

Domestication Trait Mapping and Sub-Genome Progenitor Identification in Intermediate Wheatgrass (*Thinopyrum intermedium*)

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The ecological benefits that perennial agroecosystems exhibit over annuals demand that substantial plant breeding efforts be dedicated towards their domestication and improvement for use in agriculture. Perennial agroecosystems are known to increase soil C levels, reduce N leaching, dedicate more biomass to roots, reduce soil erosion and increase N use efficiency (Zan et al., 2001; McIsaac et al., 2010; Dietzel et al., 2015; Gantzer et al., 1990). From a production standpoint, these systems require fewer inputs and reduced tillage compared to annual ones (Kantar et al., 2016). To mitigate environmental impact and improve food and ecosystem security, researchers have initiated breeding efforts for the perennial counterparts to annual crops (Glover et al., 2007; 2010). One species that has demonstrated considerable promise and attention as a perennial grain crop is intermediate wheatgrass (*Thinopyrum intermedium*). Intermediate wheatgrass

(IWG) is a rhizomatous, cool-season, outcrossing and self-incompatible allohexaploid ($2n = 6x = 42$) (Dewey, 1962). Traditionally used as a forage, IWG has an extensive root system and high forage yields (Glover et al., 2007; Wang et al., 2014).

IWG was selected for domestication as a perennial grain crop by the Rodale Institute (Kutztown, PA) in 1983 (Wagoner, 1990). Since 2003, The Land Institute (Salina, KS) has carried out pedigree and phenotype based recurrent selection (DeHaan et al., 2014). UMN joined the effort in 2011 using phenotype based recurrent selection and genomic selection (Zhang et al., 2016). Considerable gain from selection has been observed, however challenges remain. Greater understanding of the genetic control of important traits of interest and the physiological tradeoffs that exist within the breeding program will inform our breeding efforts and ultimately result in more efficient and accurate selection and improvement methodologies. We seek to identify marker trait associations for important traits, assess potential barriers to seed yield (e.g. sterility, resource allocation) and further elucidate the evolutionary history of this species. To identify marker trait associations, we developed a Nested Association Mapping (NAM) population. To investigate the evolutionary history of intermediate wheatgrass, we identified likely progenitors and seek to further validate recent findings and develop genetic linkage maps for these species.

Extensive phenotypic variation has been observed in the UMN IWG breeding program. Some families tend to reliably exhibit small or large seed size, low shattering, high fertility (big seed head) and high threshability (Zhang et al., 2016; Xiaofei Zhang, personal communication). Considering our knowledge of the genetic control of such traits in other species, we hypothesize that these important traits may be controlled by a few large effect genes (e.g. Asano et al., 2011; Li et al., 2006; Peng et al., 1999; Shomura et al., 2008; Simons et al., 2006; Taketa et al., 2008).

Linkage mapping is a common method for identifying markers adjacent to causal variants in a biparental population, however this approach is restricted by limited recombination events, low mapping resolution and underrepresentation of genetic variation in a species (Huang & Han, 2014). A Nested Association Mapping (NAM) population allows for a dual approach of both linkage and association mapping, which takes advantage of historic and recent recombination events, has a low marker density requirement, high statistical power and allows for the investigation of several traits in the same population (McMullen et al., 2009; Yu et al., 2008). An IWG NAM was initiated by crossing ten phenotypically diverse IWG genets in the UMN breeding program with one common parent. 130 F₁ progeny per family (~1,300 plants) were propagated into four clones (~5,200 total plants) planted in two reps in St. Paul, MN and Salina, KS. Plants will be phenotyped for two years for the following traits: springtime vigor, stem angle, heading score, yield, stems plant⁻¹, florets spikelet⁻¹, fertility index, spikelets head⁻¹, shattering, threshability, seed weight, width, area, length, head weight and length, maturity, flag leaf width, stem width, stem strength and height. Genotyping by sequencing (GBS) using the dual enzyme *PstI/MspI* method described by Poland et al. (2012) and optimized for IWG by Zhang et al. (2016) will be used. Libraries will be sequenced using 94-plex High Output Illumina HiSeq2500. Single nucleotide polymorphisms (SNPs) will be called against the IWG reference genome (Kevin Dorn, unpublished data). SNP markers will be tested for significant association with traits of interest.

There has been considerable effort dedicated towards unraveling the complexity of the allohexaploid IWG genome (Mahelka et al., 2011). The origin of the sub-genomes of IWG has yet to be confirmed but it is accepted that there are three distinct chromosome sets from diploid progenitor species likely from the genera *Thinopyrum* and *Pseudoroegneria* (Chen et al., 1998; Tang et al., 2011). GBS was conducted on forty-one diploid candidate species (~150 genotypes) including species from the genera *Aegilops*, *Thinopyrum*, *Dasyphyrum*, *Pseudoroegneria* and *Agropyron*. As part of an initial investigation, trimmed 100 bp reads (64-mers) from the candidates were aligned with 100% identity to the psuedochromosomes of the draft IWG reference genome and three of the species tested displayed a high level of homeology to IWG (Kevin Dorn, unpublished). Future work involves validation of the assignment using shared SNP markers between the progenitors and the IWG psuedochromosomes and testing additional genotypes of these species using a similar method. Also, we will develop linkage maps of the progenitors to assess the genome organization and identify possible translocations. The knowledge of the evolutionary history and the linkage maps will serve as valuable resources for the Triticeae plant breeding community.

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