Leveraging Novel Genomics Techniques in a New Two-Row Barley Breeding Program

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Rapid changes in the malting and brewing industries have created a need for high-quality two-row malting barley. The demand is felt strongly in Minnesota, an important barley production and malting region and a center for recent brewery proliferation. The University of Minnesota barley breeding program has expanded to meet this demand by initiating a two-row breeding effort. Our strategy, while still relying on traditional methods, is open to exploring many of the recently developed and novel genomics techniques that show promising application in crop improvement. As a composite of three different projects, my thesis will focus on these techniques or novel applications thereof for understanding and improving two-row barley.

The characteristic morphological difference between two-row and six-row barley is influenced by a single gene, *VRS1*. Functional *Vrs1* confers two-row type, while mutant *vrs1* confers six-row type (Komatsuda et al. 2007). The row types are often associated with different traits (e.g. two-row barley is associated with higher malting quality and greater resistance to *Fusarium* head blight, a devastating kernel disease). Several studies have mapped putative quantitative trait loci (QTL) for these traits to the genetic region harboring *VRS1*. However, these studies were unable to separate linkage and pleiotropy as the cause of these associations (e.g. Kjaer and Jensen 1996; Marquez-Cedillo et al. 2000; Mesfin et al. 2003). Recently-advanced genome editing technologies have shown promise in improving crops, including barley (Wendt et al. 2013), through sequence-specific gene alteration (Voytas and Gao, 2014). My objective is to take advantage of this technology to restore functional *Vrs1* in several elite six-row cultivars. Collaborating with the Dan Voytas lab, we will develop genome editing constructs that target *VRS1* and attempt to change non-functional *vrs1* to functional *Vrs1*, effectively converting the six-row line to a two-row line. We will then compare converted and non-converted lines for a variety of agronomic, malting quality, and disease resistance traits. Through this project, we hope to further understand the role of *VRS1* in row type – trait associations and potentially develop locally-adapted two-row breeding material. While this project investigates a specific genomic region, other projects will involve a more holistic application of genomics.

There is a nationwide demand for locally-grown two-row malting barley. Unfortunately, many interested growing regions do not have a recent history of barley production or are not

serviced by a breeding program. For the existing centralized breeding programs, this suggests genotype-by-environment (GxE) interactions will play a large role in developing two-row cultivars adapted to such a wide target population of environments (Bernardo 2010). Within the last few years, our program has relied extensively on genomic selection (GS), a method of using genome-wide molecular markers and a phenotyped training population to predict the performance of untested lines (Meuwissen et al. 2001; Heffner et al. 2009). While GS is not a novel technique *per se*, several recent studies have shown promise in developing novel applications of GS through the inclusion of parameters related to GxE (e.g. Helot et al. 2014; Jarquin et al. 2014). My objective is to determine methods of optimizing a training population in GS by integrating GxE components. With collaborator help, we will grow and evaluate common training and prediction populations in 54 environments from 2015 – 2017. We will use available weather data to cluster the environments and implement different optimization procedures to select suitable training populations for these clusters. Through this project, we aim to develop a robust dataset that can be used to develop regionally-superior two-row barley cultivars. In addition to a GxE extension of GS, my thesis also includes an effort to extend GS to predict population variance.

As breeding progress relies on variation for a trait, it would be advantageous to accurately predict the variance that would result from different crosses. A newly-developed GS tool, PopVar (Mohammadi et al. 2015), aims to accomplish this by simulating crosses and predicting the phenotypes of the progeny, providing information for selecting preferred crosses. My objective is to empirically test the ability of PopVar to predict the variance and superior (i.e. top 10%) mean of different crosses. Using the same training and prediction populations as in the last project, we will simulate ~390,000 crosses and predict the performance of simulated project. For several traits, we will select crosses with high and low predicted variance, high and low correlations among traits, and random crosses. We will make the selected crosses, develop populations, and evaluate. Observed data from evaluations will be used to measure the accuracy of the predicted variance, superior mean, and correlations among traits. The results of this project will help to validate a novel extension of GS with great potential utility in plant breeding.

As a nascent breeding program, our two-row initiative ought to take advantage of novel genomics methods with promise for advancing crop improvement. Genome editing and genomic selection, a newly developed tool and a developed tool with potentially new applications, are both poised to contribute significantly to this field. While my projects may be different, the common goal of my thesis work is to apply these novel techniques to answer breeding and genetics questions, while working to develop quality two-row barley cultivars for Minnesota.

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