

Extensive Genetic Variation at the *Sr22* Wheat Stem Rust Resistance Gene Locus in the Grasses Revealed through Evolutionary Genomics and Functional Analyses

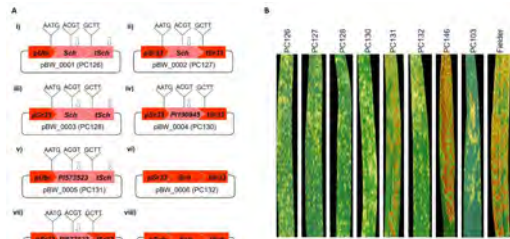
M. Asyraf Md. Hatta^{1,2}, Sreya Ghosh¹, Naveenkumar Athiyannan^{3,4}, Terese Richardson³, Burkhard Steuernagel¹, Guotai Yu¹, Matthew N. Rouse^{5,6}, Mick Ayliffe³, Evans S. Lagudah³, Guru V. Radhakrishnan¹, Sambasivam K. Periyannan^{3,4}, and Brande B. H. Wulff¹

Introduction

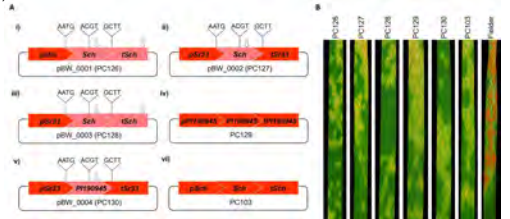
Wheat stem rust disease, caused by the fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*), has re-emerged as a major threat to wheat cultivation. The stem rust (*Sr*) resistance gene *Sr22* encodes a nucleotide-binding and leucine-rich repeat (NLR) receptor which confers resistance to the highly virulent African stem rust isolate Ug99. From our previous and present studies, we identified fourteen *Sr22* sequence variants using PCR screening and sequencing of *T. boeoticum* and *T. monococcum*, some of which were believed to confer functional *Sr22* resistance.

2. Confirmation of *Sr22* variant function in wheat transgenics

Three *Sr22* alleles (Schomburgk, PI190945, PI573523), two of which were predicted to be functional (Schomburgk and PI190945) were transformed into wheat cv. Fielder and tested for resistance function.



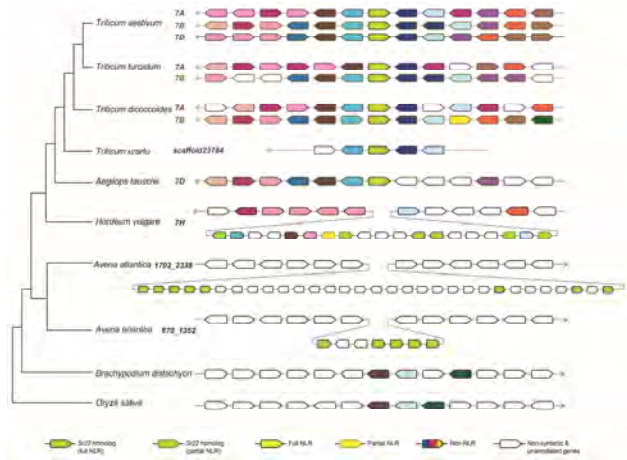
Phenotypic reaction of transgenic *Sr22* variants against Australian *Pgt* race 98-1,2,3,5,6.



Phenotypic reaction of transgenic *Sr22* variants against Australian *Pgt* race TTKSK.

4. Synteny analysis of genomic regions containing *Sr22* homologs

Sr22 is a conserved NLR found on chromosome 7 in Triticeae species.



Sr22 orthologs were not detected in *O. sativa* and *B. distachyon* but regions exhibiting conservation of microsynteny to the region containing *Sr22* in the Triticeae species investigated above could be identified. *Sr22* orthologs are coloured green. Full-length NLR genes are shown with solid outlines while partial NLR genes are shown with dashed outlines (as predicted by NLR-Annotator).

1. Nucleotide sequence analysis of *Sr22* variants

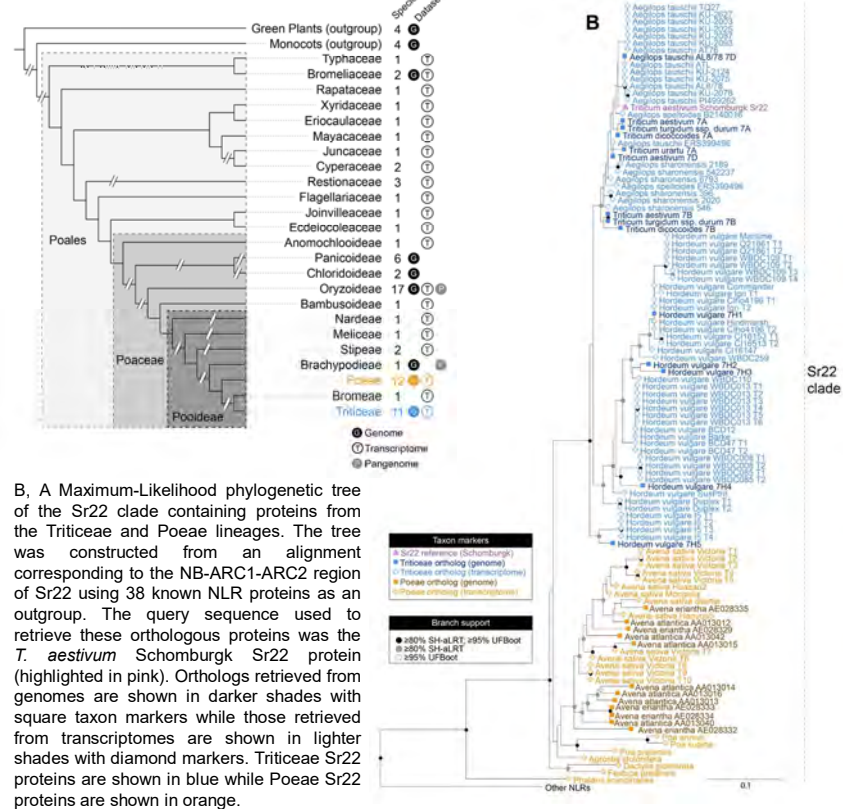
The sequence alignment of fourteen *Sr22* variants plus a possible new allele, *SrTm5* from *T. monococcum* accession PI306540 using Clustal Omega revealed evidence of historical sequence exchange in some alleles.



3. Phylogenetic analysis to identify *Sr22* orthologs

Grass genomes and transcriptomes were inspected and *Sr22* likely arose in the last common ancestor of the Triticeae and Poeae lineages, and then evolved as a single gene locus, except in barley and oat, where the locus experienced pronounced independent expansions.

A phylogenetic representation of the plant/monocot lineages from which species were examined for the presence of *Sr22* orthologs.



B, A Maximum-Likelihood phylogenetic tree of the *Sr22* clade containing proteins from the Triticeae and Poeae lineages. The tree was constructed from an alignment corresponding to the NB-ARC1-ARC2 region of *Sr22* using 38 known NLR proteins as an outgroup. The query sequence used to retrieve these orthologous proteins was the *T. aestivum* Schomburgk *Sr22* protein (highlighted in pink). Orthologs retrieved from genomes are shown in darker shades with square taxon markers while those retrieved from transcriptomes are shown in lighter shades with diamond markers. Triticeae *Sr22* proteins are shown in blue while Poeae *Sr22* proteins are shown in orange.