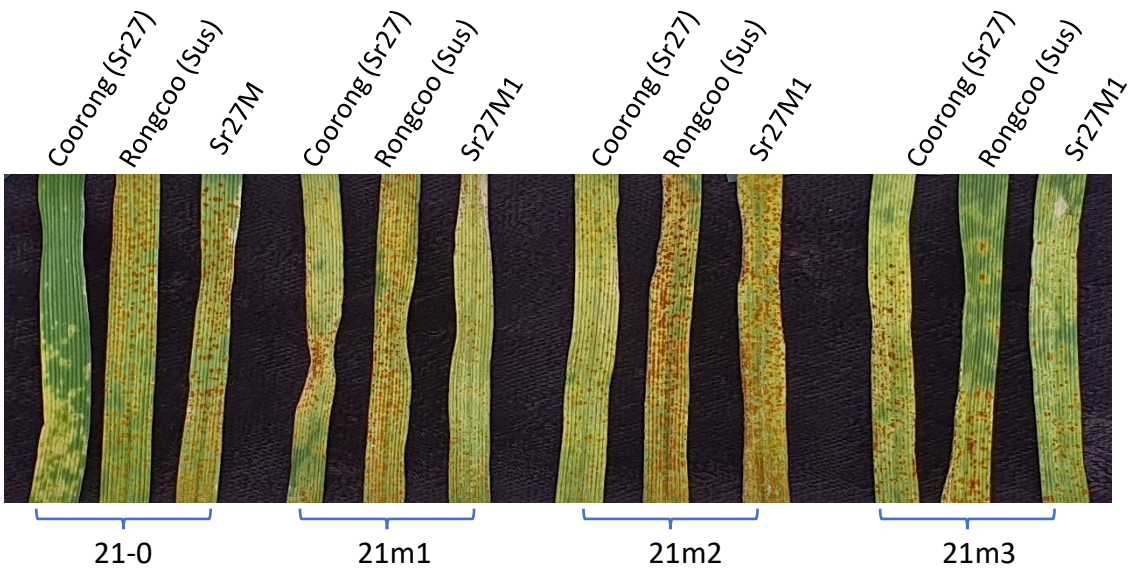


Figure S1 Infection phenotypes of Pgt21-0 and three spontaneous mutants (21m1, 21m2 and 21m3) on Triticale lines Coorong (contains *Sr27*), Rongcoo (rust susceptible) and a mutant line derived from Coorong with a loss of *Sr27* resistance gene (Sr27M1). Image was taken 14 days after inoculation of seedling leaves.

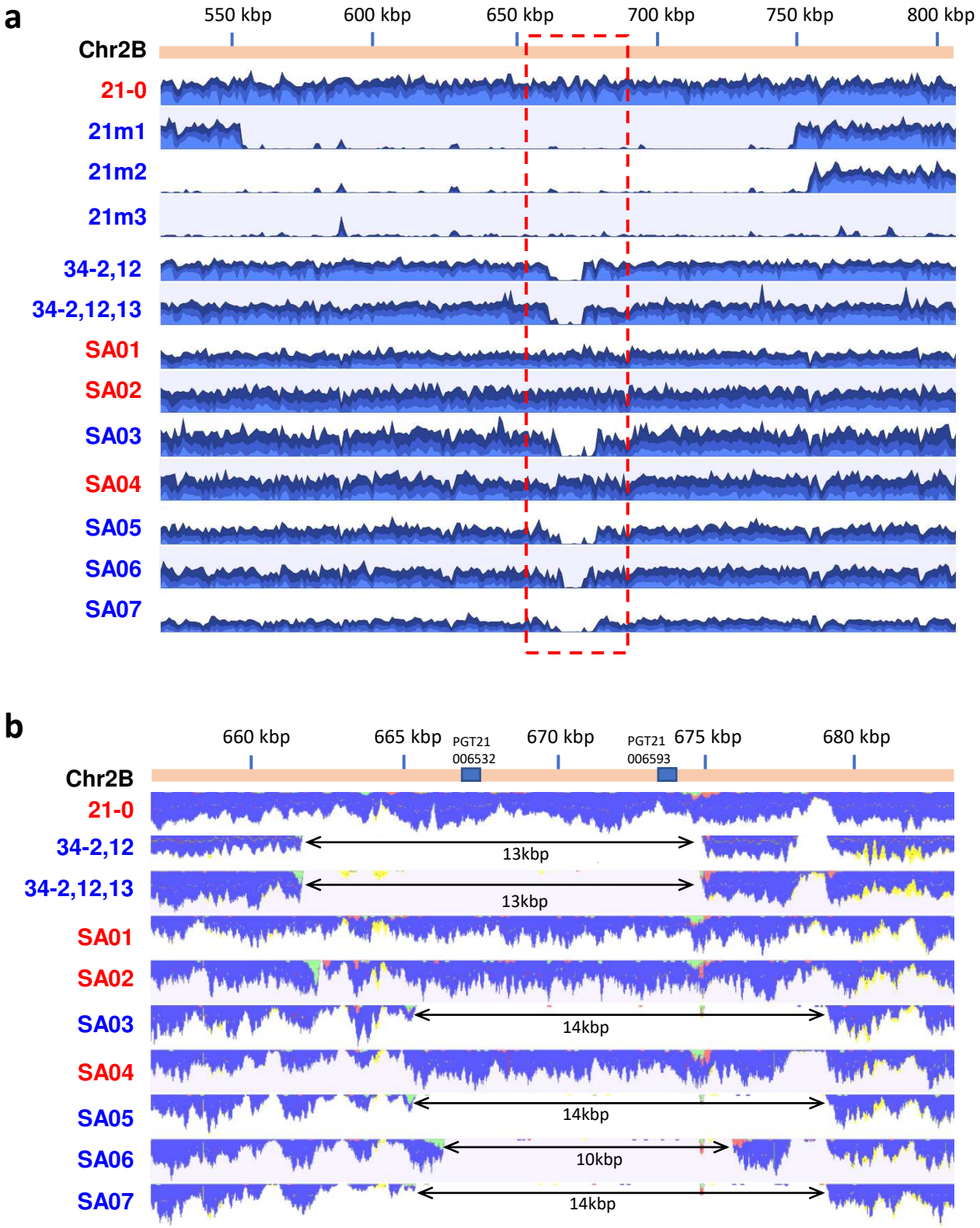
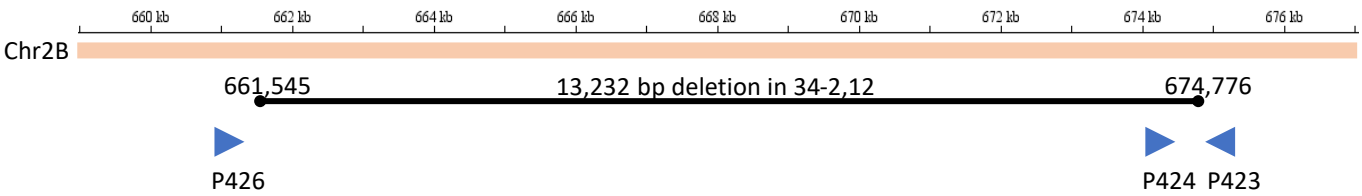


Figure S2 Field isolates of *Pgt* with virulence for *Sr27* contain small deletions on chromosome 2B. **a,** Illumina read coverage graphs for the *AvrSr27* region of chromosome 2B (orange bar) for Pgt21-0, three *Sr27*-virulent mutants of Pgt21-0 and nine field isolates of the same clonal lineage; 34,2,12 and 34,2,12,13 from Australia and SA01 to SA07 from South Africa. Isolates avirulent on *Sr27* are listed in red and virulent isolates in blue. Position on the chromosome in kbp is indicated above the graphs.

b, Close-up of read coverage graphs in boxed region of (A). Approximate sizes of the deleted regions in virulent field isolates are shown in kbp. Positions of Pgt21_006532 and PGT21_006593



Expected amplicon sizes

	21-0	34-2-12
P423/424	601bp	-ve
P423/426	-ve	531bp
P383/351	380bp	380bp

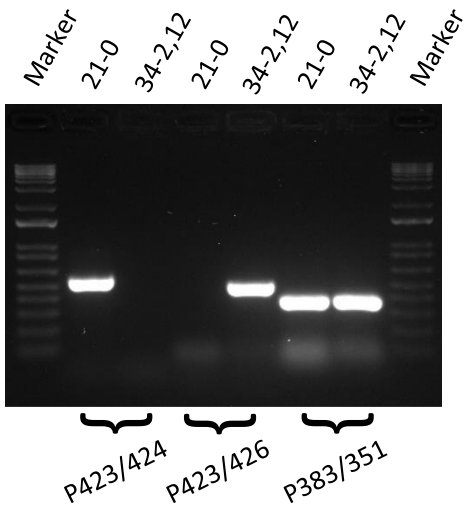


Figure S3 Confirmation of the 13.2 Kbp deletion in *Sr27*-virulent rust strain 34-2-12. The positions of primers P423, P424 and P426 on chromosome 2B around the *AvrSr27* locus are indicated (blue arrow heads) relative to the boundaries of the deletion region in 34-2-12 inferred from the genomic sequence reads. PCR amplification products from genomic DNA of Pgt21-0 and 34-2,12 are shown after separation on a 1% agarose gel. The primers P383 and P351 are designed to amplify a fragment of the *AvrSr50* gene as a control region that is identical in both isolates.

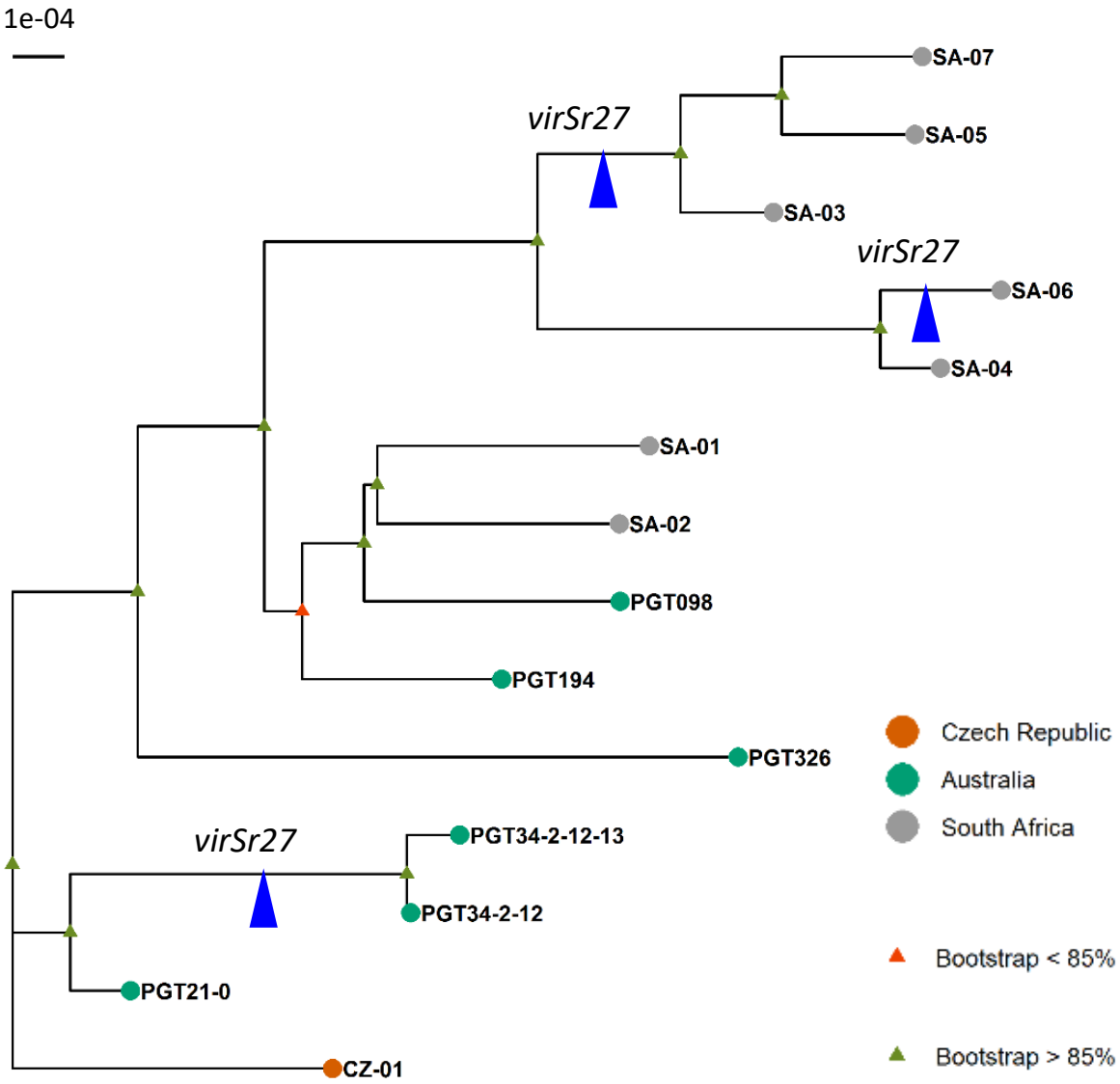
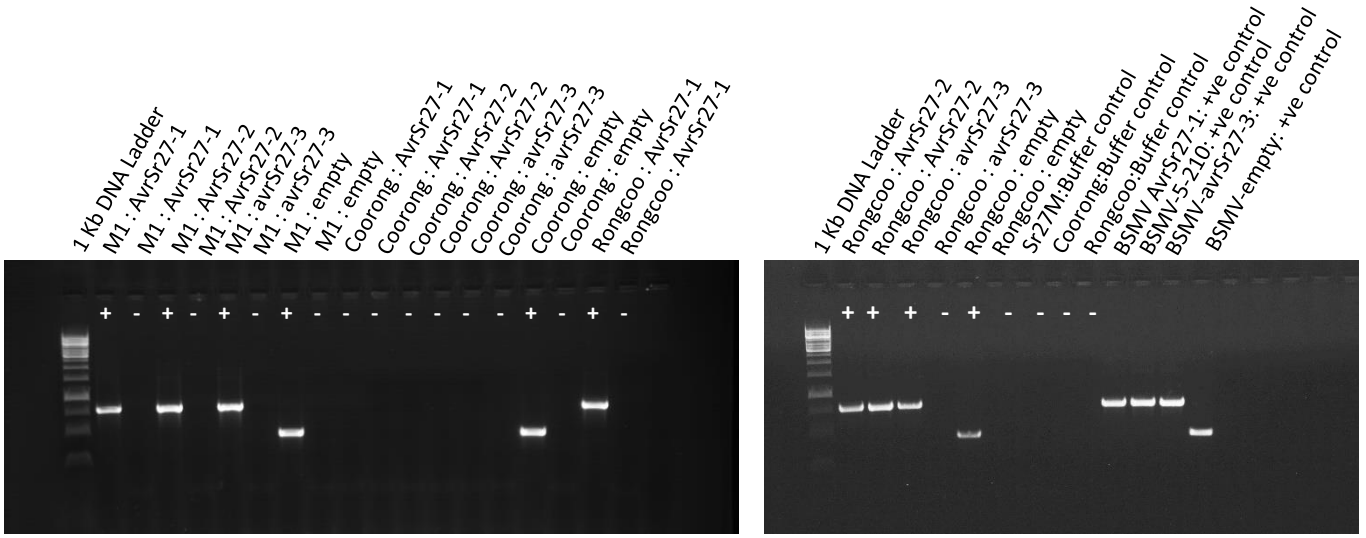


Figure S4 Independent deletions of *AvrSr27* in the race 21 clonal lineage of *Pgt*. Phylogenetic analysis of *Pgt* isolates of the race 21 lineage from South Africa, Australia and the Czech republic (indicated in colour key) using a RAxML model and biallelic SNPs called against the full dikaryotic genome of *Pgt*21-0. Blue arrowheads indicate where three independent mutations leading to virulence on *Sr27* occurred within this lineage. Scale bar indicates number of nucleotide substitutions per site. Nodes with bootstrap values greater than 85% are indicated by green triangles.

a



b

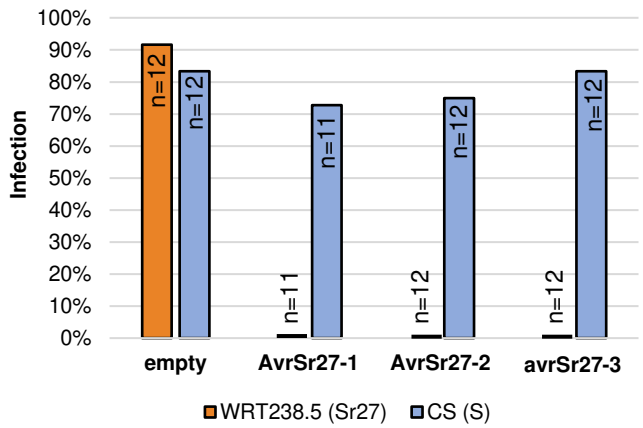


Figure S5 Infection of Triticale lines with BSMV constructs. **a,** RT-PCR assay to check the accumulation BSMV in Coorong, Rongcoo and *Sr27* mutant plants. RNA extracted from leaf samples collected at 14 days post BSMV inoculation was amplified using primers flanking the cloning site in BSMV. +/- indicates the presence or absence of virus symptoms in plants challenged with the respective BSMV construct and buffer control. pDNA of the respective BSMV constructs used as positive control. **b,** Infection of wheat lines Chinese Spring (CS) and CS WRT238.5 (carrying *Sr27* on an introgressed 3RS chromosome segment) with the *BSMV* expression vector encoding AvrSr27-1, -2, -3, or a non-coding multiple cloning site (empty). Y-axis indicates the proportion of inoculated plants that demonstrated systemic viral infection symptoms.

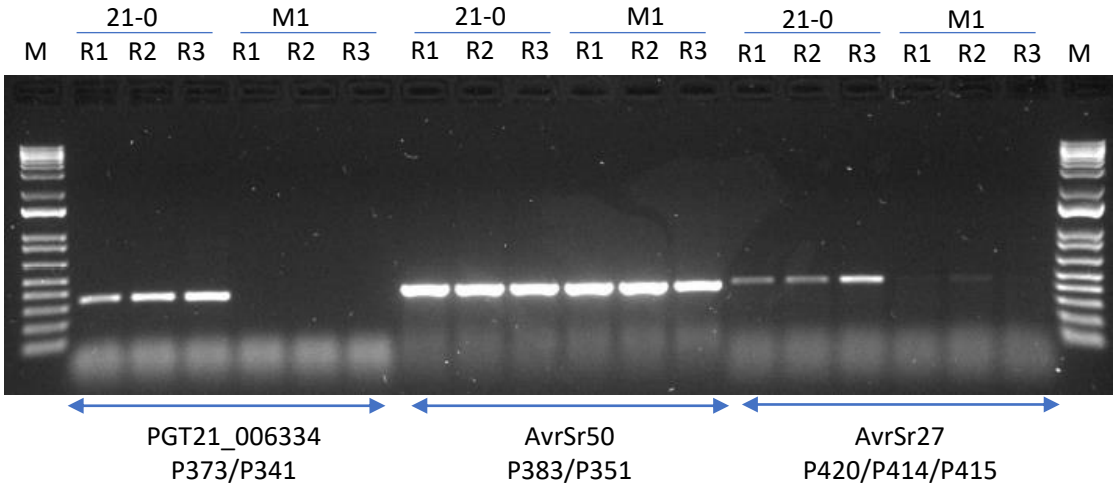


Figure S6 Differential expression of *AvrSr27* alleles. RT-PCR analysis of gene expression in Pgt21-0 and the *Sr27* virulent mutant M1. RT-PCR was performed on three replicate samples (R1 to R3) of RNA extracted from wheat infected with Pgt21-0 or the virulent mutant line (M1). Primers used targeted the transcripts from genes *PGT21-006334* (included in the deleted region of mutant 1, left lanes, primers P373/341), *AvrSr50* (not deleted in mutant 1, middle lanes, primers P383/P351) or all three *AvrSr27* variants (two of which are deleted in mutant 1, right lanes, primers P420/P414/P415).

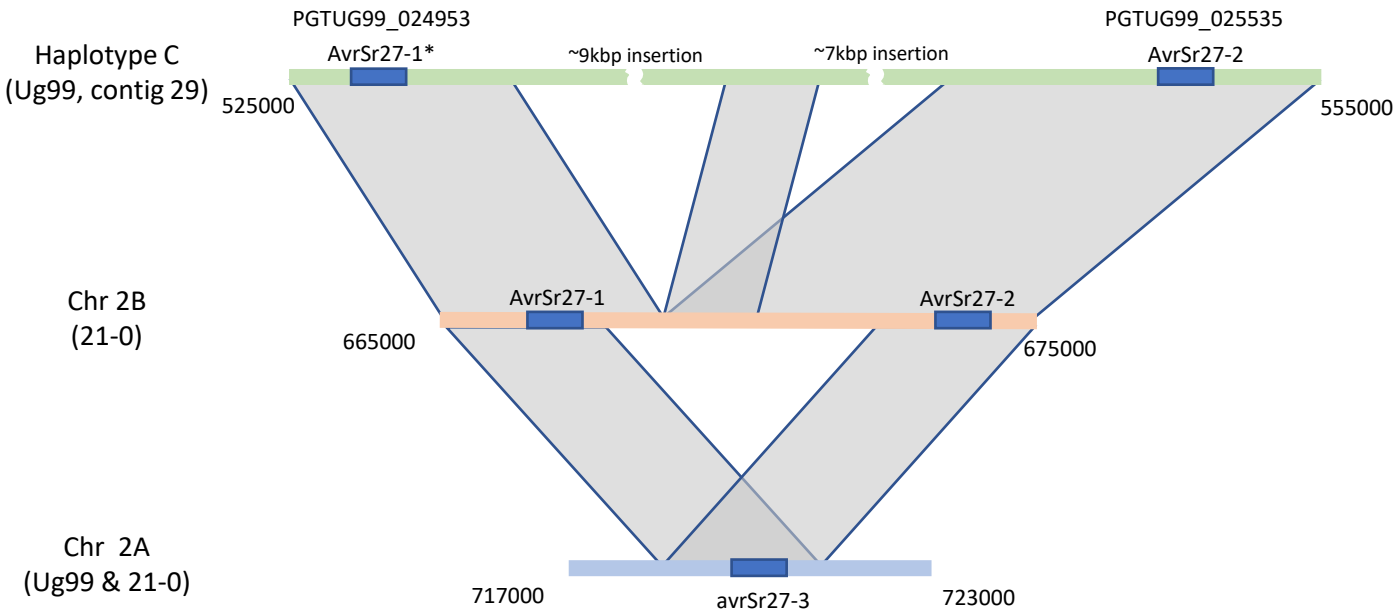


Figure S7 Schematic alignment of *AvrSr27* locus haplotypes from the A, B and C haplotypes (blue, orange and green respectively) of the Pgt21-0 and Ug99 genome assemblies. Regions of high sequence similarity (>95%) between the haplotypes are indicated by grey shading. The positions of *AvrSr27* coding sequences are indicated by the dark blue boxes. *AvrSr27-1** in haplotype C encodes a single amino acid change compared to *AvrSr27-1* on chromosome 2B. Chromosome and contig positions of the selected genome segments are indicated (bp).

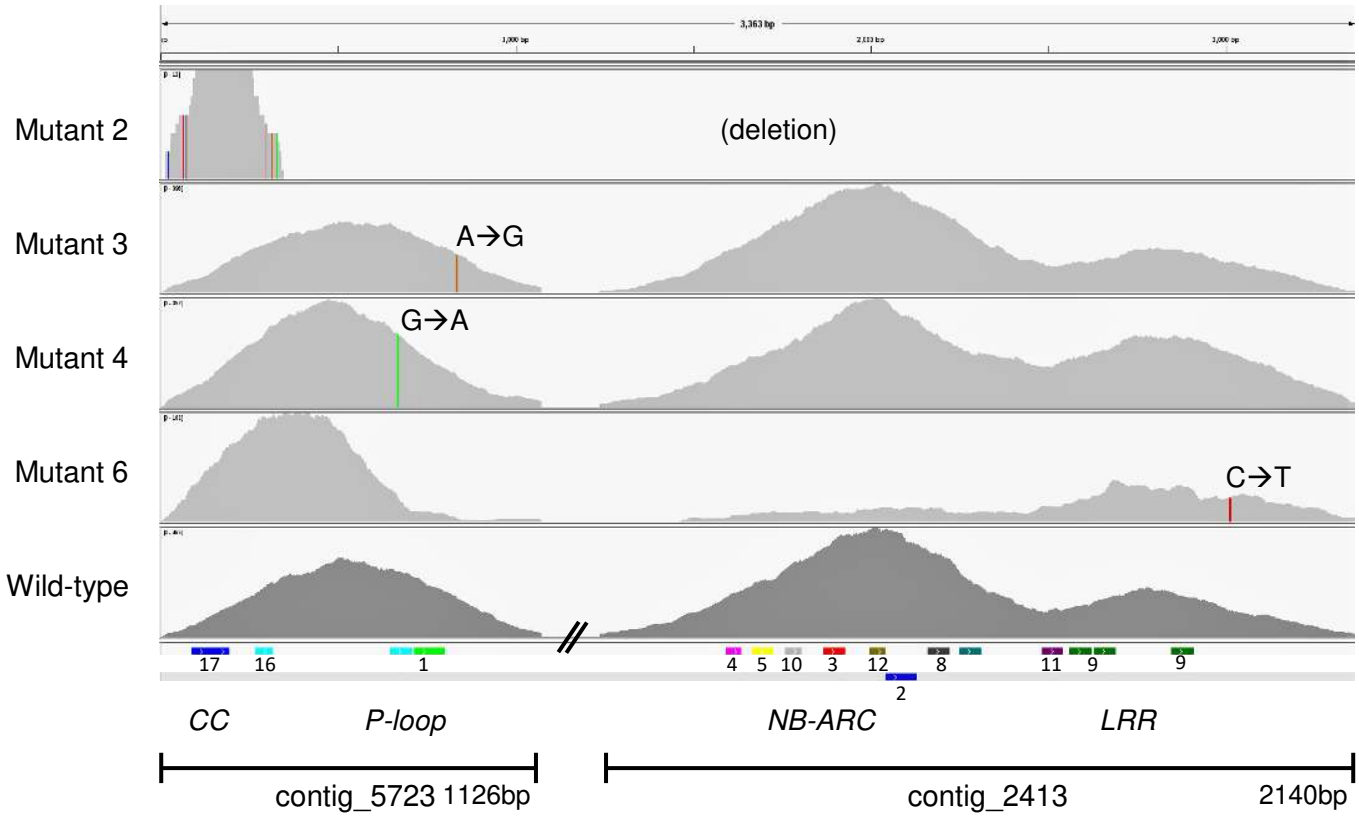


Figure S8 Detection of mutations in a candidate *Sr27* gene by NB-LRR capture and sequencing. Two contigs (#5723 and #2413) assembled from wildtype Coorong contain the 5' and 3' regions of this gene. Read coverage graphs show mapping of reads derived from the NB-LRR capture library from Coorong (wild-type) and four mutants (2,3,4 and 6) to these two contigs. The positions of single nucleotide changes are shown by the coloured bars with the specific change indicated. Mutant M2 produced no reads specific to these contigs and therefore likely contains a deletion. The positions of conserved motifs are indicated under the graphs (numbered colored bars).

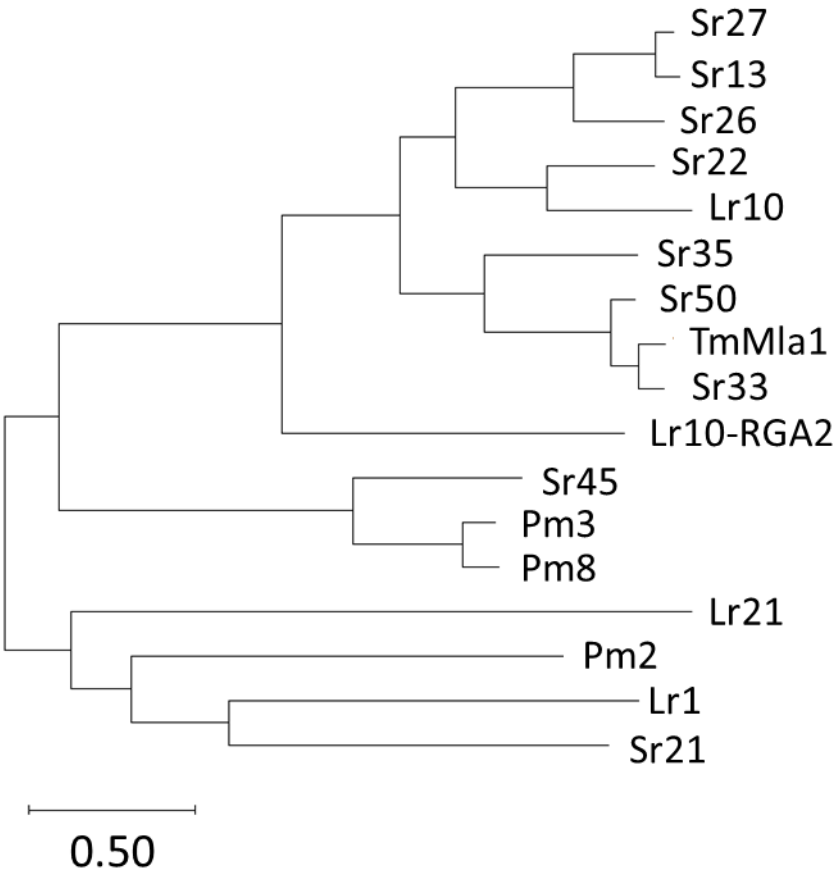
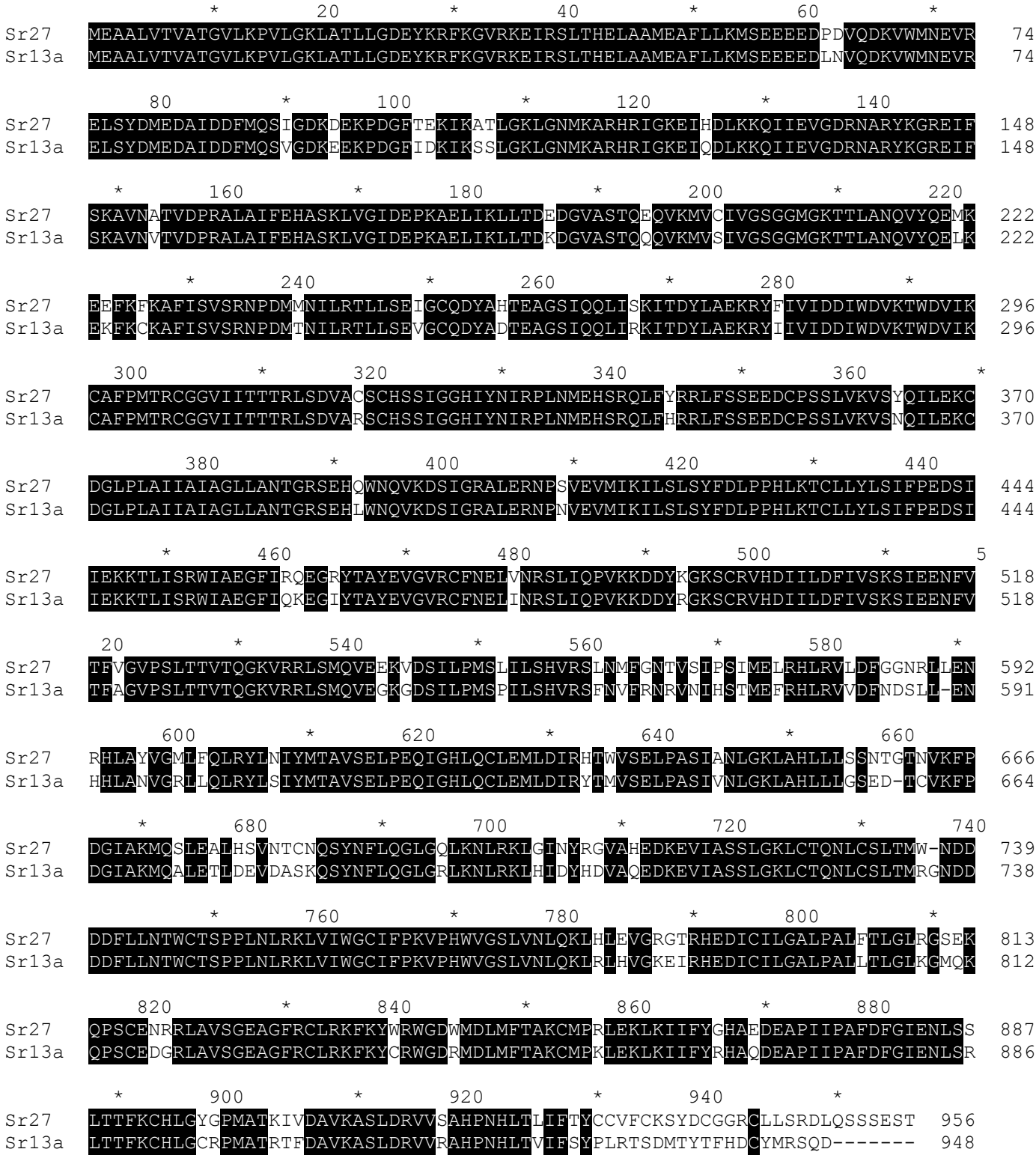
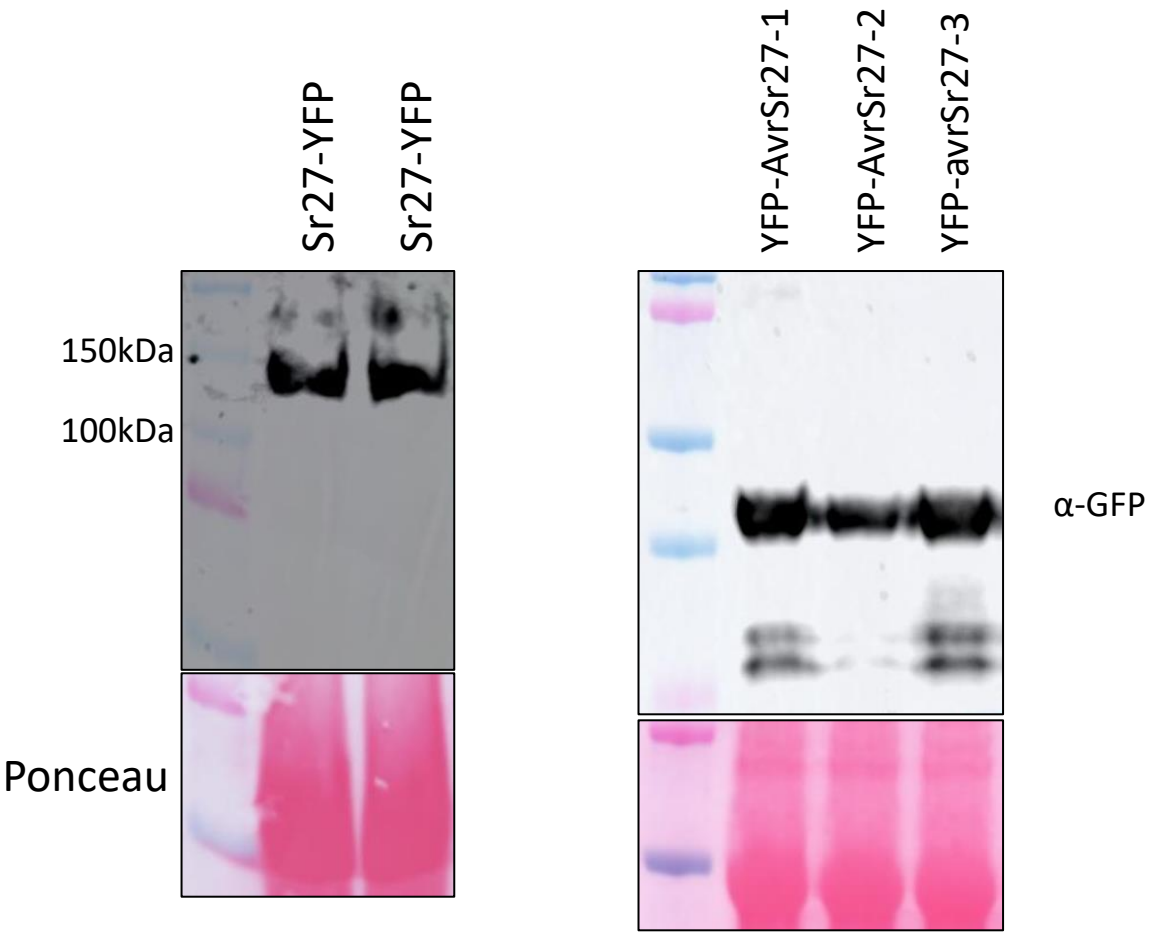


Figure S9 Maximum likelihood phylogenetic tree comparing Sr27 amino acid sequence to proteins sequences of known wheat resistance proteins. Scale bar shows amino acid sequence divergence.



Supplementary Fig. 10 Amino acid sequence alignment of the Sr27 and Sr13a resistance proteins.



Supplementary Fig. 11 Immunoblot showing protein expression of Sr27-YFP and YFP-AvrSr27 protein constructs detected using anti-GFP antibodies. Ponceau red staining of filter indicates equal loading of protein extracts.