Identification of a novel leaf rust and stripe rust resistance gene from Sharon goatgrass

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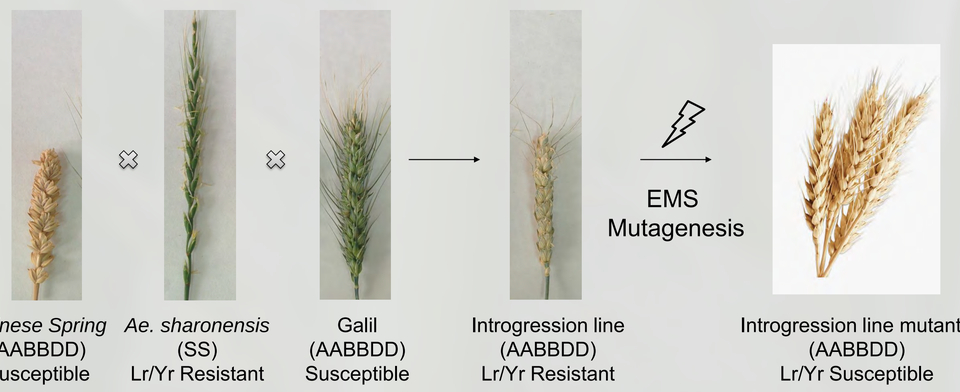
Abstract

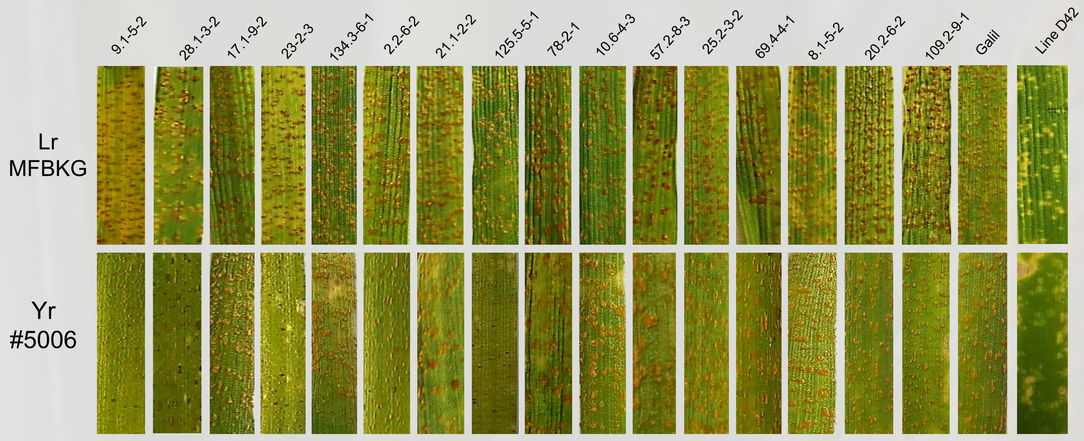
Each year, significant global wheat yield loss occurs due to diseases that affect yield quantity or quality. Breeding for resistance has been the best economic and environmentally safe approach to control wheat diseases, however many disease resistance (R) genes succumbed to the pathogens and are no longer effective. Hence, new sources of resistance are necessary to boost the wheat gene pool. The main source for such genes are species of wheat wild relatives in the secondary gene pool that contain an unexploited reservoir of novel R genes. Sharon goatgrass (*Aegilops sharonensis* Eig) is a wild diploid relative of wheat (genome $S^{sh}S^{sh}$), it is endemic to the coastal plain of Israel, and is highly resistant to rust pathogens. Among fungal diseases, rusts are the most widespread and devastating including wheat leaf rust (Lr), caused by the fungus *Puccinia triticina* Eriks, and wheat stripe (yellow) rust (Yr), caused by the fungus *Puccinia striiformis* f. sp. *Tritici*. Previously, we introgressed a segment containing leaf and stripe rust resistance from *Ae. sharonensis* into bread wheat (Millet et al., 2014). We mapped the alien region to the short arm of chromosome 6B and generated diagnostic markers (Khazan et al., 2020). Here, we report on the isolation of a candidate NLR-type R gene from the wheat introgression lines by a combination of mutational genomics, RNA-seq and chromosome sorting and sequencing.

1. Introgression of Lr / Yr resistance and EMS mutagenesis



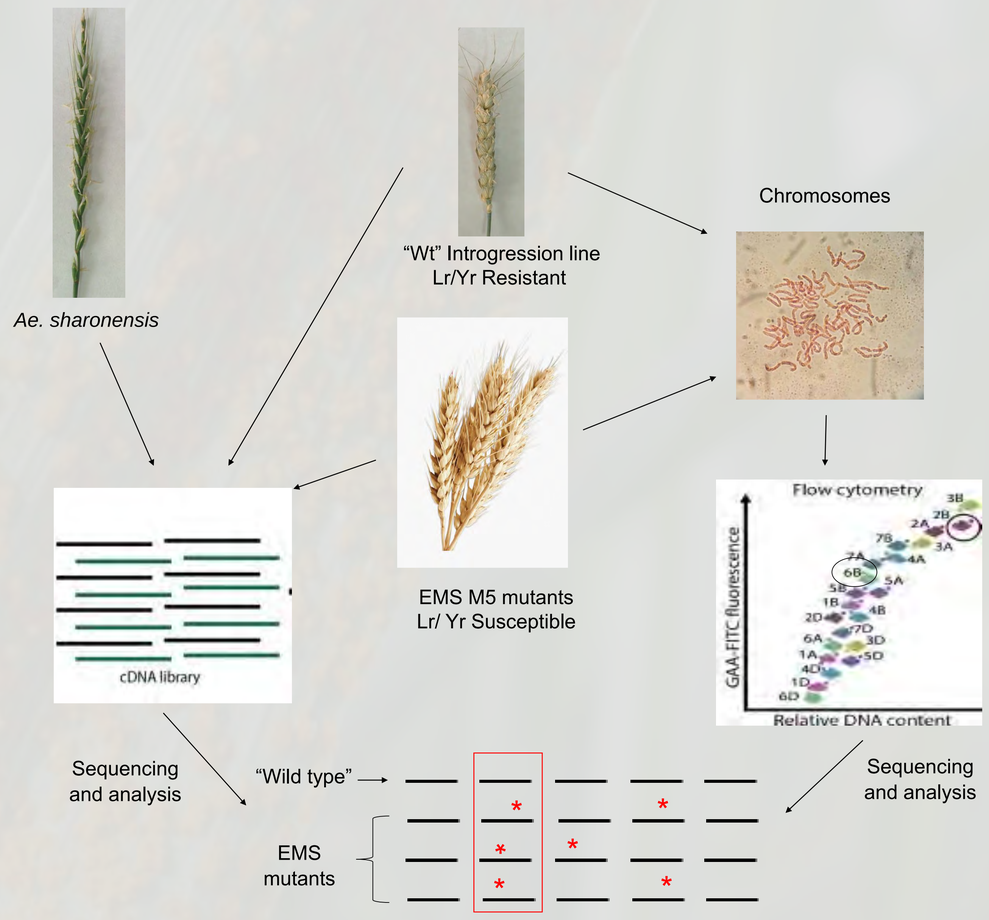
Wheat x *Ae. sharonensis* introgression lines were produced by induction of homoeologous recombination (Millet et. al., 2014). The *Ae. sharonensis* Lr/Yr resistance was translocated into chromosome 6B. We performed EMS mutagenesis of 7,397 seeds of the wheat x *Ae. sharonensis* introgression line D42, with 0.52% EMS and selected Lr/Yr sensitive lines.

2. Lr/Yr M3 susceptible mutants screening



Twenty families segregating for resistance were identified and tested in the M3 generation, revealing 16 independent susceptible mutants for both Lr (race MFBKG) and Yr (race #5006), that were verified in the M5 generation.

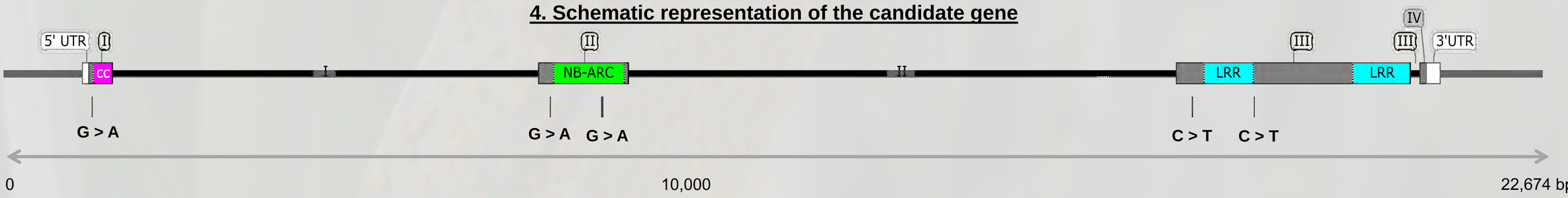
3. Chromosome sorting and sequencing (MutChromSeq) and RNA sequencing



Mitotic chromosomes were extracted from M5 roots of parental introgression line D42 and six Lr/Yr susceptible mutant lines. Fluorescent labelling of chromosomes was performed followed by flow sorting of chromosome 6B. Pure (64%-95%) chromosome preparations were amplified and sequenced. The D42 parental chromosome was *de novo* assembled, resulting in 183,708 contigs of more than 500 bp, with an N50 of 19.016 kb. Mutant reads were mapped to the assembly to search for the mutation overlaps, however no contigs were found with sufficient amount of SNPs. We further constructed and sequenced cDNA library for parental D42 introgression line, the *Ae. sharonensis* resistance accession, and 6 mutant lines. We performed RenSeq analysis combining the transcript isoforms and the previously identified DNA scaffolds data. Using this approach, we were able to identify a single 6.1-kb contig and corresponding 4.3-kb transcript with SNPs in five of the six mutants.

*Figure adapted from Hatta et al., 2019

4. Schematic representation of the candidate gene



Structure of the candidate resistance gene with predicted SNPs caused by EMS mutagenesis. Boxes represent exons, lines represent introns, CC - coiled-coil; NB-ARC - nucleotide-binding; LRR - leucine-rich repeat domains.

5. Summary

- Resistance was previously successfully transferred from *Ae. sharonensis* to wheat and mapped to chromosome 6B (Khazan et al., 2020).
- ChromSeq (Sánchez-Martín et al., 2016) together with RNASeq was used to identify and clone a candidate Lr/Yr NLR resistance gene.
- All of the EMS mutants lost resistance to both diseases suggesting tightly linked resistance genes or a single, dual resistance gene.
- Functional validation of the candidate by transformation into susceptible wheat cultivar is ongoing and will clarify the nature of the resistance.

References:

1. Millet, E., et al. "Introgression of leaf rust and stripe rust resistance from Sharon goatgrass (*Aegilops sharonensis* Eig) into bread wheat (*Triticum aestivum* L.)." Genome 57.6 (2014): 309-316.
2. Khazan, S., et al. "Reducing the size of an alien segment carrying leaf rust and stripe rust resistance in wheat." BMC plant biology 20.1 (2020): 1-13.
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4. Hatta, M. Asyraf Md, Burkhard Steuernagel, and Brande BH Wulff. "Rapid gene cloning in wheat." Applications of genetic and genomic research in cereals. Woodhead Publishing, 2019. 65-95.