N-IWYP711.1 PLANT BREEDING PARTNERSHIPS: APPLICATIONS OF CRISPR/CAS-MEDIATED GENOME EDITING FOR PRECISION BREEDING IN WHEAT.

Ed Akhunov

Goals / Objectives

Overall goal:

Our goal is to engineer novel variants of genes and their combinations in the adapted germplasm and test their effects on the wheat productivity and quality traits with the long term goal of establishing the CRISPR-Cas-based technology as a precision breeding tool for wheat improvement. The specific objectives of the project:1) Identify traits and genes for CRISPR-Cas-assisted introgression into adapted wheat cultivars; 2) Broaden phenotypic diversity of bread wheat by engineering the allelic series of the promoters of genes controlling wheat productivity and nutritional quality traits;3) CRISPR-Cas-assisted engineering and transfer of gene variants into adapted germplasm;4) Evaluate phenotypic effects of gene editing in the adapted germplasm for development, yield component and nutritional quality traits;5) Expand training opportunities for the next generation of scientists and breeders in genome editing strategies.

Project Methods

Selection of genes for CRISPR editing

In the on-going NIFA IWYP-funded project, we have identified and edited 16 genes that can positively influence yield component traits in wheat, such as grain size, grain weight, and grain number per spike. For five of these edited variants of genes (TaGW2, TaGW7, TaCKX2-1, TaGW8, TaGASR7, TaCKX2-3), our group or other published studies have confirmed positive effect on the phenotypes of interest. The natural allelic variation in many other genes was shown to have effect on yield component traits in the diversity panels, suggesting that edited variants of these genes will likely have positive effects on yield-related traits. We will test whether the introduction of our edited yield component genes (mostly affecting sink-related traits) into the germplasm expressing exceptional source-related traits (high biomass, beneficial root architecture, high spikelet number per spike) will improve wheat yield potential. We also selected three wheat genes (Rht1, TaGRF4 and TaNAM-B1) controlling plant growth, metabolism of carbon and nitrogen, and nutritional quality traits for promoter editing using CRISPR-Cas technology. Using multiplex CRISPR-based genome editing, we will create allelic series of these genes.

Design and testing of gRNA constructs

To design single guide RNA (sgRNA) constructs for Cas9 and Cpf1 endonucleases, we will combine bioinformatical design using the sgRNA Scorer 2.0 and CRISPR-DT with the high-throughput screening of sgRNAs using the wheat protoplast assay. To select gRNAs with no off-target activity, the high-scoring sgRNAs will be compared against the wheat reference genome. For targeting the gene promoters, we will design up to 20 sgRNAs targeting regions spanning 2-3 kb upstream of the gene. The editing strategies requiring the simultaneous expression of multiple sgRNAs will be implemented using the multiplex gene editing constructs where sgRNAs are separated by the tRNA spacers. All constructs will be tested in the wheat protoplasts followed by the NGS of target sites.

Generation of transgenic plants

To generate populations of wheat lines carrying different promoter alleles, T0 generation plants expressing gRNA constructs will be crossed with the wild-type plant. Due to trans-generational activity, the CRISPR-Cas9-gRNA transgenes will target in trans the wild type promoters, producing various allelic variants in the progeny of F1 plants. The F2 generation plants will be screened for editing events by the NGS of multiplexed amplicons spanning promoter regions. Two spring wheat cultivars 'Fielder' and 'Bobwhite' will be used for transformation. Mutations in the targeted regions will be identified by the NGS of barcoded PCR amplicons. Transfer of gene editing events to the recipient germplasm Adapted germplasm for transferring CRISPR-edited gene variants will be selected based on the nature of affected traits. Most of the edited genes in our on-going project influence sink-related traits (grain size/weight, number of grains per spike). We will transfer multiple edited variants of these genes into the same cultivar that also shows superior source-related traits (high root biomass or above ground biomass). To transfer gene editing events to recipient cultivar, we will use tested a strategy based on crossing cv. Bobwhite expressing Cas9 transgene at high levels with a recipient germplasm. This approach allowed for efficient gene editing of the recipient's gene copies in the F1 hybrids. The recurrent parent genome (RPG) will be recovered by two rounds of backcrossing to recipient parent (recover 87.5% of RPG), each time selecting for the presence of CRISPR-Cas-gRNA constructs, and gene editing mutations in the targeted gene.

Promoter editing: T0 generation Bobwhite or Fielder expressing the multiplexed CRISPR-Cas-gRNA constructs at high level will be identified and crossed with the recipient germplasm. The F2 progeny will be screened to identify promoter deletions in the recurrent parent's gene copy. The lines with deletions will be selected, and backcrossed twice with the recurrent parent, at each step selecting for the presence of promoter deletions and absence of Cas construct.

Phenotypic evaluation

Plant productivity traits: Transgenic lines carrying the edited version of genes will be grown in both greenhouse and field conditions. For phenotyping in the greenhouse, plants will be grown for 16 h light / 8 h dark. While plants with validated mutations will be also phenotyped at T1 and T2 generation, the effects of gene editing on phenotype will be assessed on BC2F2:3 plants in greenhouse. Seedlings of BC2F2 will be grown in 96-well trays, genotyped, and at least 20 plants with and without homozygous Cas-induced mutations will be transplanted to 1L pots and phenotyped. Phenotypes of BC2F2:3 plants will be confirmed in the next generation by growing 10 plants from at least 10 families within each group (presence or absence of mutations). Experiment will be organized according to complete randomized design, as described.

Field phenotyping of spring wheat lines will be performed on BC2F4:5 lines grown in Davis (CA) and Saskatoon (Canada). Entries will be planted in the randomized complete block design in three replicates in 4-row 2 m-long plots (260 seeds per m2). For some of the yield component traits we have already initiated crosses to transfer gene edits to the adapted germplasm.

Standard phenological data such as timing of stem elongation, date of heading, date to anthesis, date of physiological maturity, and plant height will be collected. Yield-related phenotypes collected will include yield and test weight, as well as yield components including thousand grain weight, the number of fertile tillers/spikes per plant or per unit area, average number of kernels per spike, and average kernel weight.

Nutritional quality traits: Senescence will be estimated by measuring non-destructively the relative chlorophyll content in the flag leaf of each main spike using a hand-held chlorophyll meter. Nitrogen concentration in ground tissues will be measured by NIR reflectance using a Perten DA7250 calibrated to combustion analysis. Ionomic analyses to assess the Fe and Zn concentrations in grain and flag leaves will be conducted by the facility in the Donald Danforth Plant Science Center. For each trait, we will estimate the Best Linear Unbiased Estimates (BLUEs) from a spatial model using the ASReml package in R following the previously described procedures. A model is flexible and can include the effects of environment, year, experiment design, followed by fitting the best spatial model to genotype. The BLUEs of each phenotypic trait obtained for lines with and without gene editing mutations will be compared to assess the effect of gene editing on wheat productivity and nutritional quality traits. Workshops Our project will develop educational modules for postdoctoral researchers and PhD students covering the basics of the gene editing technology. For this purpose, we will work with the KSU Integrated Genomics Facility, on incorporating gene editing training modules into the summer workshops for PhD students (2 credit course) organized on the yearly basis by the KSU IGF. Three summer workshops will be co-taught by the project team including PhD students, postdoctoral researchers, PDs and co-PDs in collaboration with the IGF personnel. The IGF routinely will conduct on-line course evaluation and provide feedback to the project team. A graduate student from the co-PD Pozniak's group will visit KSU campus each summer to participate in the workshops and discuss project activities.