N-IWYP703.1 PLANT BREEDING PARTNERSHIPS: CONTINUING TO DEVELOP AND VALIDATE THE TOOLS FOR HYBRID WHEAT.

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Goals / Objectives

With the pressing need to improve wheat (T. aestivum L.) productivity, our long-term goal is to develop the necessary knowledge base, germplasm, and heterotic pools or patterns to support the development of hybrid wheat. Toward this end, the specific objectives of this project are to:1. Validate heterosis from previously made and predicted wheat hybrids in replicated trials. 2. Continue male and female (main emphasis) parent line evaluation for characteristics needed to develop experimental and commercial wheat hybrids in a cost-efficient manner.3. Develop heterotic groups or patterns and test multiple mating designs for wheat hybrids. 4. Continue cytoplasmic male sterility (CMS) line development and identify and validate restorer genes for wheat hybrids.

Project Methods

Objective 1: Validate heterosis from previously made and predicted wheat hybrids in replicated trials (Leads: UNL and TAMU). The first step will be to use seed from the existing crossing blocks to select approximately 60 high yielding hybrids for Nebraska and Texas. In these validation trials, 50 hybrids will be selected based on their performance in Nebraska or Texas (as hybrid vigor is environmentally specific). We will also select the 10 best hybrids that have done well in both Texas and Nebraska and 10 check cultivars (previously used in our hybrid yield trials) for a total of 70 entries per trial. Each hybrid yield validation trial will be grown in an alpha lattice with three replications in three locations in three distinct ecological zones in Nebraska (Alliance, North Platte, and Lincoln) and Texas (Greenville, McGregor, and Bushland). The second part of this objective is to validate our genomic estimated breeding values (GEBVs) of the 10,475 predicted but untested hybrids (e.g., 150 parental lines = 11,175 possible hybrids - 700 tested hybrids in the field; see section 1.2 for details). We will use the ~700 tested hybrids and their spatially corrected yield values as a training dataset in the genomic prediction model and predict the yield of the remaining 10,475 hybrids (Zhao et al., 2015 and Belamkar et al., 2018 for methods). Subsequently, using the GEBVs on untested hybrids, we will select three groups of hybrids with a total of ~160 entries that includes 10 checks to connect with our previous trials. The three groups will be: 1) 90 hybrids predicted to be the highest-yielding hybrids (which should likely have high heterosis values); 2) 30 hybrids predicted to be low-yielding hybrids (which should likely have low heterosis values); and 3) 30 random sets of hybrids. We are testing these three sets of hybrids to test the accuracy of predictions. The validation hybrids will need to be made; hence, we will need to put them into our crossing block in 2019-2020 to create new hybrids for testing in 2020-2021 and possibly 2021-2022.Objective 2: Continue male and female (main emphasis) parent line evaluation for characteristics needed to develop experimental and commercial wheat hybrids in a cost-efficient manner (Leads: UNL and TAMU). We did not find the presence of major effect locus for anther extrusion. Hence, the trait architecture seems to be complex or unknown. We propose to phenotype the Freeman X Camelot (best X worst extruder) doubled haploid population comprising 175 lines for anther extrusion and pollination duration for an additional two years (2019-2020 and 2020-2021) and perform QTL mapping using GBS data to investigate the trait architecture and identify genomic regions important for this trait (for methods see Hussain et al., 2017). Additionally, at UNL and TAMU, we will continue screening advanced nurseries in the pureline program each year for anther extrusion (as part of the

breeding program efforts when possible). We will then be able to predict the male characteristics of new breeding lines without phenotyping them in the field for these traits (if we are able to get reasonably high prediction accuracy). The second part of this objective will involve screening for female characteristics, and this aspect will be emphasized in this proposal as part of the student research. The key female traits include gape date, glume angle, stigma size exsertion, and featheriness duration. Preliminary GWAS indicated the presence of major effect loci for some of these traits, as opposed to male traits where such associations could not be identified. Hence, in the proposed project, we will phenotype all the new female lines that will be part of the new crossing blocks beginning in 2019-2020. This effort will increase our population size on which the female traits are measured and allow for a robust GWAS to identify genomic regions and underlying genes associated with female characteristics. Using markers and predicting female lines is much less labor intensive than phenotyping new female lines.Objective 3: Develop heterotic groups or patterns and test multiple mating designs for wheat hybrids (Leads: UNL, TAMU, IPK, and Iowa State University [ISU]). The selection of parents to develop new hybrids will need to take into account male and female characteristics and also the GCA and SCA values. The first part of this objective is to investigate this complete matrix (representing the largest wheat growing region in the United States) for heterotic groups and patterns (using the methods built by our IPK collaborator and described in Zhao et al., 2015b). If successful, the second part of the objective would be to design the crossing block to begin investigating and developing these heterotic groups and patterns in 2021 and 2022. Based on our data so far and the analytical pipelines UNL and IPK have built, we will analyze the 2018 and 2019 field hybrid yield trials for the indication of heterotic patterns in 2019-2020, which means the first crossing block developed from these analyses will occur in 2020-2021 and the yield trials will be grown in 2021-2022. The crossing blocks will use the balanced missing design of Zhao et al. (2015b) or, if we are able to identify heterotic patterns, the designs of Guo et al. (2018). Objective 4: Continue CMS line development and identify and validate restorer genes for wheat hybrids (Leads: CIMMYT, UNL, and TAMU). In our previous research (described in section 1.2), we have focused on mapping restorer genes in T. timopheevi cytoplasm, creating a series of CMS tester lines, their maintainer lines, and a series of elite restorer lines (R-lines). Fine mapping of each major QTL/gene. The previously identified QTL regions will be saturated using high-density markers and large-size single gene mapping backcross or F2 populations. Subsequently, more diagnostic markers will be developed and validated in a diverse set of germplasm. Candidate gene sequencing. Our previous work involving QTL mapping and RFL gene family analyses has identified RFL genes in the wheat genome likely contributing to restoration ability. We will perform targeted re-sequencing (also referred to as amplicon sequencing) of these candidate RFL genes across ~96 to 192 samples and identify SNPs and indels (likely functional alleles) within these candidate genes. The samples will include selected RILs), parents and CMS-tester lines, QTL/gene specific backcross or F2 population, CARGILL R-lines, R lines developed in the ongoing project, and selected non-R lines. Method for targeted/amplicon sequencing. We will use Illumina's NextSeq Series Amplicon Sequencing Solution using the TruSeq Genotype Ne kit. Once Illumina prepares the custom oligos, they will ship the custom oligos and all reagents required for the library construction and sequencing to the University of Nebraska Medical Center (UNMC) Sequencing Core Facility led by Dr. James Eudy. The samples will be sequenced on Illumina's NextSeq machine (1 X 150 bp read lengths) to 100X depth. Comparing genetically identical CMS vs. CHA hybrids. Three crosses will be made to observe the effect of CMS/CHA on hybridity, seed set, and grain yield. The three crosses will be: 1) A-Line (CMS) x R-line; 2) R-line sprayed with CHA to become male sterile x B-line maintainer of the A-line in the first cross; and 3) B-line sprayed with CHA x R-line.