

Induction of double haploids in wheat using CENH3 mutants and genome editing

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Project Summary

Overview

Our goal is to accelerate wheat breeding by developing efficient haploid inducer lines that will produce doubled haploids (DH) as seeds. Current DH technologies in wheat are laborious and expensive, limiting their use. Here, we propose to modify the centromere-specific histone 3 (CENH3) to develop a simpler technology of haploid production in wheat. In *Arabidopsis*, single amino acid changes that result in high haploid inducing (HI) efficiency have been identified. However, the translation of these results to wheat has been complicated by the buffering effect of wheat polyploidy and the presence of two tightly linked CENH3 paralogs, designated as α CENH3 and β CENH3 in each of the wheat genomes (the B genome has two β CENH3 copies). Combined mutations in multiple CENH3 copies will likely be required to generate efficient HI lines.

Using γ -irradiated hexaploid wheat plants, we have identified deletions encompassing both α and β CENH3 paralogs in each of the three wheat genomes ($\alpha\beta$ -deletions) and generated AB, AD and BD combined deletions in hexaploid wheat. We have also transferred the A and B genome $\alpha\beta$ -deletions to tetraploid wheat. In addition, we have identified EMS induced point mutations in the A and D genomes, which have been associated with high HI frequency in *Arabidopsis*. Here, we propose to use genome editing to knockout (KO) the wild-type paralogs linked to the proposed HI mutations and combine these α mut- β KO or α KO- β mut loci with complete $\alpha\beta$ -deletions in other genomes. The resulting lines with a single functional but mutant CENH3 allele will be evaluated for HI efficiency.

This strategy will be complemented with the simultaneous editing of α/β CENH3 copies in the A genome, combined with $\alpha\beta$ -deletions in other genomes. We have confirmed high base editing efficiency in wheat protoplasts for a sgRNA and constructs have been submitted to the UC Davis transformation facility for transformation of tetraploid and hexaploid wheat. We will also evaluate guides for the other mutations. Base editing will be performed using two plant-optimized based editors (PBE) that include a catalytically inactive Cas9-D10A (nCAs9) fused to a cytidine deaminase optimized for plant codon usage (published by one of the coPIs). One point mutation change will be generated with the PBE construct that uses an NGG PAM site and the other changes with the VQR-PBE construct that uses an NGA PAM site. The selected nucleotide targets are located at protospacer positions 6-8, which are edited with high efficiency.

Finally, we will combine the most efficient HI mutations with a male sterile allele (*ms1*) to facilitate crosses and with a morphological marker (red coleoptile, *rc*) to accelerate selection of haploid plants. We have already combined the two genes in wheat.

Intellectual Merit

Although we now know the effect of several CENH3 mutations on HI frequency in *Arabidopsis*, the role of similar changes in other species is unknown. In addition, the relative HI effect of the duplicated α CENH3 and β CENH3 genes, and the effect of changes in dosage in the polyploid *Triticeae* species has not been explored so far. This project will provide useful answers to these questions.

Broader Impacts

The proposed CENH3 changes will result in a HI line that is viable, fertile, and genetically stable on self-fertilization, but prone to loss of its own genome on outcrossing, producing paternally derived haploids. Once mutant HI lines are established, they will produce haploids as seeds without tissue culture. This will result in a low-tech, high throughput, and inexpensive approach. The generation of DH from seeds will democratize the use of DH technology and accelerate wheat breeding cycles in both developed and developing countries.