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SUPPLEMENTARY INFORMATION

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

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SUPPLEMENTARY NOTE

Genetic mapping. A synteny[70,71] between genomes of wheat and *Brachypodium distachyon* was exploited for identification of a region in the fully sequenced[72] genome of *B. distachyon* v1.2 syntenic to the wheat *Stb6* locus as follows. With the help of genetic maps available for the Apache × Balance and ITMI (International Triticeae Mapping Initiative) wheat mapping populations[73,74] and their shared markers, an interval corresponding to *Stb6* was located between the single nucleotide polymorphism (SNP) markers, SyOpL2500 (wsnp_Ra_c9738_16173810) and SyOpL304 (wsnp_CAP12_c2692_1286812), present on the ITMI map. Nucleotide sequences surrounding these SNPs were subjected to similarity searches using BLASTn against *B. distachyon* genome and this allowed identification of a syntenic genome region on the *B. distachyon* chromosome 2 spanning approximately 769-kb interval containing 96 predicted gene models between *Bd2g02010.1* and *Bd2g02900.1* as shown in **Supplementary Fig. 2**. These *B. dystachyon* genes were then subjected to similarity searches using BLASTn against the different wheat databases[75,76]. Identified wheat genomic sequences were analyzed for the presence of microsatellite sequences using SSR Locator[77] and the repeat sequences were used for development of 35 new SSR markers (data not shown). Two of these, cfa3006 and cfa3010 (**Supplementary Table 9**), were polymorphic between the parents of wheat mapping populations used in this study and were assigned to the deletion bin 0.45-1.00 on 3AS. SSR genotyping was performed essentially as described[78]. The nineteen predicted RLK-like genes, *Bd2g02426.1* to *Bd2g0253 7.1*, residing close to the center of the 769-kb interval were subsequently subjected to similarity searches using BLASTn against the International Wheat Genome Sequence Consortium Chromosomes Survey Sequence (IWGSC CSS) assembly v1 (**ref. 79**). This identified 11 wheat chromosome 3A-specific genomic DNA contigs each carrying RLK-like sequences, which we partially re-sequenced from wheat Courtot, Avalon and Cadenza to identify potential polymorphic sites as follows. Fragments of these predicted genes were PCR-amplified using primers listed in **Supplementary Table 9** and AmpliTaq Gold® Master Mix (Applied Biosystems), purified using Agencourt AMPure XP (Beckman Coulter), and Sanger sequenced at GATC Biotech SARL (Konstanz, Germany). Identified single nucleotide polymorphisms (SNPs) were used for developing SNP markers (**Supplementary Table 10**). SNP genotyping was performed using the Kompetitive Allele Specific PCR (KASP™) genotyping assays (LGC Genomics) that were analyzed on the Light Cycler 480 Real-Time PCR System (Roche Applied Science). Feeding an additional available wheat genome sequencing data[71,76] identified an INDEL (INsertion/DELetion) that was used for developing a co-dominant PCR-based marker, named ctg8311. PCR using the primer pair 8311F3 / 8311R4 (**Supplementary Table 9)** produces ~550-bp and ~800-bp DNA fragments from Chinese Spring and Courtot, respectively, that are easily differentiated by size using agarose gel electrophoresis.

Physical map construction, sequencing, and annotation. Markers ctg8311 and cfn80023 co-segregating with *Stb6* as well as one of the closest flanking markers, cfn80025, were used for screening the wheat CS Tae-B-CsE BAC library available at the INRA-CNRGV Plant Genomic Resources Center, Toulouse, France using a set of 6 high-density filters each containing 55,296 BAC clones spotted in duplicates following a previously described[80] hybridization protocol. Nine positive BAC clones were detected and the largest ~100-kb BAC clone Tae-B-CsE-673A07 containing all three markers was sequenced using the PacBio RS II system (Pacific Biosciences). For this 2 µg of BAC DNA were pooled with DNA from 11 other BAC clones and one 8-12-kb DNA library was generated using the standard PacBio library preparation protocol. This library was sequenced in one Single Molecule, Real-Time (SMRT) Cell using the P6 polymerase in combination with the C4 chemistry at IGM Genomic Center (University of California, San Diego, USA). Assembly of the sequence reads was performed using the HGAP workflow of the SMRT® Analysis v2.2.0 software (Pacific Biosciences). Reads were first aligned using the BLASR (Basic Local Alignment with Successive Refinement) tool[81] against complete genome of *E. coli* strain DH10B. Identified *E. coli* reads as well as short (< 500-bp) and low quality (< 0.80) reads were excluded from sequence assembly. Vector sequences were trimmed along the assembly process and each BAC assembly was individualized by

matching their BAC end sequences using BLASTn. The BAC Tae-B-CsE-673A07 sequence assembly was then annotated automatically using the TriAnnot pipeline[82] and then manually curated. Sequences of 4 WAK-like genes (*TaWAKL1 – TaWAKL4*) predicted to reside in this BAC clone were subjected to BLASTn against the TGACv1 wheat Whole Genome Assembly (WGA) available from The Wheat Portal. One 117,845-bp scaffold Triticum_aestivum_CS42_TGACv1_scaffold_210987_3AS, overlapping with the BAC clone Tae-B-CsE-673A07 over 64-kb and containing additional markers cfn80030 and cfn80040 that flank *Stb6* was identified. Sequences of this genomic scaffold and the BAC clone Tae-B-CsE-673A07 were merged to complete the *Stb6* physical region of 155,870-bp, which was annotated using the TriAnnot pipeline[82] and then manually curated. Microsatellites identified in the long intergenic region between *TaWAKL2* and *TaWAKL3* were used for developing additional SSRs, cfa3036 and cfa3037 (**Supplementary Table 9**), for genotyping.

Very recently a new substantially improved IWGSC wheat WGA v1.0, comprising of Illumina short sequence reads produced with the DeNovoMAGIC assembly pipeline (NRGene) and further refined by the IWGSC genome assembly team, become available (Kellye Eversole and colleagues at IWGSC, unpublished). A continuous 400-kb genomic sequence containing *Stb6* and spanning the genetic interval between markers cfn80025 and cfa3010, which likely define the borders of the complete WAK-like genes cluster on 3AS, was identified and annotated (**Supplementary Fig. 3**) as follows. RNA-seq data of differently staged wheat tissues available from The Wheat Portal, wheat leaves under drought and/or heat stress from PRJNA257938, root and shoot +/- phosphorous starvation from PRJDB2496, as well as *Zymoseptoria tritici* infection time course on wheat CS (Kanyuka *et al.*, unpublished) and Riband[83] was mapped to the 400-kb genomic sequence using Hisat2 v2.0.4. 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 using BioMart tool in Ensembl Plants were aligned to the reference using Lastz v7.0 in Geneious. The 400-kb 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 to assist in curation.

Gene expression analyses, and Rapid Amplification of cDNA Ends (RACE). Total RNA extracted in duplicates from root, leaf, stem, spike, and grain at three developmental stages each from healthy wheat Chinese Spring was used for library preparation followed by sequencing by Illumina HiSeq2000 platform as reported[84]. The resulting 100-bp paired-end reads were mapped to the 4,431-bp contig IWGSC_chr3AS_ab_k71_contigs_longerthan_200_3371186 (**Supplementary Fig. 3**) containing exons 3 and 4 of *Stb6* using Tophat2 v2.0.13 (**ref. 85**) 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 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 (**ref. 86**) 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.

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

Germany). These sequences were aligned to the sequence of the BAC clone Tae-B-CsE-673A7 to determine the transcriptional initiation and termination sites of *Stb6* mRNA.

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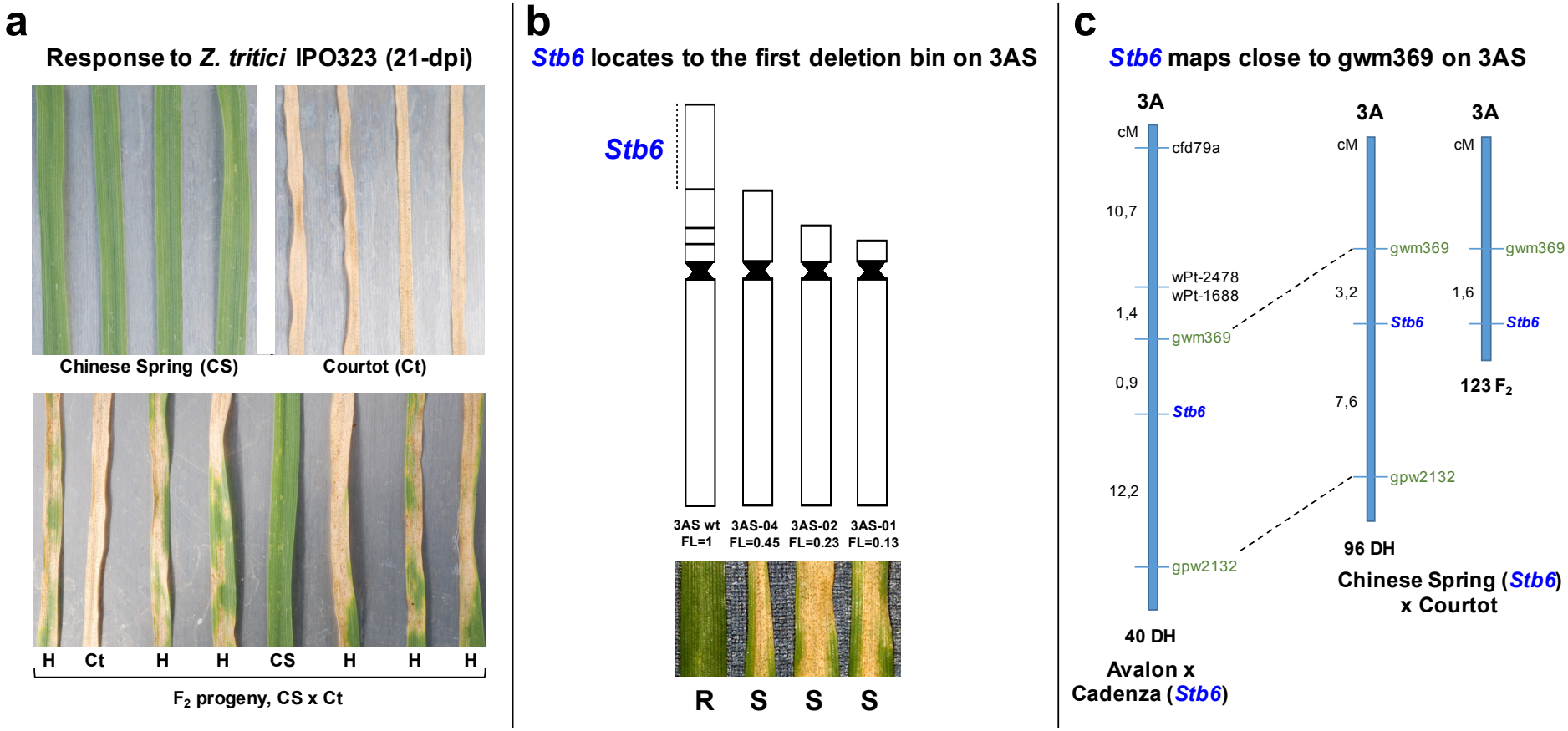
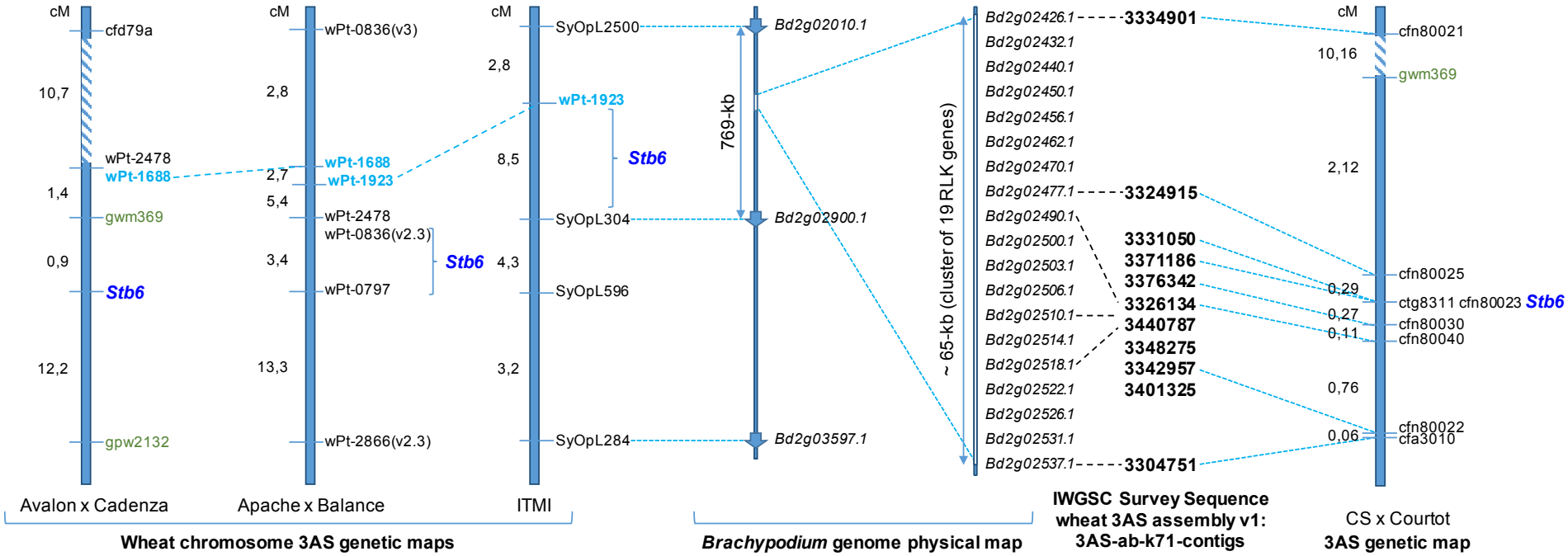
Supplementary Figure 1. *Stb6* is inherited as a semi-dominant trait and locates to a sub-telomeric region on chromosome arm 3AS in wheat Chinese Spring and Cadenza.



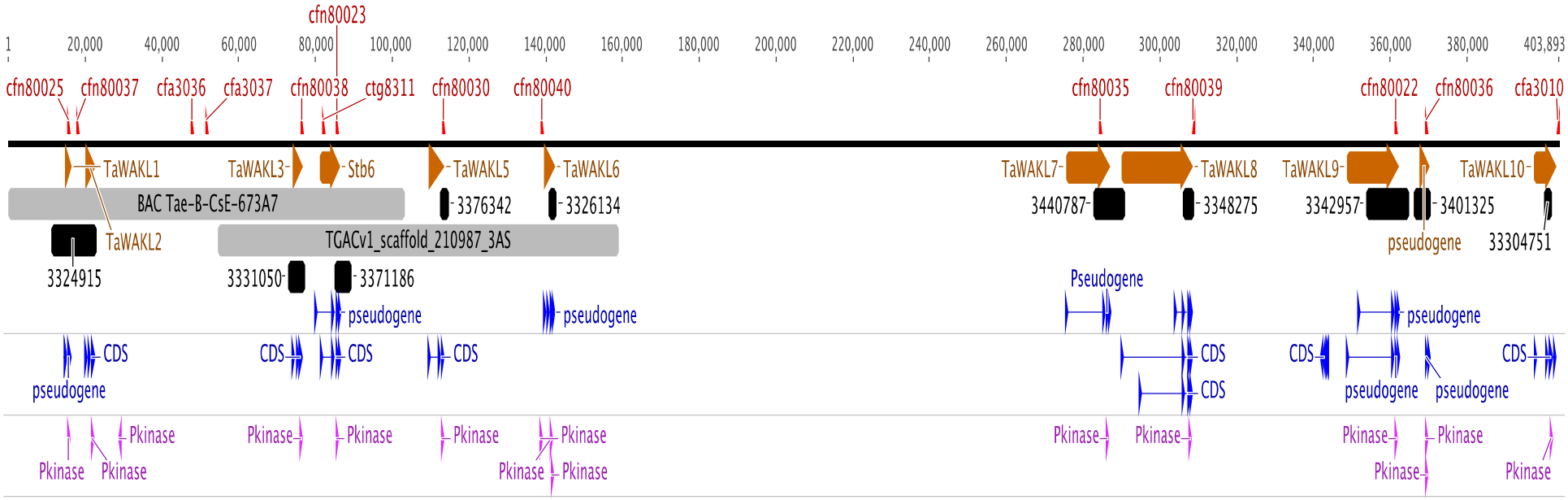
(a) Response to *Z. tritici* IPO323 of wheat cultivars Chinese Spring (CS) and Courtot (Ct), and in the F_2 progeny of a CS × Ct cross. The individuals heterozygous (H) for *Stb6* display a phenotype that is an intermediate between the phenotypes of parental cultivars, confirming that *Stb6* is a semi-dominant 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 F_2 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.



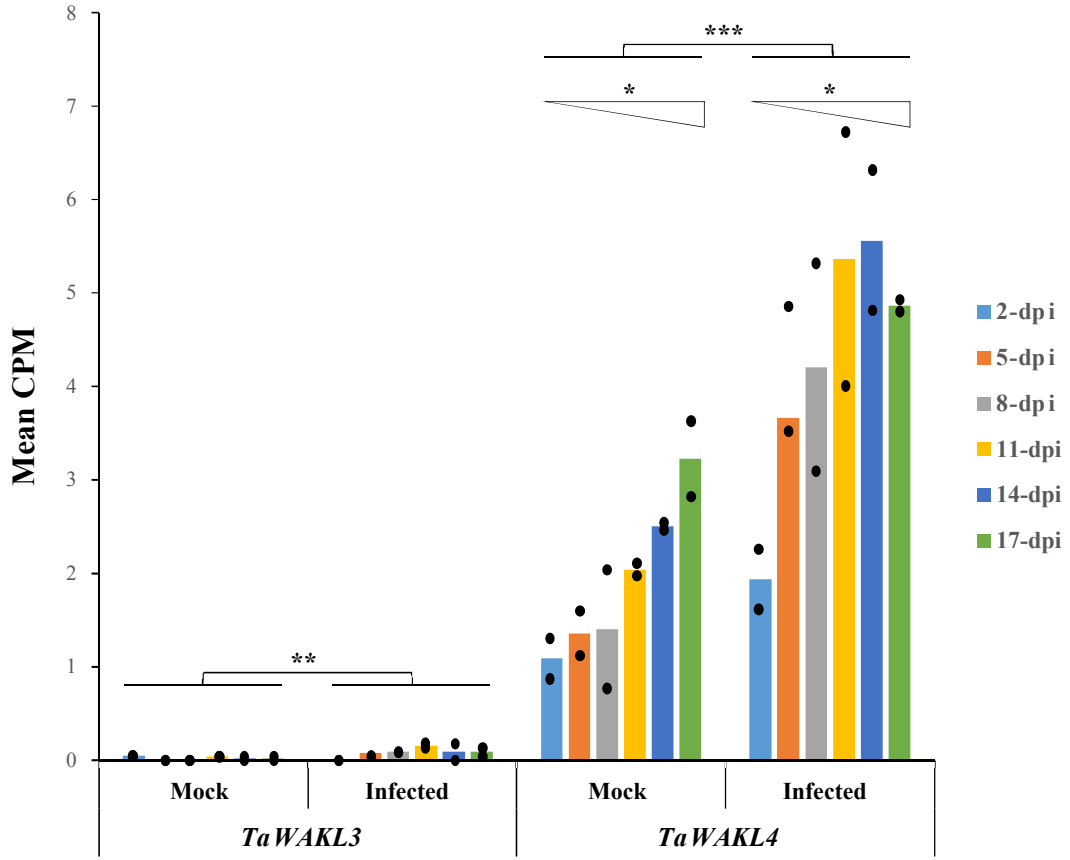
With the help of genetic maps available for the wheat mapping populations Apache × Balance and ITMI (International Triticeae Mapping Initiative) and their shared markers a genetic interval corresponding to *Stb6* was located between the sequence-characterized SyOPL markers present on the ITMI map. Homologous 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 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.

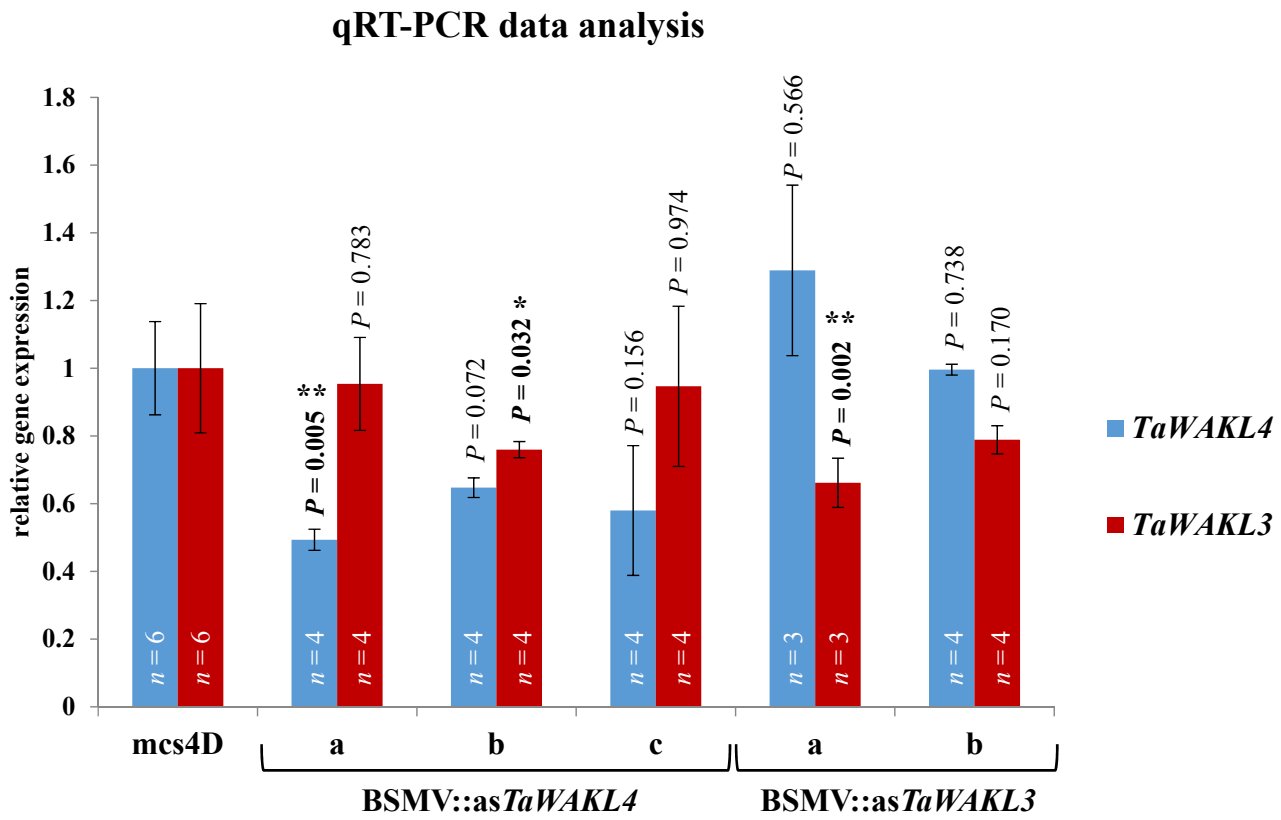


Mean counts per million (CPM) obtained from RNA-sequencing (Kanyuka *et al.,* unpublished) for transcripts of *TaWAKL3* and *TaWAKL4* genes are shown on the Y-axis. Black dots are values for each sample from the two replicated experiements. Comparison of CPM mean values using post-hoc *t*-test for *TaWAKL3* 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.

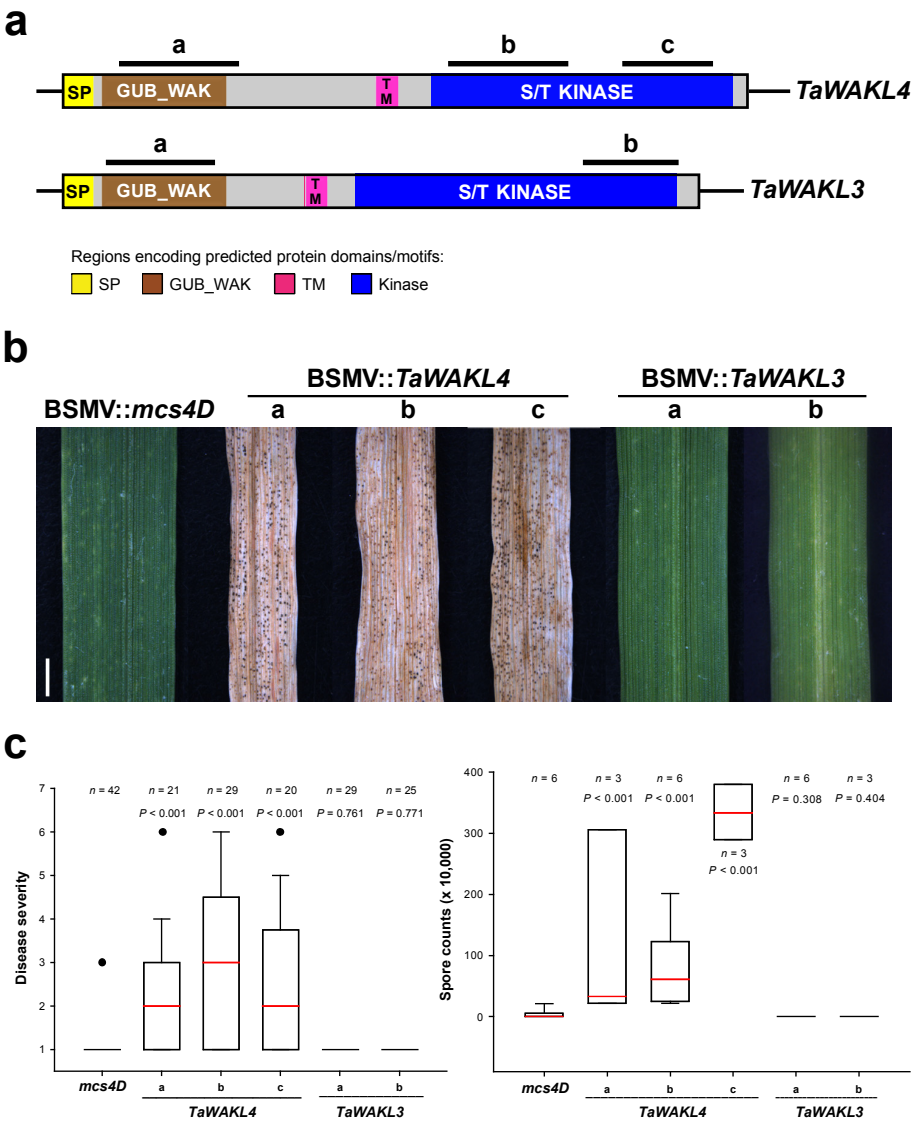
Sequences coding for predicted protein motifs and domains, such as a signal peptide (SP), a galacturonan-binding domain (GUB_WAK), a transmembrane region (TM), and a protein kinase domain, are indicated by differently colored rectangles. Only one single nucleotide polymorphism, T1340A, between the sequences 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.



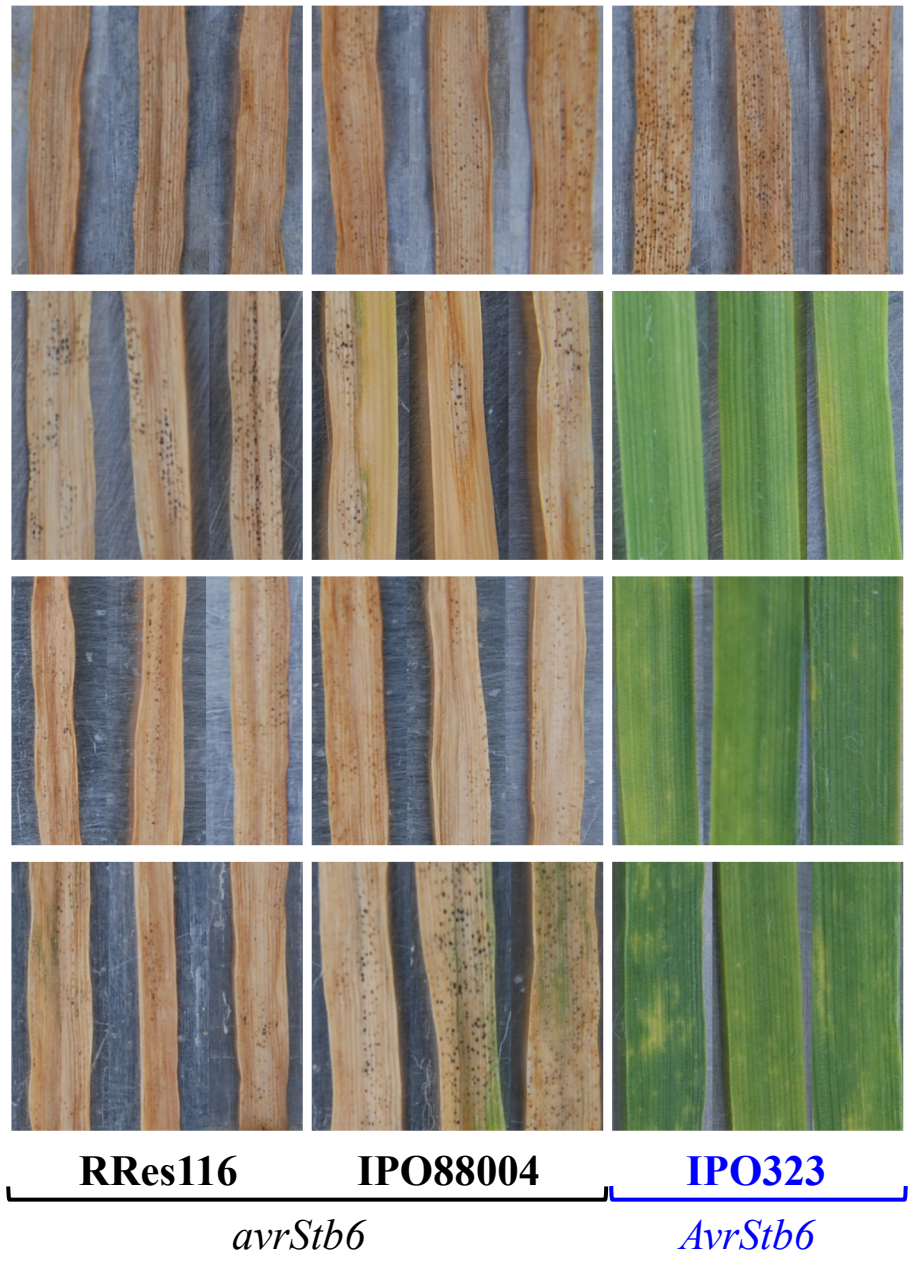
TaWAKL4 and *TaWAKL3* transcripts levels in the third leaves of wheat Chinese Spring plants infected with BSMV-VIGS constructs targeting *TaWAKL4*, *TaWAKL3* or the negative control construct BSMV::*mcs4D* as determined by quantitative RT-PCR. Error bars are mean ± standard error of the mean of n = 3-6 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.



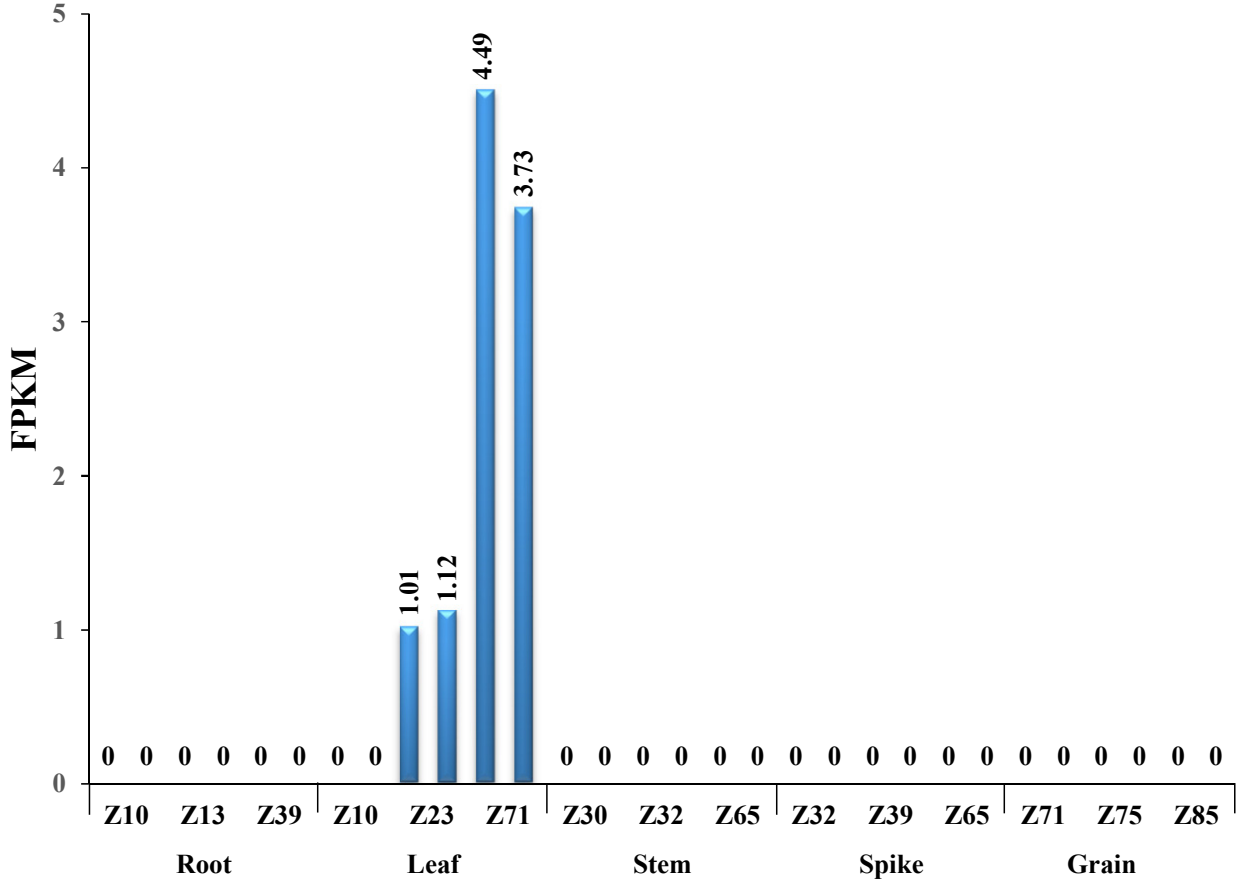
(a) Schematic representation of inferred *TaWAKL4* and *TaWAKL3* mRNAs. Coding and 5'- and 3'-non-coding sequences are shown as rectangles and solid black lines, respectively. Regions encoding predicted protein domains and motifs are labelled and shown as colored blocks: SP, signal peptide; GUB_WAK, 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* (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*.



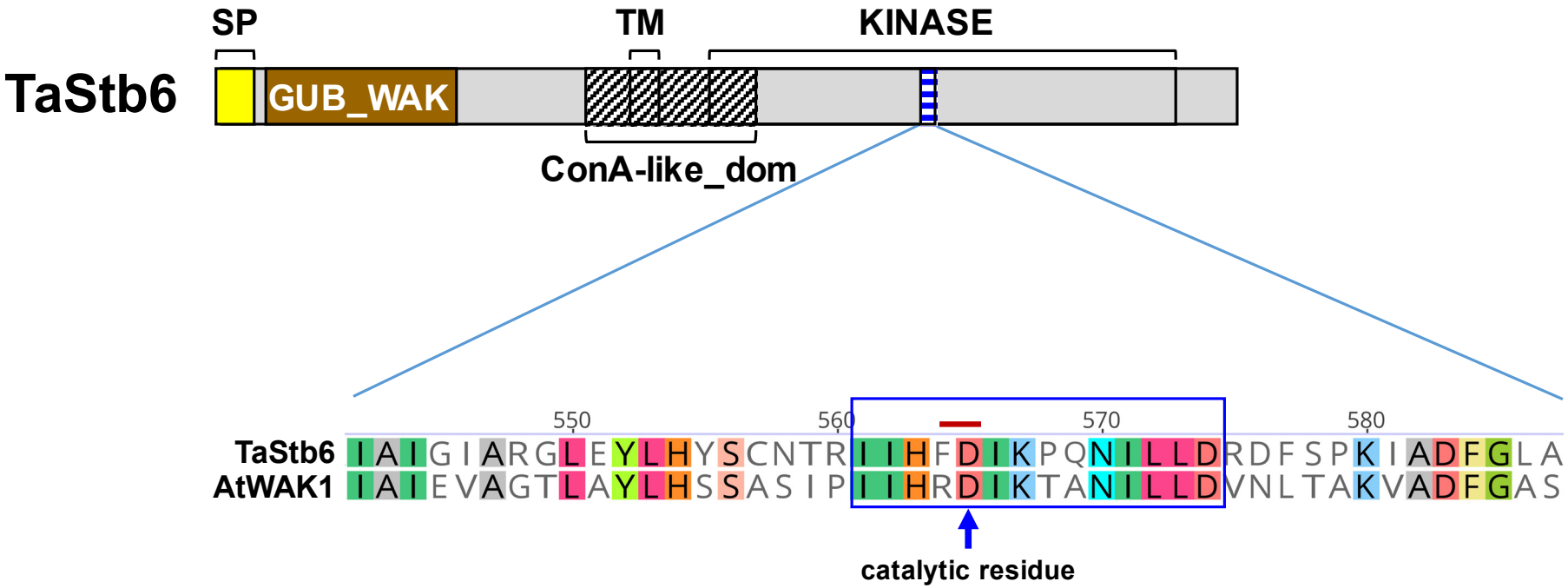
Progeny from Bobwhite wheat T_1 plants carrying *TaWAKL4* expression constructs #2 or #3 (see **Fig. 4a** for detail of constructs) were tested for resistance to *Z. tritici* isolates that are either avirulent (IPO323) or virulent (RRes116, IPO88004) on *Stb6*-containing wheat varieties. Images taken 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.



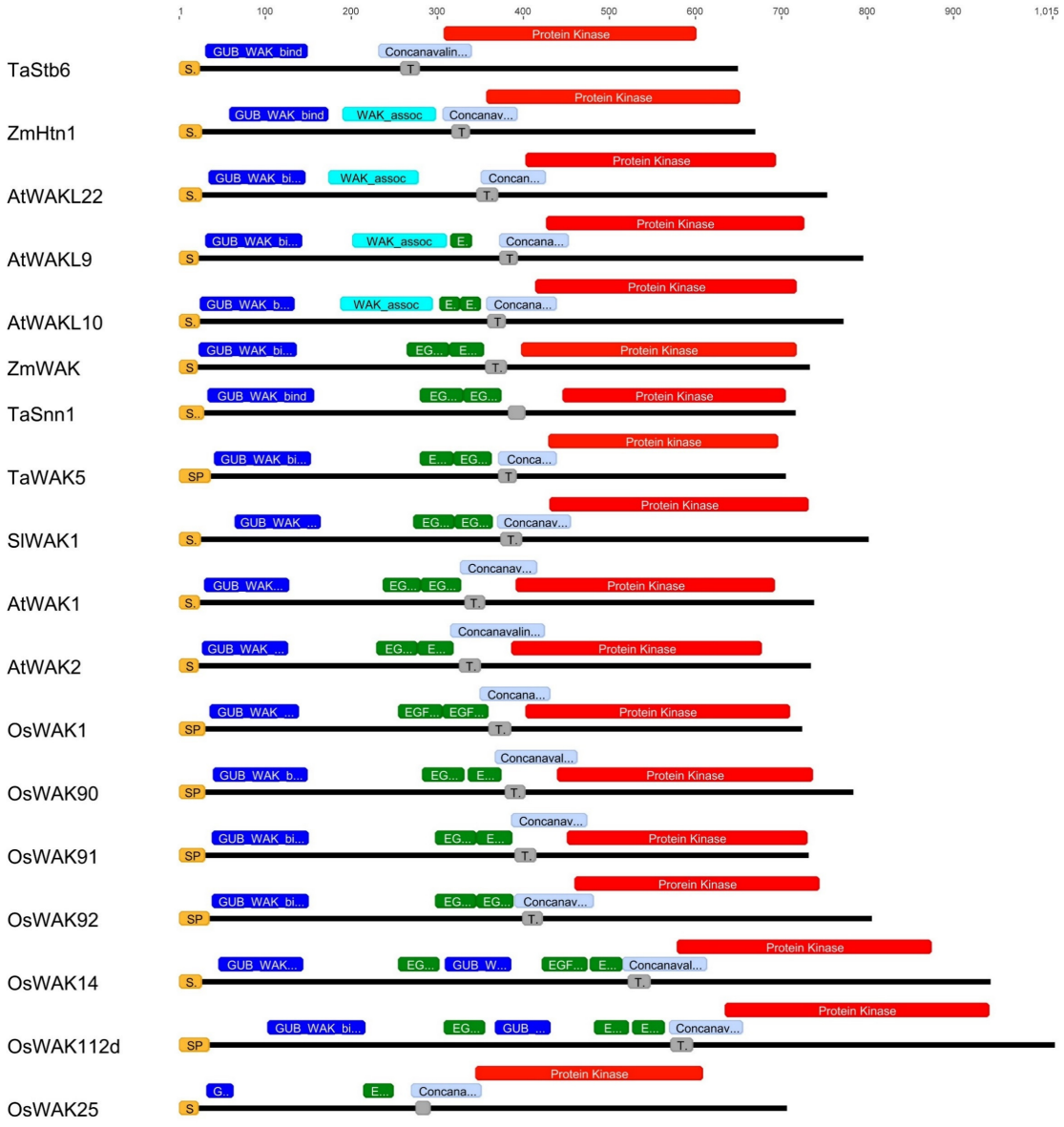
Fragments per kilobase of exon per million fragments mapped (FPKM) values obtained from the RNA-sequencing of healthy (uninfected) wheat tissues at different growth stages [Pingault, L. *et al.* Deep transcriptome sequencing provides new insights into the structural and functional organization of the 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-t*ests 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.



References‡
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Protein domains and motifs:

- Signal Peptide, SP
- Transmembrane region, TM
- Wall-associated receptor kinase galacturonan-binding domain, GUB_WAK_bind (PF13947)
- Wall-associated receptor kinase, WAK_assoc (PF08488)
- Epidermal growth factor like domain, EGF-like (PF00008)
- Concanavalin A-like lectin/glucanase domain, ConA-like_dom (IPR013320)
- Protein kinase domain (PF00069)

[*]UniProtKB or NCBI accession numbers for the WAK-like proteins analyzed:
AtWAK1 (Q39191), AtWAK2 (Q9LMP1), AtWAKL9 (Q9C9L5), AtWAKL10 (Q8VYA3), AtWAKL22 (Q8RY17), SlWAK1 (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).

[‡]References:

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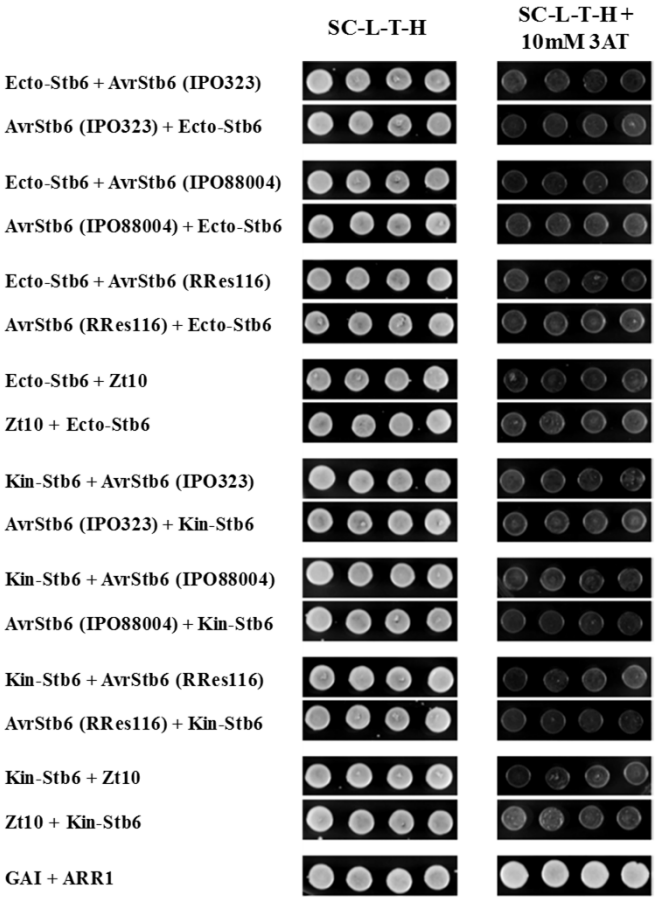
Supplementary Figure 12. No specific interaction between Stb6 and AvrStb6 proteins can be detected with the yeast two-hybrid system.



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 protein[1] of *Z. tritici* was used as a negative control. Yeast transformants containing *Arabidopsis thaliana* GAI (bait) and ARR1 (prey) proteins[2] were used as a positive protein-protein interaction control.

References:

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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.

	Pycnidial coverage classes											
	0 %		**1-20 %**		**21-40 %**		**41-60 %**		**61-80 %**		**81-100 %**	
Virus construct	**Mean[a]**	**s.e.**	**Mean**	**s.e.**	**Mean**	**s.e.**	**Mean**	**s.e.**	**Mean**	**s.e.**	**Mean**	**s.e.**
BSMV:*mcs4D*	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:as*TaWAKL4a*	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:as*TaWAKL4b*	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:as*TaWAKL4c*	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:as*TaWAKL3a*	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:as*TaWAKL3b*	10.00	1.3093	0.000	0.0012	0.000	0.0012	0.000	0.0012	0.000	0.0012	0.000	0.0012

[a]Mean 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.

	Pycnidial coverage classes											
	0 %		1-20 %		21-40 %		41-60 %		61-80 %		81-100 %	
Virus construct	**Mean[a]**	**s.e.**	**Mean**	**s.e.**	**Mean**	**s.e.**	**Mean**	**s.e.**	**Mean**	**s.e.**	**Mean**	**s.e.**
BSMV:*mcs4D*	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:as*TaWAKL4a*	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:as*TaWAKL4b*	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:as*TaWAKL4c*	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:as*TaWAKL3a*	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:as*TaWAKL3b*	8.538	1.3879	0.000	0.0008	0.000	0.0008	0.000	0.0008	0.000	0.0008	0.000	0.0008

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

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

T. durum	Durental	Cultivar	France	BRC / 33802	**13**
T. turgidum	341300	Landrace	Turkey	BRC / 33819	**13**
T. turgidum	Canoco	Landrace	Portugal	BRC / 33820	**13**
T. aestivum	Ralet	Old cultivar	France	BRC / 8048	**14**
T. dicoccoides	487255	Wild	Syria	BRC / 33779	**15**
T. monococcum	DV92	Cultivar	USA	BRC / 23861	**16**
T. boeoticum	*Triticum boeticum* 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 contaning 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**	***Stb6* 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	*Stb6* 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	ATTTTTCGGAAGCACCCAGATT	WAK resequencing
01864R1	CGCCCTGTTGATCGGATGAG	WAK resequencing
04034F1	ATGGGAGAGGCTTTACGGGAT	WAK resequencing
04034R1	CGCCTTCCGCTTCCATGATT	WAK resequencing
13609F1	CTGAAAAAAAATACGAGGCCATGA	WAK resequencing/*Stb6* re-sequencing
13609R1	GGCTTGTCTCCTCCGATCTTGT	WAK resequencing
13699F1	CCCGTTGCATGGTTCAGACTT	WAK resequencing
13699R1	TCGGCCAGACTTCTCATGTTAAC	WAK resequencing
32351_F1	TGGGGTCGATCACAAAACTAA	WAK resequencing
32351_R1	TGGCCTCGTAATTCTTCTCAGT	WAK resequencing
6342_F1	AAGCGGGTAGTCAAGTTATTAT	WAK resequencing
6342_R1	TTGCAGAAAGAGATATGTAAACTA	WAK resequencing
32351F3	TGGCTAAACCGAAGATTGTGTG	WAK resequencing
32351R3	TTCCTTCTGCTTTCTCATGTGATT	WAK resequencing
06777F2	AATGACGGGAGCTAGAGGAACA	WAK resequencing
06777R2	GCGTATCTCCTCGTATCCAGCA	WAK resequencing
13699F2	CGAGGTACGGTGAGCCCAG	WAK resequencing
13699R2	CGGCTGCTGACAATGACTACA	WAK resequencing
20822F7	GATCGCCTCTTGTCGCCTGA	WAK resequencing
20822F4	AAGCGCAGTGAAATTCGGCAT	WAK resequencing
03359F7	AGGATGTGCTCGGCGATGT	WAK resequencing
03359R7	TCTGCAGGGGTGATGAATTGA	WAK resequencing
6342_F2	GGTTTTGGTGCAGTGAGGAT	*TaWAKL5* resequencing
6342_R2	CACATGGCCTATCTGCTCAA	*TaWAKL5* resequencing
cfp5026F	CAAACTTTGCAAGCCCTCAT	BAC libray screening
cfp5026R	CAAATACAGGGAGGACGGAA	BAC libray screening
27963F1	GCTCATTAGGATTTGGCTTGTTA	BAC libray screening
27963F5	CAGCTGACTCATTTATTTTGCCTTA	*Stb6* re-sequencing
1186F1	GGAGTGGCAACGGGGTGAG	BAC library screening / *Stb6* re-sequencing
1186R1	GGGAATTGGGGGCACTACC	BAC library screening / *Stb6* re-sequencing
1186R2	GAGCAAGCTTTCAATTACAGGAG	*Stb6* re-sequencing
8311F3	CCGTTTAGCTCGTGTTGTGC	*Stb6* re-sequencing
8311F13	TGGCCCATGATGCTGTAGAG	*Stb6* re-sequencing
8311F16	GCGACATGGTAGCTCAATCAAA	*Stb6* re-sequencing
8311F19	CGCGGTTCCAGTCACATCAC	*Stb6* re-sequencing
8311R5	CTCGAAGTCCAGCGGTCCAG	*Stb6* re-sequencing
8311R9	CCAGTTCTTTTCCCCGGTGT	*Stb6* re-sequencing
8311R16	TTCCTTCCATGGTCGGTAACTT	*Stb6* re-sequencing
M13F	GTAAAACGACGGCCAGT	Plasmid insterts sequencing
M13R	CAGGAAACAGCTATGAC	Plasmid insterts sequencing
8311F12	GCGCGACCTTGAAAAAACTAT	Amplification of *Stb6* genomic region
8311R12	CTGGACGGATGTGGCGAGT	Amplification of *Stb6* genomic region
8311F11	GATTACGCCAAGCTTCGGCACGCCTGGATAAAGAA	3' RACE
8311R6	GATTACGCCAAGCTTTGGCACCCCCGCATGAACAAACGT	5' RACE
ctg8311F3	CCGTTTAGCTCGTGTTGTGC	Genotyping, co-dominant marker
ctg8311R4	GTTCACCCCGTCAATTCATCTT	Genotyping, co-dominant marker
cfa3006F	GGTTTGGTGGACTGCTACTCTGC	Genotyping, SSR marker
cfa3006R	ATCGTGCCCCTACCTACTCAGAA	Genotyping, SSR marker
cfa3010F	CCATGGATGCACACCTCTGAAA	Genotyping, SSR marker
cfa3010R	GCCCACACAACGAAAACGCA	Genotyping, SSR marker
cfa3036F	TCGGCTCTATCATATTTTGGTCT	Genotyping, SSR marker
cfa3036R	CAAGGCAGATAGGTAGAGTGGTG	Genotyping, SSR marker
cfa3037F	TCTTTCGAGCATCCAACTATATACT	Genotyping, SSR marker
cfa3037R	TCCGCCTGAGTTCATTACCT	Genotyping, SSR marker
LIC as1186a F	AAGGAAGTTTAA CAAACGTGTTGCTTGTGTTCAC	VIGS, *TaWAKL4/Stb6* specific constructs
LIC as1186a R	AACCACCACCACCGT AACAGGAAGATTATGTGGTTCGC	VIGS, *TaWAKL4/Stb6* specific constructs
LIC as1186b F	AAGGAAGTTTAA TGGGGTTTGATGTCGAAATGGA	VIGS, *TaWAKL4/Stb6* specific constructs
LIC as1186b R	AACCACCACCACCGT AGGTGGTTACGGTGTGGTTTTC	VIGS, *TaWAKL4/Stb6* specific constructs
LIC as1186c F	AAGGAAGTTTAA AGGACGATGCAAGGGTATTACT	VIGS, *TaWAKL4/Stb6* specific constructs
LIC as1186c R	AACCACCACCACCGT GCACCAGAAGTTCACTCTCGAA	VIGS, *TaWAKL4/Stb6* specific constructs
LIC as1050a F	AAGGAAGTTTAA CGCTTTCCTTCGTGCAATTGTA	VIGS, *TaWAKL3* specific constructs
LIC as1050a R	AACCACCACCACCGT CGGCAACCTGACCATCTCTAAT	VIGS, *TaWAKL3* specific constructs
LIC as1050b F	AAGGAAGTTTAA TGTATGCGACGACAAGTATGAC	VIGS, *TaWAKL3* specific constructs
LIC as1050b R	AACCACCACCACCGT CTGGAAATGGTAGGAGGAAGGA	VIGS, *TaWAKL3* specific constructs
RLK-1186 QPCR F	AGCAAGGGGATGGCTGCTT	qRT-PCR, quantification of *TaWAKL4/Stb6* transcript
RLK-1186 QPCR R	TTGCCAATCAGTGAGCCAGAAC	qRT-PCR, quantification of *TaWAKL4/Stb6* transcript
RLK-1050 QPCR F	CCTCGGCAACCTGAATCTCATC	qRT-PCR, quantification of *TaWAKL3* transcript
RLK-1050 QPCR R	GTTCCTGCACCTCACTCTTGTC	qRT-PCR, quantification of *TaWAKL3* transcript
CDC48 QPCR F2	GTCCTCCTGGCTGTGGTAAAAC	qRT-PCR, quantification of *TaCDC48* transcript

CDC48 QPCR R2	AGCAGCTCAGGTCCCTTGATAC	qRT-PCR, quantification of *TaCDC48* transcript
pCR8/GW_Stb6F1	TTGCAACAAATTGATGAGCA	Detection of the *Stb6* transgene (construct 1) in T_0 and T_1 plants
Stb6_pCR8GW_R1	CTGACTAACCCCGGTTTTGA	Detection of the *Stb6* transgene (construct 1) in T_0 and T_1 plants
R-gene-fwd	GGAAAAGGTGGTTACGGTGT	Detection of the *Stb6* transgene (construct 2 and 3) in T_0 and T_1 plants
Nos5'rev	ATCGCAAGACCGGCAACAGG	Detection of the *Stb6* transgene (construct 2 and 3) in T_0 and T_1 plants
UbiPro4	TTTAGCCCTGCCTTCATACG	Detection of the *Stb6* transgene (construct 3) in T_0 and T_1 plants
R-gene-rev	ACACCGTAACCACCTTTTCC	Detection of the *Stb6* transgene (construct 3) in T_0 and T_1 plants
bar1	GTCTGCACCATCGTCAACC	Detection of the *bar* ransgene (constructs 2 and 3) in T_0 and T_1 plants
bar2	GAAGTCCAGCTGCCAGAAAC	Detection of the *bar* ransgene (constructs 2 and 3) in T_0 and T_1 plants
Kin1186-attB1-F1	ggggACAAGTTTGTACAAAAAAGCAGGCTtcTGGCATAGAAATGGGAAAGGAC	Preparing constructs for expression of Stb6 kinase domain in *E. coli*
Kin1186-attB1-R1	ggggACCACTTTGTACAAGAAAGCTGGGTcTCACAGTTGTTGCAGGATTTTGG	Preparing constructs for expression of Stb6 kinase domain in *E. coli*
G387E-F1	GTTGTTAGCTTATTTGAGTTTTGTTTG	Preparing constructs for expression of Stb6 kinase domain in *E. coli*
G387E-R1	CAAACAAAACTCAAATAAGCTAACAAC	Preparing constructs for expression of Stb6 kinase domain in *E. coli*
E522K-F1	GAATGATGCTGCTAAAGATGGTTGGAG	Preparing constructs for expression of Stb6 kinase domain in *E. coli*
E522K-R1	CTCCAACCATCTTTAGCAGCATCATTC	Preparing constructs for expression of Stb6 kinase domain in *E. coli*
Stb6ectoF	ggggACAAGTTTGTACAAAAAAGCAGGCTtcGCCGAGGAGCAGCAAGGGGA	Preparing *Stb6* ectodomain constructs for Y2H
Stb6ectoR	ggggACCACTTTGTACAAGAAAGCTGGGTcTCACTTCTTCGCTCTCCTCTCACT	Preparing *Stb6* ectodomain constructs for Y2H
avrstb6F	ggggACAAGTTTGTACAAAAAAGCAGGCTtcAGAGTCAGTTGCGGCGGCATAG	Preparing *AvrStb6* (from *Z. tritici* IPO323 and IPO88004) constructs for Y2H
avrstb6R	ggggACCACTTTGTACAAGAAAGCTGGGTcTCACACGCAGCCACAACCAAGAAT	Preparing *AvrStb6* (from *Z. tritici* IPO323) constructs for Y2H
avrstb6R88004	ggggACCACTTTGTACAAGAAAGCTGGGTcTCACACGCAGCCACAACCACGAA	Preparing *AvrStb6* (from *Z. tritici* IPO88004) constructs for Y2H
avrstb6-116F	ggggACAAGTTTGTACAAAAAAGCAGGCTtcAGAGTCGTTTGCGGCGGCATAG	Preparing *AvrStb6* (from *Z. tritici* RRes116) constructs for Y2H
avrstb6-116R	ggggACCACTTTGTACAAGAAAGCTGGGTcTCACACGCAGCCACAACCAGGAAT	Preparing *AvrStb6* (from *Z. tritici* RRes116) constructs for Y2H
Zt10-attB1-F	ggggACAAGTTTGTACAAAAAAGCAGGCTtcCAGACGACCCAGTCTGCACCT	Preparing *Zt10* constructs for Y2H
Zt10-attB2-R	ggggACCACTTTGTACAAGAAAGCTGGGTcCTAATAGGCCGCAGAGTATCTCC	Preparing *Zt10* constructs for Y2H
dest32F	AACCGAAGTGCGCCAAGTGTCTG	Sequence verification of pDEST32-derived constructs
dest32R	AGCCGACAACCTTGATTGGAGAC	Sequence verification of pDEST32- and pDEST22-derived constructs
dest22F	TATAACGCGTTTGGAATCACT	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	GAAGGTGACCAAGTTCATGCTTGAACTGATCAACATGCATATTATAAGC	GAAGGTCGGAGTCAACGGATTGTTGAACTGATCAACATGCATATTATAAGT	CGATGTATCTGCCGTAAGTATCTCAAATA
cfn80022	GAAGGTGACCAAGTTCATGCTAAACCACTCCAAAGGTTCGCGAA	GAAGGTCGGAGTCAACGGATTAACCACTCCAAAGGTTCGCGAG	GAACAATTGGATTCATCGCCCCAGAA
cfn80023	GAAGGTGACCAAGTTCATGCTGGGGTTTGATGTCGAAATGGATGA	GAAGGTCGGAGTCAACGGATTGGGGTTTGATGTCGAAATGGATGT	CCTCGAATACTTGCACTATAGCTGTAATA
cfn80025	GAAGGTGACCAAGTTCATGCTGACACTGTCCTTGAGGTGGCAT	GAAGGTCGGAGTCAACGGATTACACTGTCCTTGAGGTGGCAC	CTAGATGACGAGTTCTGTCCCAAGAT
cfn80030	GAAGGTGACCAAGTTCATGCTGAAGTTACAAGAGAAATTGAGGAGATCA	GAAGGTCGGAGTCAACGGATTAAGTTACAAGAGAAATTGAGGAGATCG	TGCACCACAAGCCTATTATGATCATCTTT
cfn80035	GAAGGTGACCAAGTTCATGCTCATTTTTCTTGTAAACCACCTGAAT	GAAGGTCGGAGTCAACGGATTCATTTTTCTTGTAAACCACCTGAAC	AGATACACCGCTTCTGTAGATTAA
cfn80036	GAAGGTGACCAAGTTCATGCTGTCAGATGTTTATAGTTATGGGATGA	GAAGGTCGGAGTCAACGGATTGTCAGATGTTTATAGTTATGGGATGC	ATTTTACATTTCTCCTACCTCCAACC
cfn80037	GAAGGTGACCAAGTTCATGCTGATCAAAATCTAAAGAAAAGAGGA	GAAGGTCGGAGTCAACGGATTGATCAAAATCTAAAGAAAAGAGGG	TGGACTTTGACAATTTGCCAC
cfn80038	GAAGGTGACCAAGTTCATGCTGTTATGGTTACAGATCATCGTATTGCTCCG	GAAGGTCGGAGTCAACGGATTGTTATGGTTACAGATCATCGTATTGCTCCT	TTTGTCCACATAATCCTACTTTTGG
cfn80039	GAAGGTGACCAAGTTCATGCTCGGCTACAAACTACGGAGAAGCAC	GAAGGTCGGAGTCAACGGATTCGGCTACAAACTACGGAGAAGCAT	GCCCTTGGTCCTCTTCGTAG
cfn80040	GAAGGTGACCAAGTTCATGCTACTGTTTCCCAGAACTTGGCCTTGA	GAAGGTCGGAGTCAACGGATTACTGTTTCCCAGAACTTGGCCTTGG	CCTCAGGTTCACACTAGCCCATGG