

Breaking the Wheat Domestication Bottleneck by Interploidy Hybridization

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Project Description

I. Rationale

The addition of the D-genome from *Ae. tauschii* gave hexaploid wheat enhanced geographic and environmental adaptations while increasing yield capacities making wheat a staple of modern agrarian societies. Given its recent origin, D genomes chromosomes of wild *Ae. tauschii* accessions and wheat D genome chromosomes recombine freely. The perfect homology between D genomes make *Ae. tauschii* the ideal source of novel genetic variation for wheat improvement.

Limited sources of novel genetic variation in combination with intense selection during modern breeding has restricted the genetic diversity of hexaploid wheat. When a new polyploid species forms, it becomes reproductively reproductively from the progenitor diploid species. Gametes of hexaploid wheat carry three alleles from the A, B and D genomes. The diploid central cell of the *T. aestivum* female gametophyte, therefore, contains six maternal alleles. Male gametes of the diploid D genome progenitor species, *Ae. tauschii* contain only one D genome allele. Endosperm produced from crosses with *T. aestivum* as a female to *Ae. tauschii* will have a 6:1 ratio of maternal to paternal alleles and ultimately will abort due to a threefold deviation from the 2:1 ratio. Endosperm failure presents a postzygotic barrier that limits access to valuable allelic diversity in *Ae. tauschii* for wheat improvement.

Endosperm restoration in direct hybrids between hexaploid and diploid *Ae. tauschii* can be achieved by two mechanisms that augment the expression of imprinted genes in endosperm formation. First, autonomous endosperm may be produced in wheat plants carrying mutations in all three homoeoalleles of FIS-PRC2 protein complex components. Second, introducing mutations in the maternally expressed genes may counter the maternal dominance of the hexaploid female and restore the 2:1 balanced maternal:paternal allele specific expression.

II. Objectives

- 1.) Enable fertile direct hybridizations between hexaploid wheat and diploid relatives using FIS-PRC2 mutants.
- 2.) Evaluate the expression of imprinted FIS-PRC2 genes in mutant and wild type direct hybrids.

III. Experimental Plan

A. Hybridizing hexaploid FIS-PRC2 mutants with diploid *Ae. Tauschii*

1. *Mutations in Maternally and Paternally expressed genes.* Mutants of the hexaploid wheat variety 'Cadenza' (<http://www.wheat-tilling.com>) have been identified with nonsense or missense mutations in all three wheat homoeoalleles of the FIS-PRC2 components FIE, FIS2, and MEA (Table 1). Wheat

orthologues of PEGs involved in restoration of endosperm development have been identified including PEG2-1A, PEG2-1D and PEG9-3A.

2. *Interploidy hybridizations.* FIS-PRC2 mutants of hexaploid wheat will be hybridized directly with diploid *Ae. tauschii* accessions. All combinations of wild type, double and triple mutant hexaploid genotypes will be crossed with diploid *Ae. tauschii* accessions. MEG mutants will be used as females in crosses with diploids as males. PEG mutants will be used as males and diploids will be used as females.

3. *Endosperm microscopy.* The endosperm composition of crosses with FIS-PRC2 and PEG mutants will be investigated to provide insight into the mechanism of endosperm restoration.

4. *Chromosome counts.* Metaphase chromosomes will be counted in root tips of hybrids and backcross progeny to confirm ploidy levels.

5. *Backcrossing of FIS-PRC2 mutations.* A set of spring and winter wheat varieties will be crossed with FIS-PRC2 mutants to initiate transfer of the mutant alleles into elite regional wheat backgrounds. Spring wheat genotypes will include 'Seahawk' and 'Elgin'. Winter wheat genotypes will include 'Jasper', 'Hilliard', 'Overland' and 'Zenda'. A total of three backcrosses will be made with large populations of at least 300 backcross seed each generation. The BC3F1 will be self-pollinated and fixed mutants will be identified in the BC3F2. The 'Cadenza' donor background is a spring type and backcrosses will be made using spring types to avoid vernalization. For winter wheat backgrounds, winter types will be selected at the BC3F2.

B. Evaluating gene expression during compatible and incompatible hybridizations

1. *Targeting cross combinations for gene expression studies.* First, hybridizations between all wheat FISPRC2 mutants and diploid wild relatives will determine the compatible and incompatible crosses. RNASeq will be performed for both compatible and incompatible hybridizations.

2. *RNA isolation from interploidy hybrids.* RNA will be isolated from whole ground caryopses from three biological replicates of each cross. Libraries will be constructed and sequenced on an Illumina HiSeq4000 at the MSU Research Technology Support Facility (RTSF; 50 nucleotide, single end) generating a minimum of 50M paired-end reads per sample. RNA-Seq reads will be evaluated for quality using FASTQC (www.bioinformatics.babraham.ac.uk/projects/fastqc), cleaned for quality and adaptors using CutAdapt (Martin, 2017), and aligned to the Chinese Spring hexaploid reference genome (<https://www.wheatgenome.org/Projects/IWGSC-Bread-Wheat-Projects/Reference-genome/IWGSCReference-Wheat-Genome>) using TopHat2 (Trapnell et al., 2009). Normalized gene expression levels will be calculated using Cufflinks (Trapnell et al., 2010) and reported as fragments per kilobase of exon model per million fragments mapped (FPKM).

3. *DNA isolation from parental lines and determination of imprinted genes.* A 5X whole genome sequence (WGS) will be developed for the 'Cadenza' wheat parent and the *Ae. tauschii* accession TA2477. High molecular weight DNA will be isolated from young etiolated leaf tissue of the parents using a modified CTAB method and Illumina Tru-Seq Nano genomic DNA libraries constructed and sequenced on the HiSeq4000 (150 nt paired-end reads) generating 5X coverage of each genome. WGS reads will be evaluated for quality using FASTQC (www.bioinformatics.babraham.ac.uk/projects/fastqc), cleaned for quality and adaptors using CutAdapt (Martin, 2017), and aligned to the Chinese Spring hexaploid reference genome (IWGSC RefSeq v1.0) using BWA-MEM (Li, 2013). PCR duplicates will be

removed, and high-quality sequence variants called as described previously (Pham et al., 2017). To determine levels of imprinted genes in parental and hybrid endosperm, RNA-Seq reads will be filtered for uniquely mapping and high-quality alignments and read depths at biallelic positions counted as previously described (Pham et al., 2017). Imprinted genes will be defined as described previously (Yang et al., 2018).

IV. Deliverables

1.) Wheat genotypes with mutations that confer altered endosperm imprinting and fertility with *Ae. tauschii* will be made available to the global wheat community. The restoration of fertility to interploidy hybridization in wheat will link every global hexaploid wheat market class to the gene pool of *Ae. tauschii* and other diploid wild relatives. The mutations that restore compatibility to direct hybridization can be transferred by normal breeding to any wheat market class. This will allow all wheat geneticists and breeders to transfer the genome of any *Ae. tauschii* directly to their germplasm of choice.

2.) The gene expression experiments proposed in this work will elucidate the parent of origin for the maternally and paternally expressed genes during compatible and incompatible hybridizations within and across ploidy levels. The mechanism of fertile direct hybridization will be determined.