www.sciencemag.org/cgi/content/full/1166289/DC1

# Supporting Online Material for

## A Kinase-START Gene Confers Temperature-Dependent Resistance to Wheat Stripe Rust

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Published 19 February 2009 on *Science* Express
DOI: 10.1126/science.1166289

**This PDF file includes:**

## MATERIALS AND METHODS

### 1.- Plant materials and growing conditions for rust bioassays

***Mapping***: The mapping population was developed from a cross between the tetraploid wheat (*Triticum turgidum* L ssp. *durum*) cv. Langdon (LDN) and RSL65, a near isogenic line of LDN with a 30-cM segment of chromosome arm 6BS from *T. turgidum* L. ssp. *dicoccoides* (accession FA15-3, designated as DIC hereafter) (*1*).

***Targeted Induced Local Lesions IN Genome (TILLING)***: The TILLING population was developed using the hexaploid wheat (*T. aestivum* L.) breeding line UC1041+*Yr36*. UC1041 is a hexaploid spring breeding line derived from the cross Tadinia/Yecora Rojo. The DIC 6BS segment including *Yr36* was introgressed from the wheat variety Glupro (*2*) followed by six backcrosses into UC1041. UC1041 is susceptible to *Puccinia striiformis* f. sp. *tritici* (PST) race PST-113, which is virulent on the *Yr1* resistance gene present in UC1041. Seeds from UC1041+*Yr36* were mutagenized with 1% ethyl methane sulphonate (EMS) and $M_2$ plants were produced from independent $M_1$ mutants. DNAs were extracted from 1,536 $M_2$ lines and organized in 384 4-fold DNA pools that were screened using TILLING (*3*).

***Transgenics***: The hexaploid spring wheat variety 'Bobwhite' was used for the transgenic complementation experiment. This variety is susceptible to PST-113.

***Growing conditions and inoculation stages***: All the chamber experiments used long day photoperiod (8h dark, 16 h light). For the stripe rust inoculations in the controlled environment experiments, plants were placed in a dew chamber without light at $10^{o}$C

for 24 h. Plants were inoculated either at the 1-4 leaf stage ("seedling inoculation") or after flag leaves were fully emerged ("adult-plant inoculation") (Fig. S1). Plants were then moved to one of two different temperature regimes, both of which induce the expression of *Yr36* resistance. The first one had a gradual change between a minimum of 10°C at the middle of the dark period to a maximum of 35°C at the middle of the light period (referred hereafter as **10/35°C**) (*4*). This treatment was effective for the expression of *Yr36* resistance in different genetic backgrounds (*1*). In the second temperature regime, plants were kept at constant 10°C during the dark period and at constant 25°C during the light period (referred hereafter as **10/25°C**). This treatment was also effective for the expression of *Yr36* resistance (Fig. S1). When indicated, other conditions were used as described in the Materials and Methods.

In all experiments pots were randomized during infection and disease development. Plants were scored blind by two independent evaluators and either photographs or scans were taken to document the results.

Field tests were conducted in Davis (2006, 2007, and 2008) and organized in a complete randomized design (CRD, 2006 and 2007) or a randomized complete block design (RCBD, 2008). All field experiments included a border of "spreader rows" of the highly-susceptible wheat variety D6301 which was used to spread inoculum. One meter rows were used as experimental units. Seeds were sown in November and plants inoculated with PST-100, a race predominant throughout the US, were planted in the spreader rows in March. Additional races were likely present since severe natural infections were observed across the field in all three years. Infection severity was recorded twice from May to early June.

**2.- Stripe rust races, inoculation procedures, and confocal images**

Table S1 describes the PST races, their virulence profiles and the year they were first described. The PST races used in this study included some of the most virulent and predominant races from 2000 to 2007 in the U.S. For inoculation, urediniospores were mixed with laboratory-grade talcum powder (Fisher Scientific, Fairlawn, NJ, USA) and dusted on the leaf tissue. Rust severity was evaluated two to three weeks after inoculation using a 0-9 scale of infection type (IT) (*5*): 0-3 (resistant, none to trace level sporulation), 4-6 (intermediate, light to moderate sporulation), 7-9 (susceptible, abundant sporulation). Alternatively, for the mapping and TILLING experiments, the percentage of leaf surface covered with PST pustules was quantified using the digital image analysis program "pd" (Fig. S2).

***Confocal methods***: Wheat leaves were processed for fluorescence microscopy as published before (*6*). The uvitex-stained leaves were examined first with a Nikon Microphot SA fluorescence microscope with a UV-2A DM 400 filter (Nikon, Melville, NY, USA). Images shown in Fig. 2, G to J (main text), were taken on a laser point scanning confocal microscope (Olympus FV1000 spectral scanner with an UPLAPO 40× oil objective N.A. 1.0). Tissue was sequentially scanned with lasers at 405 and 543 nm to detect uvitex and autofluorescence, respectively. The laser power

at 405 nm was reduced five-fold in the compatible interaction because of the greater concentration of uvitex-stained fungus. Each image is comprised of a z-series of 98 sections at 1.2 μm steps with a 0.124 μm per pixel resolution.

**3.- High-density genetic map**

A total of 4,500 $F_2$ plants from the cross LDN × RSL65 were screened for recombination between PCR markers *Xucw71* and *Xbarc136* (*1*) (Fig. 1) and 121 lines were selected (Table S3). Selected plants were self-pollinated and recombinant substitution lines (RSLs) homozygous for the recombinant chromosomes were obtained.

Wheat ESTs with homology to single or low copy number genes in the colinear region in rice (Fig. 1, Table S2) were used to develop additional PCR markers and to further characterize the 121 critical RSLs (Table S3). Briefly, primer pairs were designed for conserved regions between rice and wheat ESTs and were used to amplify predicted introns in LDN and RSL65. PCR products were cloned into the pGEM-T vector (Promega, Madison, WI, USA). Clones from the A and B genomes were differentiated by restriction enzyme fingerprinting. Products from each genome were sequenced and polymorphisms between LDN and RSL65 were used to develop markers (Table S2). Additional markers were developed from Bacterial Artificial Chromosome (BAC) ends and BAC sequences generated during the construction of the physical map.

Seventy RSLs representing all the different recombination events present in the 121 critical lines (Table S3) were evaluated for adult plant resistance to PST-100 at 10/35°C. Thirty six RSLs were also evaluated for adult plant resistance in the field during 2006 at UC Davis. A summary of the host-pathogen interaction phenotype is presented in Table S3. To validate the mapping of *Yr36*, the 13 RSLs with the closest recombination events (0.14-cM interval between *Xucw111* and *Xucw113*) were re-tested for resistance to PST-100 in different environmental conditions and growth stages (Table S4, Fig. S3).

An additional experiment using the same temperature conditions (10/35 °C) was performed using eight different PST races that are virulent on LDN (Table S1). The experiment included control lines LDN and RSL65 and five recombinant lines with the closest recombination events flanking *Yr36* (RSL241, RSL402, RSL504, RSL17-47, RSL39-14; Table S5).

**4.- Physical map**

The physical map of the *Yr36* region was constructed using the BAC library from the resistant parent RSL65 (*7*) and a pooling PCR screening strategy that was described before (*8*). The initial screening was performed using B-genome specific primers for the distal marker *Xucw113* (Table S2). Six positive BAC clones (391M13, 400M22, 782M23, 852O1, 1129G14, and 1217L2) were identified (Fig. S4).

The BAC end sequence of clone 1129G14 was used to generate the single copy marker *Xucw125* (Table S2), which is absent in LDN and present in RSL65. This marker was mapped proximal to *Xucw113* and completely linked to *Yr36,* which oriented the contig formed by these 6 BAC clones relative to the genetic map. Screening of the BAC library with the *Xucw125* primers generated four new positive clones (508C11, 528D22, 691B11, and 984G1; Fig S4). The BAC-end sequence of BAC clone 508C11 was used to generate marker *Xucw126* (Table S2), which also was absent in LDN and present in RSL65, and was completely linked to *Yr36*. *Xucw126* was used to screen the BAC library and four new positive BAC clones were identified (651E2, 1046P23, 1070P18, and 1144M20, Fig. S4).

BAC-end sequencing of clone 1046P23 was used to generate marker *Xucw127* (LDN: 110-bp and RSL65:105-bp, Table S2). The 5-bp polymorphism was mapped proximal to *Yr36* (Table S3), which completed the physical map (Fig. S4). *Xucw127* is part of a predicted pectin lyase-like gene with an X8 domain (pfam07983).

**5.- Contig sequencing and delimitation of the *Yr36* candidate region**

Overlapping BAC clones 391M13 and 1144M20 were sequenced and a 314,057-bp contig was generated, annotated, and deposited in GenBank (EU835198, 7.5-fold coverage at Phred ≥20). This sequence includes the proximal region of BAC 391M13 and the complete sequence of BAC 1144M20. The annotated contig includes the complete 186-kb *Yr36* region flanked by markers *Xucw129* and *Xucw148*.

The proximal recombination event in RSL504 occurred between the DIC *IN BETWEEN RING finger1* (pfam01485) *IBR1* and LDN *IBR2* genes as confirmed by sequencing (FJ155069 and FJ155070). Based on the location of this recombination event the promoter and proximal 250-bp of the *IBR1* gene were excluded from the *Yr36* candidate gene region.

Over 80% of the sequence was identified as repetitive using the *Triticeae* Repeat Sequence Database (TREP http://wheat.pw.usda.gov/ITMI/Repeats/index.shtml) and the TIGR Cereal Repeat Database (http://tigrblast.tigr.org/euk-blast/index.cgi?project=tae1). The non-repetitive sequence was annotated using BLAST searches in GenBank, the wheat EST collection at GrainGenes (http://www.graingenes.org/) and the TIGR Wheat Genome Database (http://tigrblast.tigr.org/euk-blast/index.cgi?project=tae1), and the gene prediction programs Genscan (http://genes.mit.edu/GENSCAN.html) and FGENESH (http://www.softberry.com/berry.phtml) (Fig. S5).

**6.- TILLING mutants**

The UC1041+*Yr36* mutant population was screened for mutations in two regions of *WKS1* and *WKS2*. The first one included the complete kinase domain and was 1,371-bp and 1,460-bp in *WKS1* and *WKS2*, respectively. The second region included part of the START domain (pfam01852) and was 1,270-bp and 1,532-bp in *WKS1* and *WKS2*, respectively. The targeted *WKS* regions were selected using the CODDLE

program (http://www.proweb.org/coddle/), which helps Choose codons to Optimize the Detection of Deleterious Lesions. Primers specific for each of these region (Table S6) were used to screen 1,536 DNAs for *WKS1* and 768 for *WKS2*. Using the PARSESNP and Blockmaker programs (http://www.proweb.org/Tools), we selected mutations that were predicted to have the strongest effect based on Position-Specific Scoring Matrix (PSSM) differences and Sorting Intolerant From Tolerant (SIFT) scores (*9*), or that led to premature truncations (Table S7).

For each mutation, $M_3$ plants homozygous for the mutant alleles were selected. For *WKS1* lines T6-312, T6-138, and T6-567, and for *WKS2* line T6-826, $M_3$ plants homozygous for the non-mutant alleles were also selected as additional controls (Fig. 2 and Figs. S8 and S9). Resistance to race PST-113 was evaluated at 10/25°C in three separate experiments that included different mutants and controls as they became available.

***Mutant experiment 1***: In this experiment plants were inoculated at the 4$^{th}$-leaf (juvenile) stage. Fifteen days after inoculation the edge of the areas covered with pustules was marked with a black line. Five days later the same leaves were scanned to evaluate the progression of the disease beyond the mark (Fig. S8). When pustules were restricted to the marked area, plants were considered resistant and when they spread beyond the marked border they were considered susceptible (Fig. S8).

***Mutant experiment 2***: In this experiment we retested *WKS1* mutant lines T6-138 and T6-312, their corresponding non-mutant sister lines, and the susceptible and resistant control lines for PST resistance at the flag leaf (adult) stage. The percent of leaf area covered by pustules was quantified in eight leaves per line using the pd program (Fig. S2). Percentage area was log-transformed to achieve homogeneity of variance and differences were tested using ANOVA (Fig. 2). Images in Fig. 2 (main text) were obtained from plants used in this experiment.

***Mutant experiment 3***: The third experiment was performed to test mutant line T6-567 (discovered later), which has a mutation in the START domain affecting a conserved amino acid (Fig. S7A). Sister lines homozygous for the presence and absence of this mutation were compared for resistance to race PST-113 at the elongation (adult) stage (Fig. S9). Lines UC1041 and UC1041+*Yr36* were included as additional controls.

**7.- Complementation using transgenic *WKS1* plants**

To confirm that *WKS1* confers partial resistance to stripe rust, we transformed the susceptible common wheat variety Bobwhite with the pWKS1 plasmid, which includes the complete *WKS1* gene. We used the High-Fidelity DNA Polymerase Phusion™ enzyme (Finnzymes, Espoo, Finland) to amplify a 12,205-bp genomic DNA fragment from RSL65 by PCR. *Sbf*I and *Not*I restriction sites were added to the primers for cloning (YR36_S1F1/S1R4, Table S6). This fragment included 3,503-bp upstream from the *WKS1* start codon, the complete *WKS1* coding region, and 1,415-bp downstream from the stop codon.

The PCR product was cut by restriction enzymes *Sbf*I and *Not*I, recovered from a 1% agarose gel, and cloned into a *Sbf*I-*Not*I linearized pGEM®-T vector (Promega, Madison, WI, USA). To reduce the frequency of breaks within the coding sequence during transformation, the previous construct was digested with *Sbf*I and further cloned into a *Sbf*I linearized pPZP201 vector (*10*) to increase the size of the non-genic region (the pWKS1 final construct is ~22.3-kb). The *WKS1* region (12,205-bp) was sequenced and showed no differences with the wild type allele. Embryonic calluses of hexaploid spring variety Bobwhite were bombarded using a 1:1 molar ratio of pWKS1 and UBI::BAR selectable marker plasmids (15.5 µg total) coated onto Seashell 1000 nm gold particles (La Jolla, CA, USA), according to the manufacturer's instructions. Transformants were selected as previously described (*11, 12*).

In total, nine independent transgenic $T_1$ lines were obtained and positive plants were confirmed by PCR using primer pair YR36_13104F/13692R (Table S6). Transcription of the full length *WKS1* gene in the transgenic $T_1$ plants was confirmed by reverse transcriptase PCR (RT-PCR) using *WKS1* transcript specific primers WKS1_150F, 151R, and 174R (Table S6). Of the nine transgene-positive lines, only 17a and 26b yielded full-length *WKS1* cDNAs and were used for functional studies. Transcript levels of the *WKS1* transgene (all transcript variants) were determined by real-time quantitative PCR (Q-PCR) with primers WKS1_F1/R1 (Table S6) before PST inoculation. Transgenic and control lines were tested for PST resistance with race PST-113, which is virulent on Bobwhite (Figs. 3 and S10A).

Southern blots including DNAs digested with *Hind*III from eight 17a $T_1$ plants and seven 26b $T_1$ plants were hybridized with a 942-bp *WKS1* fragment derived from PCR primers YR36_PF/PR (Table S6). Radioactive probes were prepared with Prime-a-Gene® Labeling System (Promega, Madison, WI, USA) and purified by MicroSpin™ G-50 columns (Amersham, Piscataway, NJ, USA). Three 17a $T_1$ plants showed no transgene insertion and were retained as additional negative controls (Fig. S10). The construct has two *Hind*III sites flanking the probe region so a fragment of similar size is expected in different transgenic events (blue arrow, Fig. S10). Pre-hybridization, hybridization, and washing were performed as described before (*13*).

**8.- *WKS1* transcription**

***WKS1 alternative transcript variants (WKS1.#).*** Sequencing of 56 cDNA clones amplified with poly T primer and/or *WKS1* specific primers showed six alternative transcript variants (WKS1.1-6, Fig. S11). These six variants were classified into two groups based on the presence or absence of exon 11, the last exon. Transcript variant WKS1.1 included the complete gene with the poly A sequence starting 80-bp after the stop codon in exon 11. For transcript variants WKS1.2-6 the poly A tail started 80-95-bp downstream of the splicing site of exon 10, approximately 1,450-bp upstream from the start of exon 11. As a result of the exclusion of exon 11, WKS1.2-6 variants encode for shorter proteins with a truncated START domain (Fig. S11).

For the Q-PCR experiments, primers WKS1_F5/R5 were used to amplify transcript variant WKS1.1 and primers WKS1_F4/R4 to amplify transcript variants WKS1.2-6 (Table S6). The reverse primer was unique to each group with WKS1_R5 annealing to the splice junction of exons 10 and 11 (unique for WKS1.1) and WKS1_R4 annealing to the unique WKS1.2-6 sequence of exon 10 that is missing from WKS1.1 (Fig. S11). Conserved primers WKS1_F1/R1 were used to amplify simultaneously all six transcript variants (Table S6).

***Real-time quantitative PCR (Q-PCR).*** Total RNA was extracted using TRIZOL (Invitrogen, Carlsbad, CA, USA) and first strand cDNA was synthesized using the SuperScript™ First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Q-PCR was performed on an ABI PRISM 7000 SDS (Applied Biosystems, Foster City, CA, USA) using SYBR® GREEN. PCR setup and reaction conditions were as reported before (*14*). The $2^{-\Delta\Delta C_T}$ method (*15*) was used to normalize and calibrate transcript values relative to the endogenous *ACTIN* control (Table S6).

Efficiencies of each pair of primers were calculated using six 2-fold dilutions (1:1, 1:2, 1:4, 1:8, 1:16 and 1:32) in triplicates. Amplification efficiencies were higher than 95% for all three systems. The same calibrator was used for all transcript variants within each experiment so their values are comparable ($2^{-\Delta\Delta C_T}$ values represent number of RNA copies per copy in the calibrator sample).

***Effect of temperature, PST inoculation, and days post inoculation (DPI) on WKS1 transcript levels.*** Tetraploid RSL65 (resistant parental line in the mapping population) was used for this experiment. Seedlings were initially grown at a low temperature regime, which was constant 10˚C during the 8h dark period and constant 15˚C during the 16 h light period. Half of the plants were kept at low temperature and the other half were moved to the 10/35˚C temperature cycle. Chambers for all treatments were maintained at the same photoperiod (16 h of light and 8 h dark) and light intensity (145 μmol $m^{-2}$ $s^{-1}$). In all cases, samples were collected between noon and 1:00 pm.

Plants at the three-leaf stage within each temperature treatment were divided in two groups. The first group was inoculated with PST-100 and the other group was used as non-inoculated control. Six samples were collected 3, 9, and 16 days after inoculation for each of the four treatment combinations (total 72 samples). The effects of temperature, PST inoculation, days after inoculation, and their respective interactions on WKS1.1 and WKS1.2-6 transcript levels were analyzed using three-way factorial ANOVAs. Since there was a significant three-way interaction between temperature, inoculation and DPI, we analyzed separately the effect of temperature and inoculation at 3, 9 and 16 DPI. Results are summarized in Figs. 4 (main text) and S12 and in Table S8.

**9.- Distribution of the *WKS1* and *WKS2* genes among different Triticeae species.** Samples for the *Triticeae* species were generously provided by Dr. J. Dvorak (University of California Davis, USA, DV numbers Table S9), the University of Haifa (Israel) germplasm collection and the USDA National Small Grain Collection (NSGC Aberdeen, ID) (Table S9). The accessions of wild emmer (Table S10) were from the

University of Haifa collection and from the USDA- NSGC. *T. turgidum* ssp. *dicoccum* accessions were from the USDA-NSGC. Durum and bread wheat accessions were kindly provided by M.C. Sanguineti (Bologna University, Italy) or were from the UC Davis collection.

PCR conditions and sizes of the amplified products are described in the legend of Fig. S14 and primer sequences are shown in Table S6.

**10.- In-gel kinase assay**

***Plasmid construction:*** A GST fusion construct including the complete kinase domain (WKS1 amino acids 1-332, GenBank accession EU835199) was developed for the kinase activity assay. This sequence was PCR amplified from cDNA using primers GST_EcoRI_F1 and GST_XhoI_R1 (Table S6) and initially cloned into pGEM-T Easy vector (Promega, Madison, WI, USA). Restriction enzymes *Eco*RI and *Xho*I were used to clone this fragment into expression vector pGEX-6P-1 (GE Healthcare, Piscataway, NJ, USA), resulting in construct GST-WKS1_Kinase. Sequencing confirmed that no PCR errors were introduced.

***Expression and purification of fusion proteins:*** GST-WKS1_Kinase was transformed into *E. coli* strains pLysS (Gene Choice, Frederick, MD, USA) and BL21(DE3). Bacteria were grown in 50 mL of LB media containing ampicillin (100 μg/ml) to $O.D._{600}$ of 0.6-0.8. Before induction of the fusion protein, cells were collected by centrifugation at 5000 rpm for 5 minutes. Cells were resuspended in 50 mL of fresh LB media containing ampicillin (100 μg/ml) and 1 mM of isopropyl-1-thio-β-D-galactopyranoside (IPTG) and incubated at 37ºC for 6-8 hours. Cells were harvested by centrifugation and resuspended in 1x PBS buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4$, 1.47 mM $KH_2PO_4$, pH 7.4) supplemented with Complete Protease Inhibitor Cocktail Tablets (Roche, USA) and lysozyme (Sigma-Aldrich, St. Louis, MO, USA). The resuspended cells were lysed by sonication and the lysate was centrifuged at 5000g for 5 m. The GST-fusion protein was purified using glutathione-Sepharose 4B (GE Healthcare, Piscataway, NJ, USA) according to manufacturer's instructions and dialyzed overnight against 50 mM HEPES-NaOH, pH 7.4, using the Mini Dialysis Kit (GE Healthcare, Piscataway, NJ, USA).

***In-gel kinase assays*:** In-gel kinase assays were performed as described by Romeis et al. (*16*) except that the SDS-PAGE gel was co-polymerized with casein (1 mg/ml, C4032, Sigma-Aldrich, St. Louis, MO, USA) as the phosphorylation substrate and a different kinase buffer was used. The kinase buffer included 50 mM HEPES-NaOH, pH 7.4, 10 mM $MgCl_2$, 10 mM $MnCl_2$, 1 mM DTT, phosphatase inhibitors (β-glycerophosphate, NaF, $Na_3VO_4$; Cayman Chemical, Ann Arbor, MI, USA) and 75 μCi of [$\gamma$-$^{32}$P]ATP (6000 Ci/mmol, Perkin Elmer Life Sciences, Boston, MA, USA. Gels were analyzed using a Storm 860 PhosphorImager (GE Healthcare, Piscataway, NJ, USA). The size of the phosphorylated proteins was estimated by using a prestained molecular mass marker.

***Peptide Sequencing:*** To confirm the identity of the induced GST-WKS1_kinase fusion protein, we prepared the protein corresponding to the ~66-kD band for MS analysis using standard reduction, alkylation, and tryptic digest procedures (*17*). Digested peptides were analyzed by LC-MS/MS on an LTQ with Michrom Paradigm LC and CTC Pal autosampler at the UC Davis Genome Center Proteomics Core Facility (http://proteomics.ucdavis.edu/). All MS/MS samples were analyzed using Sequest (ThermoFinnigan, San Jose, CA; version SRF v. 3) to search a custom database assuming the digestion enzyme trypsin. Scaffold (version Scaffold_2_01_01, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications.

***Western blots***. GST-WKS1_kinase and GST proteins were transferred to Amersham Hybond™ ECL™ membranes by a vertical blotting unit using protein transfer buffer (1L: 3.03 g Trizma base, 14.4 g Glycine, 200 ml Methanol, pH 8.3). The presence of GST was tested using a rabbit GST antibody and detected using the ECL plus Western Blotting Detection System (Amersham Bioscience, Buckinghamshire, UK).

## SUPPORTING TEXT

**1. Adjacent kinase and START domains in Arabidopsis.** We searched the rice and Arabidopsis genomes for genes encoding for both kinase and START domains to look for a possible orthologue of *WKS1*. We did not find this combination in rice but in Arabidopsis we found putative gene *MGH6.22* (AB026645) that encodes for a 1,088 amino acid protein (BAB01397). BAB01397 has a START domain in the N-terminal region followed by multiple leucine-rich repeats (LRRs), and a protein kinase catalytic domain in the C-terminal region. There is no full-length cDNA to support this annotation, and the protein record Q9LK66 has been discontinued and replaced by two adjacent but separate genes: At3g13062 (NP_850573, an unknown protein with similarity to a START domain) and SRF4 (STRUBBELIG-RECEPTOR FAMILY 4, serine/threonine kinase) supported by multiple ESTs.

Using primers from At3g13062 and SRF4, we cloned two full-length cDNAs from *Arabidopsis* (Columbia ecotype) that include both At3g13062 and SRF4 coding sequences. However, both cDNAs have premature stop codons between the START and the LRR repeats, one of which is similar to the stop codon in At3g13062 (GenBank FJ154117 and FJ154118).

The kinase and START domains of WKS1 are in a different order than in the At3g13062-SRF4 cDNA. In addition, the two WKS1 domains are more similar to other Arabidopsis proteins than to At3g13062-SRF4 (BLASTP searches of Arabidopsis RefSeq protein database). The START domain and the adjacent 5' inter-domain region of WKS1 are more similar to Arabidopsis EDR2 (NP_193639, 56% identity over 337 amino acids, $E = 3e^{-103}$) than to At3g13062 (NP_850573, no BLASTP significant similarity). Similarly, the WKS1 kinase domain is more similar to the WALL ASSOCIATED KINASE 4 (NP_173544, 40% identity over 314 amino acids, $E = 1e^{-51}$) than to SRF4 (30% identity over 280 amino acids, $E = 1e^{-28}$). These

results indicate that the domains encoded by At3g13062 and SRF4 are not likely orthologous to the kinase and START domains in WKS1.

A BLASTP search in rice showed that the closest protein to the WKS1 kinase domain is EAY97604 (60% identical over 323 amino acids $E = 9e^{-105}$) and that the closest one to the START domain is ABB47745 (66% identical over 335 amino acids, $E = 2e^{-123}$) which is 72% identical to Arabidopsis EDR2 over its entire length ($E = 0$). These two genes (or closely related ones) are the most likely source of the shuffled domains that originated *WKS1*. The WKS1 kinase is classified as a non –RD kinase because it lacks the arginine (R) residue preceding the invariant aspartate (D) in the activation domain.

**2. START domains in plants**

START domains are lipid/sterol binding modules that are conserved from animals to plants (*18*). Although the specific ligands for some human START domain proteins are known (e.g. StAR protein binds cholesterol and CERT protein binds ceramides), a function in ligand binding has not been verified for any START plant protein so far. However, Arabidopsis plants with mutations in sterol biosynthesis genes share common phenotypes with mutants for homeodomain-START genes, which suggests that these proteins may be controlled by binding sterols (*19*). In addition, protein modeling of plant START domains based on the crystal structure of human START proteins implicates similar molecular ligands, and suggests that these proteins have retained common functions in evolution (*18*). *EDR2*, a START domain protein involved in the Arabidopsis response to powdery mildew, localizes to the endoplasmatic reticulum, plasma membrane and endosomes (*20*), which is also consistent with a role of the START domain in lipid sensing and trafficking.

Amongst the 35 Arabidopsis proteins with START domains, the EDR2 START domain is the closest to the WKS1 START domain. However, EDR2 and WKS1 differ in other conserved domains present in these proteins. WKS1 has an additional kinase domain, whereas EDR2 has additional PH (plekstrin homology) and DUF1336 (unknown function) domains. This may explain why WKS1 confers resistance, whereas EDR2 is a negative regulator of pathogen-induced disease resistance. In spite of these differences, the two genes share a late-acting resistance phenotype associated with necrotic lesions and programmed cell death (*20, 21*). Wheat plants with a mutation in the WKS1 START domain (Fig. S7) are susceptible to PST and lack the necrotic lesions characteristic of plants with wild *WKS1* alleles (Fig. S9), which indicates that this domain is necessary to trigger the hypersensitive response.

The *edr2* phenotype is very similar to the phenotype of both *edr1* and *edr1-edr2* double mutants suggesting that these two genes are part of the same signal transduction pathway (*22*). *EDR1* encodes a CTR-1 Ser/Thr kinase suggesting that EDR2 might be a phosphorylation target of EDR1 (*19*). Phosphorylation has been also shown to be important for START domain function for at least one animal protein (*23*) and to modulate the role of ceramides in programmed cell death in Arabidopsis (*24*). The presence of an active kinase and a putative START domain within a single protein (WKS1) provides a new tool to study interactions between kinases and START domains.

## SUPPORTING TABLES

**Table S1**. Races of PST used in seedling and adult plant resistance tests.

| Races [1] | Susceptible wheat differential genotypes [2] | Year isolated |
|---|---|---|
| **PST-17** | 1, 2, 3, 9, 11. | 1977 |
| **PST-37** | 1, 3, 6, 8, 9, 10, 11, 12. | 1987 |
| **PST-45** | 1, 3, 12, 13, 15. | 1990 |
| **PST-100** | 1, 3, 8, 9, 10, 11, 12, 16, 17, 18, 19, 20. | 2004 |
| **PST-113** | 1, 2, 3, 8, 9, 10, 11, 12, 14, 16, 17, 18, 19, 20. | 2004 |
| **PST-116** | 1, 3, 4, 5, 8, 9, 10, 11, 12, 14, 16, 17, 18, 19, 20. | 2005 |
| **PST-127** | 1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20. | 2007 |
| **PST-130** | 1, 3, 4, 8, 10, 11, 12, 16, 17, 18, 19, 20. | 2007 |

[1] Based on references (*5, 25-28*).

[2] Numbers indicate the wheat differential genotypes used to identify the race: 1 = Lemhi, 2 = Chinese 166, 3 = Heines VII, 4 = Moro, 5 = Paha, 6 = Druchamp, 8 = Produra, 9 = Yamhill, 10 = Stephens, 11 = Lee, 12 = Fielder, 13 = Tyee, 14 = Tres, 15 = Hyak, 16 = Express, 17 = Avocet S + *Yr8*, 18 = Avocet S + *Yr9*, 19 = Clement, and 20 = Compair.

**Table S2**. PCR markers used to produce the genetic map. Markers are listed from the telomeric to the centromeric location.

| Locus | Marker | Primers | Ann. (°C) [1] | Ext. (s) [1] | Rest. Enz. [1] | Polym. (bp) [1] | Rice homolog |
|---|---|---|---|---|---|---|---|
| *Xucw110* [2] | CAPS [3] | GGAGCAGCCACATCGTCG<br>GCCTGCTCCAACAACCATC | 57[5] | 210 | *Msp*I | L[4] ≅ 2000 | Os02g0139200 |
| *Xucw70* | CAPS | GTCTGTCCATGGGTTCTC<br>GTCATGAAGCCTTGGTTGAAG | 57[5] | 180 | *Dpn*II | L ≅ 1850<br>D ≅ 850 | Os02g0139300 |
| *Xucw112* | CAPS | GGAGTGGAACCAGAGGAGC<br>ATGATGTGCACCATGCGG | 57[5] | 120 | *Hae*III | L ≅ 390<br>D ≅ 300 | Os06g0703500 |
| *Xucw113* | CAPS | GCTGGAGGTGAGTGGTGAAT<br>AATCTCCTCCCTTCGATGCT | 57 | 30 | *Taq*I | L = 252<br>D = 175 | Os02g0139500 |
| *Xucw128* | BACs [3] | TTAGATGGAGTCCCGTGGAG<br>TGAAGCCAGCAATGAAGTTG | 58 | 40 | none | L ≅ 195<br>D = 189 | (wheat genomic) |
| *Xucw129a*[2] | BACs | AAGGACTCTGCTCCTGACGA<br>GAAGATGCTCTGAACGCACA | 58 | 130 | none | D = 1452 | (wheat genomic) |
| *Xucw129b*[2] | BACs | AAGGACTCTGCTCCTGACGA<br>TGTCGAGGGACACAATACCA | 55 | 60 | *Tsp*509I | L ≅ 760 | (wheat genomic) |
| *Xucw125* [2] | BACe [3] | CAAGCGATGTCAACATGTCC<br>TCAAATGACAGCTCCACTCG | 57 | 30 | none | D = 143 | (wheat genomic) |
| *Xucw126* [2] | BACe | GATGGTGCCTGCGATAATTT<br>GCTGTCGACATTCCCCTAGA | 57[5] | 180 | none | D = 2725 | (wheat genomic) |
| *Xucw130* [2] | BACs | CACGCAAATAAATGCTGGTG<br>TGCATAGTTTCAGCCAGGTG | 64 | 40 | none | D = 161 | (wheat genomic) |
| *Xucw148* [2] | BACs | CCCTTTGTGCCACATTTCTT<br>GGCAGGTGGAAGTCAACATT | 57[5] | 240 | *Rsa*I | D = 462[6] | (wheat genomic) |
| *Xucw127* [7] | BACe | GTACGTCCTGCTCACCATCA<br>AGAAGAACAACGGAGGACGA | 65 | 30 | none | L = 110<br>D = 105 | (wheat genomic) |
| *Xucw111* | CAPS | ACCCGTAAGATGCAATAACTTG<br>GCAGGACTGCTCTTGAAG | 59 | 30 | *Rsa*I | L ≅ 306<br>D = 215 | Os02g0139700 |
| *Xucw69* | dCAPS [3] | AGTTGTCATGTAATAGGTTGTACC<br>ATACATCAGTATKTATGTGGCATG[8,9] | 45 | 30 | *Sph*I | L = 140<br>D = 120 | Os02g0141300 |
| *Xucw103* | dCAPS | CTTTGTTTCCTGTATACGAATGCTTT[8]<br>AGAAGAATTTACAAATACACAGC | 45 | 30 | *Pst*I & *Xmn*I[10] | L = 217<br>D = 239 | Os02g0142500 |
| *Xucw65* | CAPS | GCATGTTTCAGTTTGGTTATCA<br>CTCATCATCACATCACAAAGGAA | 53 | 40 | *Nco*I | L = 418<br>D = 684 | Os02g0146600 |
| *Xucw102* | dCAPS | AACATAAGAGGGAGGTCGAG<br>GAACAAGAGCACAGCACGTTGT[8] | 59 | 30 | *Dra*III | L = 205<br>D = 188 | Os02g0148600 |

[1] Ann.: annealing temperatures, Ext.: extension time, Rest. Enz.: restriction enzyme, and Polym.: polymorphic band size.

[2] Dominant marker.

[3] CAPS: Cleavage Amplified Polymorphic Sequences, dCAPS: degenerate CAPS (*29*), $BAC_e$: BAC end sequence, $BAC_s$: BAC sequence.

[4] L: Langdon; D: *T. turgidum* ssp. *dicoccoides* accession FA15-3.

[5] Initial touch-down: 8 cycles of decreasing 1°C steps from 65°C to 57°C.

[6] The amplification product is 2.68-kb and the polymorphic digested band is 462-bp.

[7] *Xucw127* is part of a predicted pectin lyase-like gene with an X8 domain (outside *Yr36* region).

[8] Underlined letters indicate degenerate nucleotides that were introduced to generate polymorphic restriction sites.

[9] The reverse *Xucw69* primer includes a degenerate K nucleotide (G or T).

[10] Polymorphism is detected by *Xmn*I, and *Pst*I is used to reduce fragment size for convenient visualization in polyacrylamide gel.

**Table S3.** Genotypes of the 121 recombinant substitution lines (RSLs) used for genetic mapping of *Yr36*. The 13 critical RSLs with the closest recombination events flanking *Yr36* are indicated in bold, and their detailed phenotypic evaluation is presented in Table S4. Primers are listed in Table S2 except for *Xucw71, Xbarc101*, and *Xbarc136,* which were described before (*1*).

| No. of RSLs | *Xucw71* | *Xucw110* | *Xucw70* | *Xucw112* | *Xucw113* | *Xucw128* | *Xucw129* | *Xucw125* | *Yr36* | *Xucw126* | *Xucw130* | *Xucw148* | *Xucw127* | *Xucw111* | *Xucw69* | *Xucw103* | *Xbarc101* | *Xucw65* | *Xucw102* | *Xbarc136* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LDN | L[1] | L | L | L | L | L | L | L | ***S*** | L | L | L | L | L | L | L | L | L | L | L |
| 2 | L | D | D | D | D | D | D | D | ***R*** | D | D | D | D | D | D | D | D | D | D | D |
| 3 | D | L | L | L | L | L | L | L | ***S*** | L | L | L | L | L | L | L | L | L | L | L |
| 6 | L | L | D | D | D | D | D | D | ***R*** | D | D | D | D | D | D | D | D | D | D | D |
| 4 | D | D | L | L | L | L | L | L | ***S*** | L | L | L | L | L | L | L | L | L | L | L |
| **1** | **D** | **D** | **D** | **D** | **D** | **L** | **L** | **L** | ***S*** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | **L** |
| **1** | **D** | **D** | **D** | **D** | **D** | **D** | **D** | **L** | ***S*** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | **L** |
| **1** | **D** | **D** | **D** | **D** | **D** | **D** | **D** | **D** | ***R*** | **D** | **D** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | **L** |
| **1** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | ***S*** | **L** | **L** | **L** | **D** | **D** | **D** | **D** | **D** | **D** | **D** | **D** |
| **3** | **D** | **D** | **D** | **D** | **D** | **D** | **D** | **D** | ***R*** | **D** | **D** | **D** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | **L** |
| **3** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | **L** | ***S*** | **L** | **L** | **L** | **L** | **D** | **D** | **D** | **D** | **D** | **D** | **D** |
| **3** | **D** | **D** | **D** | **D** | **D** | **D** | **D** | **D** | ***R*** | **D** | **D** | **D** | **D** | **L** | **L** | **L** | **L** | **L** | **L** | **L** |
| 7 | L | L | L | L | L | L | L | L | ***S*** | L | L | L | L | L | D | D | D | D | D | D |
| 8 | D | D | D | D | D | D | D | D | ***R*** | D | D | D | D | D | L | L | L | L | L | L |
| 1 | L | L | L | L | L | L | L | L | ***S*** | L | L | L | L | L | L | L | D | D | D | D |
| 4 | L | L | L | L | L | L | L | L | ***S*** | L | L | L | L | L | L | L | L | D | D | D |
| 12 | D | D | D | D | D | D | D | D | ***R*** | D | D | D | D | D | D | D | D | L | L | L |
| 15 | L | L | L | L | L | L | L | L | ***S*** | L | L | L | L | L | L | L | L | D | D | D |
| 16 | D | D | D | D | D | D | D | D | ***R*** | D | D | D | D | D | D | D | D | L | L | L |
| 15 | L | L | L | L | L | L | L | L | ***S*** | L | L | L | L | L | L | L | L | L | L | D |
| 15 | D | D | D | D | D | D | D | D | ***R*** | D | D | D | D | D | D | D | D | D | D | L |
| RSL65 | D | D | D | D | D | D | D | D | ***R*** | D | D | D | D | D | D | D | D | D | D | D |

[1] L = Langdon, D (shaded) = RSL65, *S* = susceptible, *R* = resistant.

**Table S4.** Genotypes and phenotypes of 13 RSLs with the closest recombination events flanking *Yr36*. Race PST-100 was used for inoculations in all experiments, but additional races may have been present in the field experiments. Daily temperature cycles in the greenhouse were 10/35°C and in the chambers were 10/25°C.

| | RSL genotypes [1] | | | | | | | | | Wheat reaction to PST [2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *Yr36* | | | | | | Adult plant | | Seedling | |
| **Parents &RSL** | ***Xucw113*** | ***Xucw128*** | ***Xucw129*** | ***Xucw125*** | ***Xucw126*** | ***Xucw130*** | ***Xucw148*** | ***Xucw127*** | ***Xucw111*** | Field (R/S) | GH (IT ± SE) [3] | Chamber (R/S) | Chamber (% pustules) [3] |
| LDN | L | L | L | L | L | L | L | L | L | S | 6.8 ± 0.2 | S | 7.51 ± 0.9 |
| **39-14** | L | L | L | L | L | L | L | D | D | S | 7.0 ± 0.0 $^{S}$ | S | 7.5 ± 0.8 $^{S}$ |
| **11-19** | L | L | L | L | L | L | L | L | D | S | 6.3 ± 0.5 $^{S}$ | S | 8.0 ± 1.1 $^{S}$ |
| **291** | L | L | L | L | L | L | L | L | D | S | 6.3 ± 0.7 $^{S}$ | S | 5.9 ± 0.7 $^{S}$ |
| **324** | L | L | L | L | L | L | L | L | D | S | – | S | 9.1 ± 1.1 $^{S}$ |
| **17-47** | D | L | L | L | L | L | L | L | L | S | 6.8 ± 0.2 $^{S}$ | S | 8.8 ± 0.7 $^{S}$ |
| **504** | D | D | D | L | L | L | L | L | L | S | 6.7 ± 0.3 $^{S}$ | S | 7.0 ± 1.4 $^{S}$ |
| **241** | D | D | D | D | D | D | L | L | L | R | 4.3 ± 0.8 $^{R}$ | R | 2.8 ± 0.8 $^{R}$ |
| **3-28** | D | D | D | D | D | D | D | L | L | R | 2.5 ± 0.3 $^{R}$ | R | 3.1 ± 0.8 $^{R}$ |
| **4-36** | D | D | D | D | D | D | D | L | L | R | 2.3 ± 0.3 $^{R}$ | R | 1.2 ± 0.4 $^{R}$ |
| **402** | D | D | D | D | D | D | D | L | L | R | 2.0 ± 0.0 $^{R}$ | R | 0.2 ± 0.1 $^{R}$ |
| **22-4** | D | D | D | D | D | D | D | D | L | R | 2.0 ± 0.0 $^{R}$ | R | 1.4 ± 0.4 $^{R}$ |
| **27-15** | D | D | D | D | D | D | D | D | L | R | 2.1 ± 0.1 $^{R}$ | R | 2.7 ± 0.7 $^{R}$ |
| **28-1** | D | D | D | D | D | D | D | D | L | R | 2.0 ± 0.0 $^{R}$ | R | 1.5 ± 0.3 $^{R}$ |
| RSL65 | D | D | D | D | D | D | D | D | D | R | 2.8 ± 0.7 | R | 0.2 ± 0.1 |

[1] 'L' (white cells): alleles of the susceptible parent LDN, 'D' (shaded cells): alleles of the resistant parent RSL65. Because markers are listed in the same order as they are found on the chromosomes, changes in shading represent recombinant chromosome segments in each RSL.

[2] The 2007 and 2008 field experiments at UCD are summarized by an overall resistant (R) or susceptible (S) score. The greenhouse experiment was performed at Pullman, WA in 2006. Numbers are averages of infection scores of 6-10 plants ± SEM. R and S superscripts indicate resistant or susceptible classification based on the statistical analyses described below. In the 1st chamber experiment lines were simply classified as resistant or susceptible, whereas in the 2nd experiment leaves were scanned and the percentage of leaf area covered with PST pustules was digitally analyzed using the pd program (Fig. S2). These studies confirmed that *Yr36* is located between *Xucw129* and *Xucw148*, and linked to *Xucw125*, *Xucw126*, and *Xucw130*.

[3] After the ANOVA, each RSL was compared with LDN and RSL65 controls using Dunnett tests. Lines that were not significantly different from LDN and significantly more susceptible than RSL65 ($P$<0.01) were classified as susceptible ("S"), whereas lines that were not significantly different from RSL65 but significantly more resistant than LDN ($P$<0.01) were classified as resistant ("R"). IT= Infection type. 0-3 (resistant), 4-6 (intermediate), and 7-9 (susceptible).

**Table S5.** Effect of different PST races on infection scores in RSLs with and without *Yr36*.

| Race | Infection score[1] | | *P* value |
|---|---|---|---|
| | RSLs with *WKS1* | RSLs without *WKS1* | |
| PST-17 | 2.3 ± 0.3 | 7.0 ± 0.0 [2] | <0.0001 |
| PST-37 | 3.0 ± 0.0 | 7.0 ± 0.0 | <0.0001 |
| PST-45 | 1.0 ± 0.0 | 6.5 ± 0.6 | <0.0001 |
| PST-100 | 1.0 ± 0.0 | 7.8 ± 0.3 | <0.0001 |
| PST-113 | 1.0 ± 0.0 | 7.0 ± 0.0 | <0.0001 |
| PST-116 | 1.0 ± 0.0 | 7.0 ± 0.0 | <0.0001 |
| PST-127 | 4.3 ± 0.6 | 7.0 ± 0.0 | <0.0001 |
| PST-130 | 3.7 ± 0.6 | 7.0 ± 0.0 | <0.0001 |

[1] Scale of infection type (IT) (*5*): 0-3 (resistant, none to trace level sporulation), 4-6 (intermediate, light to moderate sporulation), 7-9 (susceptible, abundant sporulation)

[2] Some race genotype combinations showed no variation among genotypes (SE=0), resulting in 0 variance and lack of normality.

Infection scores were obtained from three RSLs with the functional *WKS1* allele (65, 241, and 402) and three RSLs with the null allele (504, 17-47, and 39-14) plus the susceptible parental line LDN. These RSLs were the critical ones used to map *Yr36* within the *Xucw129* and *Xucw148* interval (Table S3). A total of 3 to 6 plants per race-genotype combination were evaluated. Genotype averages were used as replications and individual plants were used as subsamples for the statistical analysis. Amongst races virulent on LDN, races representing a wide range of virulences (Table S1) were selected. *Yr36* resistance to PST races 100, 101 and 111 was shown before (*1*).

For all races, RSLs with the *WKS1* allele showed lower infection scores ($P<0.0001$) than RSLs without *WKS1*, indicating that the gene (s) conferring resistance to these eight PST races is located between markers *Xucw129* and *Xucw148*. This conclusion was further supported by ANOVA using different markers for genotype classification (the model included race, genotype, and race*genotype interaction). When *WKS1* was used as the classification variable, the *F* value ($F = 1,331$) was more than 30-fold higher than when flanking markers *Xucw129* ($F = 42$) or *Xucw148* ($F = 39$) were used as classification variables. These results confirmed that the gene (s) that determines the resistance to these races is located between *Xucw129* and *Xucw148*.

**Table S6.** PCR primers used for the functional characterization of *WKS* genes and for germplasm screening.

| | Gene | Function | Primer name | Primer Sequence |
|---|---|---|---|---|
| **TILLING** | *WKS1* | Kinase domain | Till_1_F1<br>Till_1_R1 | AAGAATAAAATTGGTTTTTAATTTCGGAAAAGGTC<br>ATGGAGGTGTTGGCTTTTGTGAGATGTTT |
| | *WKS1* | START domain | Till_1_F2<br>Till_1_R2 | TGCTGGAACTTGGAGCCATATAAAAATGC<br>TGAACGGAGGGAGTGTTAACTAGCATAGG |
| | *WKS2* | Kinase domain | Till_2_F3<br>Till_2_R3 | GCCATGAACAACGAACAATCACACGATA<br>TAAGTTGTTACTCAGCCCCAGCGCAATAC |
| | *WKS2* | START domain | Till_2_F4<br>Till_2_R4 | TCTGCTCCCAGACCCACCTCATACTTAAA<br>GCAAAAGAGAAAAATGTTAAGCAGCGGAAA |
| **Transgenics** | *WKS1* | Cloning pWKS1 plasmid | YR36_S1F1<br>YR36_S1R4 | AATTACCTGCAGGTGAATGTTTCGACGCG[1]<br>AATTAGCGGCCGCTCCTGGACTACCTCC |
| | *WKS1* | Transgenic screening | YR36_13104F<br>YR36_13692R | GTGGCCAAAGGGTAGATTAG<br>CATCATTGTGCACGAGCTAG |
| | *WKS1* | Confirm WKS1.1-6 transcript WKS1.2-6 length WKS1.1 | WKS1_150F<br>WKS1_151R<br>WKS1_174R | ATGGAGCTCCCACGAAACAAAC<br>GAGACTAGGACACATAACATTAATTG<br>ACTTTCACCACTTCCTGAAGAC |
| | *WKS1* | Probe for Southern blot hybridization | YR36_PF<br>YR36_PR | ATCGTCTCAGGCCGTGGTA<br>CCACTTTGCCTTTGCCTTTA |
| **Transcription** | *WKS1* | Q-PCR all WKS1.1-6 variants in transgenics | WKS1_F1<br>WKS1_R1 | AATCAACATCCATTATTGCGAAGA<br>ATACTTCGTCAGGGCCTCCTATG |
| | *WKS1* | Q-PCR WKS1.1 | WKS1_F5<br>WKS1_R5 | CACAAGTACAATACCTTATGAAGATGG<br>CCTGAGCCCAGCAATACTGT |
| | *WKS1* | Q-PCR WKS1.2-6 | WKS1_F4<br>WKS1_R4 | CTCCACTGAAAACCCGTAATG<br>AACCAAGAGTTTTACCAGCAATACTG |
| | *WKS2* | Q-PCR | WKS2_F1<br>WKS2_R1 | ATCACGAACGTTTGTTTAGTCAAGAA<br>GAGGACCATTTGCAATTGATGTT |
| | *ACTIN* | Q-PCR | Actin_F<br>Actin_R | ACCTTCAGTTGCCCAGCAAT<br>CAGAGTCGAGCACAATACCAGTTG |
| **Germplasm screen** | *WKS1*<br>*WKS2* | Kinase domain | WKS_K_F<br>WKS_K_R | ATCCATTGCCAAGTCAACCAC<br>TCACTTCCATGAAGGAGGTC |
| | *WKS1* | Inter domain | WKS1_I_F<br>WKS1_I_R | CGAAGAAAATCAACATCCATTATT<br>GTGTGGCCATCTACCTCCTC |
| | *WKS2* | Inter domain | WKS2_I_F<br>WKS2_I_R | GAAAAATCAGAAATATTTTACGTGGA<br>AGCTGCAGTCCCACCTAAAA |
| | *WKS1*<br>*WKS2* | START domain | WKS_S_F<br>WKS_S_R | GGCCACACTGCAATACTATACC<br>CACAAATCCTGGCTGTGGAC |
| **Kinase** | *WKS1* | Construct for GST-kinase fusion protein | GST_EcoRI_F1<br>GST_XhoI_R1 | TTGAATTCATGGAGCTCCCACGAAACA[2]<br>TCACTTCCATGAAGGAGGTC |

[1] Sequences highlighted in gray are *Sbf*I and *Not*I restriction sites for cloning. The underlined bases correspond to the target sequence. [2] Sequences highlighted in grey are *Eco*RI and *Xho*I restriction sites for cloning

**Table S7.** *WKS1* and *WKS2* mutants evaluated for PST resistance.

| Gene | Screened region | Allele | Line ID | Nucleotide change[1] | Effect on amino acid[2] | PSSM | SIFT | Reaction to PST |
|---|---|---|---|---|---|---|---|---|
| *WKS1* | Kinase | *wks1a* | T6-569[3] | G 163 A | V 55 I | **11.5** | **0.00** | Susceptible |
| | | --- | T6-89 | G 508 A | D 170 N | **10.4** | 0.46 | Resistant |
| | | *wks1b* | T6-312[3] | G 595 A | G 199 R | **19.7** | **0.00** | Susceptible |
| | | *wks1c* | T6-480-1[3] | C 632 T | T 211 I | **12.6** | **0.01** | Susceptible |
| | | *wks1d* | T6-138[3] | G 914 A | R 305 H | **13.6** | **0.01** | Susceptible |
| | START | *wks1e* | T6-567[3] | G 4437 A | D 477 N | **12.3** | **0.00** | Susceptible |
| *WKS2* | Kinase | --- | T6-960 | C 13 T | R 5 * | -[4] | - | Resistant |
| | | --- | T6-480-2[3] | G 72 A | W 24 * | - | - | Resistant |
| | START | --- | T6-826 | G 2221 A | W 379 * | - | - | Resistant |

[1] The first letter indicates the wild-type nucleotide, the number its position from the ATG start codon, and the last letter the mutant nucleotide.

[2] The first letter indicates the wild-type amino acid, the number its position from the start methionine, and the last letter the mutant amino acid.

[3] Complete *WKS1* or *WKS2* coding regions were sequenced. No additional mutations were found.

[4] PSSM and SIFT scores are not reported for mutations that cause premature stop codons.

From the 117 mutations affecting the kinase and START domains, we selected six in *WKS1* and three in *WKS2*. The three mutations in *WKS2* resulted in premature stop codons, but no such mutations were available for *WKS1*. The *WKS1* mutations were ranked using the bioinformatics programs SIFT (Sorting Intolerant From Tolerant) (*30*) and ParseSNP (Project Aligned Related Sequences and Evaluate SNPs) (*31*) which estimate the severity of each missense change. High PSSM (>10) and low SIFT scores (<0.05) predict mutations with severe effects on protein function.

The $M_2$ line T6-480 was heterozygous for mutations in both *WKS1* (C632T) and *WKS2* (G72A) in repulsion. Homozygous $M_3$ progenies containing mutants for one or the other gene were selected and designated T6-480-1 (*WKS1*) and T6-480-2 (*WKS2*).

Five of the 6 selected *WKS1* mutants were susceptible and were assigned allele names *wks1a* through *wks1e*. T6-89 was the only *WKS1* mutation tested with a resistant phenotype. This mutation has the lowest PSSM value and a non-significant SIFT score, suggesting that the mutated amino acid may not be essential for resistance.

None of the *WKS2* mutations affected PST resistance suggesting that the gene responsible for resistance is *WKS1*. The C13T and G72A mutations are upstream of the kinase domain and the G2221A mutation is in the inter-domain region.

**Table S8. Effect of temperature and PST inoculation on WKS1.1 and WKS1.2-6 transcript levels at 3, 9 and 16 days post inoculation (DPI).** Numbers in the body of the Table are *P* values of the two-way ANOVAs.

| | WKS1.1 | | | WKS1.2-6 | | |
|---|---|---|---|---|---|---|
| Source | 3* | 9* | 16 | 3 | 9* | 16* |
| Temperature (Temp.) | <.0001 | <.0001 | 0.016 | 0.05 | <.0001 | 0.004 |
| Inoculation (Inoc.) | 0.15 | <.0001 | 0.0002 | 0.0003 | <.0001 | <.0001 |
| Temp. * Inoc. | 0.02 | 0.92 | 0.24 | 0.36 | 0.13 | 0.79 |

* Data was transformed to meet assumptions of normally distributed errors and homogeneity of variance.

A 3-way factorial ANOVA showed significant effects of temperature, inoculation and DPI on WKS1.1 ($P<0.0001$) and WKS1.2-6 ($P<0.0001$) transcript levels. The three way interaction of these main effects was also significant in both WKS1.1 ($P<0.01$) and WKS1.2-6 ($P<0.05$) analyses. Therefore the analysis for each transcript variant was conducted separately for each DPI.

The interactions between temperature and PST inoculation were not-significant for all 2-way ANOVAs except for WKS1.1 at 3 DPI. At this early stage WKS1.1 was significantly up-regulated ($P<0.05$) in the inoculated samples at low temperature, whereas no significant differences were detected between control and inoculated samples at high temperature (Fig. 4A). At all other time points PST inoculation consistently down-regulated both transcript variants. The effect of temperature was also consistent across DPI. Higher temperatures significantly increased transcript levels of WKS1.1, whereas those from WKS1.2-6 significantly decreased with higher temperature.

An independent experiment including 12 inoculated and 12 control plants at low temperature 3DPI confirmed the increase of WKS1.1 after inoculation (38%) but the values were more variable and therefore the difference was not significant ($P=0.10$).

**Table S9.** Distribution of *WKS1* and *WKS2* among different *Triticeae* species. Presence or absence of *WKS1* and *WKS2* was assessed by PCR for three different regions of the gene (Table S6) and by sequencing the PCR products (GenBank accessions FJ154103 to FJ154116).

| ***WKS1*** | ***WKS2*** | **Species** |
|---|---|---|
| **Detected** | **Detected** | *Aegilops longissima*[1], *T. turgidum* ssp. *dicoccoides*[2]. |
| **Detected** | Not detected | *Dasypyrum villosum*[3], *Lophopyrum elongatum*[4], *Pseudoroegneria gracillima*[5], *Thinopyrum bessarabicum*[6]. |
| Not detected | **Detected** | *Ae. comosa*[7]. |
| Not detected | Not detected | *Ae. bicornis*, *Ae. crassa*, *Ae. markgrafii*, *Ae. juvenalis*, *Ae. mutica*, *Ae. searsii*, *Ae. sharonensis*, *Ae. speltoides*, *Ae. tauschii*, *Ae. umbellulata*, *Ae. vavilovii*, *Agropyron cristatum*, *Eremopyrum orientale*, *Heteranthelium piliferum*, *Psathyrostachys juncea*, *Pseudoroegneria libanotica*, *P. spicata*, *P. strigosa*, *Secale cereale*, *Taeniatherum caput-medusae*, *Triticum aestivum*, *T. monococcum*, *T. turgidum* ssp. *dicoccum*, *T. turgidum* ssp. *durum*, *T. urartu*. |

[1] Present in G509 (J.G. Waines, FJ154103 and FJ154104) and absent in DV1252 (J. Dvorak).

[2] See Table S10 for intraspecific variation in *WKS1* and *WKS2* distribution.

[3] Present in DV1062 (J. Dvorak, FJ154105 and FJ154106) and absent in D-2990 (D. Dewey).

[4] Present in e3 (J. Dvorak, FJ154107 to FJ154109) and absent in e2 (J. Dvorak).

[5] Present in PI 440000 (FJ154110 and FJ154111).

[6] Present in D-3483 (D. Dewey, FJ154112 and FJ154113). Only kinase and inter-domain PCR products were observed in DV013 and DV727 (J. Dvorak).

[7] Present in G1288, G1289, and G5029 (J. G. Waines, FJ154114 to FJ154116) and absent in G659, G601, G5036, and G5307 (J. G. Waines). The LINE retrotransposon insertion detected in RSL65 in WKS2 intron 10 was not detected in *WKS2* from *Ae. comosa.*

Amongst accessions tested in this study, most species with *WKS1*, *WKS2*, or both genes showed intraspecific variability for the presence and absence of these genes. Therefore, other accessions of the species listed in the group with no detected *WKS* gene may still carry one or both *WKS* genes. Despite this uncertainty, the results above are sufficient to conclude that the duplication that originated *WKS1* and *WKS2* predated the divergence of the *Triticeae* species listed above, and that these two genes have been deleted repeatedly in several *Triticeae* lineages.

**Table S10.** *T. turgidum* and *T. aestivum* germplasm used in the allelic diversity study.

| Wheat | No. | *WKS1/2* | Germplasm Number [1] / Variety Name |
|---|---|---|---|
| *T. turgidum* ssp. *dicoccoides* Southern population [2] | 16 | Present | PI428015, PI428113, PI487252, PI503315, PI538672, PI538673, PI538678, PI538688, PI538697, PI538699, 5-61, 7-4, 8-12, 9-36, 19-14, 30-22 |
| | 24 | Deleted | PI352324, PI428107, PI428111, PI428117, PI428119, PI428123, PI428126, PI428130, PI428135, PI428139, PI428141, PI428143, PI470981, PI470984, PI487264, PI503313, PI503314, PI538681, PI538719, PI560697, PI560872, 1-22, 27-37, 28-50 |
| *T. turgidum* ssp. *dicoccoides* Northern population [3] | 28 | Deleted | PI428016, PI428028, PI428036, PI428041, PI428047, PI428055, PI428058, PI428061, PI428065, PI428070, PI428072, PI428079, PI428082, PI428087, PI428089, PI428098, PI428145, PI503310, PI538626, PI554580, PI554581, PI554582, PI554583, PI554584, PI560874, 42-8736, 43-8811, 44-8821 |
| *T. turgidum* ssp. *dicoccum* Domesticated emmer | 23 | Deleted | PI182743, PI254158, PI254180, PI319868, PI319869, PI347230, PI352329, PI352347, PI352352, PI352357, PI352367, PI355454, PI355496, PI355498, PI470737, PI470738, PI470739, PI606325, PI94626, PI94627, PI94640, CItr17675, CItr17676 |
| *T. turgidum* ssp. *durum* Cultivated durum [4] | 40 | Deleted | Aconchi 89, Adamello, Altar 84, Appio, Appulo, Capelli, Ciccio, Cirillo, Colorado, Colosseo, Duilio, Durfort, Exeldur, Inrat 69, Karel, Karim, Khiar, Kronos, L35, Langdon, Latino, Maier, Messapia, Mexicali 75, Nefer, Neodur, Ofanto, Produra, Rugby, Russello Sg7, San Carlo, Saragolla, Trinakria, Valbelice, Valforte, Valnova, Varano, Vitron, Wb 881, Zenit |
| *Triticum aestivum* Bread wheat | 45 | Deleted | Bobwhite [5], Caledonia, Cayuga, RSI5, Express, Pio 26R61, Kanqueen, CO940610, Eltan, Finch, Foster, Grandin*5/ND614, Harry, Heyne, IDO444, IDO556, Jagger, Jaypee, Jupeteco, KS01HW163-4, Louise, McCormick, McNeal, NY18/Clark's Cream 40-1, OR9900553, Penawawa, Pio 25R26, Pioneer 26R46, PI 610750, PI610752, P91193, P92201, Platte, Reeder/Bw-277, Rio Blanco, Stephens, SS550, TAM 105, Thatcher, UC1110, USG3209, Weebill, Wesley, Zak, 2174. |
| | 5 | Present [6] | Glupro, Lassik, Farnum, ND683, PI 638740. |

[1] PI and CItr germplasm correspond to Germplasm Resources Information Network (GRIN) numbers. Other numbers correspond to 'Location-Genotype' identification numbers from the University of Haifa wheat germplasm collection (*32*).

[2] Wild emmer from Israel, Lebanon, and Syria (*33*).

[3] Wild emmer from Iran, Iraq and Turkey. *WKS1* was not found in this sub-population. Tetraploid wheat was domesticated from the Northern populations explaining the absence of *Yr36* from the domesticated forms.

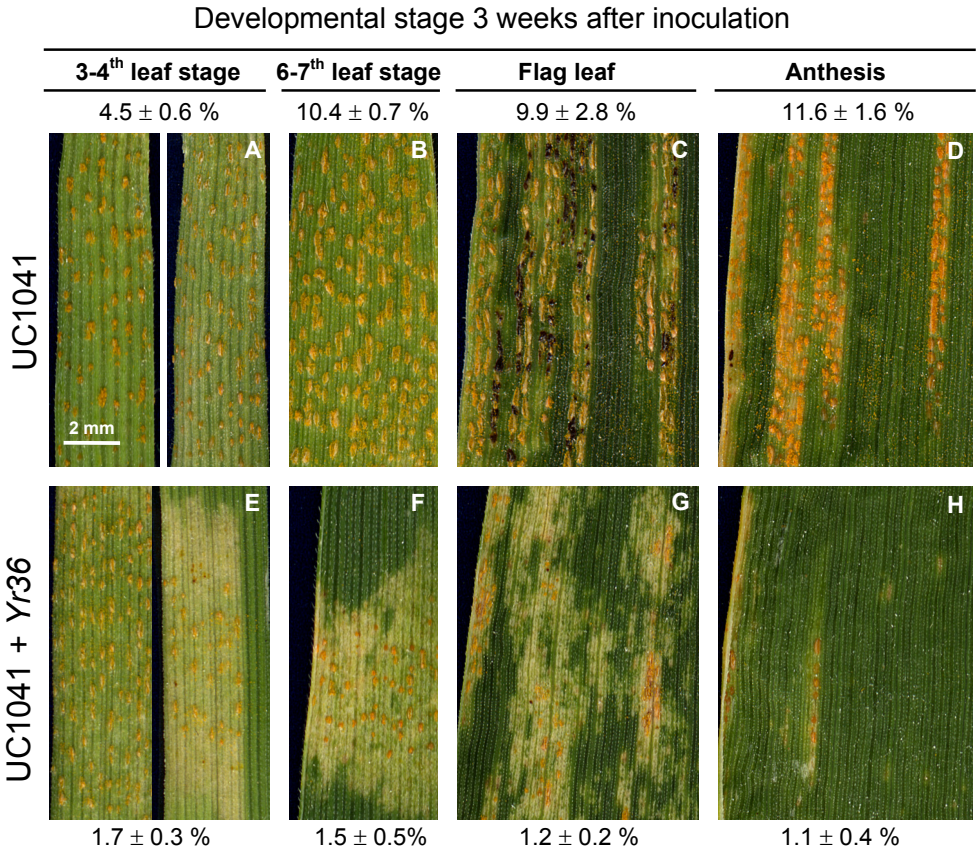
[4] The country of origin of the cultivated durum varieties is described in (*12*).

[5] Deletion of *WKS1* and *WKS2* in hexaploid Bobwhite was confirmed by Southern blot (Fig. S10).

[6] *Yr36* has been found only in varieties selected for the closely linked *GPC-B1* gene. Lassik and Farnum are new *Yr36* stripe rust resistant varieties from California and Washington (USA), respectively.

## SUPPORTING FIGURES

**Figure S1. Effect of plant age on *Yr36* resistance response (10/25°C).** UC1041 near isogenic plants with and without *Yr36* at different developmental stages were inoculated on the same day with race PST-113 and the percent leaf area covered with pustules was evaluated three weeks later using the pd program (Fig. S2). **(A and E)** Inoculated at 1st leaf and scored at 3rd-4th leaf. **(B and F)** Inoculated at 4th leaf and scored at 6-7th leaf. **(C and G)** Inoculated at elongation stage and scored at flag leaf emergence. **(D and H)** Inoculated at heading and scored at anthesis. Plants at the first two developmental stages are classified as juvenile plants, whereas those at the two later stages are classified as adult plants. The black areas in (C) are teliospores. Values are averages of four plants ± SEM of the percentage area covered with PST pustules.




The wheat - *P. striiformis* interaction differs in juvenile and adult plants. Part of the difference is due to changes in leaf anatomy. In younger leaves, a single infection event leads to fungus growth both between and across the veins giving rise to a wide lesion (Fig. S1, A, B, E, and F). In contrast, in adult leaves a single infection event is

primarily limited in growing between veins, which results in a "stripe" pattern of sporulation (Fig. S1, C, D, G, and H).

In addition to this *Yr36*-independent developmental difference, there is also a *Yr36*-dependent difference between adult and juvenile plants. In juvenile resistant plants inoculated at the 1st leaf stage, necrotic patches appeared a few days later than the necrotic stripes observed in adult plants inoculated at later stages. Consequently, in some juvenile leaves, profuse sporulation was observed before any necrosis was detected (E). Later, necrotic patches encompassed the region with pustules and limited the growth of the pathogen, i.e., there was no further expansion of the region of sporulation (Fig. S1, E and F, see also Fig. S8). These necrotic regions were not observed in susceptible UC1041 plants at comparable developmental stages (Fig. S1, A and B). The *Yr36* resistance response at juvenile stages differs from a hypersensitive response in the delayed appearance of the necrotic regions and the incomplete control of sporulation within these necrotic patches.

**Figure S2. Quantification of the percentage of leaf area covered with PST pustules. (A and E)** Scanned images of 5-cm segments of wheat leaves. **(C and G)** Each pixel in (A) and (E) was categorized by the pd program as either leaf (green), *P. striiformis* (red) or background (black). Images in the right column are an enlargement of the image in the rectangle in the left column. **(A to D)** Susceptible RSL 11-19 with pustules covering 6.9% of the surface area in the segment shown in (A). **(E to H)** Resistant RSL3-28 with pustules only on 0.9% of the segment shown in (E). Contrast and brightness were manipulated in (B) and (F) to better show pustules. The white areas in (E) are necrotic patches. Bar = 5 mm. The pd program is freely available at http://plantpathology.ucdavis.edu/faculty/epstein/ .

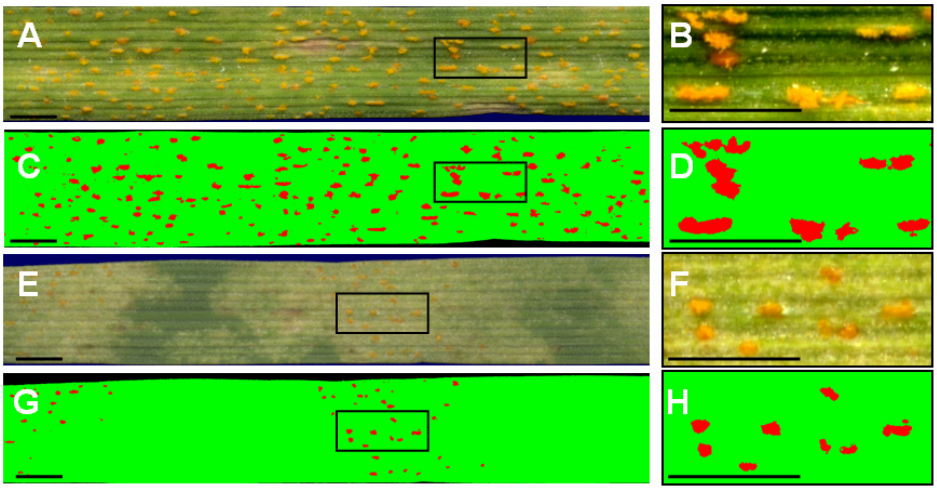


**Figure S3. Reaction to PST in parental and 13 critical RSLs used to map *Yr36*.** Quantification of the percentage leaf area covered by PST pustules is presented in Table S4. Plants at the 3-leaf stage were inoculated with PST-100 and then incubated in a growth chamber with a daily 10/25°C cycle. Susceptible leaves have prodigious sporulation in the orange pustules. Resistant leaves have necrotic regions with reduced sporulation. Images of the progeny leaves are perpendicular to the parental leaves. Bars = 1 mm.

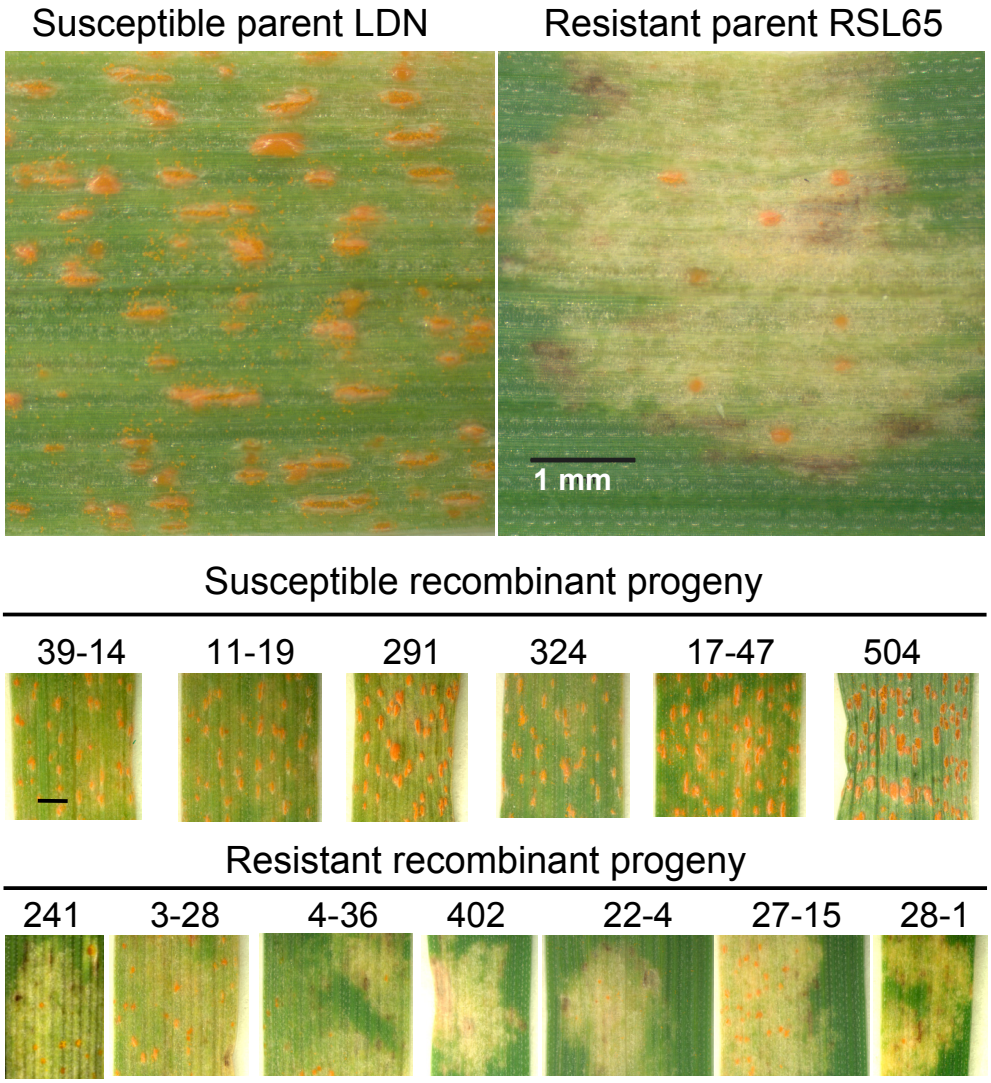


**Figure S4. Physical contig of the *Yr36* region.** The top line summarizes the information from the *Hind*III fingerprinting of the B genome BACs listed below and the sequencing of BACS 391M13 and 1144M20 (in blue). Colored ovals represent markers used for the fine mapping of *Yr36*, whereas colored arrows represent genes *WKS1*, *WKS2*, *IBR1*, and *IBR2* linked to *Yr36*. Markers *Xucw129* and *Xucw148* flank the *Yr36* region (186-kb, yellow shaded square). Markers *Xucw125*, *Xucw126*, and *Xucw130* and genes *WKS1*, *WKS2*, and *IBR1* were not amplified in Langdon, suggesting the presence of a large deletion (between 149 and 183-kb long based on current markers).

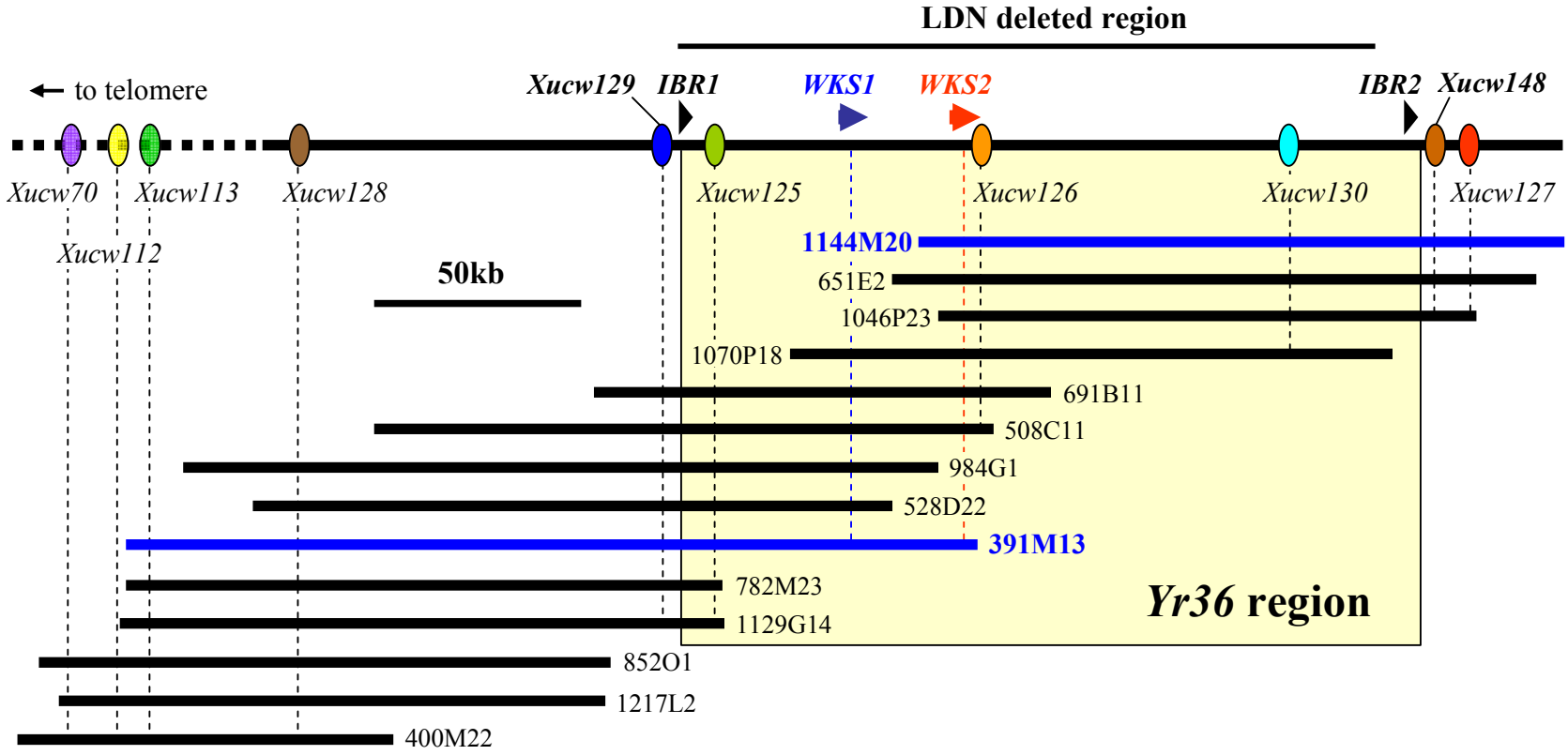


**Figure S5. Sequence annotation of the *Yr36* region**. **(A)** Graphic representation of the annotation of the 314-kb sequenced contig. Two large direct duplications were identified: 1a/1b and 2a/2b. The 1a and 1b duplicated regions each include a putative gene which encodes for a protein with an 'in between RING finger' domain (IBR), designated as *IBR1* and *IBR2*. The 2a and 2b regions include genes *WKS1* and *WKS2,* respectively. The *X8* putative gene (=*Xucw127*) is outside the *Yr36* critical region defined by markers *Xucw129* and *Xucw148*. Boxes outside the duplicated regions represent transposable elements. Half-arrows indicate putative genes. The 262-bp overlap between the 2b and 1b duplication is indicated by a bright blue box. **(B)** Detail of the duplicated regions. The 1a (64.5-kb) and 1b (32.3-kb) regions are 96% identical across 31.3-kb. The 1a region has four unique retrotransposon insertions and a series of repeats absent in the 1b segment. The 2a (19.0-kb) and 2b (94.9-kb) regions are 81% identical across 7.9-kb of shared sequence, suggesting that this duplication is older than the 1a/1b duplication. The 2b region is larger than the 2a region because of a 70.2-kb insertion of multiple nested retrotransposons (shown in different scale). Conserved sequences between the corresponding duplicated regions are represented by similar colors. GenBank accession EU835198 includes a detailed annotation of the region.

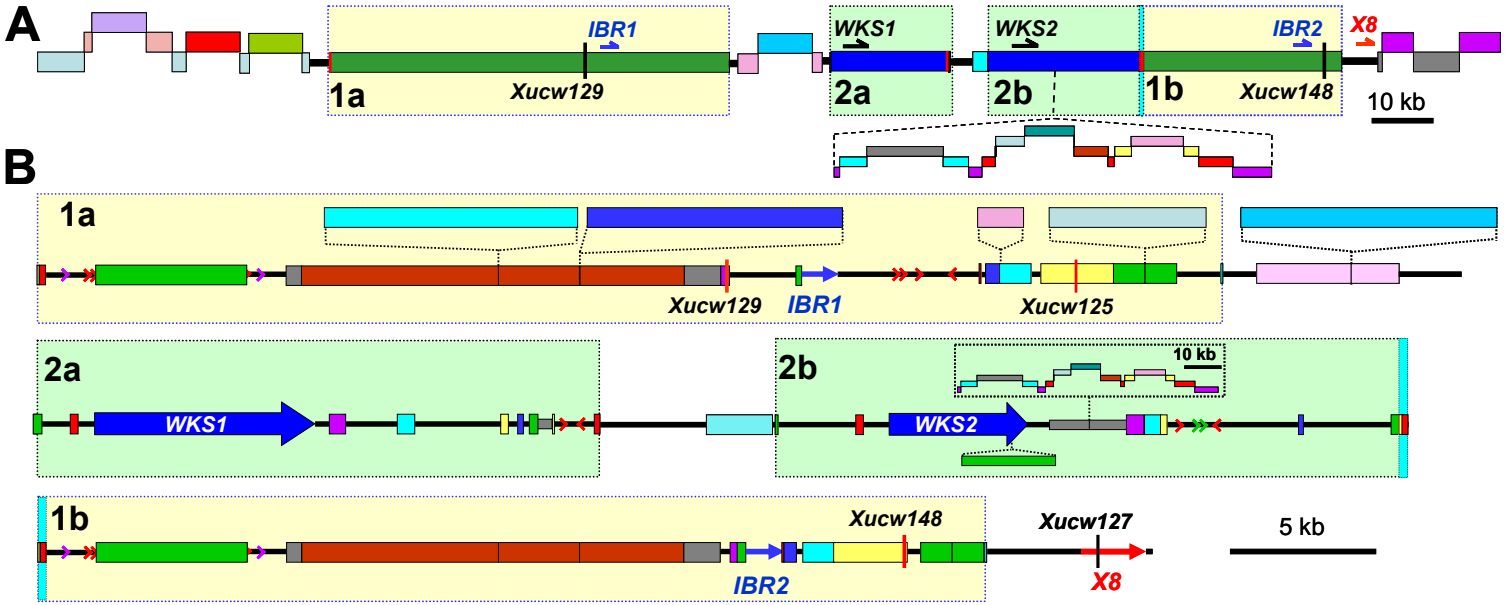


**Figure S6. Selected mutations in the WKS1 kinase domain.** CLUSTALW alignment of the kinase domains (smart00219) from the three closest rice and *Arabidopsis* homologues to WKS1 and WKS2. Amino acid residues that form the ATP (▲) and substrate (*) -binding pocket are labeled. Residues that form part of the catalytic or activation loop are underlined. The red diamond indicates the site of the conserved arginine (R) residue within the catalytic loop that is used to classify kinases as either RD on non-RD. WKS1 has a glycine (G) residue at this position, and is therefore a non-RD kinase. Amino acids affected by the mutations resulting in *WKS1* loss of function are indicated by bold orange letters. The TILLING mutant line designation and the amino acid change in that line is indicated above the *WKS1* sequence.

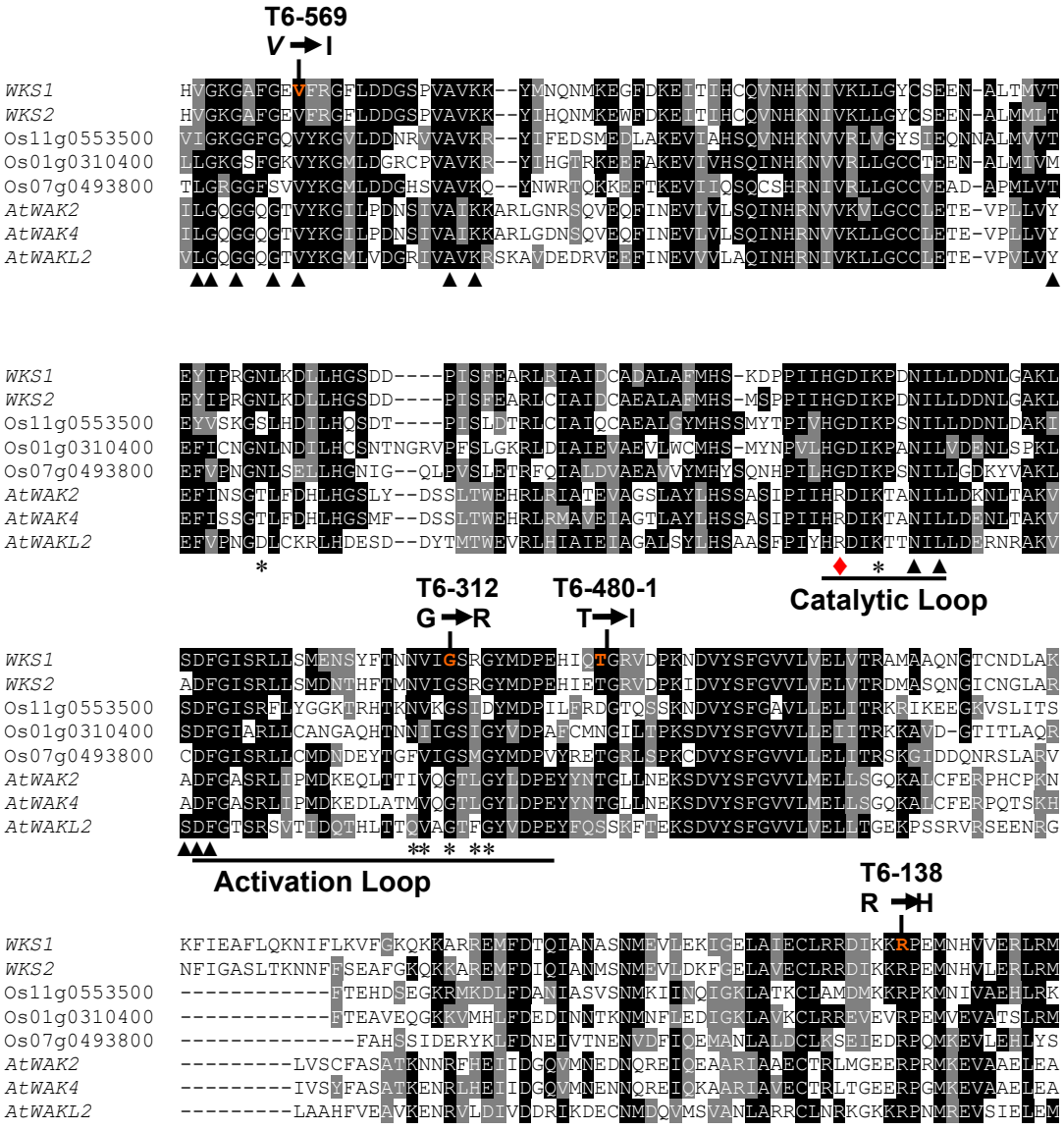


**Figure S7**: **WKS1 START domain. (A)** CLUSTALW alignment of the START-domain region surrounding the D477N mutation in T6-567 (bold orange letter). The aspartic acid residue (D) is conserved across all plant species examined as well as across the closest human START genes (StarD6, D14, D15; not shown). The alignment includes the closest plant homologues from *Arabidopsis* (At), rice (Os), *Poa*, *Populus*, *Vitis*, *Sorghum* (Sb), *Zea*, and moss *Physcomitrella patens* (Pp). Complete sequences are available in Genbank or Phytozome (http://www.phytozome.net/). **(B)** Partial alignment of the START domain C-terminus region. The orange arrow indicates the last common amino acid residue between WKS1 transcript variants WKS1.1 and WKS1.2.

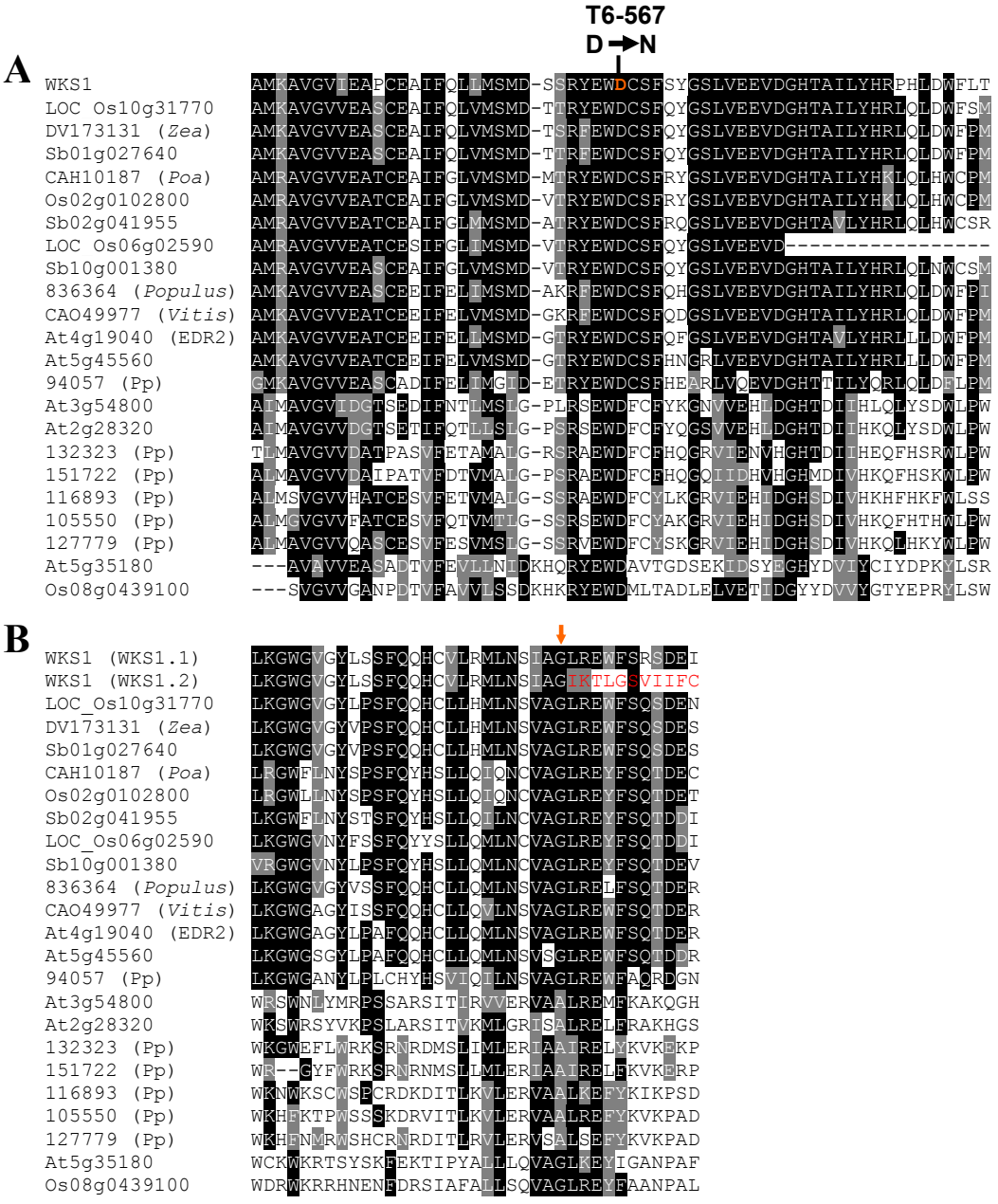


**Figure S8. Effect of *WKS1* and *WKS2* mutations on PST resistance (Mutant experiment 1).** Three *WKS2* and 5 *WKS1* mutants (Table S7) were inoculated at the 4th-leaf (juvenile) stage with race PST-113. The susceptible wheat line UC1041 (without *Yr36*) and the isogenic line UC1041+*Yr36* were used as controls. Sister lines of mutants T6-826 and T6-312 homozygous for the absence of the mutation also were included as controls. The margins of the leaf regions with pustules were marked with a black marker 15 days after inoculation and pictures were recorded five days later.

All the *WKS2* mutants with premature stop codon disrupting the kinase or START domains (Table S7) had a response similar to the resistant control indicating that *WKS2* is not necessary for resistance. On the contrary, four of the five lines with selected mutations in the *WKS1* kinase domain (Fig. S6) were as susceptible as the susceptible control. Only T6-89 (non-significant SIFT score) showed a resistant response suggesting that the D170N mutation did not alter *WKS1* function. Taken together these results indicate that *WKS1* is *Yr36* and that the kinase domain is required for its function.

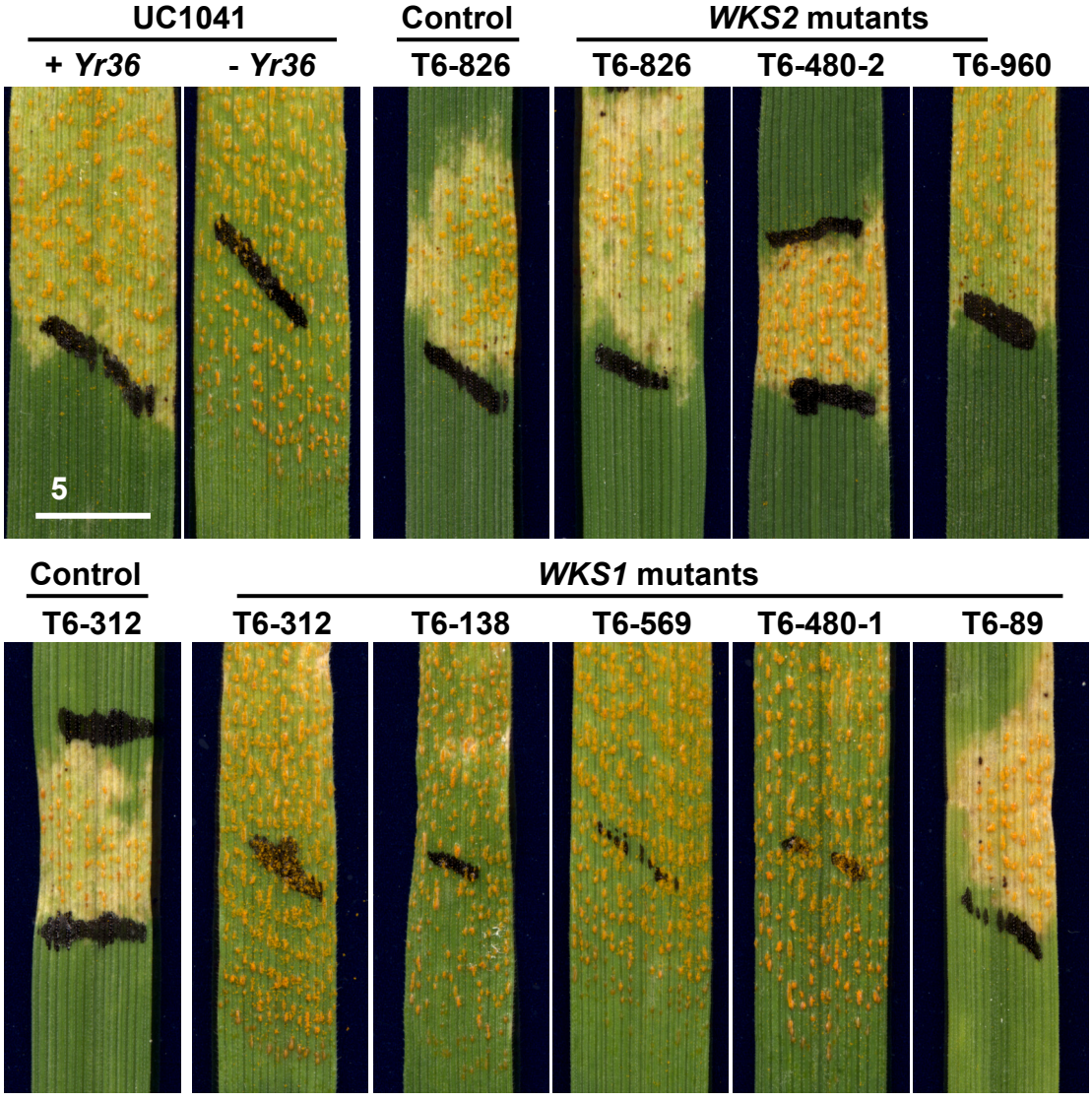


**Figure S9. Loss of PST resistance in *WKS1* START mutant T6-567 (Mutant experiment 3).** The T6-567 line (discovered after Mutant experiments 1 and 2) has a mutation in a conserved codon in the START domain (Fig. S7A). PST infection in this mutant line was compared with a sister line homozygous for the absence of this mutation. Susceptible UC1041 and resistant UC1041+*Yr36* also were tested as controls. Plants were inoculated with race PST-113 at the stem elongation (adult plant) stage.

The T6-567 mutant had a susceptible response similar to UC1041 (null *Yr36*) and was more susceptible than its sister control line. This result indicates that a functional START domain in *WKS1* is necessary for stripe rust resistance.

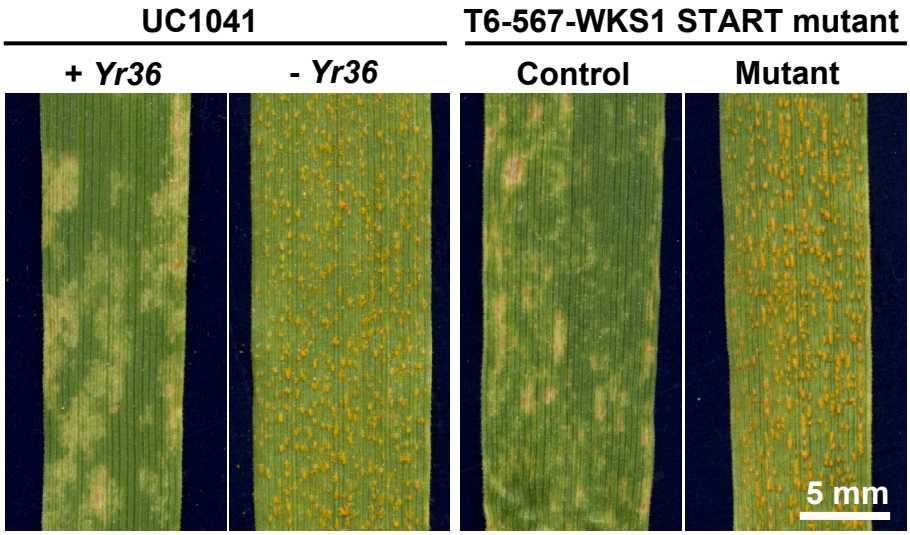


**Figure S10.** ***WKS1*** **transgenic lines.**

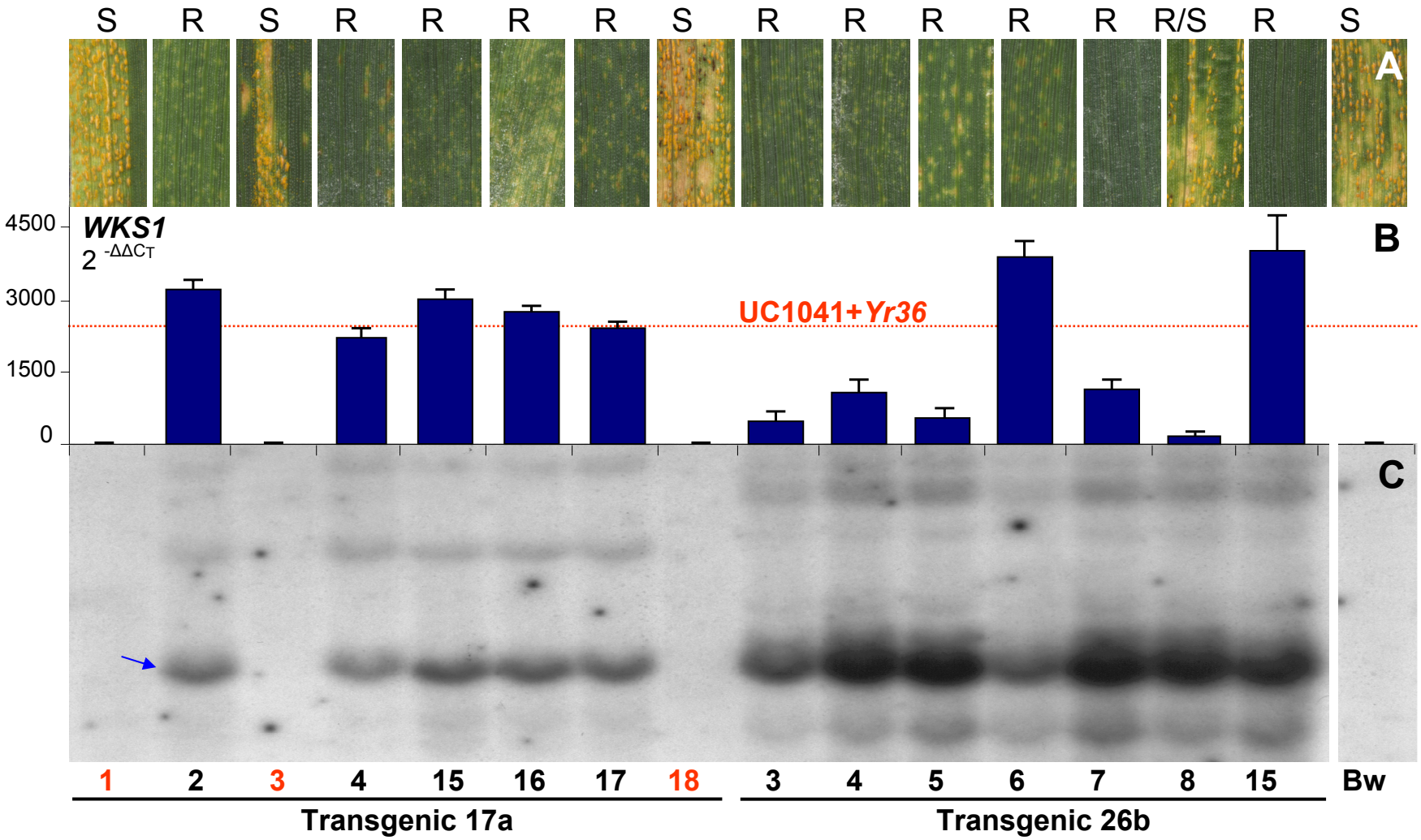


**Figure S10. Continuation.**

Characterization of $T_1$ plants from independent transgenic events 17a and 26b in hexaploid wheat variety Bobwhite (Bw).

**(A)** Resistance (R) or susceptible (S) response on leaves after inoculation with race PST-113.

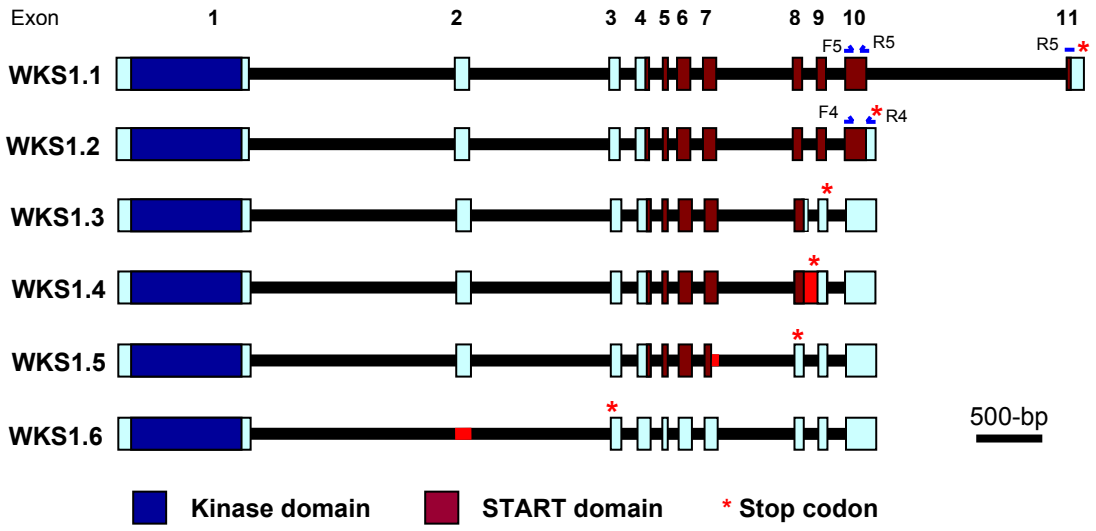
**(B)** *WKS1* transcript levels before infection as determined by Q-PCR. Values are averages of 6 leaves ± SE of the means. The red dotted line indicates *WKS1* transcript level in a non-transgenic UC1041+*Yr36* positive control[a].

**(C)** Southern blots hybridized with *WKS1* showed absence of the transgene in 17a-1, 17a-3 and 17a-18 (in red) and presence in the other five 17a lines. Transgenic 26b lines showed a stronger hybridization signal than transgenic 17a lines, suggesting higher copy number of the transgene. Lines 26b-6 and 26b-15 showed high transcript levels (B) and strong resistance (A). The blue arrow indicates the expected size of the restriction fragment that hybridizes with the *WKS1* probe.

The 17a lines without the transgene were as susceptible as the negative Bobwhite control. Transgenic lines with *WKS1* transcript levels similar or higher than the positive control UC1041+*Yr36* (dotted red line)[a] were resistant to PST-113 and showed little or no sporulation. Line 26-8, which had an intermediate resistance reaction (R/S), had the lowest *WKS1* transcript levels of the transgenic lines.

[a] Since the endogenous *WKS1* in UC1041+*Yr36* is in a different genetic background, its transcript levels should be considered just as an approximate positive control.

**Figure S11. *WKS1* alternative transcript variants.** Cloning and sequencing of 56 *WKS1* cDNA clones revealed six transcript variants designated WKS1.1 to WKS1.6. Primers WKS1_F5/R5 and WKS1_F4/R4 were used to amplify transcript variant WKS1.1 and WKS1.2-6, respectively. Primer WKS1_R5 anneals to the WKS1.1 exon 10-11 splice junction whereas primer WKS1_R4 anneals to the WKS1.2-6 sequence of exon 10 that is missing from WKS1.1.




**WKS1.1** is the only variant with 11 exons coding for a complete START domain.

**WKS1.2-6** transcript variants do not include exon 11 and have an alternative polyadenylation signal located upstream from this exon.

**WKS1.2** transcripts continue beyond the GT splicing site after exon 10 until a stop codon 57-bp after this splicing site.

**WKS1.3** transcripts have an alternative GT splicing site located 4-bp after exon 8. This change in reading frame generates a premature stop codon in exon 9.

**WKS1.4** transcripts continue through the GT splicing site at the end of exon 8 until a stop codon in intron 8 (marked in red).

**WKS1.5** transcripts have a premature splicing site in exon 7 (56-bp before the conserved GT splice site, marked in red), which changes the reading frame and generates a stop codon within exon 8.

**WKS1.6** transcripts do not include the second exon (marked in red). This difference generates a change in reading frame and a premature stop codon in exon 3.

Several amino acids from the C terminal end of the START domain are well conserved from vascular plants to mosses (Fig. S7B). Deletions of the last 10 amino acids of the human StARD protein result in non functional proteins, indicating that this region is critical for its normal function. In the human START proteins, the C terminal α4 helix opens and closes the steroid binding pocket in the hydrophobic tunnel and is able to interact with lipid membranes. Therefore, it is possible that the elimination of the conserved C terminal region in WKS1.2-6 might alter or eliminate function.

**Figure S12. Effect of temperature and infection with PST on *WKS1* transcript variants.** The effect of high (10/35°C) and low (10/15°C) temperatures on transcript levels of WKS1.1 (black bars) and WKS1.2-6 (gray bars) was determined by Q-PCR. Leaf-samples from RSL65 plants were collected 3, 9 and 16 days post inoculation (DPI) with race PST-100. Half of the plants were not inoculated. Each datapoint is the average of 6 samples ± SE of the mean. Since the ANOVAs showed significant interactions between transcript variants and temperatures, simple effects for transcript variants within each temperature are presented above each pair of bars.

**(A to C)** Inoculated. **(D to F)** Non-inoculated. At high temperature, transcript levels of WKS1.1 were significantly higher than those of WKS1.2-6 for all the ANOVAs. At low temperature, no significantly differences were detected between transcript levels of WKS1.1 and WKS1.2-6 except for D. In the non-inoculated samples 3DPI the transcript levels of WKS1.2 were significantly higher than those of WKS1.1. **: $P<0.01$, ***: $P<0.001$, and NS: not significant.

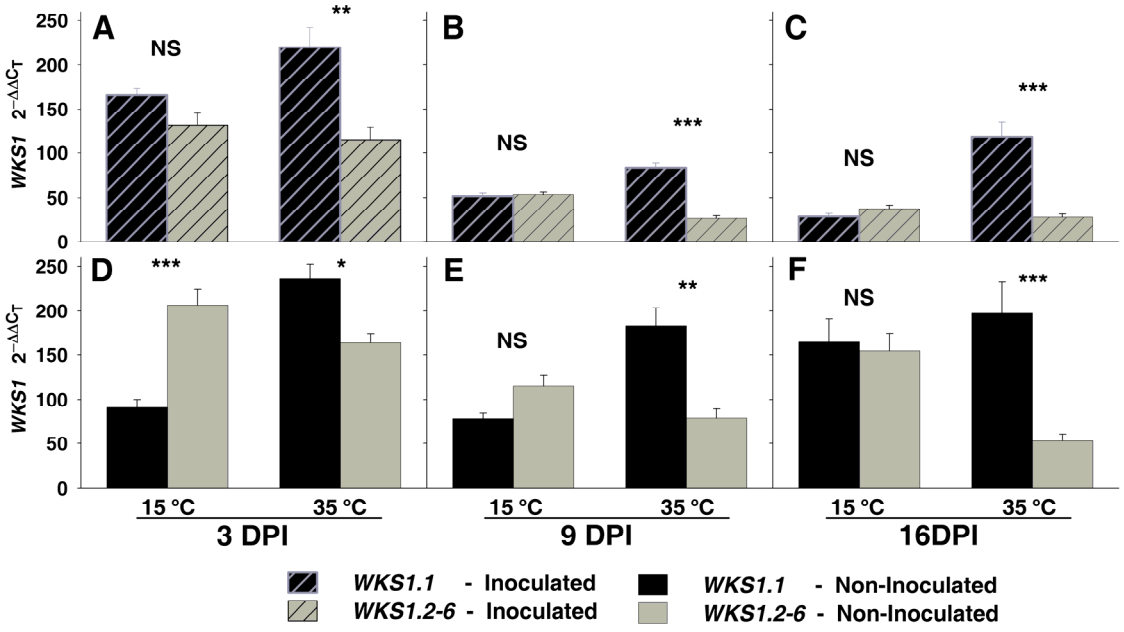


**Fig. S13. In-gel kinase activity of GST-WKS1_Kinase.** (A) Coomassie Blue stained gel of purified proteins from GST-WKS1_Kinase fusion protein (KIN, red arrow) and the GST control vector (GST). (B) In-gel kinase activity assay using casein (1 mg/mL) as a phosphorylation substrate. Radioactive bands were visualized using a Storm 860 PhosphorImager. The in-gel kinase assay was repeated twice with independently expressed fusion proteins with identical result. (C) The presence of the GST-WKS1_Kinase fusion protein was confirmed by Western blot using a GST-antibody and by peptide sequencing of the ~66-kD protein band (WKS1-Kinase protein sequence coverage was >91%).The Western blot also shows that the strong band of ~28 kD in the GST-WKS1_Kinase construct (white arrow) has the GST protein and is likely the result of partial cleavage of the GST-WKS1_Kinase fusion protein in *E. coli*. As expected, this cleaved protein did not show kinase activity (panel B). The ~80-kD protein band present in both KIN and GST is likely an *E. coli* protein that has kinase activity (B) but lacks GST (C). This experiment was repeated with identical results.

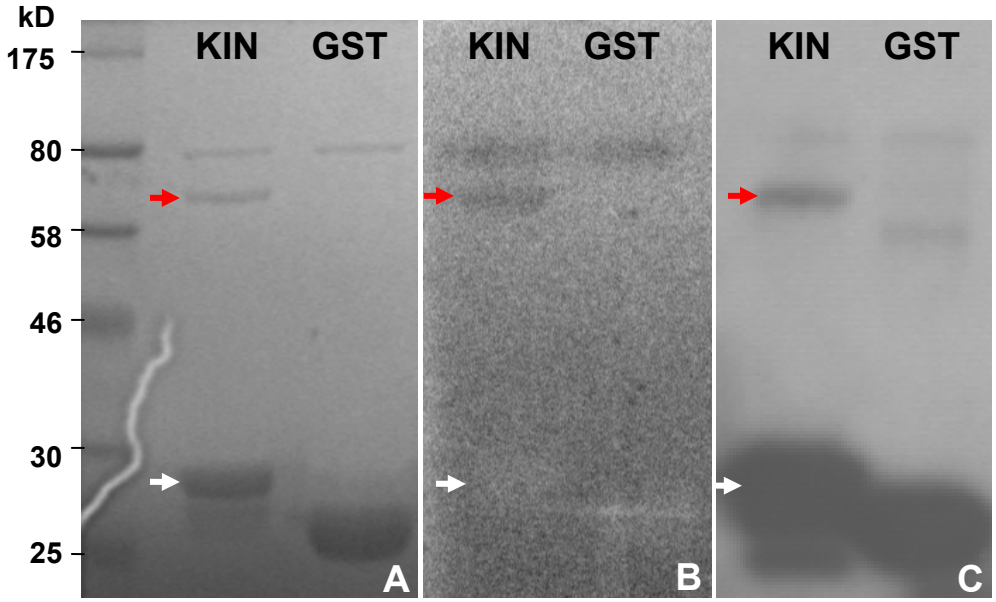
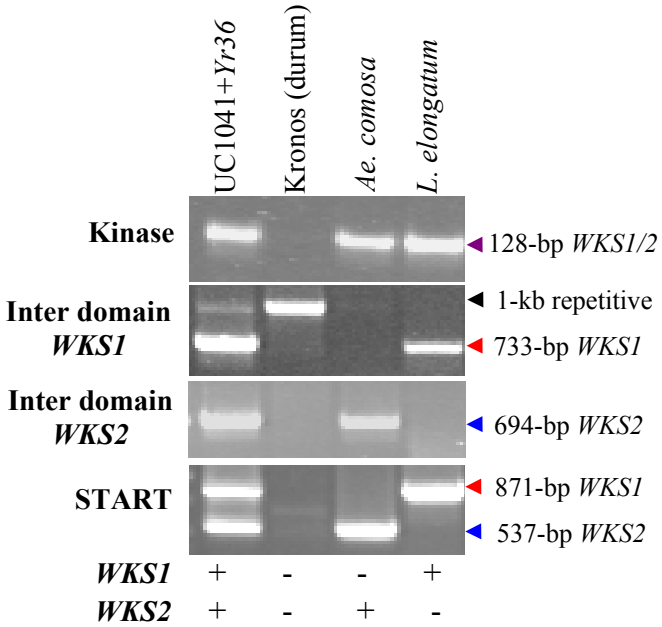


**Fig. S14. PCR markers used to determine *WKS1* and *WKS2* distribution among different *Triticeae* species.**

Primers for the kinase domain (Table S6) amplify fragments of 128-bp for both *WKS1* and *WKS2*. These primers require an initial touchdown with a decrease of 0.5°C per cycle from 70 to 66°C (30 s per cycle).

Two pairs of primers, each specific for one of the *WKS* genes, were used to determine the presence of the inter-domain region (Table S6). The first one amplifies a product of 733-bp when *WKS1* is present and no product in its absence. The second inter-domain primer pair amplifies a product of 694-bp when *WKS2* is present and no product when it is absent. These primers require an initial touchdown with a decrease of 0.5ºC (60 s) per cycle from 65 to 60ºC for the first pair and from 64 to 59ºC for the second pair (60 s per cycle).

The primer pair specific for the START domain amplifies products of 871-bp from *WKS1* and 537-bp from *WKS2* (Table S6). Size differences are partially due to the insertion of a MITE in the *WKS1* intron amplified by these primers. These primers require an initial touchdown with a decrease of 0.5ºC per cycle from 70 to 65ºC (60 s per cycle).



**Acknowledgements for Supplemental Materials**

We thank J. Dvorak for the DNA samples from different *Triticeae* species used in Table S9, the National Small Grain Collection, E. Nevo and M. Sanguineti for germplasm used in this study, L. Comai and R. Tran for their assistance with the TILLING screen, L. Penman and A. Wan for assistance with stripe rust testing, M. Fon, H. Garces, S. Braybrook and J. Harada for assistance with the kinase activity assay, T. Jeoh for her help with FPLC, and R. Eigenheer and B. Phinney at the UC Davis Proteomics Facility for LC−MS/MS analysis.

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