Supplementary Information Appendix

Identification and characterization of *Sr13*, a tetraploid wheat gene that confers resistance to the Ug99 stem rust race group

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This file includes:

SI Appendix, Methods

SI Appendix, Method S1. Map-based cloning of *Sr13*

To generate additional markers in the *Sr13* region, we identified the colinear region in the genome of *Brachypodium distachyon* (L.) P. Beauv. (2.9 Mb), identified the genes in this region (*Bradi3g60550* to *Bradi3g56757*, Fig. 1A), and found the closest wheat sequences (Fig. 1B). Using genome specific primers described in *SI Appendix*, Table S1, we mapped these markers in the 45 lines carrying informative recombination events and delimited the *Sr13* region to a 0.08 cM interval (0.01 cM distal to marker *CJ641478* and 0.07 cM proximal to marker *CJ671993*). Within this region, we identified a marker completely linked to *Sr13*, that we designated *EX24785*. This marker was developed from transcriptome fragment EX_c24785 (Kansas State University wheat transcript assembly http://129.130.90.211/snp/), identified using an NLR gene from Chinese Spring chromosome arm 6AL as query in a BLAST search (Fig. 1B).

We used the completely linked marker *EX24785* to screen a bacterial artificial chromosome (BAC) library of the *Sr13*-resistant durum wheat variety Langdon. We identified BAC 156P14 (Fig. 1C), sequenced it, and developed four markers for two complete NLR genes (*CNL1* and *CNL3*) and two NLR pseudogenes (*cnl2* and *cnl4*). Two recombination events were identified between distal marker *CNL1* and *cnl2* and one recombination event between *cnl2* and the linked group of markers *CNL3-cnl4-EX24785-Sr13* (Fig. 1D). We initiated a chromosome walk from both ends that resulted in the identification of nine overlapping BACs covering a region of roughly 955 kb (Fig. 1C). The complete region was sequenced, annotated and deposited in GenBank as accession KY924305.

SI Appendix, Figures

Figure S1. *CNL3* and *CNL13* Mutants Inoculated with Races TKTTF, JRCQC and TRTTF. Kronos: *Sr13*-resistant control, Rusty: susceptible control. *CNL3* mutants T4-403, T4-1065, and T4-3715: resistant to races TKTTF (**A**) and JRCQC (**B**). *CNL13* mutants T4-476, T4-771, and T4-3102: susceptible to races TKTTF and JRCQC. All mutants were resistant to TRTTF (**C**), likely due to a second gene in Kronos resistant to this race (see *SI Appendix*, Fig. S2). Numbers below leaves indicate average pustule sizes (n = 3) and superscripts indicate significance of differences with Kronos (ns = not significant, * = $P < 0.05$). Results for TTKSK are in Fig. 2.

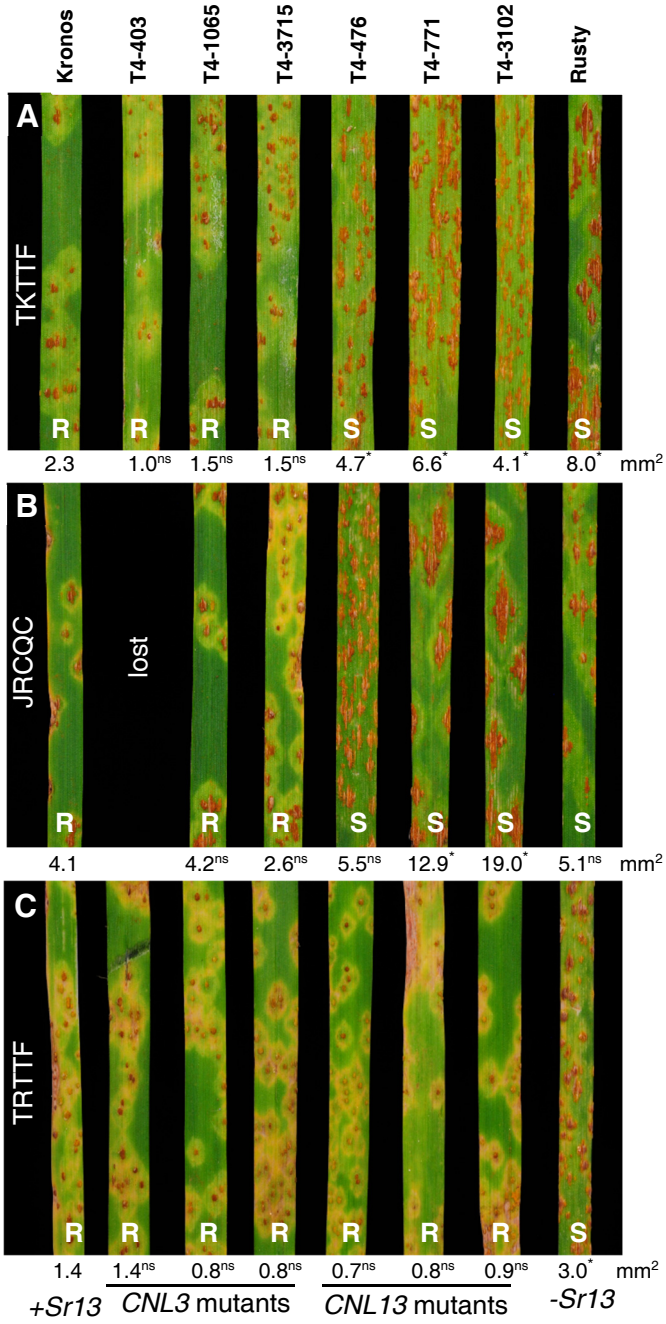


Figure S2. Kronos × Rusty Plants Segregating for *CNL13*. 1) Kronos, 2) Rusty, 3-5) homozygous plants for resistant (++) allele, 6-14) homozygous plants for susceptible *CNL13* allele (--). *CNL13* alleles and phenotypes showed perfect co-segregation for races TTKSK (**A**) and JRCQC (**B**). However, some plants homozygous for *CNL13* susceptible allele in lanes 7, 9, and 14) showed resistance to race TRTTF (**C**). This result confirmed the presence of a second resistance gene to race TRTTF in Kronos (suggested by *CNL13* mutants in *SI Appendix*, Fig. S1).

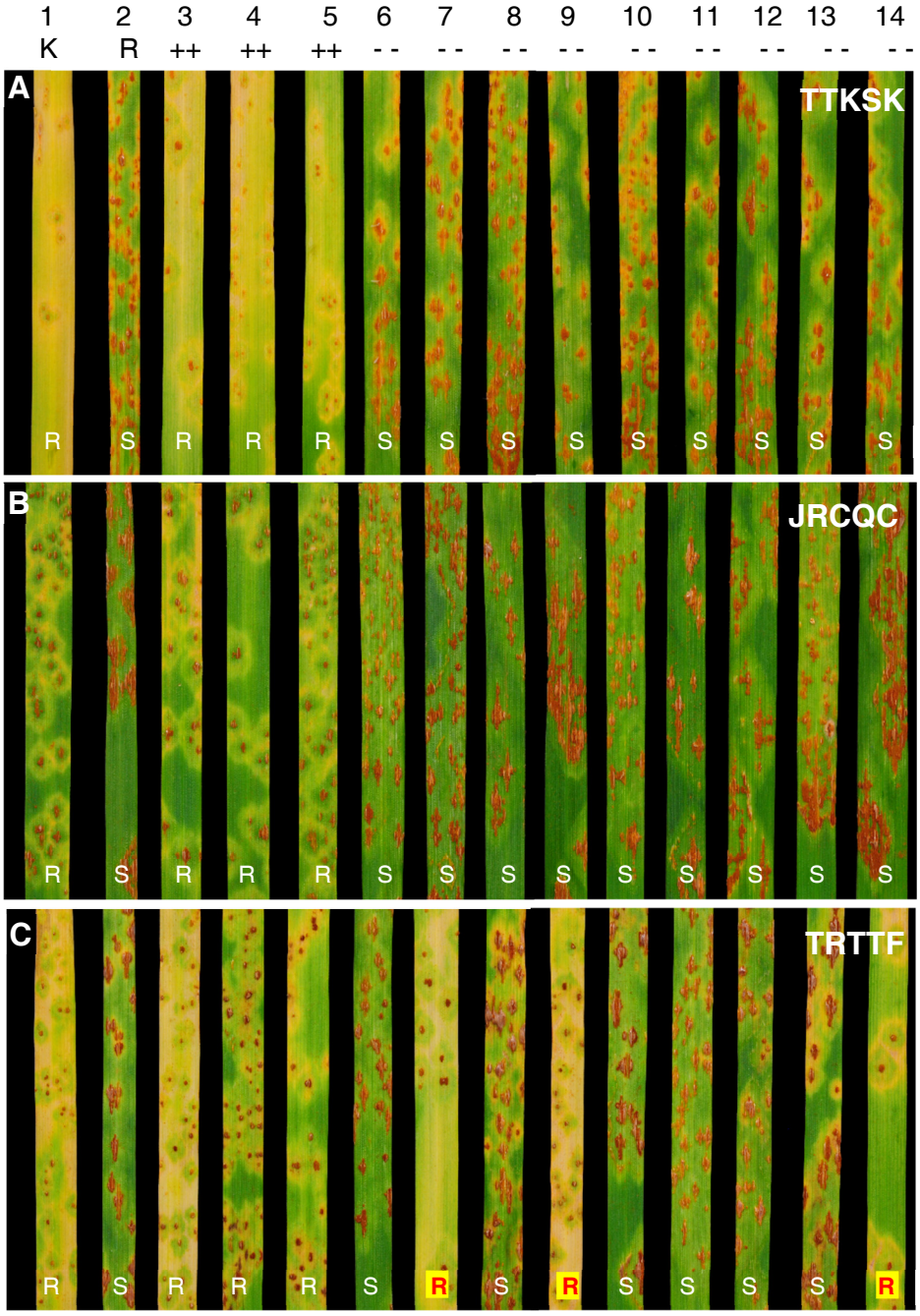


Figure S3. **Induced Mutations in the LRR Domain**. Reaction of four Kronos mutant lines with non-synonymous mutations in the LRR domain. The mutant line T4-771 carrying a premature stop codon in the LRR domain (*SI Appendix*, Fig. S1) was included as a susceptible control. Among these four LRR mutants, T4-4367 (A717V) was the only one susceptible to TTKSK. Numbers below leaves indicate average pustule sizes (n = 3). Different letters indicate significant differences among lines based on Tukey's test ($P < 0.001$).

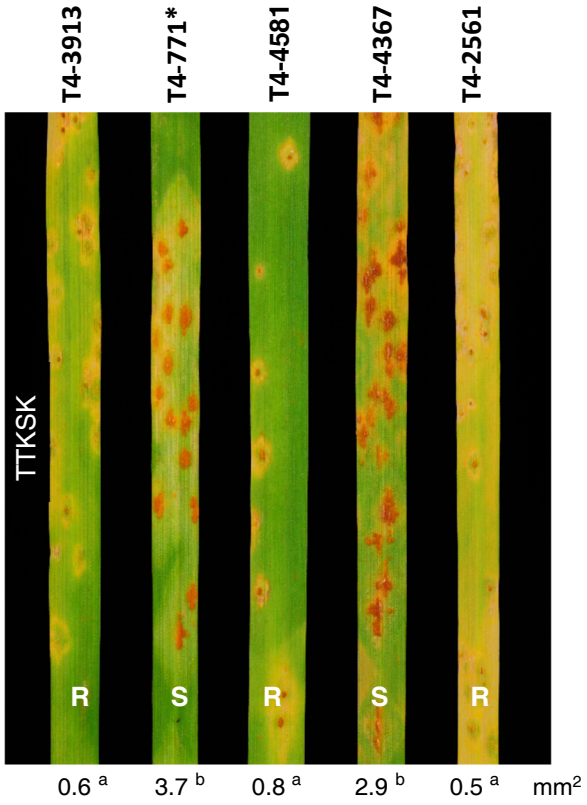


Figure S4. *CNL13* T_2 Transgenic Plants Inoculated with Races TTKSK, TKTTF and TRTTF. (**A**) TTKSK, (**B**) TKTTF, and (**C**) TRTTF. 1) Khapstein/*9 LMPG (resistant control), 2) Fielder (susceptible control), 3-10) T_2 transgenic plants in a Fielder background, 3-4) T_2Sr13-1, 5-6) T_2Sr13-2, 7-8) T_2Sr13-3, and 9-10) T_2Sr13-4. All lines are hexaploid. Numbers below leaves indicate average pustule sizes (n = 3) and superscripts indicate significance of differences between transgenic and Fielder (ns = not significant, ** = $P < 0.01$, *** = $P < 0.001$).

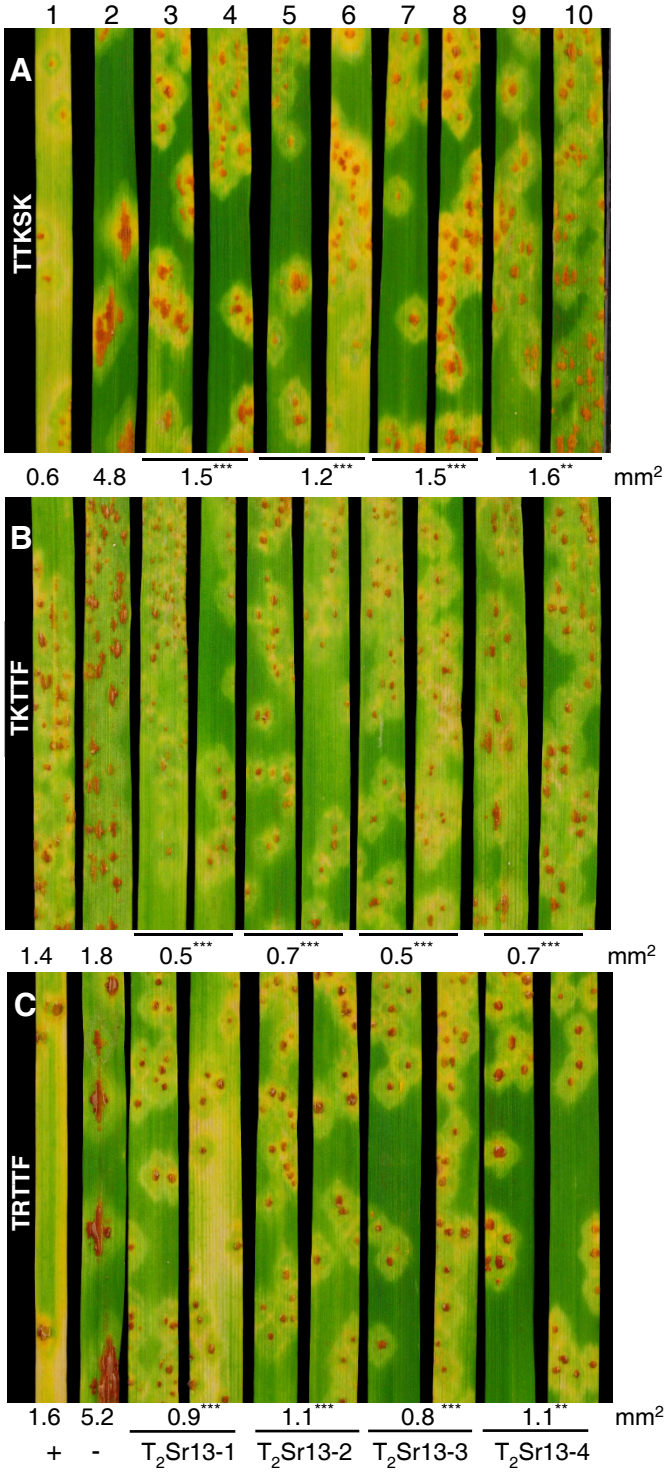


Figure S5. Reactions to *Puccinia graminis* f. sp. *tritici* Race TTKSK at Two Temperature Regimes (18 °C day / 15 °C night and 25 °C day / 22 °C night). Both experiments were performed under-long-days (16 h light / 8 h dark). 1-2) Hexaploid. 3-4) Tetraploid. 1) LMPG (no *Sr13* = S). 2) Khapstein/9*LMPG (*Sr13* = R). 3) Rusty (no *Sr13*= S). 4) Kronos (*Sr13* = R). Numbers below the leaves indicate average pustule sizes ± their standard error of the means (n = 3). A three-way ANOVA of a larger experiment (n = 8) is presented in (*SI Appendix*, Table S3).

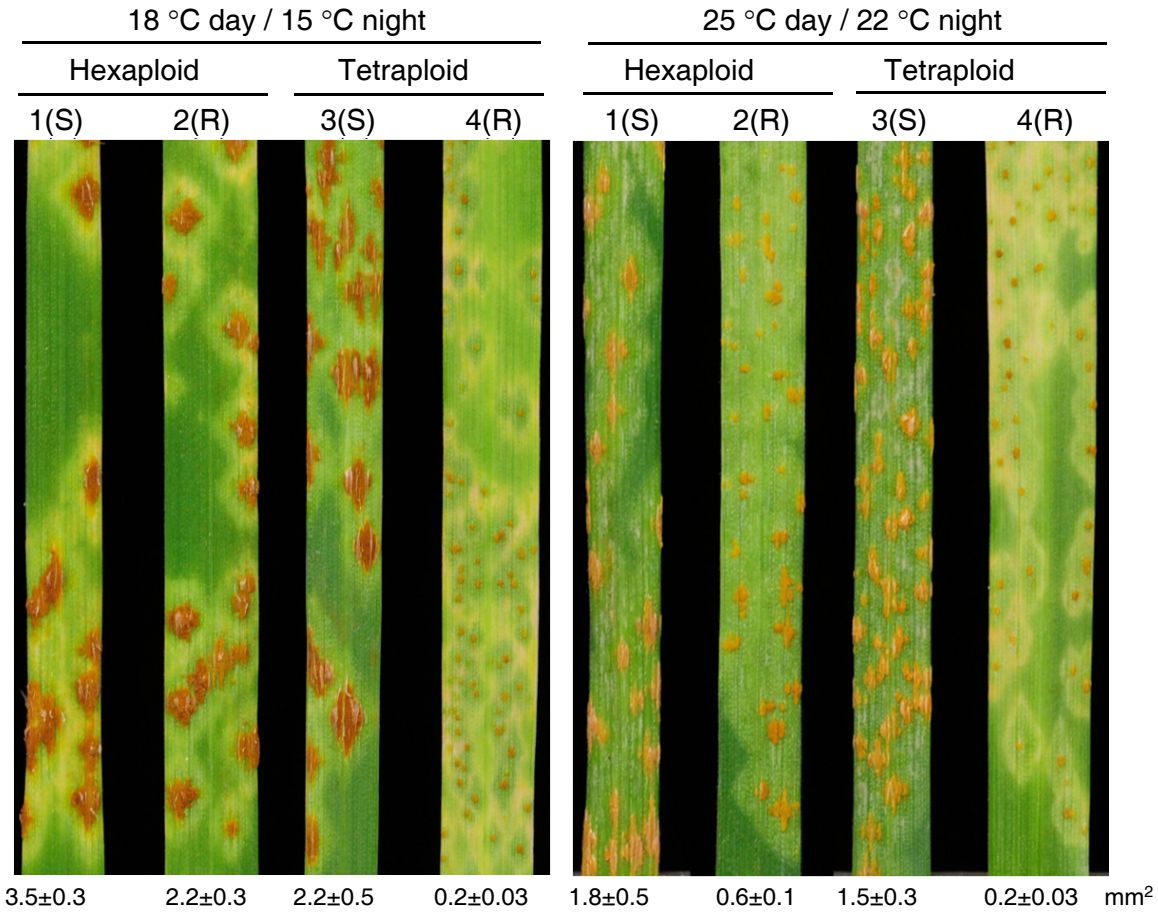


Figure S6. Effect of *Sr13* on *Pgt* Race BCCBC Growth Five Days post Inoculation (dpi). White bars are 2 mm. The average growth area of a single infection was 1.42 ± 0.09 mm^2 for LMPG (no *Sr13*) and 0.42 ± 0.04 mm^2 for Khapstein/9*LMPG (LMPG-*Sr13*). The 70.8% decrease in *Pgt* growth area five dpi was highly significant ($P = 0.0004$). n = 3 leaves (10 individual infection areas measured per leaf). Leaves were cleared with KOH (37 °C, 12 h) and stained with WGA-FITC (L4895-10MG; Sigma–Aldrich).

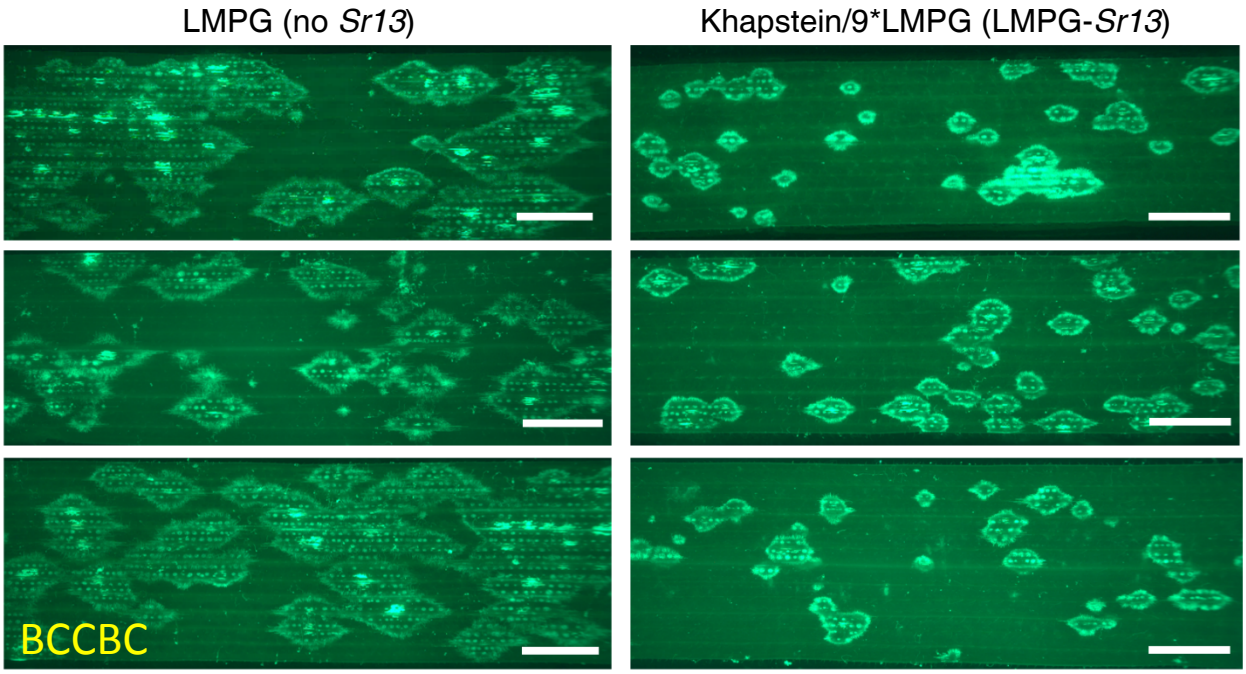


Figure S7. **Effect of Temperature and Inoculation with *Pgt* Race TTKSK on *CNL13* Transcript Levels**. Leaves were collected from Kronos one, two, four and six days post inoculation with TTKSK. Transcript levels were expressed as fold-*ACTIN*. The statistical analysis is presented in *SI Appendix*, Table S5.

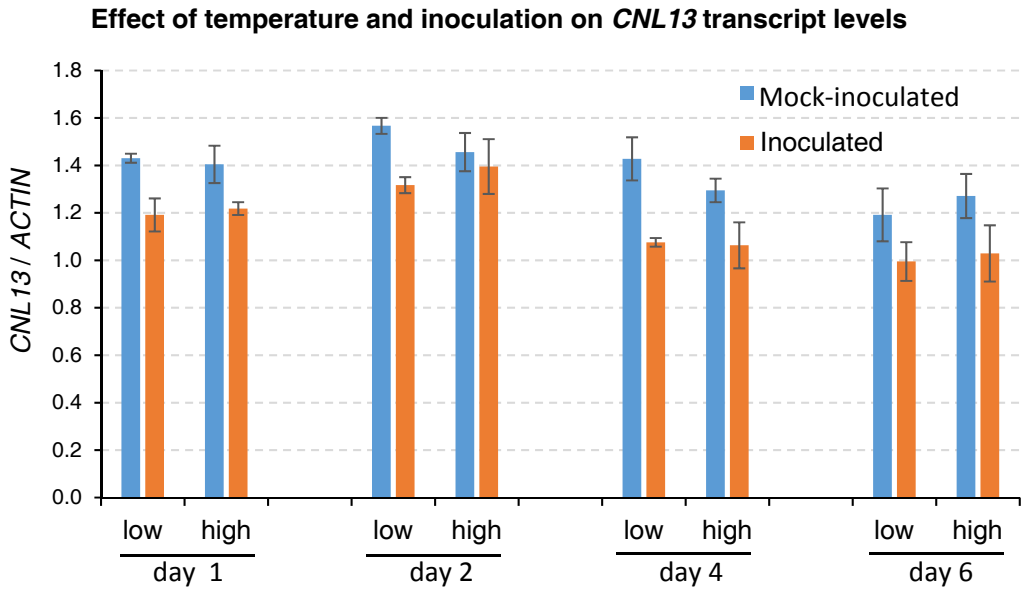


Figure S8. **Transcript Levels of Pathogenesis-Related (*PR*) Genes in LMPG.** Transcript levels of *PR* genes *PR1*, *PR2*, *PR3*, *PR4*, *PR5*, and *PR9*. Interaction graphs between genotype (susceptible common wheat LMPG *vs.* resistant isogenic lines LMPG-*Sr13*) and inoculation (TTKSK *vs.* mock). Transcript levels relative to the *ACTIN* endogenous control were calculated using the ΔCT method. Error bars indicate standard errors of the means. All interaction were highly significant ($P < 0.001$). n = 6. Samples were collected five days post inoculation. Statistical analyses are presented in *SI Appendix*, Table S6.

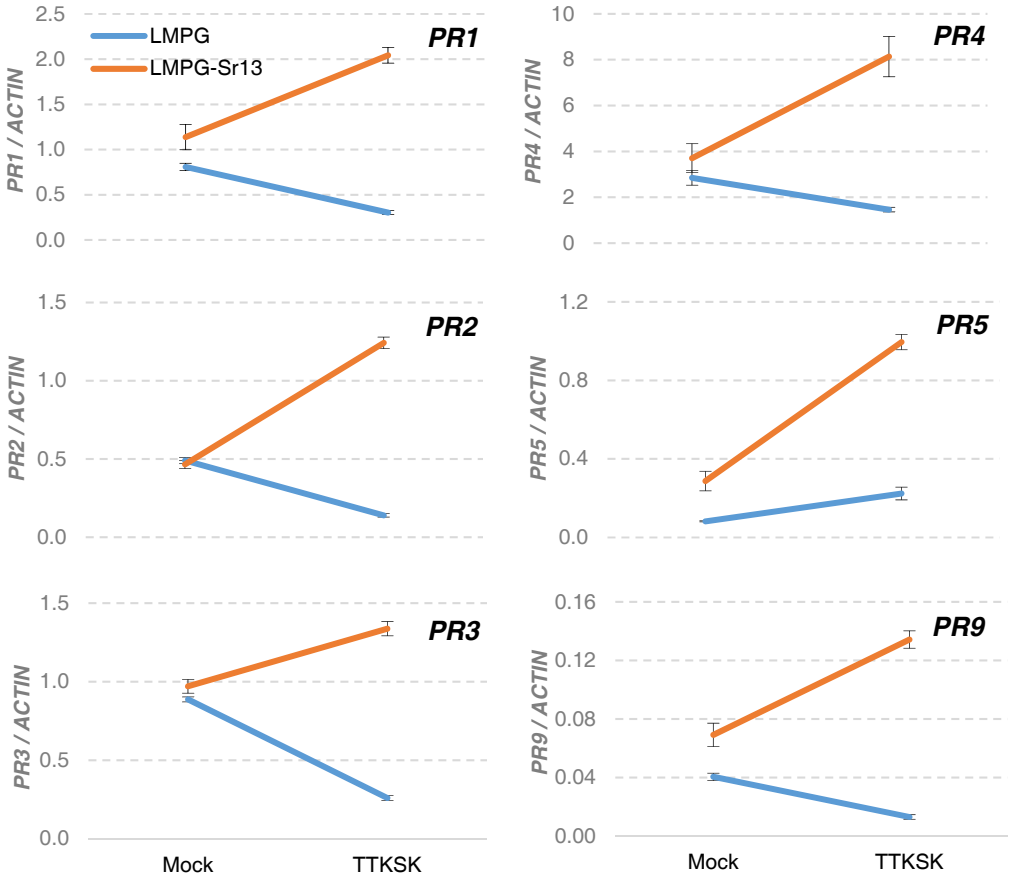


Figure S9. **Transcript Levels of *PR* Genes in Transgenic Fielder Plants.** Transcript levels of *PR* genes *PR1*, *PR2*, *PR3*, *PR4*, *PR5*, and *PR9*. Interaction graphs between genotype (susceptible common wheat Fielder *vs.* transgenic line T_1Sr13-2) and inoculation (TTKSK *vs.* mock). Transcript levels relative to the *ACTIN* endogenous control calculated using the ΔC_T method. Error bars indicate standard errors of the means. All interaction were highly significant ($P < 0.001$). n = 6. Samples were collected five days post inoculation. Statistical analyses are presented in *SI Appendix*, Table S6.

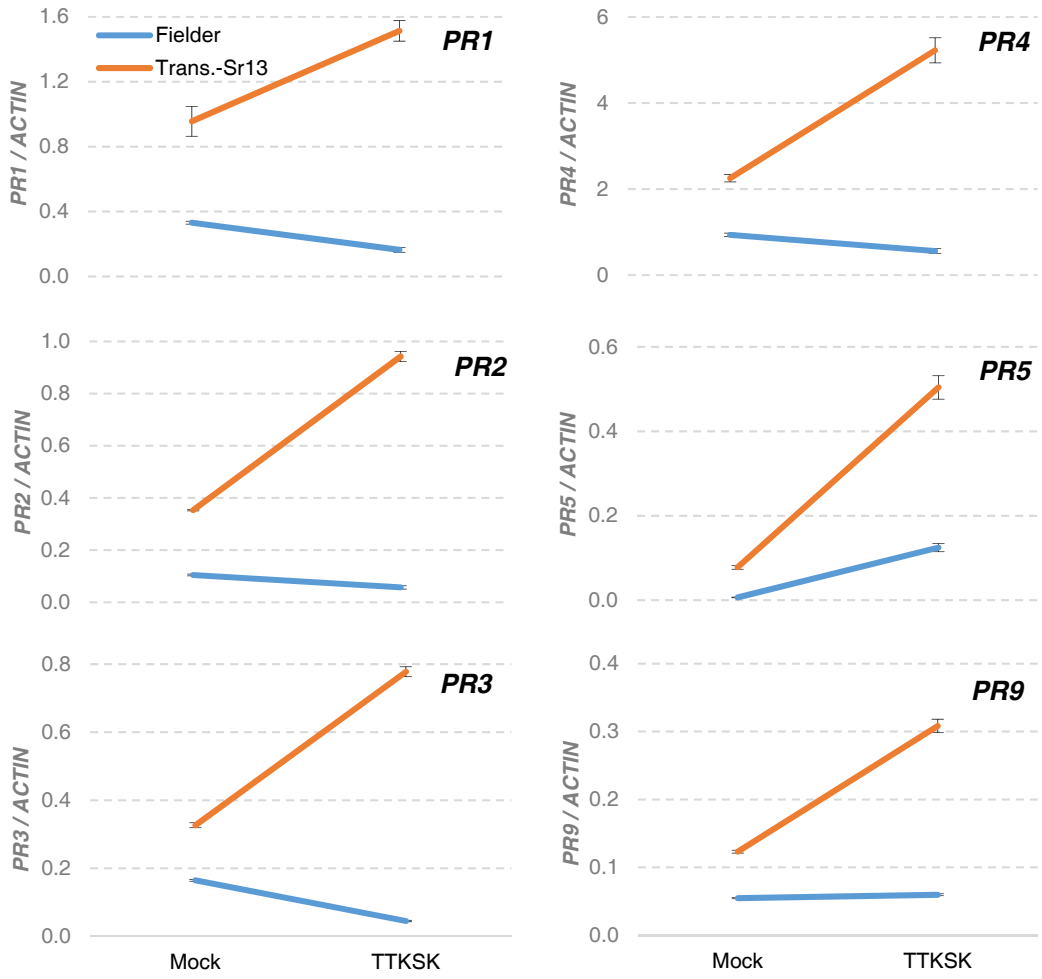


Figure S10. **Induction of Pathogenesis Related Genes in Kronos.** *PR1*, *PR2*, *PR3*, *PR4*, *PR5*, and *PR9* transcript levels relative to *ACTIN* in samples collected 4 and 6 days post inoculation (dpi) with TTKSK or mock inoculation. Kronos plants were grown at low (18 C° day / 15 C° night) and high (25 C° day / 22 C° night) temperatures. Values were calculated using the ΔCT method using *ACTIN* as endogenous control. Error bars indicate standard errors of the means. All interaction were highly significant ($P < 0.001$). n = 3.

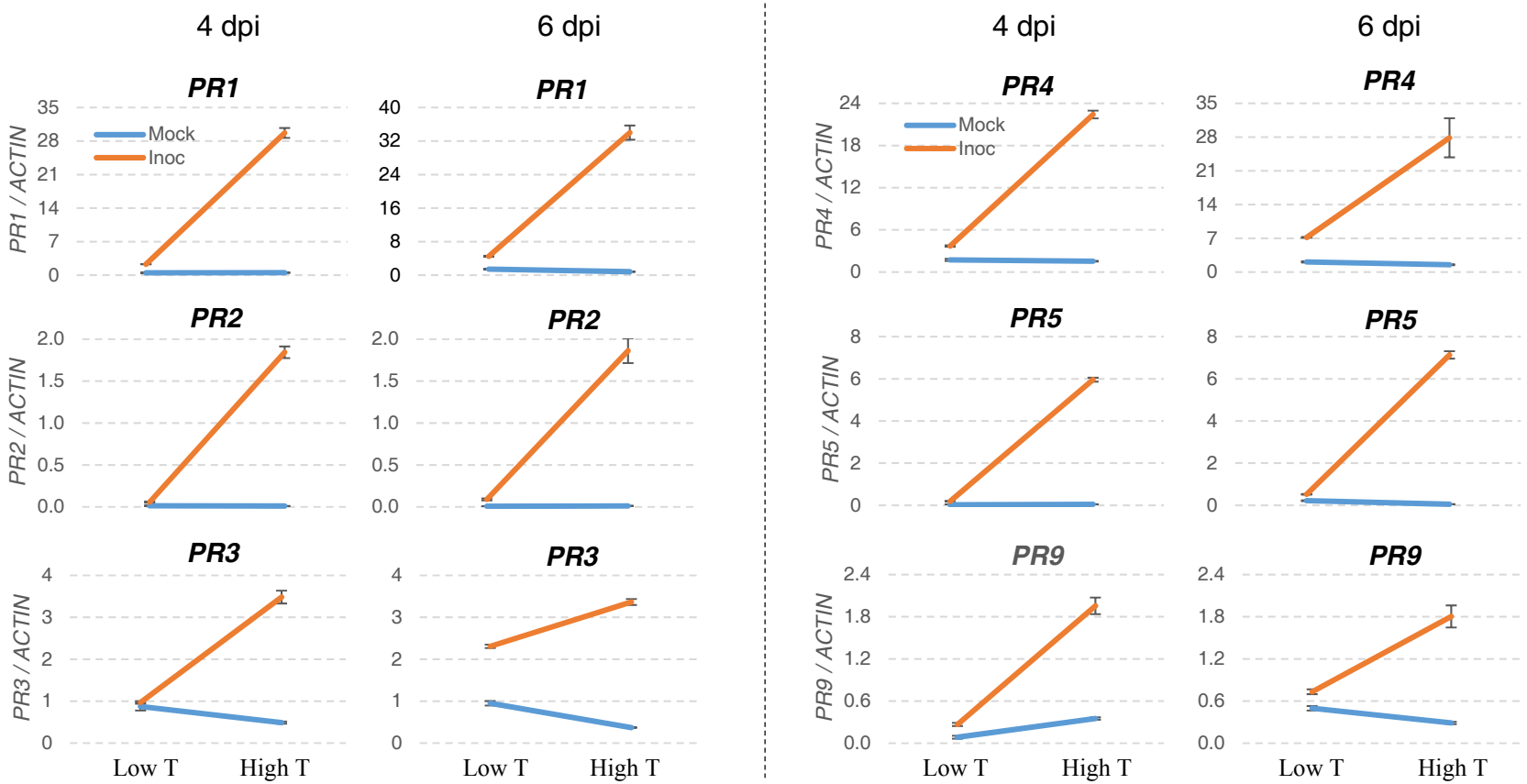


Figure S11. **Natural Variation in *CNL13***. DNA (top) and protein (bottom) polymorphisms among *CNL13* haplotypes in the LRR (right panels) and non-LRR regions (left panels). Red= present in a single haplotype. Blue= shared by multiple haplotypes. Orange/green= perfectly linked with the *Sr13* resistance. "-" = deletion. Re = resistant, Su = susceptible, black border= synonymous variant.

DNA polymorphisms

	DNA polymorphisms Non-LRR									DNA polymorphisms LRR																			
						1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	**S**
		2	5	9	9	0	4	6	6	9	9	9	0	2	2	2	3	3	4	5	5	5	5	6	6	7	**R**
	8	9	5	5	8	8	8	5	5	2	3	6	3	0	7	7	0	4	2	1	1	8	8	0	8	9	**1**
	9	2	9	5	3	2	0	2	8	2	8	3	3	0	2	8	6	4	5	5	7	8	9	3	6	8	**3**
R1	G	C	A	C	A	A	A	T	C	C	T	C	A	C	C	T	C	C	A	T	G	T	A	C	G	C	Re
R2	G	C	A	C	A	A	A	T	C	C	T	G	A	C	G	T	C	C	A	T	G	T	A	C	G	A	Re
R3	G	C	A	C	A	A	A	T	C	C	T	G	A	C	G	T	C	C	A	T	T	T	A	C	G	C	Re
S1	G	C	A	T	A	A	A	T	T	C	T	G	A	T	G	T	C	C	A	T	G	T	A	C	G	C	Su
S2	G	C	A	C	A	A	A	T	C	C	T	G	A	T	G	T	C	T	G	G	G	T	A	C	G	C	Su
S3	G	C	A	C	A	A	A	T	C	C	T	G	A	T	G	C	C	T	A	T	G	T	A	A	G	C	Su
S4	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Su
S5	G	C	A	C	A	A	A	T	C	C	T	G	A	T	G	T	C	T	A	T	G	T	A	C	G	C	Su
S6	G	C	A	C	A	A	A	T	C	C	T	G	A	T	G	T	T	T	A	T	T	T	A	C	G	C	Su
S7	G	C	A	T	G	A	A	C	T	C	T	G	G	T	G	T	C	T	A	T	C	T	A	C	G	C	Su
S8	A	C	G	T	A	G	T	T	T	A	C	G	A	T	G	T	C	T	A	T	G	T	G	C	A	C	Su
S9	G	T	A	C	A	A	A	T	C	C	T	G	A	T	G	T	C	C	A	T	G	G	A	C	G	C	Su
S10	G	C	A	T	A	A	A	C	T	C	T	G	A	T	G	T	C	T	A	T	T	T	A	C	G	C	Su

Protein polymorphisms

	Protein polymorphisms Non-LRR									Protein polymorphisms LRR																**S**
			1	3	3	3	4	5	5	6	6	6	7	7	7	7	7	7	8	8	8	8	8	9	**R**
	3	9	8	1	2	6	9	5	5	4	5	7	3	5	6	6	6	8	0	3	6	6	9	3	**1**
	0	8	7	9	8	1	4	1	3	1	5	8	4	8	0	3	9	2	9	9	3	8	6	3	**3**
R1	R	P	K	R	H	K	R	M	P	A	R	E	R	L	W	-	P	R	K	W	I	A	G	T	Re
R2	R	P	K	R	H	K	R	M	P	A	G	E	R	V	W	-	P	R	K	W	I	A	G	K	Re
R3	R	P	K	R	H	K	R	M	P	A	G	E	R	V	W	-	P	R	K	C	I	A	G	T	Re
S1	R	P	K	C	H	K	R	M	L	A	G	E	W	V	W	-	P	R	K	W	I	A	G	T	Su
S2	R	P	K	R	H	K	R	M	P	A	G	E	W	V	W	-	P	C	E	G	I	A	G	T	Su
S3	R	P	K	R	H	K	R	M	P	A	G	E	W	V	R	-	P	C	K	W	I	D	G	T	Su
S4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Su
S5	R	P	K	R	H	K	R	M	P	A	G	E	W	V	W	-	P	C	K	W	I	A	G	T	Su
S6	R	P	K	R	H	K	R	M	P	A	G	E	W	V	W	-	L	C	K	C	I	A	G	T	Su
S7	R	P	K	C	R	K	R	T	L	A	G	G	W	V	W	-	P	C	K	C	I	A	G	T	Su
S8	H	P	E	C	H	R	W	M	L	D	G	E	W	V	W	-	P	C	K	W	M	A	S	T	Su
S9	R	S	K	R	H	K	R	M	P	A	G	E	W	V	W	-	P	R	K	W	R	A	G	T	Su
S10	R	P	K	C	H	K	R	T	L	A	G	E	W	V	W	C	P	C	K	C	I	A	G	T	Su

Figure S12. Differential Responses of Resistant Haplotypes R1, R2 and R3 to JRCQC. (A) *T. turgidum* ssp. *durum* accessions carrying *CNL13* haplotypes R1, R2 and R3 inoculated with *Pgt* race JRCQC. 1) Rusty (S4 haplotype, susceptible control), 2) Medora (R2), 3) D99656 (R2), 4) Kronos (R1), 5) Maier (R1), 6) Renville (R1), 7) Langdon (R3). **(B)** Two *Sr13* homozygous resistant and two homozygous susceptible $F_{2:3}$ families from the cross Kofa (R2) × UC1113 (S2) inoculated with races TTKSK, TRTTF and JRCQC. 1-2) F_3 plants homozygous for the *CNL13* R2 resistant allele (++), 3-4) F_3 plants homozygous for the *CNL13* S2 susceptible allele (--). Accessions carrying the R2 (*Sr13b*) haplotype were susceptible to JRCQC, whereas those carrying the R1 and R3 (*Sr13a*) haplotypes were resistant.

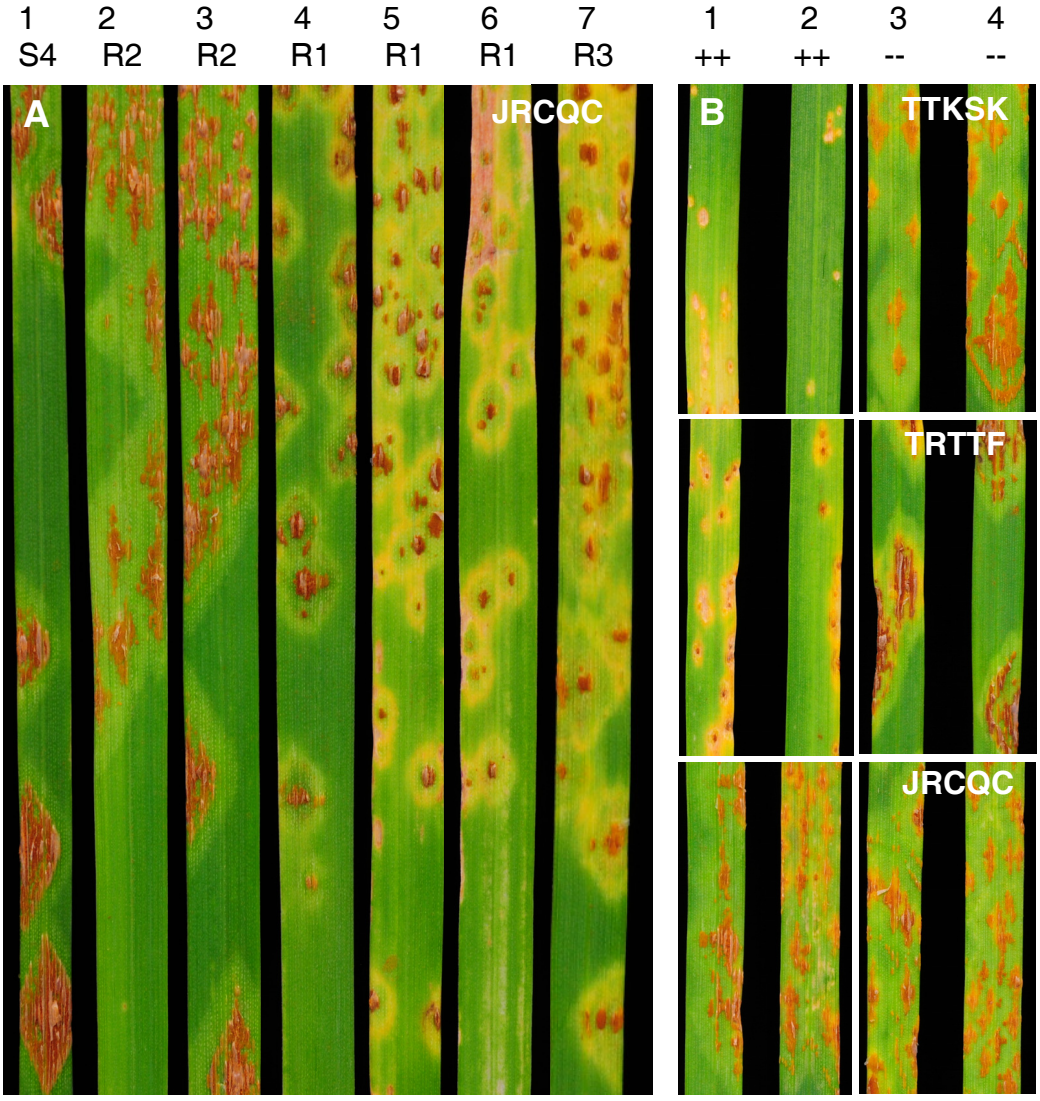


Figure S13. **Geographic Distribution of Accessions Carrying the Different *CNL13* Haplotypes**. Only *T. turgidum* ssp. *dicoccoides* and *T. turgidum* ssp. *dicoccon* accessions with available collection information at GRIN (www.ars-grin.gov/npgs/searchgrin.html) were included in this map. The areas are approximate since many accessions lack precise coordinates. Susceptible haplotype S4 (deletion) is not shown because it was found in all the regions (except Serbia-Italy, S8, *SI Appendix*, Table S9-10).

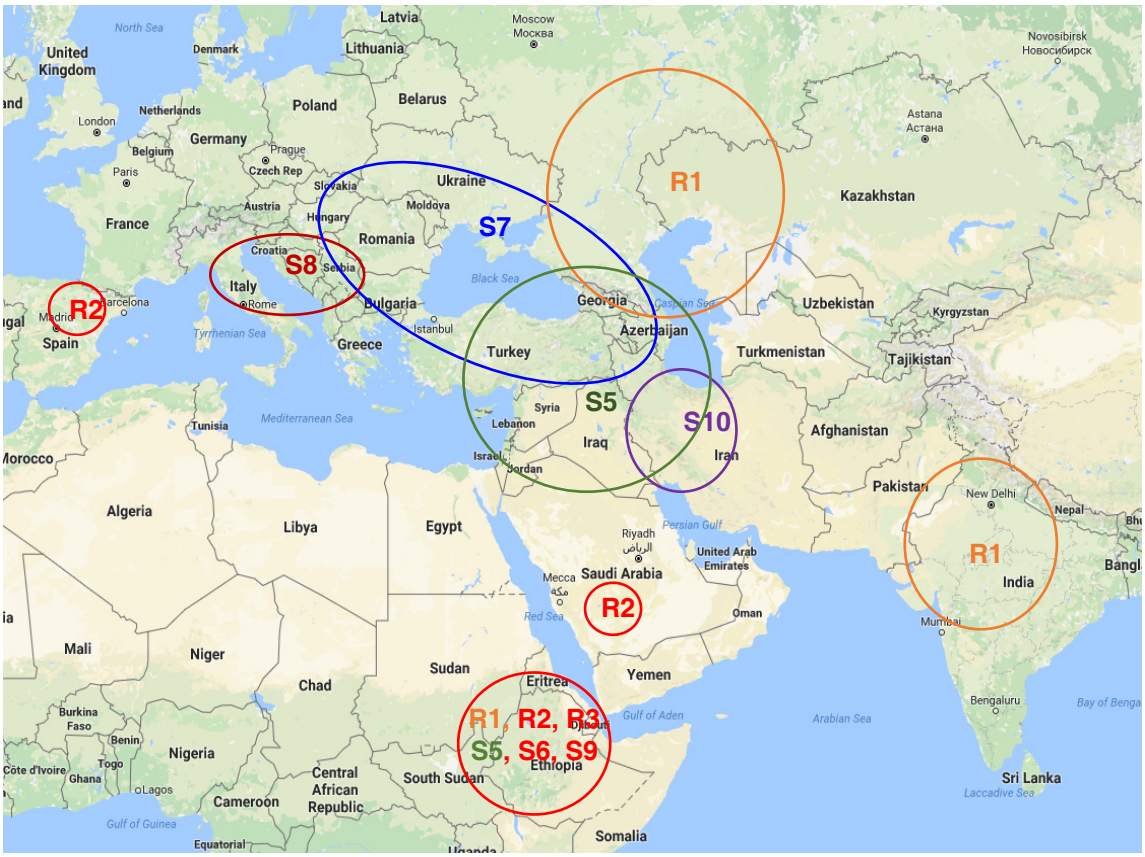


Figure S14. Characterization of Susceptible *CNL13* Haplotypes S5 to S10. Accessions carrying different *CNL13* haplotypes were inoculated with *P. graminis* f. sp. *tritici* race TTKSK. Plants were grown at 25 °C day / 22 °C night temperatures with a 16 h photoperiod. 1) Kronos (R1, *Sr13* positive control), 2) Kofa (R2, *Sr13* positive control), 3) Rusty (S4, *Sr13* negative control), 4) PI 254164 (S5), 5) PI 94623 (S5), 6) PI 273980 (S6), 7) PI 168679 (S6), 8) PI 94615 (S7), 9) PI 272531 (S7), 10) PI 94648 (S8), 11) PI 377655 (S8), 12) PI 384332 (S9), 13) PI 352364 (S10). These results confirmed that haplotypes S5 to S10 were susceptible to TTKSK.

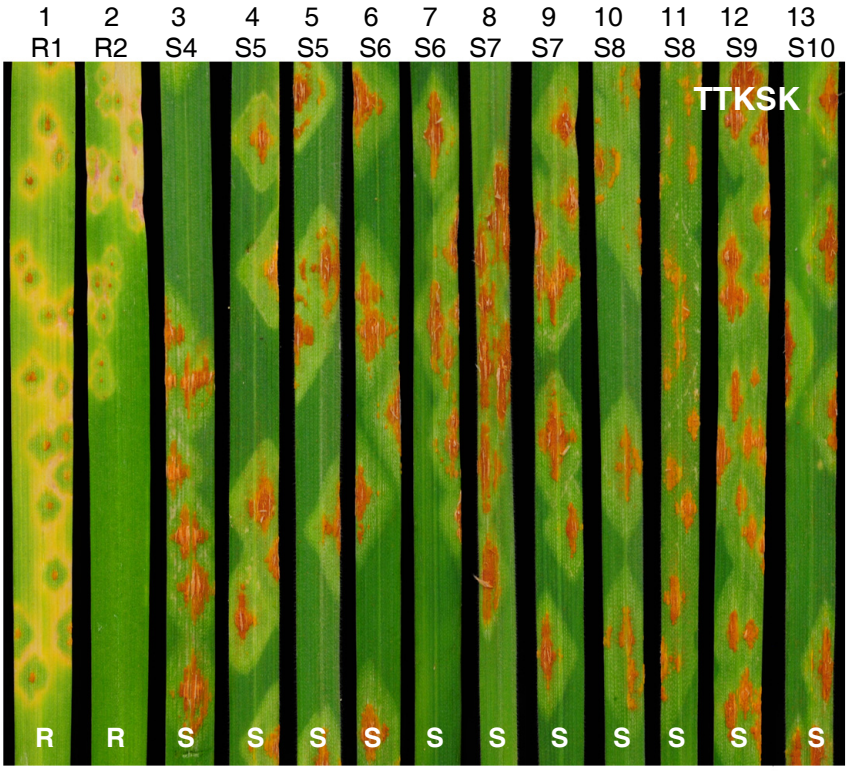
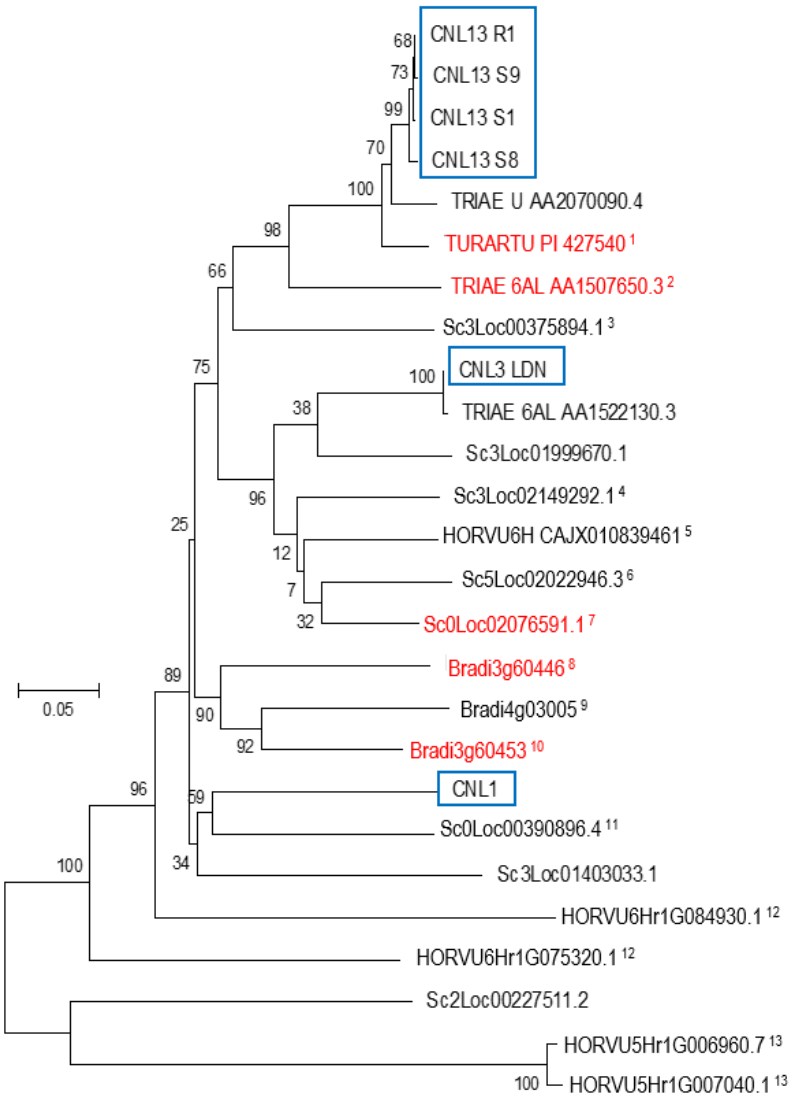


Figure S15. **Phylogenetic Tree of CNL13 Related Proteins**. Neighbor Joining tree including CNL13 (Sr13) protein alleles, linked CNL3 and CNL1 proteins (blue squares), and the closest predicted proteins from *Triticum aestivum* (TRIAE), *T. urartu* (TURARTU), *Secale cereale* (Sc), *Hordeum vulgare* (HORVU), and *Brachypodium distachyon* (Bradi) calculated using MEGA. The tree is based on the MUSCLE alignment of the conserved CC and NBS domains (first 494 amino acids). Numbers on the nodes indicate bootstrap values based on 1000 iterations. Truncated genes are indicated in red. Frame shifts and repetitive element insertions in the truncated genes were removed to maximize similarity of the predicted proteins (superscripts describe the specific changes). Pseudogenes diverge faster than genes and, therefore, their position in the tree can be distorted. It was not possible to determine if the rye or barley 6H contigs were in colinear regions.



[1] One bp deletion ("C") at cDNA position 932. For this alignment, a "C" was inserted to restore the original frame and similarity of the predicted protein to CNL13.

[2] Frame shift mutation after amino acid 530 in second exon. Elimination of 2-bp in this region restores similarity in rest of the protein. Different from current annotation.

[3] Only 516 amino acids available. Source rye Lo7_v2_contig_1376058 is 3' truncated before end of the gene

[4] Only 654 amino acids available. Source rye Lo7_v2_contig_91046 is 3' truncated before end of the gene.

[5] Barley MLOC_5371. Bowman_6HL_CAJX010839461.

[6] Based on rye DNA contig Lo7_v2_contig_68142. Missing LRR and four last amino acids of the aligned region used for tree. Different start exon 2 from current annotation Sc5Loc02022946.3.

[7] Likely a rye pseudogene. There is no similarity with CNL3 after amino acid 690.

[8] Colinear *Brachypodium* pseudogene due to premature stop codon at amino acid 231. Protein similarity continues after stop codon. Different from current annotation.

[9] Non-colinear *Brachypodium* NLR gene

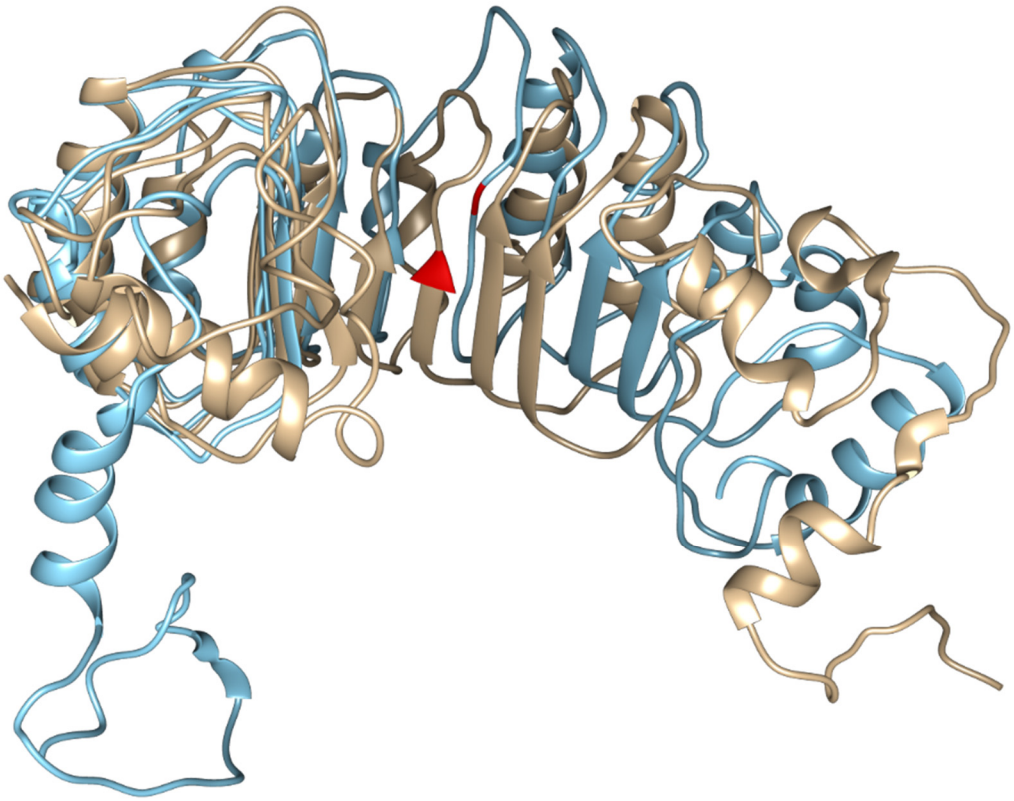
[10] Colinear *Brachypodium* pseudogene. Repetitive element insertion flanked by duplicated TGTTGACAGAA and frame shift mutation after 292 amino acids were both corrected to restore the ancestral frame.

[11] Lo7_v2_contig_1379036 is 5' truncated and is missing the first 47 amino acids of the alignment.

[12] Barley chromosome 6H.

[13] Barley non-colinear chromosome 5H (outgroup).

Figure S16. Effect of the W734R Mutation on the Structure of the CNL13 LRR Domain. Phyre 2 (1) prediction of the LRR structure in resistant haplotype R3 (734R, gold) superimposed to the same haplotype with a 734W amino acid (light blue). The changed amino acid is indicated in red. Only the LRR domain from amino acid 590 to 948 was used in the prediction. The C-terminal end of the protein is on the left side of the figure. Note the displacement of the proximal β-sheets in the interior side of the horseshoe fold.



SI Appendix, Tables

Table S1. Primers used for fine mapping, BAC library screening (physical mapping), allelic variation (haplotype), marker-assisted selection (MAS), semi-quantitative PCR, qRT-PCR (*CNL13* and *PR* genes), 5' RACE, production and screening of transgenic lines, copy number assays, and pathogen growth evaluation (fungal / host DNA).

Marker	Primer sequence 5'-3' (Forward)	Primer sequence 5'-3' (Reverse)	Enz.	Function
CJ666008	CGTCACCATCATTATATCTCTCCACT	CACAAAGGCTTTTAGGTCTTCCA	*Bsr*I	Fine map
CJ671993	TAGGAGCTCTTGAGAGGTTCC	GCAAATGGACACTGATTACCAC	*Pvu*I	Fine map
EX24785	GAAGAATCAAGTCACTGAGGC	ACAAGGAAACTAGAGATCTTGG		Fine map
CJ641478	AAGCCTACGTCGAGGACGAA	GAGTGTGAAAATCGCAAAACG	*Xho*I	Fine map
CD926040	GCTCATAACACCAGTCGGTG	AAACAGTGGTGCGTTAATACATG		Fine map
861TF1/TR1	CACGACCAGTTTTGGTAGTAAG	ATCTTCTGGGAACAGACTCAG		Physical map
449TF2/TR2	CCTTCTGCATTCGCATCTACC	GAGAATTGTACCGTAGGAAACC		Physical map
468MF3/MR3	TAAGGAAACATGGTGCCACTG	CTCGTAGAGTCATCAAATGCAC		Physical map
1225MF3/MR3	GTATGCTCACAGACAAGGTTC	GTGCTAGTTCTACGTATGTAGG		Physical map
952MF8/MR8	TGCTACCTATCTGTCTGTCTG	GAGACGTATCAGTAACCTATAC		Physical map
1062TF2/TR2	GTTGTGTGTGGGAATGATAGG	CGCATGTTCTTTCAATCTCCC		Physical map
6ACNL2F1/R1	GCATAGTGGCTTACTTCCATC	CTCGGAGTATGTATTCTCACG		Physical map
6ACNL13F7/R2	ACGCTTCGTTTTAACACATCAC	GTAAGTGTGCCAGTTTGCCAAGATTGAC		Haplotype
6ACNL13F4/R7	ATATCTTGAGAACCCTCCTTAG	CGGAGCTGAATAATCTTCTATG		Haplotype
6ACNL13F3/R3	GACAGTCTACTGGAAAACCACCATCTTG	GAGGCAGGATTCGTCCTTCGTACAAAC		Haplotype
6ACNL13F3/R8	GACAGTCTACTGGAAAACCACCATCTTG	CATACTCTCTGAAAGAGCAGC		Haplotype
6ACNL13F5/R6	CTAGACTTGGCGCAAACTTTCCTG	GATGTAACTTTCTATGGGTGAGAAG		Haplotype
RAR1	TGTATTTGATCTTAAACATTTTTG	GCTAATCCCTAATCGGTGA		Control marker
Sr13F/R	TTCTTGGCTCAGAAGACACATG	AAGTCATCATCATCATTCCCGC	*Hha*I	MAS/ qPCR
Sr13RTF1R1	GAAGTTGGGTGTCAAGATTATGC	TATACCTTTTTTCTGCTAGGTAGTCG		Exp. analysis
Sr13RACE5'-out-R1		AAACGCTTGTACTCGTCGCCGAGC		5' RACE
Sr13RACE5'-in-R2		GAACAGGAAAGTTTGCGCCAAGTC		5' RACE
Sr13TransF1R1	CACCAAGCTTCTTGATCATCCAAGCTGCTG	GTGCAGCTTCCTCAGATTCTTTAGCCG		Transgenic
Sr13TransF2R2	CACCGTTGAAGGGAAAGGGGATTC	AATGTGCTAGCTTCCATGAAAAGTGGACACG		Transgenic
M13-pEntry-F/R	GTAAAACGACGGCCAG	CAGGAAACAGCTATGAC		Transgenic
Hptmiki-F/R	GGCCTCCAGAAGAAGATGTTGG	GAGCCTGACCTATTGCATCTCC		Transgenic
SR13TaqmanF1/R1	CCTTTGTTCTCTAACTATGC	TATGACAAGTTTTCGGAGGT		Copy No.
SR13-Probe	FAM-ATGATGACTTCTTGCTGAATACA-BHQ1			Copy No.
CO2F1/R1control	TGCTAACCGTGTGGCATCAC	GGTACATAGTGCTGCTGCATCTG		Copy No.
CO2-Probe control	CY5-CATGAGCGTGTGCGTGTCTGCG-BHQ3			Copy No.
PhytochelatinF1/R1	GTATGTCCTCCTACCTCACGAAGT	CGCTGCTGCGATAATCTGCT		Wheat DNA
STD-Actin-F/R	GGGGGCTTCAGTCAATAGGA	CCTATCGAACACGGGATTGT		Fungal DNA
PR1-F/R	CTGGAGCACGAAGCTGCAG	CGAGTGCTGGAGCTTGCAGT		PR1 (2)
PR2-F/R	CTCGACATCGGTAACGACCAG	GCGGCGATGTACTTGATGTTC		PR2 (3)
PR3-F/R	AGAGATAAGCAAGGCCACGTC	GGTTGCTCACCAGGTCCTTC		PR3 (4)
PR4-F/R	CGAGGATCGTGGACCAGTG	GTCGACGAACTGGTAGTTGACG		PR4 (5)
PR5-F/R	ACAGCTACGCCAAGGACGAC	CGCGTCCTAATCTAAGGGCAG		PR5 (6)
PR9-F/R	GAGATTCCACAGATGCAAACGAG	GGAGGCCCTTGTTTCTGAATG		PR9 (7)

Table S2. Estimated copy number of transgenic insertions in each transgenic event based on T_1 plants and a TaqMan® copy number assay of the transgenic plants relative to Kronos.

Transgenic Family	*Sr13* carrier[1]	*Sr13* Null [1]	χ^2 *P* 1 copy	χ^2 *P* 2 copies	χ^2 *P* 3 copies	Ratio	TaqMan Tr./Kronos	Copy No.
T_1Sr13-1	32	10	0.8586	<0.0001	<0.0001	3:1	0.94 [2]	1
T_1Sr13-2	80	0	<0.0001	0.0235	0.2995	63:1	3.33 [3]	3
T_1Sr13-3	48	14	0.6600	<0.0001	<0.0001	3:1	1.04 [2]	1
T_1Sr13-4	37	11	0.7389	<0.0001	<0.0001	3:1	1.05 [2]	1

[1] Genotyped with marker 6ACNL13F3/R3 (*SI Appendix*, Table S1) and statistically tested by χ^2.

[2] The average copy number values obtained for the transgenic plants were divided by the average (for homozygous plants) or ½ the average copy number value (for heterozygous plants) obtained from Kronos plants (all homozygous *Sr13*), respectively.

[3] For the T_1Sr13-2 event carrying multiple copies, copy number was estimated by dividing the average of the two highest copy values (expected to be homozygous for the largest number of copies) by the Kronos average copy number value.

Table S3. Differences in average pustule size between lines with *Sr13* (tetraploid Kronos and hexaploid LMPG-*Sr13*) and without *Sr13* (tetraploid Rusty and hexaploid LMPG). Plants were grown at two different temperatures (low= 18°C day / 15°C night and high= 25°C day / 22°C night) and samples were collected 13 days post inoculation (dpi) with TTKSK. Data was analyzed using a three-way factorial ANOVA using ploidy level, temperature and presence of *Sr13* as factors. Data was transformed to restore normality of residuals. Leaves from eight different plants were analyzed for each of the eight treatment combinations (except for Rusty low temperature, for which only seven plants were available).

Dependent Variable: Average pustule size (in mm^2).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	2.267	0.324	79.97	<.0001
Error	55	0.223	0.004		
Corrected Total	62	2.490			

R^2= 0.911

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sr13	1	1.456	1.456	359.51	<.0001
Temp	1	0.204	0.204	50.30	<.0001
Ploidy	1	0.149	0.149	36.68	<.0001
Temp*Ploidy	1	0.063	0.063	15.55	0.0002
Temp*Sr13	1	0.279	0.279	68.97	<.0001
Ploidy*Sr13	1	0.101	0.101	24.97	<.0001
Temp*Ploidy*Sr13	1	0.002	0.002	0.37	0.5448

Level of Temp	Level of Ploidy	Level of Sr13	N	PZ Mean	PZ Std Dev
High temperature	Hexaploid	No-Sr13	8	10.657	0.971
High temperature	Hexaploid	Sr13	8	2.379	0.421
High temperature	Tetraploid	No-Sr13	8	7.227	1.501
High temperature	Tetraploid	Sr13	8	1.241	0.186
Low temperature	Hexaploid	No-Sr13	8	7.804	4.132
Low temperature	Hexaploid	Sr13	8	4.684	1.833
Low temperature	Tetraploid	No-Sr13	7	8.627	1.482
Low temperature	Tetraploid	Sr13	8	3.137	0.999

Table S4. Differences in fungal growth between lines with and without *Sr13* estimated by fungal DNA relative to host DNA, average *Pgt* infection area (fluorescence), and average sporulation area. Plants were grown at two different temperatures (low= 18°C day / 15°C night and high= 25°C day / 22°C night) and samples were collected five days post inoculation (dpi) with TTKSK for the DNA and fluorescence data and 13 dpi for the sporulation area. Data was transformed to restore normality of residuals and was analyzed using a two-way factorial ANOVA.

		LMPG *vs.* LMPG-*Sr13*	Fielder *vs.* *Sr13* transgenics[1]	Kronos *vs.* s*r13*-mutants[1]
Fungal DNA relative to host DNA	Replications[2]	n= 6 vs. 6	n= 6 *vs.* 24	n= 6 *vs.* 8
	Genotype	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
	Temperature	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
	Interactions G x T	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Average individual growth area (mm^2)	Replications[2]	n= 4 vs. 4	n= 8 *vs.* 32	n= 8 vs. 16
	Genotype	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
	Temperature	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
	Interactions G x T	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Average individual sporulation area (mm^2)	Replications[2]	n= 8 *vs.* 8	n= 8 *vs.* 32	n= 8 *vs.* 14
	Genotype	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
	Temperature	$P = 0.0220$[3]	$P < 0.0001$	$P = 0.7053$[3]
	Interactions G x T	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

[1] Data from different transgenic events or *sr13*-mutants have been combined.

[2] Number of replications per genotype per temperature. Numbers are in the same order as the lines listed in the title of the respective column.

[3] Differences between temperatures were significant within genotypes (in opposite direction).

Table S5. Three-way ANOVA for *CNL13* transcript levels at two different temperatures (low= 18°C day / 15°C night and high= 25°C day / 22°C night), two inoculation treatments (TTKSK and mock), and four collection times post inoculation (1, 2, 4, and 6 dpi). Different plants were used at each collection time. Contrasts between the first two and last two sampling points are presented separately for the inoculated and mock-inoculated samples.

Dependent Variable: Transcript levels of *Sr13*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	1.3187	0.0879	4.86	<.0001
Error	32	0.5787	0.0181		
Corrected Total	47	1.8974			

R-Square
0.695017

Source	DF	Type I SS	Mean Square	F Value	Pr > F
T	1	0.0008	0.0008	0.04	0.8372
Inoc	1	0.5797	0.5797	32.06	**<.0001**
T*Inoc	1	0.0188	0.0188	1.04	0.3150
Day	3	0.6431	0.2144	11.85	**<.0001**
T*Day	3	0.0256	0.0085	0.47	0.7036
Inoc*Day	3	0.0282	0.0094	0.52	0.6717
T*Inoc*Day	3	0.0225	0.0075	0.41	0.7437

Contrast **Mock inoculated**	DF	Contrast SS	Mean Square	F Value	Pr > F
D1-2 *vs.* D4-6	1	0.1700	0.1700	9.86	**0.0063**
% difference 11.5%					

Contrast **TTKSK inoculated**	DF	Contrast SS	Mean Square	F Value	Pr > F
1-2 *vs.* 4-6	1	0.3449	0.3449	18.22	**0.0006**
% difference 18.7%					

Table S6. Effect of inoculation (TTKSK *vs.* mock) and genotype (Kronos *vs. sr13*-mutant T4-476, LMPG *vs.* LMPG-*Sr13*, and Fielder *vs.* transgenic T_1Sr13-2) on *PR* transcript levels (5 dpi). Two-way ANOVAs with six replications per genotype-inoculation combination (data was transformed to restore normality of the residuals). Interaction graphs with the corresponding averages and standard errors are presented in Figure 6 and *SI Appendix*, Figs. S8 and S9.

		PR1	*PR2*	*PR3*	*PR4*	*PR5*
Kronos	Genotype (G)	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
vs.	Inoculation (I)	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
mutant-*sr13*	G x I	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
LMPG-*Sr13*	Genotype (G)	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
vs.	Inoculation (I)	$P = 0.0026$[1]	$P < 0.0001$	$P = 0.0009$[1]	$P = 0.7515$[1]	$P < 0.0001$
LMPG	G x I	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Trans. T_1Sr13-2	Genotype (G)	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
vs.	Inoculation (I)	$P = 0.0053$[1]	$P < 0.0001$	$P < 0.0001$	$P = 0.2189$[1]	$P < 0.0001$
Fielder	G x I	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

[1] In the hexaploid genotypes (LMPG and Fielder), several *PR* genes (except *PR5*) showed opposite responses in resistant and susceptible genotypes, resulting in lower *P* values for inoculation. For all *PR* genes, the differences between inoculation treatments were highly significant within each genotype ($P < 0.001$, except for Fielder where $P = 0.0148$)

Table S7. Three-way ANOVA for transcript levels of six *PR* genes in Kronos leaves 4 and 6dpi. Plants were inoculated with TTKSK or were mock-inoculated at high and low temperatures. Data was transformed to normalize the residuals. Interaction graphs with the means and standard errors are presented in *SI Appendix*, Fig. S10.

Source	*PR1*	*PR2*	*PR3*	*PR4*	*PR5*	*PR9*
Day	<0.0001	0.3693	<0.0001	0.0004	<0.0001	<0.0001
Inoculation	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001
Day*Inoculation	<0.0001	0.0013	<0.0001	0.0599	0.0010	0.4106
Temp.	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001
Day*Temp.	<0.0001	0.0318	<0.0001	0.0008	<0.0001	<0.0001
Inoculation*Temp.	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001
Day*Inoculation*Temp.	0.1045	<0.0001	<0.0001	0.3814	0.0001	0.4218

Table S8. Distribution of *Sr13* haplotypes in diploid, tetraploid and hexaploid wheat accessions.

Haplotype	Accession or Name
R1	Khapli[b] (CItr4013[b], PI 101971[b], PI 355477[b], PI 310471[b]), CItr12213[b], CItr14919[b], PI 41024[b], PI 94747[b], PI 101971[b], PI 154582[b], PI 164578[b], PI 168673[b], PI 193879[b], PI 217637[b], PI 217639[b], PI 217640[b], PI 272533[b], PI 324076[b], PI 355477[b], PI 532305[b]; CItr15326[c], Kronos[c], Maier[c], Renville[c]; Khapstein/9*LMPG[d], W2691Sr13[d], Combination VII[d]
R2	PI 58789[b], PI 94631[b], PI 94664[b], PI 194042[b], PI 194375[b], PI 244341[b], PI 254147[b], PI 273981[b], PI 275996[b], PI 480460[b]; D98530[c], D99656[c], Karim[c], Kofa[c], Medora[c], Sceptre[c], WB881[c], Zenit[c], Cirillo[c], Ward[c], Leeds[c]
R3	PI 352548[b]; Altar84[c], Langdon[c], L35[c], Aconchi89[c], ST 464[c]
S1	2174[d], Caledonia[d], Cayuga[d], CIMMIT III[d], Express[d], Fielder[d], Finch[d], GRN*5/ND614-A[d], Harry[d], IDO444[d], IDO556[d], Jaypee[d], Jupeteco[d], Louise[d], Penawawa[d], Eltan[d], PI 610750[d], Pioneer 26R46[d], Platte[d], Rio Blanco[d], RSI5[d], SS550[d], TAM105[d], Thatcher[d], USG3209[d], Wesley[d]
S2	Adamello[c], Colorado[c], Dilse[c], Divide[c], Duilio[c], Exeldur[c], Mexicali75[c], Nefer[c], Saragolla[c], San Carlo[c], UC1113[c], Valforte[c], Valnova[c], Vanavo[c], Ciccio[c], Neodur[c], Latino[c], Appio[c], PI 478298[c], Vitron[c], PI 532239[c]
S3	LMPG[d]
S4	05[a], 07[a], 09[a], 10-85[a], 11[a], 17[a], 19[a], 20[a], 24[a], 27[a], 28[a], 29[a], 30[a], 32[a], 40[a], 41[a], 42[a], 43[a], 44[a], PI 355455[a], PI 355458[a], PI 355459[a], PI 362036[a], PI 428017[a], PI 428020[a], PI 428036[a], PI 428041[a], PI 428047[a], PI 428055[a], PI428058[a], PI 428061[a], PI 428086[a], PI 470997[a], PI 471033[a], PI 471815[a], PI 487255[a], PI 538701[a], PI 538718[a], PI 560877[a], PI 654334[a], PI 656872[a], PI 428016[a], PI 554584[a], PI 560697[a], PI 560872[a], PI 560877[a], PI 654311[a]; CItr 12214[b], CItr 14133[b], CItr 14135[b], CItr 14621[b], CItr 14916[b], CItr 14970[b], PI 94616[b], PI 94621[b], PI 94638[b], PI 94656[b], PI 94657[b], PI 193877[b], PI 193880[b], PI 193882[b], PI 193883[b], PI 194041[b], PI 197259[b], PI 197260[b], PI 197481[b], PI 197485[b], PI 197486[b], PI 197489[b], PI 197492[b], PI 197493[b], PI 197495[b], PI 221400[b], PI 254165[b], PI 254189[b], PI 273978[b], PI 276013[b], PI 298582[b], PI 298586[b], PI 319869[b], PI 355507[b], PI 362696[b], PI 377656[b], PI 377657[b]; Capelli[c], Colosseo[c], Durfort[c], Messapia[c], Mindum[c], Produra[c], Russello SG7[c], Rusty[c], Valbelice[c], PI 94701[c]; Clark's Cream[d], CO940610[d], Foster[d], Heyne[d], Jagger[d], KS01HW163-4[d], McCormick[d], McNeal[d], NY18/CC 40-1[d], OS9[d], P91193[d], P92201[d], Pioneer26R61[d], Reeder/Bw-227R[d], Reeder/Bw-227S[d], Zak[d], Chinese Spring[d], Golden Ball Derivative W3504[d]; PI 428183[e], PI 428210[e], PI 538724[e], PI 428231[e], PI 428211[e], PI 428195[e], PI 538728[e], PI 503319[e], PI 428235[e], PI 428193[e], PI 428213[e], PI 428217[e], PI 428212[e], PI 428216[e], PI 428196[e], PI 428203[e], PI 428238[e], PI 428198[e], PI 428187[e], PI 538730[e], PI 428200[e], PI 538725[e], PI 428188[e], PI 428223[e], PI 428240[e], PI 428239[e], PI 428222[e], PI 428277[e], PI 428285[e], PI 428290[e], PI 428228[e], PI 428278[e], PI 428247[e], PI 428279[e], PI 428287[e], PI 428291[e], PI 538733[e], PI 428280[e], PI 538740[e], PI 428292[e], PI 428275[e], PI 428282[e], PI 428288[e], PI 428293[e], PI 428276[e], PI 428283[e], PI 428294[e], PI 428295[e], PI 428304[e], PI 428321[e], PI 428327[e], PI 428296[e], PI 428305[e], PI 428322[e], PI 428298[e], PI 428299[e], PI 428302[e], PI 428303[e], PI 428306[e], PI 428307[e], PI 428308[e], PI 538744[e], PI 428315[e], PI 428319[e], PI 538747[e], PI 428320[e], PI 428323[e], PI 428324[e], PI 428325[e], PI 428336[e]
S5	23[a], 33[a]; CItr7687[b], CItr14637[b], PI 94623[b], PI 225332[b], PI 254164[b]; Ofanto[c], Appulo[c], Trinakria[c]
S6	PI 168679[b], PI 273980[b]
S7	PI 74108[b], PI 94614[b], PI 94615[b], PI 94624[b], PI 94626[b], PI 94635[b], PI 94666[b], PI 94674[b], PI 94675[b], PI 94738[b], PI 254190[b], PI 272531[b], PI 349043[b], PI 349046[b]
S8	PI 94648[b], PI 377655[b]
S9	PI 384332[b]
S10	PI 352364[b]

[a]*T. turgidum* subsp. *dicoccoides*, [b]*T. turgidum* subsp. *dicoccon*, [c]*T. turgidum* subsp. *durum*, [d]*T. aestivum*, [e]*T. urartu*. PI and CItr numbers correspond to Germplasm Resources Information Network (GRIN) numbers. Other numbers correspond to 'Location-Genotype' identification numbers from the University of Haifa wheat germplasm collection (8)

Table S9. PCR amplification results for 182 accessions classified as haplotype S4 (*SI Appendix*, Table S8) with five additional pairs of primers.

Hapl.	*Triticum*	*Triticum*	*T. turgidum* ssp.			Total	Amplified	% id.	bp
	urartu	*aestivum*	*dicoccoides*	*dicoccum*	*durum*		Region [1]	R1	
S4a	67	15	21	9	9	121	None	-	0
S4b	3	0	0	1	0	4	Intron 1	100	575
S4c	0	0	1	0	0	1	Intron 1	97.9	575
S4d	0	2	2	1	1	6	Exon 1	95.7	833
S4e	0	0	1	0	0	1	Exon 1	96.2[2]	834
S4f	0	0	0	1	0	1	Exon 1	97.0	833
S4g	0	0	2	0	0	2	Exon 1	97.1	833
S4h	0	0	2	0	0	2	Exon 1	99.2	833
S4i	0	0	4	1	0	5	Exon 1	99.3	833
S4j	0	1	0	0	0	1	Exon 1	99.5	833
S4k	0	0	0	1	0	1	Exon 1	100	833
S4l	0	0	3	0	0	3	Exon 1+Intron 1[3]	97.3	1457
S4n	0	0	1	0	0	1	Exon 1+Intron 1[3]	98.9	1458
S4o	0	0	7	1	0	8	Exon 1+Intron 1[3]	98.9	1458
S4p	0	0	1	0	0	1	Exon 1+Intron 1[3]	99.2	1456
S4q	0	0	1	0	0	1	Exon 1+Intron 1[3]	99.1	1456
S4t	0	0	1	1	0	2	3 regions	95.1[4]	2519
S4u	0	0	0	1	0	1	3 regions	98.4[5]	2519
S4v	0	0	0	20	0	20	3 regions	98.2[6]	3595

[1] Exon 1 region amplified with primers F_5-R_6, intron 1 amplified with primers F_4-R_7, first part Exon 2 amplified with primers F_7 -R_2 and last part of Exon 2 and Exon 3 with primers F_3 and R_3/R_8 (Fig. 2A).

[2] Truncated gene with frame shift mutation.

[3] Intron 1 in these sequences (and in haplotypes S1and S8) includes a 48 bp insertion flanked by AGTA direct repeats

[4] Truncated gene with frame shift mutations in Exon 2.

[4-5] For these accessions we amplified exon1, intron 1 and the first part of exon 2 (primers F_7-R_2). The end of exon 2 and exon 3 was not amplified with two different primer combinations F_3 - R_3 and F_3 -R_8.

[6] For these accessions we amplified exon1, intron 1, the first part of exon 2 (primers F_7-R_2) and the end of the gene (primers F_3 -R_8). This is a truncated gene with a premature stop codon at the end of exon 2 and frame shift mutations in exon 3.

Table S10. Geographic distribution of the different haplotypes in *T. turgidum* subsp. *dicoccoides*, and *T. turgidum* subsp. *dicoccon*. Countries in gray letters in parenthesis represent likely secondary distribution locations.

Haplotype	Geographic distribution
T. turgidum* subsp. *dicoccoides	
S4	Israel (22), Turkey (17), Lebanon (3), Armenia (1), Iran (1), Syria (1), Romania (1), Russia (1)
S5	Israel (2)
T. turgidum* subsp. *dicoccon	
R1	India (8), Ethiopia (1), Georgia (1), Russia (1), Hungary (1), (Taiwan, USA, Canada, Venezuela)
R2	Ethiopia (8), Spain (1), Saudi Arabia (1)
R3	Ethiopia (1)
S1	(Only in *T. aestivum*)
S2	(Only in *T. turgidum* subsp. *durum*)
S3	(Only in *T. aestivum*)
S4	Ethiopia (20), Serbia (5), India (1), Iran (2), Russia (1), Turkey (2), Armenia (1), Georgia (1), Montenegro (1), Spain (1), (USA)
S5	Iran (3), Ethiopia (1), Russia (1)
S6	Ethiopia (2)
S7	Georgia (5), Ukraine (2), Russia (3), Iran (2), Turkey (1), Hungary (1)
S8	Serbia (1), Italy (1)
S9	Ethiopia (1)
S10	Iran (1)

Table S11. Z-test for positive selection (Ka/Ks) as implemented in MEGA 6. Lower diagonal matrix: probabilities of positive selection based on Z-test. Highlighted = $P<0.05$, Highlighted and bold= $P<0.01$. Upper diagonal matrix in blue: Ka/Ks values (9). Ka= non-synonymous changes per non-synonymous sites, Ks= synonymous change per synonymous sites, Inf= infinite (Ks=0).

	R1	**R2**	**R3**	**S1**	**S2**	**S3**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
R1		Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	3.91	2.11	Inf.
R2	0.021		Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	3.61	1.81	Inf.
R3	0.021	0.040		Inf.	2.71	Inf.	Inf.	Inf.	2.11	3.61	1.81	Inf.
S1	**0.007**	0.012	0.012		Inf.	Inf.	Inf.	Inf.	Inf.	2.40	1.81	Inf.
S2	**0.004**	**0.007**	0.094	**0.007**		Inf.	Inf.	2.11	4.53	3.30	2.11	3.31
S3	**0.004**	**0.007**	**0.007**	**0.007**	0.012		Inf.	Inf.	Inf.	3.30	2.11	Inf.
S5	0.012	0.021	0.021	0.021	0.040	0.040		Inf.	Inf.	2.70	1.50	Inf.
S6	**0.004**	**0.007**	0.021	**0.007**	0.137	0.012	0.040		1.81	3.30	2.11	Inf.
S7	**0.000**	**0.001**	0.098	**0.007**	0.028	**0.001**	**0.004**	0.131		3.31	3.32	0.60
S8	0.013	0.019	0.019	0.074	0.027	0.027	0.054	0.027	0.027		1.62	2.70
S9	0.099	0.131	0.131	0.132	0.099	0.099	0.171	0.099	0.027	0.108		2.71
S10	**0.001**	**0.002**	**0.007**	0.021	0.064	**0.004**	0.012	0.012	0.500	0.054	0.053	

References *SI Appendix*

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