Gene Editing for Durable Wheat Rust Resistance

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Wheat Rust : A formidable foe!

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

Fig. 2: Geographic distribution of wheat rust.

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

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

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).

Fig. 5: Lr67 functions as homo- and hetero-dimer. (Moore et al. 2015).

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

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. 6: Phylogenetic analysis of all wheat Sugar transporters.

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.

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

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

Table 1: List of Lr67 gene edited lines

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<tr>
<th>Plant Line</th>
<th>A Mutation</th>
<th>B Mutation</th>
<th>D Mutation</th>
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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 edited plants 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


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