

Grant Code: New

Title: Developing bunt resistant wheat cultivars by gene pyramiding using classical and marker assisted selection breeding approaches

Personnel: G. S. Dhillon (PI), J. Chen (CO-PI and supervisor), P. Joshi (Grad Student), new breeder at USU (Collaborator)

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Justification/ Rationale: Wheat is a vital crop for Idaho farmers, contributing significantly to the state's economy. Organic farming, striving to address modern agricultural challenges, faces threats to crop yields, particularly from dwarf bunt (DB) caused by *Tilletia controversa* and common bunt (CB) caused by *T. caries* and *T. laevis*. Historically devastating, bunt has re-emerged as a concern for organic agriculture due to the absence of systemic fungicides. The limited breeding for bunt resistance over the past decades has left organic farmers with few cultivars offering protection. Even minimal bunt infections can lead to quality reduction and substantial yield losses, emphasizing the urgency for efficient control measures and thus there is a renewed focus on diverse resistance sources. The emergence of new and aggressive pathogens also poses a threat to wheat production. This research aims to continue the effort from the current NIFA-AFRI funded project but focus on enhancing the bunt resistance by pyramiding multiple QTL through classical breeding, marker-assisted selection, and new sequencing technologies. We have made significant progress in the current NIFA-AFRI grant and identified molecular markers and candidate genes associated with two major QTL on chromosome arms 6DL and 7DS. Recently, we identified additional QTLs on chromosomes 4A, 4B, 6B, and 7DL using bulk segregant analysis (BSA) in two highly resistant lines PI178210 and PI554106, respectively, using populations developed by USU breeder and Wheat 90K SNPchip data in the resistant and susceptible RILs. The PI178210 and PI554106 were claimed as Bt8 and Bt12 differential lines. It is important to understand the association of the QTL on 4A, 4B, 6B, and 7DL with Bt8 and Bt12. These QTLs represent major genes that have been deployed in the UI and USU winter wheat germplasm for many decades. The markers associated with these QTL will facilitate resistance genes transfer in adapted winter and spring wheat lines from Idaho, Utah, Washington, Colorado, and other wheat growing areas.

- Objectives:**
1. Validation of resistance QTL identified in PI178210 and PI554106
 2. Stacking multiple QTLs in adapted susceptible cultivars through backcrossing and marker assisted selection

Methods/Plan of Work:

Objective 1: Validation of resistance QTL identified in PI178210 and PI554106. Dwarf bunt resistance for the two recombination inbred lines (RILs) populations ((HeinesVII, PI201195, Bt0) X PI178210 (Yayla, Bt8) and HeinesVII (HeinesVII, PI201195, Bt0) X PI554106 (Bt12)) have been previously assessed in four years (2015, 2016, 2022 and 2023) by USU program. Resistant and susceptible RILs were recently genotyped with Wheat 90K SNP iSelect platform by Dr. Chen's program and used in a Bulk Segregation Analysis (BSA). Two QTL on chromosome 4A and 6B were tentatively identified in PI178210 and two QTLs on 4B and 7DL in PI554106. In the proposed objective, DB resistance of the two populations will be further

assessed in the DB nursery in Logan, Utah for the season 2023-24. Phenotypic data would be generated and stability of resistance in the resistant genotypes would be checked. Tightly linked SNP will be converted to KASP markers and validated in a diverse panel of 180 winter wheat lines consisting of advanced breeding lines and released cultivars of PNW. Genotypic and phenotypic data of the 180 lines will be retrieved from the current NIFA dwarf bunt project. Highly resistant RILs with two or more QTLs will be selected as donors for transferring the QTLs to known high performing cultivars in objective 2.

Objective 2: Stacking multiple QTLs in adapted winter and spring wheat cultivars through backcrossing and marker assisted selection. Five spring wheat UI Platinum, UI Gold, IDO1902S, IDO2103 (new FHB resistant line), Jefferson, and five winter wheat cultivars/new lines will be used as the recurrent parents. DB donor parents will include highly resistant RILs from the two populations in this proposal and resistant doubled haploid lines from 7DS and 6DL populations previously published (Chen et al., 2016; Wang et al., 2018). All donor lines have good agronomic traits which will facilitate the release of new resistant cultivars. Marker assisted selection (MAS) will be performed in the BC_1F_1 , BC_1F_2 , BC_2F_1 and BC_2F_2 generations. Selected lines will be selfed using bulk or single seed decent methods. The lines selected through marker assisted selection and advanced to F_4 and F_5 generations will be planted for phenotypic evaluation of disease resistance at USU and agronomic traits in multiple locations in ID and Utah.

Duration: 3 years

Cooperation/Complementation: Currently DB resistance genes are being mapped and fine mapped, donated through other genetic sources. We have collaboration with USU for dwarf bunt resistance screening along with collaboration for gene mapping in different populations. We have developed international collaborations for single race screening of our resistant germplasm. Sharing genotypic and phenotypic data along with germplasm exchange would help us to incorporate multiple resistance genes in our germplasm which can be further used as starting breeding material for germplasm development in PNW region. By working together with different universities and oversea collaborators, this research aims to enhance the competitiveness of regional wheat producers and contribute to the development of a more sustainable and resilient agricultural system.

Anticipated Benefits/Expected Outcomes: Utilizing diverse resistance sources for developing new cultivars with improved/increased resistance against the bunt pathogen and pyramiding multiple resistance sources aligns with the overarching goal of ensuring sustainable and resilient wheat cultivation. The new germplasm or cultivars developed through this study would benefit both conventional and organic farmers, strengthening competitiveness of Idaho wheat in the market. The need for effective control measures and the economic/environmental advantages of deploying resistance cultivars addresses the goal of sustainable agriculture. Many benefits are anticipated with the proposed research. Firstly, it will lead to a deeper understanding of the wheat plant's resistance mechanisms against DB, a significant disease that affects wheat yields worldwide. Additionally, the development of MAS tools will enable breeding programs to introgress resistance genes into elite wheat varieties more efficiently and accurately. This will result in improved resistance in wheat varieties, reducing crop losses and enhancing food security. Furthermore, this research will foster collaboration between public and private research

sectors, contributing to sustainable agriculture and environmental protection. The expected outcomes of this research include the identification and validation of novel resistance genes or QTLs, the development of markers linked to these resistance QTLs, and the successful introgression of resistance genes into elite wheat varieties using marker-assisted selection. Moreover, the research will evaluate and select improved wheat lines with enhanced resistance against targeted pathogens, ensuring their performance in diverse environments.

What is the potential economic impact: Significant economic impact of this research can be attributed to improving resistance of the wheat varieties against DB. The resistant varieties developed would protect the farmers from significant yield losses and economic damage due to quality loss. Deploying resistant wheat varieties would lead to reduced crop losses, lower production cost, improved food security, and boost trade and commerce.

Transfer of Information/Technology: The projects' findings and data will be shared amongst the collaborators and with other researchers, breeders and stakeholders through publications, workshops and conferences. This would further promote collaborations and advancement of wheat research in the field of bunt resistance. The resistance wheat varieties developed through this project will be shared with will be disseminated to farmers and seed companies.

Literature Review: Dwarf Bunt (DB), induced by *T. controversa*, and Common Bunt (CB), caused by *T. caries* and *T. laevis*, pose a significant threat to wheat production due to the destructive nature of these diseases, leading to the formation of bunt balls filled with spores (Muellner et al., 2021). The de-emphasis on resistance selection in breeding programs, particularly for common and dwarf bunt, followed the introduction of seed treatments (Gordon et al., 2020). However, the recent upswing in organic farming and concerns for sustainable agriculture have revived interest in bunt host resistance. This renewed focus is prompted by the limitations of organic-certified seed treatments and the challenges posed by soil-borne and seed-borne infections (Matanguihan et al., 2011). The *Tilletia* species responsible for these diseases share genes (Bt) in a gene-for-gene system, with 36 pathogenic races of *T. caries*, 15 races of *T. laevis*, and 19 races of *T. controversa* identified (Goates, 1996). Despite this, molecular markers linked to Bt genes have been proposed in only a few bunt resistances, and their effectiveness in Marker-Assisted Selection (MAS) remains unexplored.

Compared to other wheat traits, research on the genetic control of bunt resistance is confined to a limited number of QTL mapping studies. QTLs associated with resistance have been reported on wheat chromosomes 1B, 7A, 7B, 5B, 4D, 6D, and 7D for common bunt resistance. Notably, the 6D QTL was linked to the gene Bt10 (Muellner et al., 2021). For dwarf bunt resistance, Chen et al. (2016) identified QTLs on chromosomes 1A, 2B, and 7D in the resistant line IDO444, with QDb.ui-7DS explaining a substantial portion of phenotypic variation. In 2019, two major QTLs on chromosomes 6D and 7A were identified, elucidating the intricate genetic architecture. Fine-mapping these QTLs, particularly Bt9 and Bt12 associated with dwarf and common bunt resistance, respectively, is essential for map-based cloning and the development of bunt-resistant cultivars. Lunzer et al. (2023) identified a major QTL on chromosome 6D with five small effect QTLs on other genomic regions.

Marker-assisted selection (MAS) has proven beneficial for combining major-effect resistance genes for dwarf and common bunt. Nevertheless, it encounters challenges with minor-gene quantitative resistance. Mapping genes and QTLs with major and minor effects enables us to deploy them into new backgrounds and pyramiding them. Using marker-assisted selection saves both time and resources by aiding in the selection of genotypes with desired traits in the early life

cycle of the plant and saving resources for phenotyping (Miedaner et al., 2012). MAS is especially critical for traits like bunt, where phenotyping is usually done at the physiological maturity of the plant.

Two mapping populations using highly resistant lines PI178210 and PI554106 had been developed by USU breeder. These populations have been screened for dwarf bunt resistance, two QTL on chromosome 4A and 6B were tentatively identified in PI178210 and two QTL on 4B and 7D in PI554106. The validation of these results and the development of molecular markers will expedite marker-assisted transfers, hastening the development of highly resistant wheat cultivars.

References:

- Chen J, Guttieri MJ, Zhang J, Hole D, Souza E, Goates B. A novel QTL associated with dwarf bunt resistance in Idaho 444 winter wheat. *Theor Appl Genet*. 2016 Dec;129(12):2313-2322. doi: 10.1007/s00122-016-2783-2. Epub 2016 Sep 28. PMID: 27681089; PMCID: PMC5121181.
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- Gordon, T., Wang, R., Hole, D. et al. Genetic characterization and genome-wide association mapping for dwarf bunt resistance in bread wheat accessions from the USDA National Small Grains Collection. *Theor Appl Genet* 133, 1069–1080 (2020). <https://doi.org/10.1007/s00122-020-03532-0>
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- Miedaner T, Korzun V. Marker-assisted selection for disease resistance in wheat and barley breeding. *Phytopathology*. 2012 Jun;102(6):560-6. doi: 10.1094/PHYTO-05-11-0157. PMID: 22568813.
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- Muellner, A.E., Buerstmayr, M., Eshonkulov, B. et al. Comparative mapping and validation of multiple disease resistance QTL for simultaneously controlling common and dwarf bunt in bread wheat. *Theor Appl Genet* 134, 489–503 (2021). <https://doi.org/10.1007/s00122-020-03708-8>.

**FY2025
COMMODITY COMMISSION BUDGET
Principal Investigator: G. Dhillon**

Allocated by _____ (Commission/Organization)	during FY2023	\$	-
Allocated by _____ (Commission/Organization)	during FY2024	\$	-

REQUESTED SUPPORT Budget Categories	<u>Awarded for FY2024</u>	<u>Requested for FY2025</u>
(10) Salary <i>(staff, post-docs, et NOTE: Faculty salary/fringe NOT allowed)</i>	\$ -	\$ 39,628
(12) Temporary Help/IH	\$ -	\$ 16,380
(11) Fringe Benefits	\$ -	\$ 21,444
(20) Travel	\$ -	\$ 7,785
(30) Other Expenses	\$ -	\$ 950
(40) Capital Outlay >\$5k	\$ -	\$ -
(45) Capital Outlay <\$5k	\$ -	\$ -
(70) Graduate Student Tuition/Fees	\$ -	\$ -
TOTALS	\$ -	\$ 86,187

TOTAL BUDGET REQUESTED FOR FY2025:	\$ 86,187
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BREAKDOWN FOR MULTIPLE INDEXES:					
Budget Categories	<i>(Insert Co-PI Name)</i>	<i>(Insert Co-PI Name)</i>	<i>(Insert Co-PI Name)</i>	<i>(Insert Co-PI Name)</i>	
(10) Salary <i>(staff, post-docs, et</i>	\$ -	\$ -	\$ -	\$ -	-
(12) Temporary Help	\$ -	\$ -	\$ -	\$ -	-
(11) Fringe Benefits	\$ -	\$ -	\$ -	\$ -	-
(20) Travel	\$ -	\$ -	\$ -	\$ -	-
(30) Other Expenses	\$ -	\$ -	\$ -	\$ -	-
(40) Capital Outlay >\$5k	\$ -	\$ -	\$ -	\$ -	-
(45) Capital Outlay <\$5k	\$ -	\$ -	\$ -	\$ -	-
(70) Graduate Student Tuition/Fees	\$ -	\$ -	\$ -	\$ -	-
TOTALS	\$ -	\$ -	\$ -	\$ -	-
Total Sub-budgets					\$ -