

Washington Grain Commission Wheat and Barley Research Final Report

Project #: 3019-3009

Progress Report Year: 3 of 3

Title: Improving Barley Varieties for Feed, Food and Malt

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Executive Summary: During my first field season as the WSU barley breeder and PI of this grant we made excellent progress considering the difficulties imposed by COVID-19. The Washington Grains Commission (WGC) funds were directed at enhancing the WSU spring malt barley breeding program and establishing a winter malt barley breeding program. The major goal of both spring and winter classes of malt barley is the development of varieties that make the American Malting Barley Association (AMBA) recommended list.

To quickly achieve greater efficiency in the WSU malt barley breeding programs we needed to add or upgraded equipment, instrumentation and technologies. The most important addition to instrumentation and capabilities included the development of the state-of-the-art WSU Malt Quality Lab (WMQL) that was funded by the WGC. This facility is projected to be analyzing 2019 field season material in February, 2020, which will provide much greater efficiency when selecting early and late generation materials for malt quality. To aid the rapid early generation selection we also equipped the WSU barley breeding and molecular lab with the capability to run PCR genotype-by-sequencing libraries for amplicon sequencing and added the computational power to run the bioinformatics analysis. In collaboration with Dr. Deven Cee's USDA cereal genotyping lab we developed a panel of 100 DNA markers associated with malt quality traits initially focused on malt extract, low protein, and low beta-glucan. This will allow us to utilize marker assisted selection (MAS) and genomic selection (GS) strategies to further our capability to make early generation quality predictions and select progeny for malt quality characteristics. Thus, the early generation spring head rows (~10,000/year), advanced single rep yield trials (~500/year) and multi rep yield trials (~50/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 capability will also be important in the winter malt barley selection processes as we begin advancing these materials in the program which will double our materials in the field each season.

To aid in the efficient later generation selection the major piece of equipment and upgrade was our new "Wintersteiger classic plus" plot combine with the Harvest Master H2-Classic weighing system. The new combine provides us with real time yield, test weight and moisture data, saving time and funding previously dedicated to threshing and analysis. This will allow for greater field capacity of later generation lines, which will be very important as we add winter field nurseries and trials to the breeding program. 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 of both the advanced spring malt barley lines and future advanced winter barley lines.

Impact: In the 1.5 years as the PI on this WGC barley breeding grant the WSU barley program has continued advancing feed, food and malting populations and lines in the field at Spillman Farm and greenhouses on the WSU campus. In the last year we started shifting the focus of the WSU breeding program mainly to malt barley variety development including both spring and winter classes of barley. In my first field season and PI on this grant we made over 200 new crosses focused on malt quality and targeted agronomic traits and disease resistance, screened approximately 12,000 single head plots and selected nearly 400 candidate malt barley progeny lines for single rep yield trials for the 2021 field season. All lines have been harvested and processing to collect data for selection and advancement of elite material. In the 2020-2021 greenhouse the second spring malting barley crossing blocks were planted with selected high quality malting parental lines and high yielding adapted PNW germplasm selected with increased malt quality. Ten elite crosses were picked and are currently being advanced to plant an additional 10,000 F₃₋₄ head rows in the field for evaluation in the 2021 field season.

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 2019 we planted over 600 elite and advanced winter malting lines provided by Dr. Pat Hayes, the Oregon State University winter malt barley breeder, to begin the process of identifying parental materials for the WSU breeding program that perform well at Spillman farm in terms of both quality and yield. 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 are being evaluated by genotyping with malt quality molecular markers. In collaboration with Dr. Deven See we are developing a new amplicon sequencing PCR targeted Genotype-By-Sequencing (PCR-GBS) platform adapted to his Illumina sequencer. This was necessary because the USDA-ARS cereal genotyping lab quit supporting their Ion Torrent system for which we had designed our previous panels described in the previous WGC grant and report. We are currently utilizing this platform to screen 150 two-rowed malting parental lines and F₁ progeny to validate the system and once this training set is validated will begin screening our early generation selections with the markers associated with low grain protein, increasing malt extract, and reduced wort β -glucan. Once a training population has been selected and genotyped from the WSU 2020 field lines with malting data collected by our WMQL we will test prediction accuracy. Then we can begin utilizing the marker panel for future marker assisted selection and genomic selection.

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 identified 10 isolates that are highly virulent on both *Rpg1* and *rpg4/Rpg5*. This type of virulence has 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.

As we further optimize and streamlining our selection processes including high throughput genotyping and phenotyping procedures this infrastructure investment early in the development of my program will have a measurable impact on optimizing the return on the WGC's funding investment. This investment in time and resources now will expedite the development of AMBA recommended malt barley varieties in the future. As we move the breeding program forward these investments will have measurable 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 water use efficiency, disease resistance and ultimately yield.

The most measurable impact(s) this project had in the most recent funding cycle is the transformation of the WSU barley breeding program into a spring and winter malt barley breeding program. This includes the addition of important infrastructure and support instrumentation that will allow us to quickly shift gears towards the goal of premium AMBA recommended spring and winter malt barley varieties. Although, I may not report on the development and release of these varieties in the short term, the goal of my career at WSU is to transform the program into a top malt barley breeding program and see a major increase in barley acreage to feed the domestic supply of quality malting barley as well as international markets. Considering that this is my first year as the barley breeder at WSU, I believe we have made considerable progress towards achieving our long term goals.

Outputs and Outcomes:

Objective	Deliverable	Progress	Timeline	Communication
1	Release of the malting barley cultivar Palmer suited to brewing and distilling	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	2021-2022	Talks and presentations at field days; distribution of informative variety rack cards; Wheat Life articles; Pod Casts

2	Release of a second IMI-tolerant barley variety with high yield and excellent disease resistance to complement Survivor in the coming two years.	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.	2022	Talks and presentations at field days; distribution of informative variety rack cards; Wheat Life articles; Pod Casts
3	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	2021-2022	Talks and presentations at field days; distribution of informative variety rack cards; Wheat Life articles; Pod Casts
4	One new two-row feed spring barley variety released with superior yield disease resistance, protein and agronomic characteristics	Two two-row spring varieties were released in 2013. The elite breeding lines are showing excellent promise to exceed these and other existing varieties.	2021-2022	Talks and presentations at field days; distribution of informative variety rack cards; Wheat Life articles; Pod Casts

Communications continued

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

Peer-Reviewed Publications/Preprints (16 total) and Book Chapters (3 total):

Published, Accepted or Submitted from December 1, 2019 – December 31, 2020

Peer-Reviewed Publications/Preprints:

1. Tamang, P., Richards, J.K., Solanki, S., Ameen, G., Sharma Poudel, R., Deka, P., Effertz, K., Gross, T., Hegstad, J., Rehman, S., Bezbaruah, A., Li, X., Horsley, R.D., Friesen, T.L. and **Brueggeman, R.S. (2020)** The barley *HvWRKY6* transcription factor is required for resistance against *Pyrenophora teres f. teres*. *Frontiers in Genetics* 11:601500. doi:10.3389/fgene.2020.601500
2. Restrepo-Montoya, D., **Brueggeman, R.S.**, McClean, P., and Osorno, J.M. (2020) Computational identification of receptor-like kinases “RLK” and receptor-like proteins “RLP” in legumes. *BMC Genomics* 21(1) DOI: 10.1186/s12864-020-6844-z.

3. Solanki, S., Ameen, G., Zhao, J., Flatten, J., Borowicz, P., and **Brueggeman, R.S. (2020)** Visualization of spatial gene expression in plants by modified RNAscope fluorescent in situ hybridization. *Plant Methods* 16(1) DOI: 10.1186/s13007-020-00614-4
4. Sanyal, D., Solanki, S., Ameen, G., **Brueggeman, R.S.**, and Chatterjee, A. (2020) Understanding the expression dynamics of symbiont rhizobial nifH and nitrogen assimilatory NR and GS genes in dry bean (*Phaseolus vulgaris* L.) genotypes at various growth stages. *Legume Science* DOI: 10.1002/leg3.26.
5. Gyawali, S., Reda, A., Verma, R.P.S., **Brueggeman, R.S.**, Rehman, S., Belqadi, L., Arbaoui, M., Tamang, P., and Singh, M., (2020) Seedling and adult stage resistance to net form of net blotch (NFNB) in spring barley and stability of adult stage resistance to NFNB in Morocco. *Journal of Phytopathology* 168(39) DOI: [10.1111/jph.12887](https://doi.org/10.1111/jph.12887).
6. Aoun, M., Kolmer, J.A., Breiland, M., Richards, J., **Brueggeman, R.S.**, Szabo, L.J., and Acevedo, M. (2020) Genotyping-by-Sequencing for the Study of Genetic Diversity in *Puccinia triticina*. *Plant Disease* 104(3) DOI:10.1094/PDIS-09-19-1890-RE.
7. Ameen, G., Solanki, S. Drader, T., Sagar-Bittara L., Steffenson, B.J., Kleinhofs, A., Vogiatzis, C., and **Robert S. Brueggeman (2020)** A wall-associated kinase gene is the spot blotch susceptibility gene *rsc5* in barley. *bioRxiv* doi:<https://doi.org/10.1101/2020.04.13.040238>
8. Jin, Z., Solanki, S., Ameen, G., Gross, T., Sharma Poudel, R., Borowicz, P., **Brueggeman, R.S.**, and Schwarz, P. (2020) Localization of hyphal growth associated with mycotoxin production during the malting of Fusarium head blight infected grains. *bioRxiv* doi:<https://doi.org/10.1101/2020.06.06.126979>.
9. Ameen, G., Solanki, S. Drader, T., Sagar-Bittara L., Steffenson, B.J., Kleinhofs, A., Vogiatzis, C., and **Robert S. Brueggeman (2020)** A wall-associated kinase gene is the spot blotch susceptibility gene *rsc5* in barley. *Proceedings of the National Academy of Sciences, USA*, (Submitted).
10. Ameen G., Bittara, L., Ameen G., Richards, J., Tamang, P., Solanki, S., Friesen, T.L, and **Brueggeman, R.S. (2020)** The *Nec3* gene is a putative negative regulator of pathogen induced programmed cell death in barley. *New Phytologist* (Submitted).
11. Zhang, Z, Running, K.L.D., Seneviratne, S., Peters-Haugrud, A. R., Szabo-Hever, A., Shi, G., Luo, M.-C., **Brueggeman, R.S.**, Xu, S.S., Friesen, T.L., and Faris J.D. (2020) A protein kinase-major sperm protein gene hijacked by a necrotrophic fungal pathogen triggers disease susceptibility in wheat. *The Plant Journal* (Submitted).
12. Capo-chich, L., Eldridge, S., Elakhdar, A., Kubo, T., Kamamaru, T., **Brueggeman, R.S.**, and Anyla, A.O. (2020) QTL Mapping and Phenotypic Variation for Seedling Vigour Traits in Barley (*Hordeum vulgare* L.). *Frontiers in Plant Science* (Submitted).
13. Jin, Z., Solanki, S., Ameen, G., Gross, T., Sharma Poudel, R., Borowicz, P., **Brueggeman, R.S.**, and Schwarz, P. (2020) Localization of hyphal growth associated with mycotoxin production during the malting of Fusarium head blight infected grains. *Molecular Plant Microbe Interactions* (Submitted)
14. Sharma Poudel, R., Richards, J., Shetha, S., Solanki, S., and **Brueggeman, R.S. (2019)** Transcriptome-wide association study identifies putative elicitors/suppressor of *Puccinia graminis* f. sp. *tritici* that modulate barley *rpg4*-mediated stem rust resistance. *BMC Genomics* 20(1) doi: 10.1186/s12864-019-6368-7.
15. Clare, S., Wyatt, N., **Brueggeman, R.S.** and Friesen, T. (2019) Research advances in the *Pyrenophora teres*–barley interaction. *Molecular Plant Pathology* 21(2) DOI:10.1111/mpm.12896.
16. Haque, M.E., Bloomquist, M., Bhuiyan, M.Z.R., Gross, T., Hakk, P., Leng, Y., Liu, Y., Zhong, S., **Brueggeman, R.S.** Sharma Poudel, R., and Kahn, M. (2019) First Report of Alternaria Leaf Spot Caused by *Alternaria tenuissima* on Sugar Beet (*Beta vulgaris*) in Minnesota, USA. *Plant Disease* <https://doi.org/10.1094/PDIS-03-19-0603-PDN>.

Book Chapters:

1. Solanki, S., Ameen, G., Sanyal, D., Jain, S., Elakhdar, A., Lall, S., Chittem, K., Brueggeman, L., Kumar, A., and **Brueggeman, R.S. (2020)** Friends and Foes: Phyto-Microbial Interactions in Molecular Perspective. In: Kumar M., Kumar V., Prasad R. (eds) Phyto-Microbiome in Stress Regulation. Environmental and Microbial Biotechnology. Springer, Singapore. https://doi.org/10.1007/978-981-15-2576-6_5.
2. **Brueggeman, R.**, Solanki, S., Ameen, G., Effertz, K., Sharma Poudel, R., and Karakaya, A., (2020) Fungal Diseases Affecting Barley. In: Fox, G.P. and Li, C. (eds) [Achieving sustainable cultivation of barley](#). Burleigh Dodds Science Publishing, UK. DOI: 10.19103/AS.2019.0060.16.
3. Effertz, K.M., Clare, S.J., Harkins, S.M., and **Brueggeman, R.S. (2020)** 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)