Gene Editing for Durable Wheat Rust Resistance

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Wheat Rust : A formidable foe!

Annual yield loss: US $4.3 – 5.0 bn

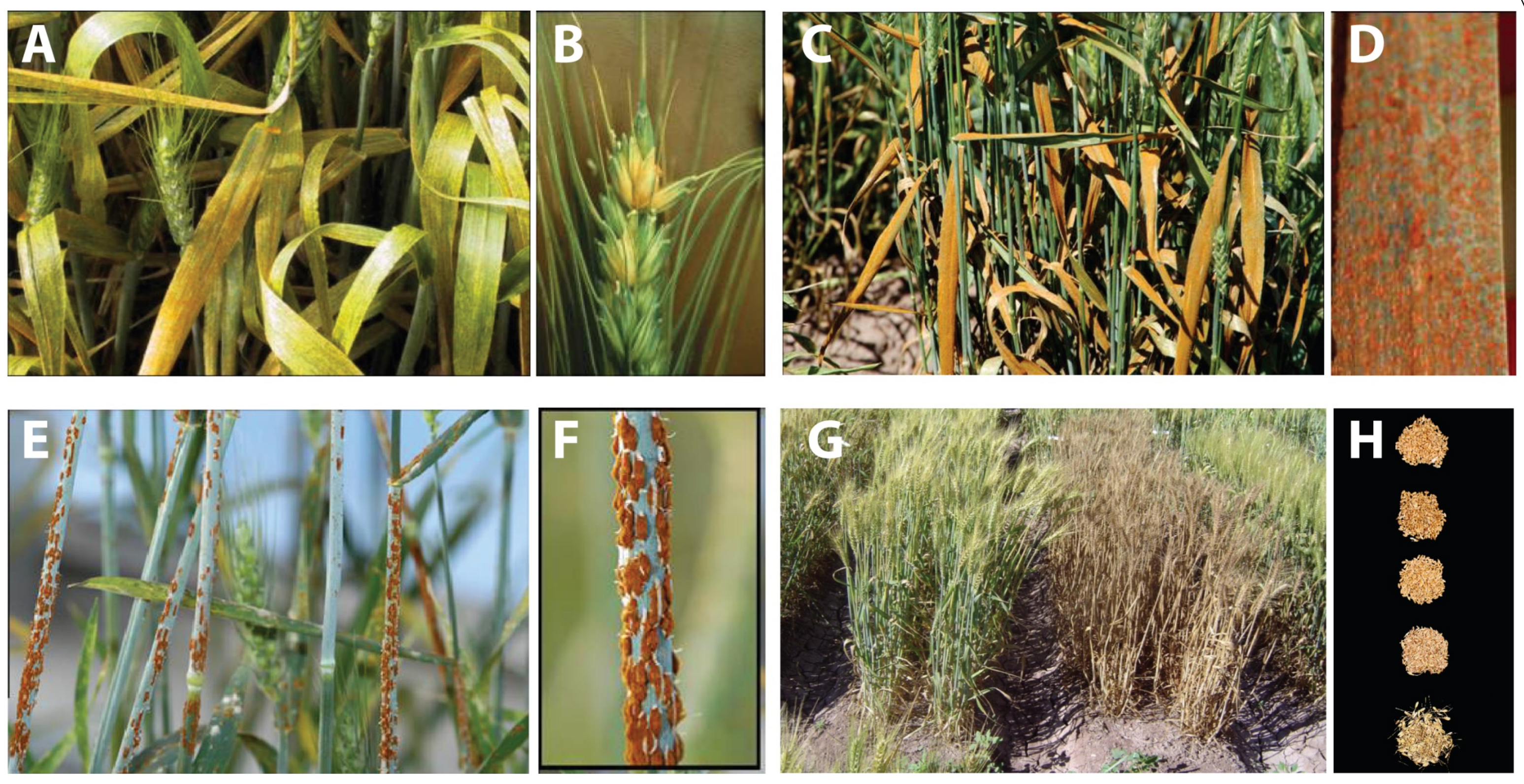


Fig. 1: Different kind of rust diseases affect different parts of the plant. Sometimes the yield loss can be upto 100%

Wheat rust probably started as early as the domestication of wheat. This disease is caused by a parasitic fungus of genus *Puccinia* that can be spread by wind over large areas through microscopic spores or by seed.

Evolution of new rust pathotypes such as UG99 is breaking down the single R-gene mediated resistance and putting wheat cropping at risk.

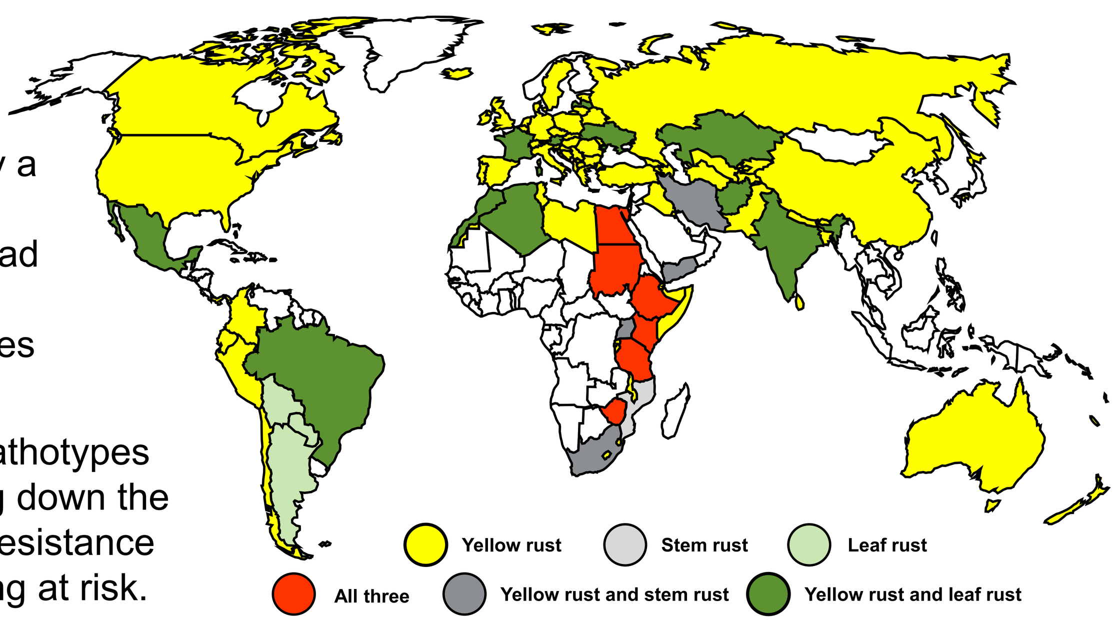


Fig. 2: Geographic distribution of wheat rust

Fig. 3: Field evaluation of advanced breeding lines in Kenya/Ethiopia

Adult plant resistance gene Lr67 provides durable partial resistance to Leaf rust, stripe rust (yellow rust), stem rust and powdery mildew in adult plants

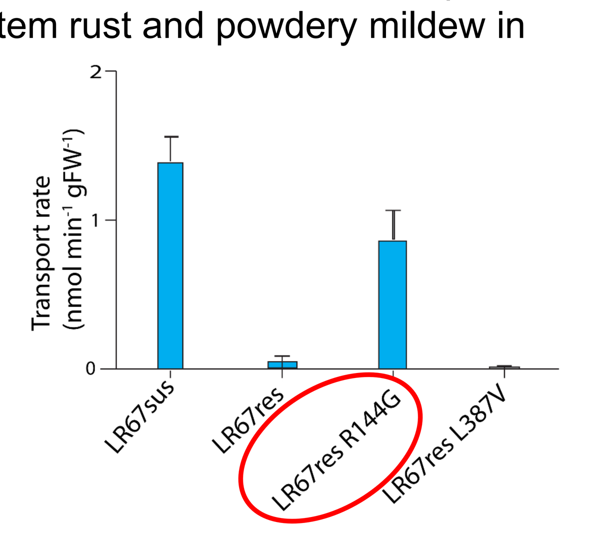


Fig. 4: The resistance allele was unable to transport sugar in yeast. However, The R144G mutation restores the sugar transport in yeast (Moore *et al.* 2015).

Gene cloning and expression in yeast indicated that only a single point mutation G144R converts the susceptible allele to resistant (Moore *at al.* 2015).

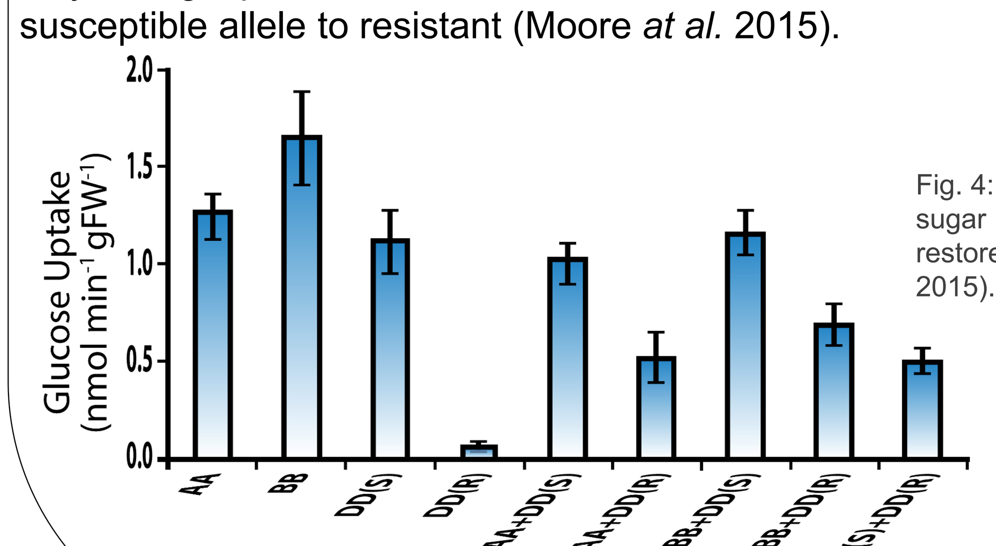


Fig. 5: Lr67 functions as homo- and hetero-dimer

All three homoeologs are capable of sugar transport with different capacity and function as homo- and hetero-dimers (Moore *at al.* 2015).

Phylogenetic analysis indicated that all three homoeologs are very similar to each other in sequence.

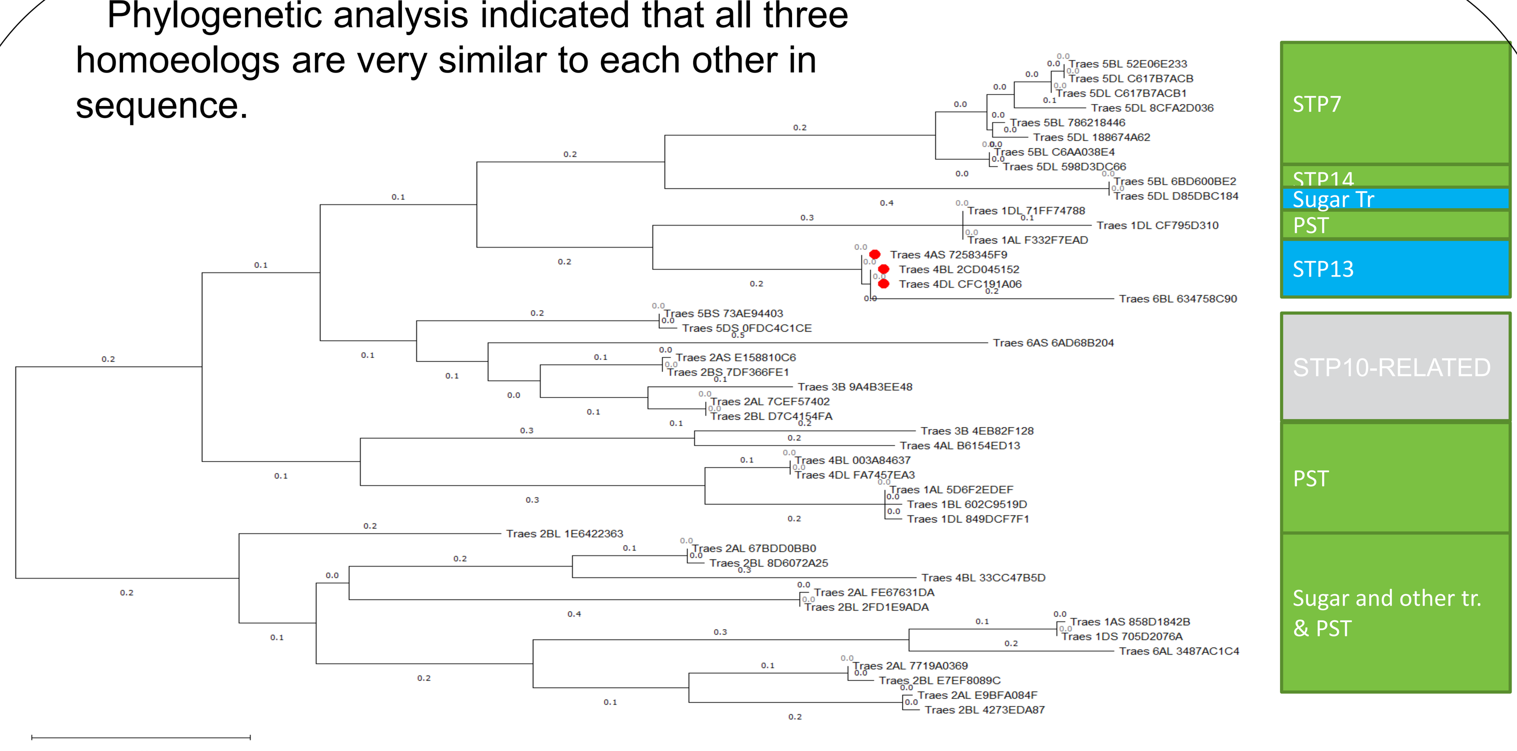


Fig. 6: Phylogenetic analysis of all wheat Sugar trans-porters

TMHMM analysis indicated that there are 11 transmembrane domains in Lr67.

Using sequence homology, we designed two CRISPR guide RNAs to target at positions indicated by 'X' in Fig. 7. Both guide RNAs target all three Lr67 homoeologs simultaneously.

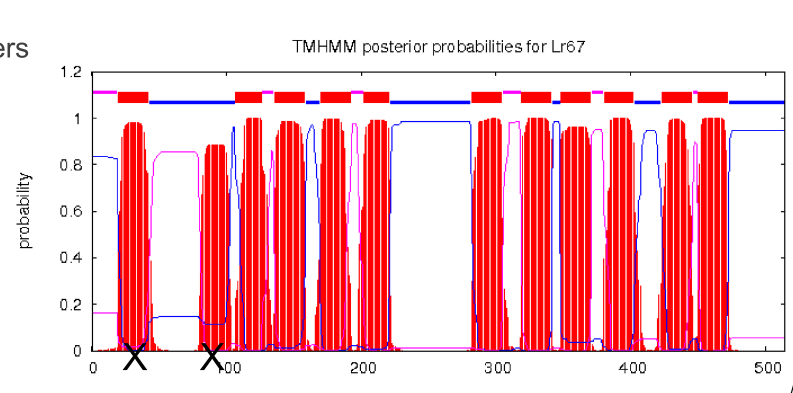


Fig. 7: TMHMM analysis of Lr67 protein sequence and targets of CRISPR-mediated gene editing

Results

We used biolistic particle delivery method to genetically transform experimental wheat lines such as Fielder as well as elite wheat lines such as Reedling, Baj and Kachu with our CRISPR constructs.

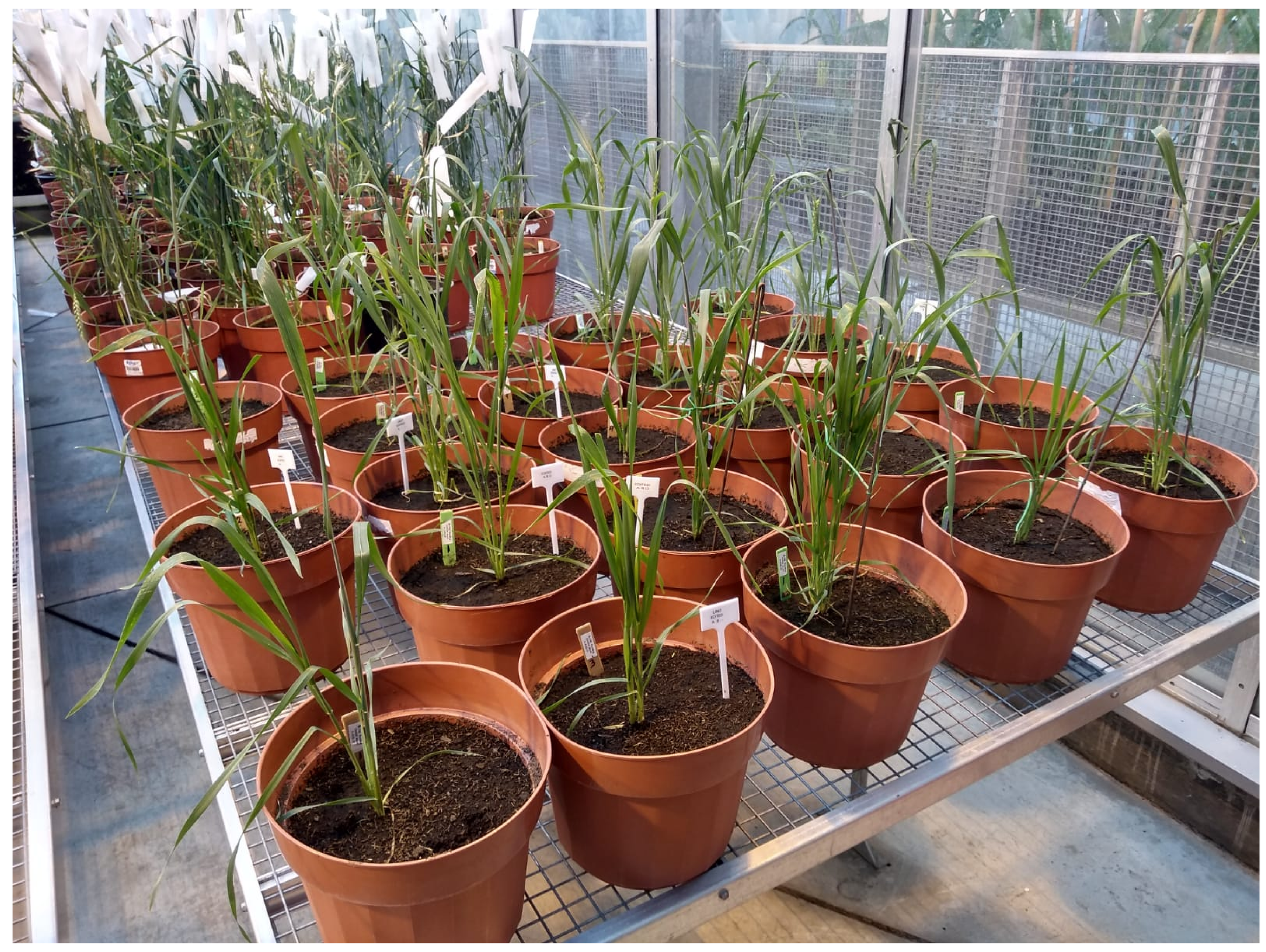
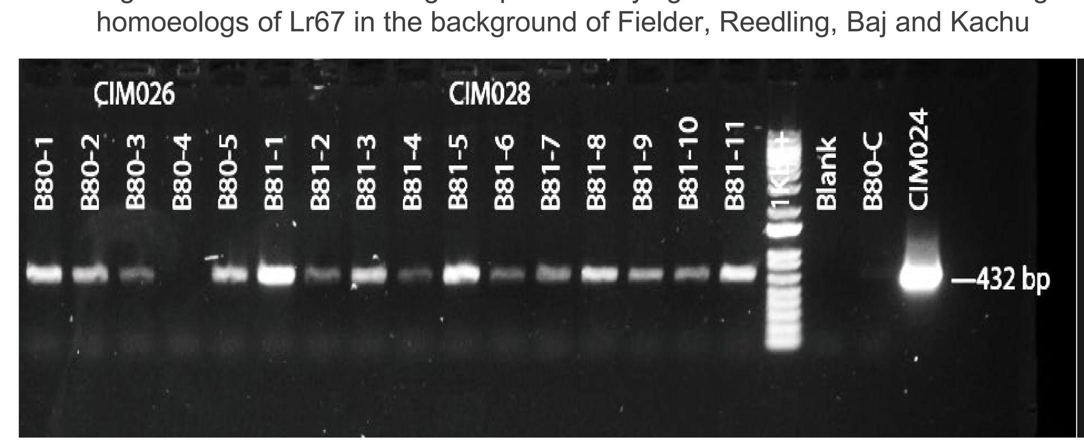


Fig. 8: Generation of transgenic plants carrying the T-DNA with Cas9 and sgRNA for editing of all three homoeologs of Lr67 in the background of Fielder, Reedling, Baj and Kachu



The putative transgenic lines were analyzed by PCR using gRNA and Cas9 specific primers.

Fig. 9: Confirmation of T-DNA integration by PCR

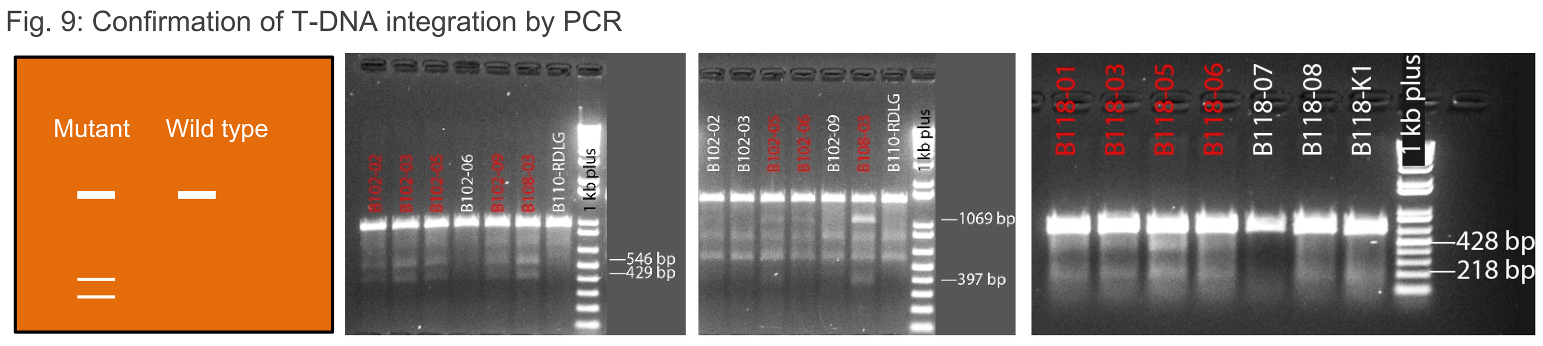


Fig. 10: Analysis of mutants by Surveyor mutation detection kit®

The mutation in target gene/homeologs were analyzed by Surveyor mutation detection kit and Sanger sequencing. We have generated plants for single, double and triple mutants for all three homoeologs.

Fig. 11: T0/G0 lines growing in our biosafety greenhouse

Plant	A mutation	B mutation	D mutation
Lr67-01	Y		
Lr67-02	Y		
Lr67-03	Y		
Lr67-04	Y		
Lr67-05	Y	Y	
Lr67-06	Y		Y
Lr67-07	Y		Y
Lr67-08		Y	Y
Lr67-09		Y	Y
Lr67-10		Y	Y
Lr67-11	Y	Y	Y
Lr67-12	Y	Y	Y
Lr67-13	Y	Y	Y

Table 1: List of Lr67 gene edited lines indicating type of mutation

Over 150 independent transgenic lines with single, double and triple edits were generated. The T1/G1 lines are being challenged against leaf rust fungus in our biosafety contentment facility.

Conclusion

- Based on the available literature, this is the first report of generating mutant alleles for all three homeologs of Lr67.
- Our *prima-facie* observation shows improved resistance of gene-eited lines against rust pathogen without any other physiological variation.

Future direction

- We are eager to determine whether 4A and 4B homeologs of Lr67 (4D) phenocopy the latter and, if so, whether
- their combinations further augment resistance

References

- Moore, J. W., *et. al.* (2015) Nat. Genetics, 47(12), 1494–1498.
- Debernardi, J. M., *et al.* (2020) Nat. Biotechnology, 38(11), 1274–1279.

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