

Washington Grain Commission Barley Research Report

Project #: 3019-3200

Progress Report Year: 1 of 3

Title: Improving Spring and Winter Barley Varieties for Malt, Feed and Food

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Executive Summary: During the first year of this grant and my second full season as the WSU barley breeder excellent progress was made towards improving WSU barley varieties for malt quality. The Washington Grains Commission (WGC) funds were integral in transitioning the WSU breeding program into a spring and winter malt barley breeding program. With the infrastructure now in place for malt quality analysis the bar is set to develop both spring and winter adjunct and all malt barley varieties with consistent quality and agronomics to make the American Malting Barley Association (AMBA) recommended list.

To quickly transition to a fully integrated malt barley breeding programs we established the state-of-the-art WSU Malt Quality Lab (WMQL) which was accomplished through funding provided by the WGC. The fully operational WMQL is producing excellent data as compared to samples provided by Hartwick College. The correlation between the labs is excellent ($R^2 = 0.988$) and is well within the tolerance for reproducibility. We are currently malting and analyzing elite 2021 field season yield trials material prioritized by high yielding experimental lines derived from elite-by-elite malt barley parental crosses. The quality data which we can now generate in house weeks from harvest provides great efficiency when selecting early and late generation materials for malt quality. To aid the rapid early generation selection we also developed a new association mapping panel of 364 elite malting lines from the 2021 WSU yield trials. The parents of these lines included eight adapted experimental lines with decent malt quality crossed with seven high quality varieties (Bentley, CDC Copeland, CDC Kindersley, CDC Meredith, AC Metcalfe, Merem, and Cerveza). All 364 lines are being genotyped utilizing the 50K SNP panel and malt quality analysis is currently being generated in the WMQL. The data will be utilized to develop a panel of ~150 DNA markers associated with malt quality traits initially focused on malt extract, alpha amylase, protein, and beta-glucan composition. We are currently developing a new marker system utilizing the Oxford Nanopore MinION DNA sequencing technology to genotype our early generation materials in house. Thus, the early generation spring head rows (~12,000/year), advanced single rep yield trials (~600/year) and multi rep yield trials (~60/year) will be enriched for malt quality so we can concentrate on selecting for farmer traits including yield, height, standability and disease resistance in the field utilizing traditional selection strategies. This will provide much more efficient use of the WGC funds and will allow for greater capacity to analyze later generation material that have already been screened for quality in the WMQL and via marker assisted selection.

Impact: In the first year of this WGC grant we have now transitioned the program to a fully integrated malt barley breeding program with the genetics, genotyping and phenotyping capabilities in place with the focus on the development of both spring and winter classes of malt barley varieties. We identified five promising advanced spring malt barley lines with excellent agronomics across diverse environments with preliminarily data suggesting that they have excellent malt quality. The current plan is to fast track these lines into the WSU variety testing program and collect one more year of malt quality data at two locations then enter them into the AMBA pilot program in 2023. If the data allows we would like to release the best performing line as an all malt craft variety then pursue AMBA recommendation if they make it through the AMBA testing program.

In our efforts to maintain the spring malt barley development pipeline we made over 100 new crosses focused on malt quality and targeted agronomic traits and disease resistance. Ten elite-by-elite crosses were selected and progeny advanced to plant an additional 12,000 F₃₋₄ head rows in the field for evaluation in the 2022 field season. In the 2021 field season we screened approximately 12,000 single head plots from our 2020 crossing block and selected 623 candidate malt barley progeny lines for single rep yield trials for the 2022 field season. All lines have been harvested and processing to collect data for selection and advancement of elite material. In the 2021-2022 greenhouse the third spring malting barley crossing blocks were planted. The parental lines were selected based on quality and additional crosses are currently being made.

Winter malting and feed barley lines were also selected and planted to begin crossing for the WSU winter barley breeding program that was initiated in the Fall of 2020. In Fall 2020 we planted over 600 elite and advanced winter malting lines provided by Dr. Pat Hayes, the Oregon State University winter malt barley breeder. We identified 70 parental lines for the WSU breeding program that perform well at Spillman farm in terms of yield and will assess them for quality once the spring materials have been completed. Because of the adverse year we experienced in the 2021 field season (heat and drought) many of these lines were planted again in Fall of 2021 to generate a second year of evaluation and malt quality data in 2022. We are also continuing to evaluate and advance IMI-tolerance in multiple classes of selected elite barley lines.

To better utilize molecular marker selection current parental lines and breeding material from the WSU breeding program were to be evaluated utilizing genotyping with the malt quality molecular markers developed in collaboration with the USDA-ARS cereal genotyping lab, Pullman, WA. However, the data was not sufficient for the analysis, thus we are forging a new path for early generation genotyping. To aid the rapid early generation selection we developed a new association mapping panel of 364 elite malting lines from the 2021 WSU yield trials. The parents of these lines include eight adapted experimental lines with decent malt quality crossed with seven quality varieties (Bentley, CDC Copeland, CDC Kindersley, CDC Meredith, AC Metcalfe, Merem, and Cerveza). All 364 lines are currently being genotyped utilizing the 50K SNP panel (USDA-ARS cereal genotyping lab, Fargo, ND) and malt quality analysis is being performed in the WMQL with nearly 100 of the lines already malted. We are developing a new DNA marker system utilizing the Oxford Nanopore MinION DNA sequencing technology. We have two MinION DNA sequencing instruments and experience utilizing the technology. However, now we will utilize this technology for an adapted amplicon sequencing strategy to generate genotyping data of over 2,000 experimental lines each year for early generation malt quality selection. Once this AM mapping data is generated, and a training set is validated on the MinION DNA sequencing technology we will select a training population from the WSU 2021

field lines genotyped with the 50K SNP panel and with the malting data generated in the WMQL we will test prediction accuracy. Once this research is completed we can begin utilizing the marker panel for marker assisted selection strategies early in our selection process with the markers associated with low grain protein, increasing malt extract, high alpha amylase and reduced wort β -glucan.

We collected a virulent population of *Puccinia graminis* f. sp. *tritici* (*Pgt*) from three Washington barley fields and from the alternate hosts Mahonia and barberry from locations in Spokane, Colfax, and Pullman, WA and Moscow and Potlatch, ID . We generated nearly 200 hundred single pustule isolates and inoculated them onto lines containing the only two effective barley stem rust resistance genes, *Rpg1* and *rpg4/Rpg5*. We determined through the phenotyping of the entire population that ~10% of the isolates collected were highly virulent on both *Rpg1* and *rpg4/Rpg5*. This virulence had never been reported as the combination of the two genes has always provided resistance to all known isolates collected worldwide. These data show that this *Pgt* population contains the most virulence isolates in the world in regards to barley virulence and pose a potential issue to barley and wheat production in Washington and the region. We conducted phenotypic and genotypic screening of the World Barley Core Collection (WBCC) with the most virulent isolate Lsp21 and utilized association mapping to identify new sources of resistance on barley chromosomes 2H and 5H that are effective against the Washington State *Pgt* virulent isolates. In our 2020 field barley crossing block we hybridized lines containing these novel resistance sources with malt barley parental lines and are using a backcrossing scheme to introduce these resistances into our breeding materials. In 2021 we established an adult plant stem rust screening nursery in the field at Spillman farm and included the lines from the WBCC that had seedling resistance as well as barley lines from the WSU variety testing program and lines from the OSU barley breeding program that contained novel stem rust resistance genes. Alarming, the seedling resistance identified from the WBCC did not translate to adult plant resistance in the field and all WSU experimental materials and WSU variety testing lines were highly susceptible to the cocktail of six PNW isolates identified as representing the broadest virulence on both barley and wheat resistance genes. However, six lines from the OSU breeding program showed a high level of adult plant resistance. We are currently utilizing these lines in our 2022 crossing block to introduce these new sources of adult plant resistance into the WSU breeding program.

As we further optimize and streamline selection utilizing high throughput genotyping and phenotyping the infrastructure put into place early in the development of my program will have a measurable impact on optimizing the return on the WGC's investments. This investment in time and resources will expedite the development of AMBA recommended malt barley varieties in the future. As we move the breeding program forward these investments will have an impact on the ability to make more precise selections from intensive hybridization and screening of larger numbers of recombinant individuals. Our major focus will be on fixing malt quality in the program while increasing agronomic traits such as water use efficiency, heat tolerance, stature and lodging resistance, disease resistance and ultimately yield.

The most measurable impact(s) this project has had in this funding cycle was that we can now fully utilize the infrastructure and instrumentation investment by the WGC to transform the WSU barley breeding program into a self-sustained spring and winter malt barley breeding program. It became apparent where the bottlenecks were in the program, specifically genotyping, and we are alleviating these hindrances so we can move malt barley variety development forward in an expedited fashion. Although, it may take a few years before I can report on the release of

AMBA varieties, with the tools in hand it is only a matter of hard work to achieve this goal. We will focus on the goal of transforming the program into a top malt barley breeding program that will help increase barley acreage to feed the domestic supply of quality malting barley as well as international markets.

Outputs and Outcomes:

Objective	Deliverable	Progress	Timeline	Communication
1	Release of a craft malting barley cultivar suited to brewing with enhanced flavor profile	Several of the advanced malting barley breeding lines have performed well in malting end use quality tests and agronomically in Variety Testing. A second WSU malting line should be released in the coming year	2022-2023	Talks and presentations at field days; distribution of informative variety rack cards; Wheat Life articles; Pod Casts
2	Release of a WSU winter malting barley variety suited to adjunct and or all malt craft brewing	We are selecting parental lines and making crosses from material selected from the OSU breeding program that performs well in eastern Washington	2027-2029	Talks and presentations at field days; distribution of informative variety rack cards; Wheat Life articles; Pod Casts
3	Release of a second IMI-tolerant barley variety with high yield and excellent disease resistance to complement Survivor. This could also be in the food or malt market class	We have thousands of known IMI-tolerant barley lines in our breeding pipeline. These have and will continue to undergo greenhouse and field trials as well as multi-location yield trials to identify the superior breeding lines available.	2024	Talks and presentations at field days; distribution of informative variety rack cards; Wheat Life articles

4	Hulless, waxy food barley variety release to support non-waxy high beta glucan varieties Havener and Meg's Song	Our hulless, high Beta-glucan breeding lines are performing well in the advanced breeder trials and will be included in variety testing trials	2023-2024	Talks and presentations at field days; distribution of informative variety rack cards; Wheat Life articles; Pod Casts
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Communications continued

Publications are listed here as the block above was too small.

Peer-Reviewed Publications (12 total) and Book Chapters (1 total):

Published, Accepted or Submitted from January 1, 2021 – December 31, 2021

Peer-Reviewed Publications:

1. Ameen, G., Solanki, S., Sager-Bittara, L., Richards, J., Tamang, P., Friesen, T.L., and **Brueggeman, R. (2021)** Mutations in a barley cytochrome P450 gene enhances pathogen induced programmed cell death and cutin layer instability. *PLOS Genetics* (Accepted).
2. Clare S.J., Duellman, K.M., Richards, J.K., Sharma Poudel, R., Friesen, T.L. and **Brueggeman, R.S. (2021)** Association mapping reveals A reciprocal virulence/avirulence locus within diverse US *Pyrenophora teres* f. *maculata* isolates. *BMC Genomics* (Accepted).
3. Upadhyaya, A., Upadhyaya, S., and **Brueggeman, R.S. (2021)** The wheat stem rust (*Puccinia graminis* f. sp. *tritici*) population from Washington State contains the most virulent isolates reported on barley. *Plant Disease* DOI: 10.1094/PDIS-06-21-1195-RE.
4. Alhashel, A., Sharma Poudel, R., Fiedler, J., Carlson, C., Rasmussen, J., Baldwin, T., Friesen, T., **Brueggeman, R.**, and Yang, S. (2021) Genetic mapping of host resistance to the *Pyrenophora teres* f. *maculate* isolate 13IM8.3. *Genes Genomes Genetics/G3* DOI: 10.1093/g3journal/jkab341.
5. Clare, S.J., Celik Oguz, A., Effertz, K., Sharma Poudel, R., See, D., Karakaya, A., and **Brueggeman, R.S. (2021)** Genome wide association mapping of *Pyrenophora teres* f. *maculata* and *Pyrenophora teres* f. *teres* resistance loci utilizing natural Turkish wild and landrace barley populations. *Genes Genomes Genetics/G3* DOI: 10.1093/g3journal/jkab280.
6. Capo-chichi, L.J.A., Eldridge, S., Elakhdar, A., Kubo, T., **Brueggeman, R.**, and Anyia, A.O. (2021) QTL Mapping and Phenotypic Variation for Seedling Vigour Traits in Barley (*Hordeum vulgare* L.). *Plants*, DOI: 10.3390/plants10061149.
7. Tamang, P., Richards, J. K., Solanki, S., Ameen, G., Sharma Poudel, R., Deka, P., Effertz, K., Clare, S., Hegstad, J., Bezbaruah, A., Li, X., Horsley, R. D., Friesen, T. L., **Brueggeman, R. S. (2021)**. The barley HvWRKY6 transcription factor is required for resistance against *Pyrenophora teres* f. *teres*. *Frontiers in Genetics* DOI: 10.3389/fgene.2020.601500
8. Zhang, Z., Running, K.L.D., Seneviratne, S., Peters Haugrud, A.R., Szabo-Hever, A., Shi, G., **Brueggeman, R.**, Xu, S.S., Friesen, T.L., Faris, J.D. (2021) A protein kinase-major sperm protein gene hijacked by a necrotrophic fungal pathogen triggers disease susceptibility in wheat. *The Plant Journal* DOI: 10.1111/tpj.15194
9. Jin, Z., Solanki, S., Ameen, G., Gross, T., Sharma Poudel, S., Borowicz, P., **Brueggeman, R.S.**, and Schwarz, P. (2021) Expansion of internal hyphal growth in *Fusarium* Head Blight

infected grains contribute to the elevated mycotoxin production during the malting process. *Molecular Plant-Microbe Interactions* DOI: 10.1094/MPMI-01-21-0024-R.

10. Deka, P., , **Brueggeman, R.**, and Bezbaruah, A. (2021) Sequestration of Zinc Nanoclusters in Root Vacuoles of *Spinacia oleracea* exposed to Nanoscale Zinc. *Nanotechnology for Environmental Engineering* (Accepted).

11. Clare S.J., Duellman, K.M., Richards, J.K., Sharma Poudel, R., Friesen, T.L. and **Brueggeman, R.S.** (2021) Association mapping reveals A reciprocal virulence/avirulence locus within diverse US *Pyrenophora teres* f. *maculata* isolates. *BMC Genomics* (Accepted).

12. Amezrou, R., Rehman, S., Pal Singh Verma, R., **Brueggeman, R.S.**, Belquadi, L., Arbaoui, M., and Gyawali, S. (2021) Identification of SNP markers associated with *Pyrenophora teres* f. *maculata* resistance/susceptibility loci in barley (*Hordeum vulgare* L.). *Euphytica* (Submitted).

Book Chapters:

1. Effertz, K.M., Clare, S.J., Harkins, S.M., and **Brueggeman, R.S.** (2021) Understanding plant-pathogen interactions in net blotch infection of cereals. In Oliver, R. (ed) Achieving durable resistance in cereals. Burleigh Dodds Science Publishing, UK (In Press)