

Complementation of the BED domain containing wheat stripe rust resistance gene candidates *Yr5*(*Yr5a*) and *YrSP*(*Yr5b*)

Jianping Zhang¹, Terese Richardson¹, Dhara Bhatt¹, Robert A. McIntosh², Peng Zhang², Evans Lagudah^{1,2}

¹CSIRO Agriculture & Food, Canberra, ACT, Australia.

²Plant Breeding Institute, School of Life and Environmental Sciences, University of Sydney, Cobby, NSW, Australia.



Validation of gene candidate through complementation experiment is crucial and also considered as the gold standard proof of gene identity. Here we report the complementation results through wheat transformation¹ of the BED domain containing wheat stripe rust resistance gene candidates *Yr5*(*Yr5a*) and *YrSP*(*Yr5b*) that were isolated in our previous study².

Extending the UTR region through comparative genomic strategy

A comparative genomic strategy was utilised for extending of the UTRs of *Yr5* and *YrSP*. We used the *Yr5* and *YrSP* sequences to identify haplotypes present in the sequenced genomes of the wheat cultivars Cadenza and Claire. Sequence alignments facilitated the design of conserved primers across the predicted UTR regions. Amplification products were then generated from the wheat isolines (Avocet +*Yr5* and Avocet+*YrSP* wild type genotypes) based on a combination of conserved and *Yr5*/*YrSP* candidate gene specific primers. The PCR products amplified from each wildtype genomic DNA were then subcloned into *E. coli* in order to obtain intact sequence. The final confirmed native 5' regulatory element (RE) for *Yr5* and *YrSP* candidate genes were 744bp and 671bp, respectively, and the 3' RE for *Yr5* and *YrSP* were 1,500bp and 2,092bp, respectively.

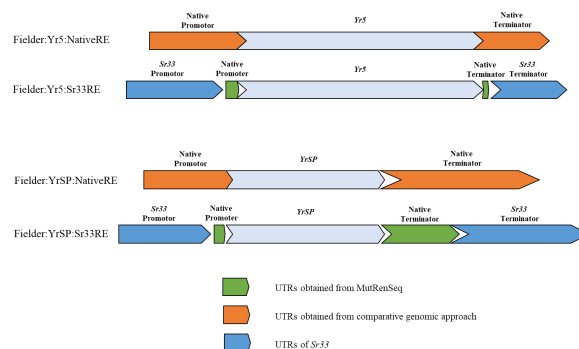
Designing of the constructs

To validate the candidate gene of *Yr5* and to ensure its expression, two constructs were used to generate transgenic wheats in the cultivar Fielder, because the 5' RE obtained through comparative genomic approach was considered to be suboptimal (744bp). One construct was assembled with the native 5' and 3' REs, 744bp and 1,500bp, respectively, and designated as Fielder:Yr5:NativeRE. The second construct, designated as Fielder:Yr5:Sr33RE, was designed by fusing the short native 5' (225bp) and 3' Res (47bp) initially obtained via MutRenSeq (Mutagenesis and Resistance gene Enrichment and Sequencing), with the gene regulatory elements from *Sr33* (1,531bp for 5' RE and 996bp for 3' RE) (Figure 1). We obtained 18 and 13 independent primary transgenic T₀ plants carrying the Fielder:Yr5:NativeRE and Fielder:Yr5:Sr33RE, respectively. All independent T₀ plants with either Fielder:Yr5:NativeRE or Fielder:Yr5:Sr33RE showed resistance to stripe rust pathotype 134 E16 A+17+27+ (PBI rust Culture No. 617), while all the empty vector-transformed Fielder controls were susceptible (Figure 2).

A similar approach was also adopted with two *YrSP* gene constructs for transformation. One construct was assembled with the native 5' and 3' RE, 671bp and 2,092bp, respectively, and designated as Fielder:YrSP:NativeRE.

The second construct, designated as Fielder:YrSP:Sr33RE, fused the native 5' and 3' REs obtained from MutRenSeq, 225bp and 2,092bp, respectively, together with the gene RE from *Sr33* (1,531bp at 5' UTR and 996bp at 3' UTR) (Figure 1). We obtained 18 and 20 independent T₀ plants carrying the Fielder:YrSP:NativeRE and Fielder:YrSP:Sr33RE, respectively. All independent T₀ plants with Fielder:YrSP:NativeRE and Fielder:YrSP:Sr33RE showed resistance to stripe rust pathotype 134 E16 A+17+27+, while all the empty vector-transformed Fielder controls were susceptible (Figure 2).

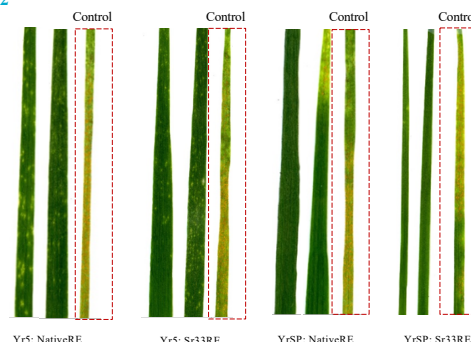
Figure 1



Conclusions

Our transformation results confirmed the candidates for both *Yr5* and *YrSP*, and not only the native full-length regulatory elements, but also the heterologous REs are sufficient to confer resistance.

Figure 2



1. Ishida, Y., M. Tsunashima, Y. Hiei and T. Komari (2015). Wheat (*Triticum aestivum* L.) Transformation Using Immature Embryos. *Agrobacterium Protocols*, Vol. 1, 3rd Edition 1223: 189-196.
2. Marchal, C., J. Zhang, P. Fenwick, B. Steuernagel, N. M. Adamski, L. Boyd, R. McIntosh, B. B. H. Wulff, S. Berry, E. Lagudah and C. Usary (2018). BED-domain-containing immune receptors confer diverse resistance spectra to yellow rust. *Nat Plants* 4: 662-668.