**Grant Code**: AP4538

**Title:** Breeding Durable and High Level of Dwarf Bunt Resistance Using Molecular Marker-Assisted Selection

**Personnel:** Dr. Jianli Chen (PI), Dr. R. Chowdhury, Mr. J. Wheeler, Mr. Joshi Pabitra

**Collaborators (non-funded):** Dr. Tyler Gordon, Dr. David Hole, and Dr. Juliet Marshall

**Address:** Dr. Jianli Chen, University of Idaho (UI) Aberdeen Research & Extension Center, Aberdeen, ID 83210; 208-397-4162, ext. 229; jchen@uidaho.edu.

**Justification:** Dwarf bunt (DB) caused by *T. contraversa* J.G. Kühn, and common bunt (CB) caused by *T. caries* (DC.) Tul. & C. Tul. (=*T. tritici*) and *T. foetida* (Wallr.) Liro (=*T. laevis*) are among the world's most potentially damaging diseases of wheat. These diseases reduce grain yield and quality because they destroy the wheat grains by forming bunt balls filled with dark, foul smelling spores. With the advent of seed treatments over the past two decades, most breeding programs de-emphasized bunt resistance selection. Over 95% of winter wheat cultivars currently used in U.S. production have neither dwarf bunt nor common bunt resistance. However, bunt host resistance has regained world-wide interest due to the increase in organic farming and concerns for more sustainable production methods in agriculture (Matanguihan et al., 2011). Lack of effective organic certified seed treatments has led to increasing incidence and losses from common bunt. Development of organic certified treatments for dwarf bunt is even more difficult due to the timing of soil born infections and the necessity of systemic anti-fungal activity that can persist throughout a lengthy infection period. The three *Tilletia* species that cause the two bunt diseases are closely related (Bao, et al., 2010) and dwarf and common bunt resistances are partly controlled in wheat by shared genes (*Bt*) in a gene-for-gene system (Goates, 1996; Goates, 2012). Fifteen resistance genes (*Bt1* to *Bt15*) were proposed based on phenotypic evaluations of differential lines (Goates, 2012), but none of the genes have been well characterized at the molecular level. Current dwarf bunt resistance breeding efforts are mainly based on phenotypic selection, which is extremely difficult in the field since disease development requires a period of snow cover and low temperature of 3-8 °C for spore germination (Goates, 1996) and the disease can only be assessed when plants are mature. It is essential to identify quantitative trait loci (QTL) and tightly linked molecular markers to facilitate cultivar improvement for dwarf and common bunt resistances and conduct gene cloning to understand resistance gene mechanisms.

**Hypothesis & Objectives:** Current genomics enabled technologies, such as QTL mapping and next generation of sequencing (NGS), can facilitate gene cloning and molecular marker assisted selection for cultivar development and germplasm enhancement. Mutagenesis followed by sequence-comparison of multiple independently derived mutants has been successfully applied to identify genes from a wide array of organisms including worms, flies, and plants. Mutagenesis

has also widely used in cultivar improvements in crops. The objectives of this study are to: 1) fine map two dwarf bunt resistance QTL (6DL and 7DS) with the new sequencing technology (Targeted Gene Capture) and develop KASP markers for MAS, 2) pyramid multiple resistant QTL and develop novel germplasm and varieties using marker assisted selection, 3) advance a UI Silver EMS population for use in future candidate gene identification.

**Procedures:**

**Objective 1.1 Fine map *QDB.ui-7DS* and develop KASP markers for MAS**

The 7DS QTL was previously identified in the RioBlanco x IDO444 derived population (Chen et al. 2016). The QTL was flanked by six markers (one DArT marker and five SNP markers) in a 25 cM region. In FY20, this region was reduced to 2.4 cM after seven KASP markers were added to the genetic map. In FY21, we genotyped 2800 individual plants from 10 $F_9$ heterozygous inbreed families (HIF) with the seven KASP markers and assessed the plants for dwarf bunt resistance. In fall 2020, we space-planted a total of 24,900 seeds from eight HIF-derived $F_3$ populations, comprised of 498 families and50 seed per family... Additional markers will be designed and genotyped in the 498 families to reduce the target region. In summer 2021, we will assess dwarf bunt resistance for these materials and select critical recombinants to identify candidate gene based on the correlation between marker haplotype and the second year of disease resistance data. In 2022, we will use CRISPER-CAS9 to edit the candidate gene.

**Objective 1.2. Fine map *QDB.ui-6DL* and develop KASP markers for MAS**

The 6DL QTL was identified in two bi-parental populations IDO835 x Moreland (IMDH) and UI Silver x Shaan89150 (SSDH) (Wang et al., 2019) and in a diverse panel from National Small Grain Collection (NSGC) (Gordon et al., 2020). To fine map and develop markers for the *QDB.ui-6DL*, we created two $F_2$ mapping populations between two resistant and two susceptible lines within the SSDH population, SSDH-128 (R) x SSDH-097 (S) and SSDH-048 (R) x SSDH-036 (S). The two large $F_2$ populations will be genotyped with more KASP markers to fine map the QTL region. The $F_3$ of the two populations will be planted in a field nursery in Logan, UT in fall 2021 for dwarf bunt testing.

**Objective 2. Marker-assisted breeding for multiple resistance QTL to dwarf bunt**

Molecular markers identified in Objective 1 will be used in genotyping elite lines in winter wheat. Based on correlation analysis between marker data and disease data, we will assess the efficiency of the markers in predicting the resistance. In FY21, sixty doubled haploid lines (DHLs) were developed from the cross IDO1906 x IDO1506. The two parents both have marker alleles for QTL on the 7DS but have alternative allele for the QTL on the 6DL. These DHLs were planted in fall 2020 for seed increase. In FY22, we will select DHLs that have resistant alleles for both QTL and test them for dwarf bunt resistance in a disease nursery in Logan UT and simultaneously assess agronomic performance in field nurseries in multiple locations. Because both parents are elite lines, within five years it will be possible to release high yielding lines from this cross that have durable resistance to dwarf bunt.

**Objective 3. Advance UI Silver EMS population**

UI Silver is adapted to dryland production. It has excellent resistance to dwarf bunt and premium baking quality. However, UI Silver's tall plant height and susceptibility to stripe rust limit its utility for the irrigated production. To facilitate gene cloning for dwarf bunt resistance, UI Silver

seeds (breeder seeds) were used in EMS mutagenesis and an optimum dose for LD50 was applied in 2020. Two thousand M0 seeds were space-planted in the bunt nursery in the fall of 2020. The susceptible M1 will be sequenced in 2021. An additional two thousand M2 will be used in TILLING in spring 2021 and planted in the dwarf bunt nursery in fall 2021. Based on marker haplotypes, UI Silver has three QTL on the 6DL, 7AL, and 7DS. We expect to select single knockout mutants as well as double and triple knockout mutants in UI Silver mutant population. Dwarfing is a common mutation in EMS. Thus, it should be possible to identify for release short mutant lines that maintain DB resistance but have better agronomic performance.

**Duration:** 5 years (FY2022 falls in year 3 of the 5-year project)

**Cooperation:** Dr. Chen is the supervisor and PI for this project. Dr. R. Chowdhury and graduate student Ms. Joshi Pabitra will be main persons to conduct the project. Dr. David Hole, a wheat breeder at Utah State University, has been our collaborator on dwarf bunt research over the past ten years. His program establishes the dwarf bunt nursery and plants our materials each year. We will work together on assessing disease at the plant maturity stage. Dr. Tyler Gordon at the USDA-ARS will also collaborate with us and assess dwarf bunt resistance. To complement this project, Dr. Marshall and her student will work on a molecular assay to track bunt infection during plant development.

**Anticipated benefits, expected outcomes and impacts, and transfer of information:** The application of molecular marker-assisted selection will significantly enhance dwarf bunt resistance breeding by reducing time and labor costs. We will be able to select some highly resistant DHLs, which can be released as either germplasm or cultivars. In the long term, growers, especially the organic farming growers, will benefit from growing the highly resistance cultivars and increase their profit. In addition, the anticipated knowledge about the major bunt resistance genes studied in this project will enable us to further identify and pyramid multiple resistance genes using the most advanced technology. Pyramiding multiple resistance genes will greatly increase the likelihood that the resistance will prove durable over time. Our research findings will be published in *Idaho Grain Magazine*, refereed journals, and presented at different growers and professional meetings. One postdoctoral fellow will be partially funded and trained through this project.

**Literature Review:** QTL mapping is a practical method to identify molecular markers associated with the traits of interest. Compared to other traits in wheat, few QTL mapping studies have been conducted on resistance to dwarf bunt and common bunt, likely due to the stringent conditions required for disease resistance assessment. Very few disease nurseries are available for disease screening. Chen et al. (2016) was the first to identify a major effect QTL on the distal end of chromosome 7DS. Wang et al. (2019) identified two major-effect QTL for dwarf bunt on chromosomes 6DL and 7AL, respectively. Soon after, Gordon et al. (2020) confirmed the presence of the 6DL QTL in 28 resistant accessions in a diversity panel of 292 wheat accessions. Muellner et al. (2020a and 2020b) identified a major effect QTL associated with both dwarf and common bunt resistance on the 7DS in proximity to the 7DS QTL reported by Chen et al. (2016). Muellner et al. (2020b) also identified a QTL on the 7AL associated with both resistances in proximity to the 7AL QTL reported by Wang et al. (2019). To develop durable dwarf bunt resistant cultivars using molecular marker assisted selection, it is essential to

dissect the three chromosome regions and identify candidate genes and diagnostic markers and use them in cultivar development.

**References:**


Bao, X. 2010. Host specificity and phylogenetic relationships among *Tilletia* species infecting wheat and other cool season grass. Doctoral dissertation, Washington State University, Pullman

Chen J., Guttieri M.J., Zhang J., Hole D., Souza E., Goates B. 2016. A novel QTL associated with dwarf bunt resistance in Idaho 444 winter wheat. Theor. Appl. Genet. 129:2313-2322

Goates B.J. 1996. Common bunt and dwarf bunt. In: Wilcoxson RD, Saari EE (eds) Bunt and smut disease of wheat: concepts and methods of disease management, CIMMYT, Mexico, D.F.

Goates B.J. 2012. Identification of new pathogenic races of common bunt and dwarf bunt fungi, and evaluation of known races using an expanded set of differential wheat lines. Plant Dis. 96:361–369.

Gordon, T., Wang, R., Hole, D., Bockelman, H., Bonman, J.M., and Chen, J. 2020. 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. https://doi.org/10.1007/s00122-020-03532-0.

Matanguihan, J.B., Murphy, K.M., Jones, S.S. 2011. Control of common bunt in organic wheat. Plant Dis. 95:92-103.

Muellner, A.E., Eshonkulov B., Hagenguth, J., Pachler, B., Michel, S., Buerstmayr, M., Hole, D., and Buerstmayr. H. 2020.Genetic mapping of the common and dwarf bunt resistance gene *Bt12* descending from the wheat landrace PI119333. Euphytica 216: 83.

Muellner, A.E., Buerstmayr, M., Eshonkulov B., Hole, D., Michel, S., Hagenguth, J., Pachler, B., Pernold, R., Pachler, B., and Buerstmayr. H. 2020. Comparative mapping and validation of multiple disease resistance QTL for simultaneously controlling common and dwarf bunt bread wheat. Theor. Appl. Genet. doi.org/10.1007/s0012 2-020-03708 -8.

Wang, R., Gordon, T., Hole, D., Zhao, W., Isahm, K., Bonman, J.M., Goates, B., and Chen, J. 2019. Identification and assessment of two major QTLs for dwarf bunt resistance in winter wheat line 'IDO835'. Appl. Genet. doi.org/10.1007/s00122-019-03385-2.

## IDAHO WHEAT COMMISSION - BUDGET FORM

**Principal Investigator: Jianli Chen**

| | | | | | |
|---|---|---|---|---|---|
| **If applicable,** | **Allocated by** | **Idaho Wheat Commission** | **during FY 20:** | **$53,329** | **$ -** |
| **If applicable,** | **Allocated by** | **Idaho Wheat Commission** | **during FY 20:** | **$40,067** | **$ -** |

**REQUESTED FY2022 SUPPORT:**

| Budget Categories | (10) Salaries (*staff, post-docs, etc.*) | (12) Temp Help | (11) Fringe | (20) Travel | (30) OE | (70) Graduate Tuition/ Fees | TOTALS |
|---|---|---|---|---|---|---|---|
| Idaho Wheat Commission | $ 24,320 | $ 8,798 | $ 8,127 | $ 2,000 | $ 8,000 | $ 5,889 | $ 57,134 |

**TOTAL BUDGET REQUEST FOR FY 2022:** $ 57,134

**BREAKDOWN FOR MULTIPLE SUB-BUDGETS:**

| Budget Categories | *Chen* | *(Insert CO-PI Name)* | *(Insert CO-PI Name)* | *(Insert CO-PI Name)* |
|---|---|---|---|---|
| (10) Salaries | $ 24,320 | $ - | $ - | $ - |
| (12) Temp Help | $ 8,798 | $ - | $ - | $ - |
| (11) Fringe Benefits | $ 8,127 | $ - | $ - | $ - |
| (20) Travel | $ 2,000 | $ - | $ - | $ - |
| (30) Other Expenses | $ 8,000 | $ - | $ - | $ - |
| (70) Graduate Student Tuition/Fees | $ 5,889 | $ - | $ - | $ - |
| TOTALS | $ 57,134 | $ - | $ - | $ - |

**Total Sub-budgets** $ 57,134

**Brief Explanatory Comments: (see FY2022 RFP for guidance)**
**$24,320 is requested for a senior technician and a MS student 50% salary.**
**$8798 is requested for the MS student summer salary.**
**$8127 is requested to cover 50% benefits for the senior technician and student.**
**$2,000 is requested for travels to off-campus disease nursery.**
**$8,000 is requested to cover Lab reagents for KASP assay and harvesting needed stuff.**
**$5889 is requested to cover 50% of student fees.**

***FY2022 Version***

## ANNUAL REPORT

**Grant Code:** AP4538
**Title**: Breeding Durable and High Level of Dwarf Bunt Resistance Using Molecular Marker-Assisted Selection
**Personnel:** Drs. J. Chen, R. Chowdhury, R. Wang, Mr. J. Wheeler
**Collaborators:** Drs. D. Hole, J. Marshall, T. Gordon
**Address:** Dr. Jianli Chen, University of Idaho (UI) Aberdeen Research & Extension Center, Aberdeen, ID 83210; 208-397-4162, ext. 229; jchen@uidaho.edu.

**Accomplishments**
The level of infection in our 2020 dwarf bunt nursery was excellent. A total of 776 headrows were assessed for dwarf bunt resistance. Dwarf bunt severity ranged from 0 to 90%. Test materials included 300 breeding lines from the elite trials and 476 genetic materials being used for fine-mapping and marker development for the 7DS QTL. Of the 476 headrows, dwarf bunt severity was assessed in individual plants in 140 headrows, 20 plants per headrow. Approximately 2800 plants were harvested and genotyped with KASP markers to dissect the 7DS QTL. The homozygous recombinants were selected and planted again this fall in the dwarf bunt nursery in Logan, UT. We developed a new line, 'IDO1906', that has exceptionally high resistance to dwarf bunt and good resistance to stripe rust. IDO1906 could potentially be released in one to two years. Sixty doubled haploid (DH) lines derived from IDO1906 x IDO1506 were produced. These lines combined dwarf bunt resistance alleles from QTL on 6DL, 7AL, and 7DS. We also generated good genetic materials and preliminary results to support a NIFA grant application in spring 2021.

### Objective 1. Fine map *QDB.ui-7DS* and develop KASP markers for MAS

To fine-map the 7DS QTL, we used KASP markers closely linked to the 7DS QTL to select 10 heterozygous inbreed families (HIF, HIF-34, HIF-63, HIF-90, HIF-149, HIF-107, HIF-143, HIF-184, HIF-171, HIF-111, HIF-128) from the $F_5$ generation of the original population derived from RioBlanco x IDO444. Approximately 200 seeds for each HIF were space-planted in the bunt nursery in Logan, UT in fall of 2019. Individual plants were assessed for bunt infection and genotyped with seven markers in the 7DS QTL region. Correlation analysis between disease data and marker haplotype was conducted. We tentatively pin-pointed a candidate gene in a 3.8 MBp segment (physical distance from 3.8 to 7.6 Mbp) from within the original the 25 cM region. To confirm the candidate region, we planted eight HIF-F3 generation lines, three of which (HIF-149, -063, -090) were replicated twice. Homozygous HIF-derived F3 NILs from the other five HIF families were planted without replication. In each headrow, 50 wheat seeds mixed with 50 rice seeds were planted to achieve space-planting. We also planted 600 plants of an $F_2$ generation derived from RB-659(R) x RioBlanco in the bunt nursery to construct a large fine-mapping population for gene cloning. This population has markers segregating in the 7DS 2.5-2 candidate gene region.

**Objective 2. Fine map *QDB.ui-6DL* and develop KASP markers for *MAS***

The 6DL QTL was identified in the IDO835 x Moreland (IMDH) derived doubled haploid population and was mapped in a 2.11 cM region. This QTL was also validated in UI Silver x Shaan89150 derived DH population (named as SSDH) (Wang et al., 2019) and in a diverse panel from National Small Grain Collection (Gordon et al., 2020). Based on the map location, this QTL was tentatively identified as *Bt9*. To fine map the *QDB.ui-6DL*, we used a different strategy than that used in objective 1. Because no heterozygous inbred family (HIF) were available, we created two $F_2$ populations to fine map the *QDB.ui-6DL*. Two selected highly resistant DH lines were crossed to two highly susceptible DH lines and the $F_1$ seeds (SSDH-128/SSDH-097 and SSDH-048/SSDH-036) were obtained in 2019. This summer we harvested $F_2$ plants in greenhouse. In the spring of 2021, we will screen progeny from these $F_2$ plants for common bunt resistance in the greenhouse.

**Additional activities not included in the original proposal**

We initiated another approach using knock-out mutants to identify multiple candidate genes for resistance to dwarf bunt. First, we mutagenized the widely grown DB-resistant cultivar UI Silver with ethyl methane sulfonate (EMS). Based on marker haplotypes, UI Silver has three QTL on the 6DL, 7AL, and 7DS. We expect to select single knockout mutants as well as double and triple knockout mutants in the UI Silver mutant population. Simultaneously, we can identify for release any UI Silver mutant lines that maintain DB resistance but have improved agronomic performance.

**Projections:** 1) We will design more KASP markers to saturate the 6DL and 7DS QTL regions this winter and next spring to identify the candidate gene in the winter in 2021. 2) We will aim to develop high yielding winter wheat cultivars with multiple resistance genes to dwarf bunt using molecular marker assisted selection. 3) We will submit a USDA AFRI proposal in the spring 2021 to identify candidate genes underlying the two QTL on the 6DL and 7DS.

**Publications:**

Gordon, T., R. Wang, D. Hole, H. Bockelman, J. M. Bonman, and **J. Chen*.** 2020. 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. https://doi.org/10.1007/s00122-020-03532-0.