
ctg8311R4	GTTCACCCGTCGAATTCATCTT	Genotyping, co-dominant marker
cfa3006F	GGTTTGGTGGACTGCTACTCTGC	Genotyping, SSR marker
cfa3006R	ATCGTGCCCTACCTACTCAGAA	Genotyping, SSR marker
cfa3010F	CCATGGATGCACACCTCTGAAA	Genotyping, SSR marker
cfa3010R	GCCCCACACAACGAAAACGCA	Genotyping, SSR marker
cfa3036F	TCGGCTTATCATAITTTGGTCT	Genotyping, SSR marker
cfa3036R	CAAGGCAGATAGGTAGAGTGGTG	Genotyping, SSR marker
cfa3037F	TCTTTCGAGCATCCAATAATACT	Genotyping, SSR marker
cfa3037R	TCCGCTGAGTTCAATACCT	Genotyping, SSR marker
LIC as1186a F	AAGGAAAGTTAA CAAACGTGTTGCTTGTGTTAC	VIGS, <i>TaWAKL4/Stb6</i> specific constructs
LIC as1186a R	AACCACCACCACCGT AACAGGAAAGATTATGTGGTTCGG	VIGS, <i>TaWAKL4/Stb6</i> specific constructs
LIC as1186b F	AAGGAAAGTTAA TGGGTTTGGATGTCGAAATGGA	VIGS, <i>TaWAKL4/Stb6</i> specific constructs
LIC as1186b R	AACCACCACCACCGT AGGTGGTACGGTGTGGTTTTTC	VIGS, <i>TaWAKL4/Stb6</i> specific constructs
LIC as1186c F	AAGGAAAGTTAA AGGACGATGCAAGGGTATTACT	VIGS, <i>TaWAKL4/Stb6</i> specific constructs
LIC as1186c R	AACCACCACCACCGT GCACCAGAAGTTCACCTCTCGAA	VIGS, <i>TaWAKL4/Stb6</i> specific constructs
LIC as1050a F	AAGGAAAGTTAA CGTTCCTTCGTGCAATTGTA	VIGS, <i>TaWAKL3</i> specific constructs
LIC as1050a R	AACCACCACCACCGT CGGCAACCTGACCATCTCTAAT	VIGS, <i>TaWAKL3</i> specific constructs
LIC as1050b F	AAGGAAAGTTAA TGTATGCGACGACAAGTATGAC	VIGS, <i>TaWAKL3</i> specific constructs
LIC as1050b R	AACCACCACCACCGT CTGGAAATGGTAGGAGGAAGGA	VIGS, <i>TaWAKL3</i> specific constructs
RLK-1186 QPCR F	AGCAAGGGGATGGCTGCTT	qRT-PCR, quantification of <i>TaWAKL4/Stb6</i> transcript
RLK-1186 QPCR R	TTGCCAATCAGTGAGCCAGAAC	qRT-PCR, quantification of <i>TaWAKL4/Stb6</i> transcript
RLK-1050 QPCR F	CCTCGCAACCTGAATCTCATC	qRT-PCR, quantification of <i>TaWAKL3</i> transcript
RLK-1050 QPCR R	GTTCTGACCTCACTTGTCT	qRT-PCR, quantification of <i>TaWAKL3</i> transcript
CDC48 QPCR F2	GTCTCTGGCTGTGGTAAAAAC	qRT-PCR, quantification of <i>TaCDC48</i> transcript

CDC48 QPCR R2	AGCAGCTCAGGTCCTTGTATAC	qRT-PCR, quantification of <i>TaCDC48</i> transcript
pCR8/GW_Stb6F1	TTGCAACAAATTGATGAGCA	Detection of the <i>Stb6</i> transgene (construct 1) in T ₀ and T ₁ plants
Stb6_pCR8GW_R1	CTGACTAACCCCGTTTGTGA	Detection of the <i>Stb6</i> transgene (construct 1) in T ₀ and T ₁ plants
R-gene-fwd	GGAAAAGGTGGTTACGGTGT	Detection of the <i>Stb6</i> transgene (construct 2 and 3) in T ₀ and T ₁ plants
Nos5'rev	ATCGCAAGACCGCAACAGG	Detection of the <i>Stb6</i> transgene (construct 2 and 3) in T ₀ and T ₁ plants
UbiPro4	TTTAGCCCTGCCTTCATACG	Detection of the <i>Stb6</i> transgene (construct 3) in T ₀ and T ₁ plants
R-gene-rev	ACACCGTAACCACCTTTTCC	Detection of the <i>Stb6</i> transgene (construct 3) in T ₀ and T ₁ plants
bar1	GTCTGCACCATCGTCAACC	Detection of the <i>bar</i> ransgene (constructs 2 and 3) in T ₀ and T ₁ plants
bar2	GAAGTCCAGCTGCCAGAAAC	Detection of the <i>bar</i> ransgene (constructs 2 and 3) in T ₀ and T ₁ plants
Kin1186-attB1-F1	ggggACAAGTTTGTACAAAAAAGCAGGCTcTGGCATAGAAATGGGAAAGGAC	Preparing constructs for expression of Stb6 kinase domain in <i>E. coli</i>
Kin1186-attB1-R1	ggggACCACCTTTGTACAAGAAAGCTGGGTcTCACAGTTGTTGCAGGATTTTGG	Preparing constructs for expression of Stb6 kinase domain in <i>E. coli</i>
G387E-F1	GTTGTAGCTTATTTGAGTTTGTGTTG	Preparing constructs for expression of Stb6 kinase domain in <i>E. coli</i>
G387E-R1	CAAACAAAACCTCAAATAAGCTAACAAC	Preparing constructs for expression of Stb6 kinase domain in <i>E. coli</i>
E522K-F1	GAATGATGCTGCTAAAGATGGTTGGAG	Preparing constructs for expression of Stb6 kinase domain in <i>E. coli</i>
E522K-R1	CTCCAACCATCTTTAGCAGCATCATTC	Preparing constructs for expression of Stb6 kinase domain in <i>E. coli</i>
Stb6ectoF	ggggACAAGTTTGTACAAAAAAGCAGGCTcGCCGAGGAGCAGCAAGGGGA	Preparing <i>Stb6</i> ectodomain constructs for Y2H
Stb6ectoR	ggggACCACCTTTGTACAAGAAAGCTGGGTcTCACTTCTCGCTCTCCTCTCACT	Preparing <i>Stb6</i> ectodomain constructs for Y2H
avrstb6F	ggggACAAGTTTGTACAAAAAAGCAGGCTcAGAGTCAGTTGCGCGGCATAG	Preparing <i>AvrStb6</i> (from <i>Z. tritici</i> IPO323 and IPO88004) constructs for Y2H
avrstb6R	ggggACCACCTTTGTACAAGAAAGCTGGGTcTCACACGCAGCCACAACCAAGAAT	Preparing <i>AvrStb6</i> (from <i>Z. tritici</i> IPO323) constructs for Y2H
avrstb6R88004	ggggACCACCTTTGTACAAGAAAGCTGGGTcTCACACGCAGCCACAACCAAGAA	Preparing <i>AvrStb6</i> (from <i>Z. tritici</i> IPO88004) constructs for Y2H
avrstb6-116F	ggggACAAGTTTGTACAAAAAAGCAGGCTcAGAGTCGTTTTCGGCGGCATAG	Preparing <i>AvrStb6</i> (from <i>Z. tritici</i> RRes116) constructs for Y2H
avrstb6-116R	ggggACCACCTTTGTACAAGAAAGCTGGGTcTCACACGCAGCCACAACCAAGAA	Preparing <i>AvrStb6</i> (from <i>Z. tritici</i> RRes116) constructs for Y2H
Zt10-attB1-F	ggggACAAGTTTGTACAAAAAAGCAGGCTcCAGACGACCCAGTCTGCACCT	Preparing <i>Zt10</i> constructs for Y2H
Zt10-attB2-R	ggggACCACCTTTGTACAAGAAAGCTGGGTcCTAATAGGCCGCAGAGTATCTCC	Preparing <i>Zt10</i> constructs for Y2H
dest32F	AACCGAAGTGCGCCAAGTGTCTG	Sequence verification of pDEST32-derived constructs
dest32R	AGCCGACAACCTTGATTGGAGAC	Sequence verification of pDEST32- and pDEST22-derived constructs
dest22F	TATAACGCGTTTGAATCACT	Sequence verification of pDEST22-derived constructs

Supplementary Table 10. Primers used for SNP genotyping.

Marker name	Allele-specific FAM-labelled forward primer sequence	Allele-specific HEX-labelled forward primer sequence	Common reverse primer sequence
cfn80021	GAAGGTGACCAAGTTCATGCTTGAAGTATCAACATGCATATTATAAGC	GAAGGTCGGAGTCAACGGATTGTTGAAGTATCAACATGCATATTATAAGT	CGATGTATCTGCCGTAAGTATCTCAAATA
cfn80022	GAAGGTGACCAAGTTCATGCTAAACCACTCCAAAGGTTGCGCAA	GAAGGTCGGAGTCAACGGATTAACCACTCCAAAGGTTGCGCAG	GAACAATTGGATTCATCGCCCAGAA
cfn80023	GAAGGTGACCAAGTTCATGCTGGGGTTTGATGTCGAAATGGATGA	GAAGGTCGGAGTCAACGGATTGGGGTTTGATGTCGAAATGGATGT	CCTCGAATACTTGCACTATAGCTGTAATA
cfn80025	GAAGGTGACCAAGTTCATGCTGACTGTCCTTGAGGTGGCAT	GAAGGTCGGAGTCAACGGATTACTGTCCTTGAGGTGGCAC	CTAGATGACGAGTCTGTCCCAAGAT
cfn80030	GAAGGTGACCAAGTTCATGCTGAAGTTACAAGAGAAAATTGAGGAGATCA	GAAGGTCGGAGTCAACGGATTAAGTTACAAGAGAAAATTGAGGAGATCG	TGCACCACAAGCCTATTATGATCATCTTT
cfn80035	GAAGGTGACCAAGTTCATGCTCATTTTTCTTGTAAACCACCTGAAT	GAAGGTCGGAGTCAACGGATTCATTTTTCTTGTAAACCACCTGAAC	AGATACACCGCTTCTGTAGATTAA
cfn80036	GAAGGTGACCAAGTTCATGCTGTCAGATGTTTATAGTTATGGGATGA	GAAGGTCGGAGTCAACGGATTGTCAGATGTTTATAGTTATGGGATGC	ATTTTACATTTCTCCTACCTCCAACC
cfn80037	GAAGGTGACCAAGTTCATGCTGATCAAAATCTAAAGAAAAGAGGA	GAAGGTCGGAGTCAACGGATTGATCAAAATCTAAAGAAAAGAGGG	TGGACTTGACAATTTGCCAC
cfn80038	GAAGGTGACCAAGTTCATGCTGTTATGGTTACAGATCATCGTATTGCTCCG	GAAGGTCGGAGTCAACGGATTGTTATGGTTACAGATCATCGTATTGCTCCT	TTTGTCCACATAATCCTACTTTTGG
cfn80039	GAAGGTGACCAAGTTCATGCTCGGCTACAACTACGGAGAAGCAC	GAAGGTCGGAGTCAACGGATTGCGGCTACAACTACGGAGAAGCAT	GCCCTTGGTCTCTTCGTAG
cfn80040	GAAGGTGACCAAGTTCATGCTACTGTTTCCAGAACTTGGCCTTGA	GAAGGTCGGAGTCAACGGATTACTGTTTCCAGAACTTGGCCTTGG	CCTCAGGTTCACTAGCCCATGG