Grant Code: AP2708

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

Personnel: Dr. Rui Wang, Dr. Jianli Chen, Dr. Sam Hunter, Mr. Tyler Gordon, Dr. David Hole, Dr. Michael Bonman, and Dr. Juliet Marshall

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Justification/Rationale: Dwarf bunt (DB) caused by *T. contraversa* J.G. Kühn can severely reduce wheat yield and quality when epidemic occurs because the grains in the infected spikes become bunt balls filled with brown-black, unpleasantly smelling spores. Dwarf bunt has not been paid a lot of attention in the past two decades due to the application of the fungicide seed treatment. However, the recent years' interest on expanding organic wheat production makes it essential to develop durable bunt resistance since the fungicide seed treatment is not allowed in such important cropping system. The current dwarf bunt resistance breeding mainly based on phenotypic selection, which is extremely difficult in the field since the 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. Molecular marker-assisted selection (MAS) is an alternative of selection method and an efficient way of pyramiding multiple genes to achieve durable and high level of resistance. Currently, sixteen resistance genes (*Bt1* to *Bt15*, and *Btp*) were proposed based on phenotypic evaluations in the differential lines (Goates 2012), but none of them have been well characterized in molecular levels. Among limited QTL mapping studies, we published one novel major QTL on chromosome 7DS that contributed up to 53 % of phenotypic variation (Chen et al. 2016). In the ongoing project, we also found additional two major QTL on chromosome 6DL and 7AL associated with dwarf bunt resistance in other two populations (Table 1). The proposed project will be based on such solid preliminary results and focus on the development of breeder-friendly molecular markers (KASP markers) that can be directly used to select each QTL/gene and pyramid multiple QTL/genes to develop durable and high level of bunt resistance. To better understand the molecular host-pathogen interaction, the proposed research will also assign each QTL to specific bunt resistance gene using newly established greenhouse protocols and conduct transcriptome analysis through RNA-seq experiment.

Table 1. Major QTL identified in the three bi-parental populations.

Population	QTL	Chromosome	LOD	R^2	Possible *Bt* gene
IRRIL	*QDB.ui-7DS*	7DS	35.2	53.4	Unknown *Bt* gene
IMDH	*QDB.ui-6DL*	6DL	20.5	52.7	*Bt9*
	QDB.ui-7AL	7AL	13.0	37.5	Possible *Bt14* or *Bt15*
SSDH	*QDB.ui-6DL*	6DL	3.7	10.9	*Bt9*

Hypothesis & Objectives: The traditional method for bunt resistance breeding is phenotypic selection, which is a time and labor consuming process and only one season can be done per year. In this study, we will develop user-friendly molecular markers and use them in MAS to

breed durable and high level of dwarf bunt resistance. The objectives of this study are: 1) to fine map the 7DS QTL with the new sequencing technology (Targeted Gene Capture) and develop KASP markers for MAS; 2) to develop KASP markers for QTLs on chromosome 6DL and 7AL for MAS; 3) to understand the gene expression profile in the resistant line during infection; 4) to assign each QTL to specific dwarf bunt gene. The ultimate goal is to develop molecular markers that associated with major *Bt* genes and use these markers to develop durable and high-level resistant wheat cultivars.

Procedures/Plan of Work:

Objective 1. Fine map *QDB.ui-7DS* and develop KASP markers for MAS (Year 1 and 2)

The 7DS QTL was identified in the IRRIL population. The QTL was flanked by six markers (one DArT marker and five SNP markers) in a 25 cM region. To saturate this region and develop breeder-friendly markers, we propose to use a new sequencing technology (Targeted Gene Capture). For this method, two parents and selected 20 resistant and 10 susceptible RILs will be used in DNA library construction and sequencing process, which will be conducted by Sam Hunter at Institute for Bioinformatics and Evolutionary Studies (IBEST). Meanwhile, a Near-Isogenic Line population (NILs) will be developed using heterogeneous inbred family (HIF) analysis method based on the developed KASP markers (Year 1). After this, the NIL population will be phenotyped and genotyped to refine the location of resistance gene (Year 2).

Objective 2. Fine map *QDB.ui-6DL* and develop KASP markers for *QDB.ui-6DL* and *QDB.ui-7AL* for MAS (Year 1 to 3)

The major QTL on chromosome 6DL and 7AL were identified using Illumina 90K SNP assay in two DH populations derived from the crosses IDO835 x Moreland and UI Silver x Shaan89150. The genetic map of the two QTL were better saturated than the 7DS QTL, which were approximately flanked by a dozen of markers in about 5 cM regions. The first step is to convert SNP peak markers to KASP markers and to validate them in a set of differential lines and breeding lines with known dwarf bunt resistance (Year 1). The second step, to fine map the major QTL on 6A (*Bt9*), we will develop a couple of F_2 populations by crossing DH lines from two DH populations and more KASP markers for more phenotyping and genotyping (Year 2 and 3).

Objective 3. Understand the gene expression profiles in the resistant line during infection (Year 2 and 3)

One pair of NILs for *QDB.ui-7DS* will be selected and inoculated in the greenhouse. Plant samples will be collected in four different time points after inoculation. The RNA library preparation, sequencing, and related data analysis will be conducted by Sam Hunter at IBEST.

Objective 4. Assign identified QTL to specific dwarf bunt genes (Year 3)

Upon the completion of the above objectives, we will be able to select a set of lines that contain individual QTL or multiple QTL among the 6DL, 7DS, and 7AL. This set of materials and dwarf bunt differential lines will be inoculated by specific race of dwarf bunt in greenhouse. Based on gene-for-gene system, we will be able to assign each QTL to specific gene and propose potential genes in each resistant line.

Duration: 3 years (FY2020 falls in year 2 of the 3-year project)

Cooperation/Collaboration: Dr. Chen is a supervisor of this project. Dr. Rui Wang will serve as a PI coordinate and implement this project. Dr. Sam Hunter at IBEST, University of Idaho, Moscow is a co-PI of this proposal and will conduct sequencing related work. Dr. David Hole, a wheat breeder at Utah State University, has been our collaborator on dwarf bunt research in the past ten years. His program establishes dwarf bunt nursery and plant our materials each year. We will work together on assessing disease at plant maturity stage. Dr. Michael Bonman and his support scientists Tyler Gordon will also collaborate with us and assess dwarf bunt resistance in a world-collected germplasm in Dr. Hole's nursery. Tyler and Rui will work together on race-specific inoculation study and development of F_2 population segregating for *QDB.ui-6DL* (*Bt9*) in greenhouse at Aberdeen, ID. Molecular markers developed in this project will be used to genotype materials being planted in variety trials that Dr. Marshall leads.

Anticipated Benefits, Expected Outcomes and Impacts, and Transfer of Information: The application of molecular marker-assisted selection will significantly promote the dwarf bunt resistance breeding by reducing the time and labor cost. We will be able to select some high resistant DHLs in the two DH populations, which can be released as germplasm or cultivars based on their agronomic performance. In long term, growers, especially the organic farming growers, will benefit from growing the durable and high-level resistance cultivars and increase their profit. In addition, the anticipated knowledge about the major bunt resistance genes in this project will enable us to further identify and pyramid multiple resistance genes using the most advanced technology, as reported in IWC Press (*New gene-detecting technology could lead to 'super wheat'*; http://www.idahowheat.org/media/press.aspx?id=177) (Steuernagel et al. 2016; Witek et al., 2016). Our research findings will be published in Idaho Grain Magazine, referred journals, presented at different growers and professional meetings. One Postdoctoral Fellow will be partially funded and trained through this project.

Literature Review: Dwarf bunt (DB) caused by *T. contraversa* J.G. Kühn, and common bunt (CB) caused by *Tilletia caries* (DC.) Tul. & C. Tul. (=*T. tritici*) and *T. foetida* (Wallr.) Liro (=*T. laevis*) are considered to be among the world's most potentially damaging diseases of wheat that reduce grain yield and quality because they destroy the wheat grains by forming bunt balls filled with brown-black, unpleasantly smelling spores (Martens et al. 1984). With the advent of seed treatments, most breeding programs de-emphasized common bunt and dwarf bunt resistance selection in the past two decades. However, bunt host resistance has regained world-wide interest due to the increase in organic farming and concerns for more sustainable agriculture (Matanguihan et al. 2011). Lack of efficient organic certified seed treatment lead to increasing incidence and damages due to 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 these two bunt diseases are closely related (Bao, et al. 2010), to the extent that dwarf and common bunt resistances are partly controlled in wheat by shared genes (*Bt*) in a gene-for-gene system (Goates 1996; Goates 2012). Currently, 36 pathogenic races of *T. caries*, 15 races of *T. foetida* and 19 races of *T. contraversa* have been identified based on their reaction to 14 wheat differential lines that each putatively contains one of 14 postulated bunt resistance genes, *Bt1* through *Bt13*, and *Btp* (Goates, 2012). Currently, *Bt* gene linked molecular markers were only proposed in a few bunt resistance studies (Dumalasová et al. 2012;

Knox et al. 2013; Muellner et al. 2018; Wang et al. 2018) but none of their usefulness in MAS was evaluated. We currently identified three major QTL/genes conferring resistance to dwarf bunt. They will be well studied in the development of molecular selection markers and understanding of the molecular host-pathogen interaction, which will ultimately enable us to breed the super wheat.

FY2020

IDAHO WHEAT COMMISSION - BUDGET FORM

Principal Investigator: Rui Wang

Allocated by	**Idaho Wheat Commission**	**during FY 2018**	**$**	**-**
Allocated by	**Idaho Wheat Commission**	**during FY 2019**	**$**	**53,259**

REQUESTED FY2020 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	**$ 23,754**		**$ 7,862**	**$ 3,000**	**$ 18,713**		**$ 53,329**
Post Doc (50%)							

TOTAL BUDGET REQUEST FOR FY 2020: **$ 53,329**

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

Budget Categories	*Rui Wang*	*Sam Hunter*	*(Insert CO-PI Name)*	*(Insert CO-PI Name)*
(10) Salaries	$ 23,754	$ -	$ -	$ -
(12) Temp Help			$ -	$ -
(11) Fringe Benefits	$ 7,862		$ -	$ -
(20) Travel	$ 3,000	$ -	$ -	$ -
(30) Other Expenses	$ 8,000	$ 10,713	$ -	$ -
(70) Graduate Student Tuition/F		$ -	$ -	$ -
TOTALS	$ 42,616	$ 10,713	$ -	$ -

Total Sub-budgets $ 53,329

Explanatory Comments:

Fall 2018 Version

ANNUAL REPORT

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Accomplishments:

Overall, two winter wheat DH populations IMDH (IDO835 x Moreland) and SSDH (UI Silver x Shaan89150), 15 dwarf bunt differentia lines, 300 NSGC accessions, and around 200 breeding lines were assessed for dwarf bunt resistance in the nursery in Logan, UT. QTL was re-analyzed using dwarf bunt data from all years and the two major QTLs (*QDB.ui-6DL* and *QDB.ui-7AL*) detected in our preliminary analysis were confirmed. KASP markers associated with the three major QTLs (*QDB.ui-7DS, QDB.ui-6DL,* and *QDB.ui-7AL*) were developed and tested in three bi-parental populations and 15 dwarf bunt differentia lines and other resistant resources. Details of the progress are described below:

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

Three additional KASP markers were developed and used to dissect the *QDB.ui-7DS* in the IRRIL (IDO444 x Rio Blanco) population, which reduced the original QTL region from 25 cM to current 8.12 cM. Selected KASP markers flanking *QDB.ui-7DS* were used to develop a 2400 near-isogenic line (NIL) population from six F_6 heterozygous inbreed families (HIFs). This fine mapping population was planted in the nursery at Logan in 2018 fall and will be phenotyped in 2019 summer. In addition, the peak KASP marker for *QDB.ui-7DS* was screened in dwarf bunt differential lines and other resistant resources, indicating some *Bt* gene candidates for this QTL (Table 1).

Under collaboration with Sam Hunter in IBEST, we have picked the target region for gene capture, constructed DNA library, and synthesized the baits. The sequencing is ongoing and we expect to have capture data to be presented at IWC research review in Feb. 2019. When capture sequence data becomes available, more KASP markers will be developed and used in genotyping of the large NIL population to refine the QTL region.

Objective 2. Fine map *QDB.ui-6DL* and develop KASP markers for *QDB.ui-6DL* and *QDB.ui-7AL* for MAS

Using dwarf bunt infection data from this and previous years, QTL was re-analyzed in the two DH populations (IMDH and SSDH). The *QDB.ui-6DL* was consistently detected in both DH populations with large effects (Fig. 1) and mapped to a 2.11 cM region. *QDB.ui-7AL* was mapped to a 4.66 cM region but only in the IMDH population. KASP markers for both *QDB.ui-7AL* and *QDB.ui-6DL* were developed and screened in dwarf bunt differential lines and other resistant resources, indicating some *Bt* gene candidates for these two QTL (Table 1).

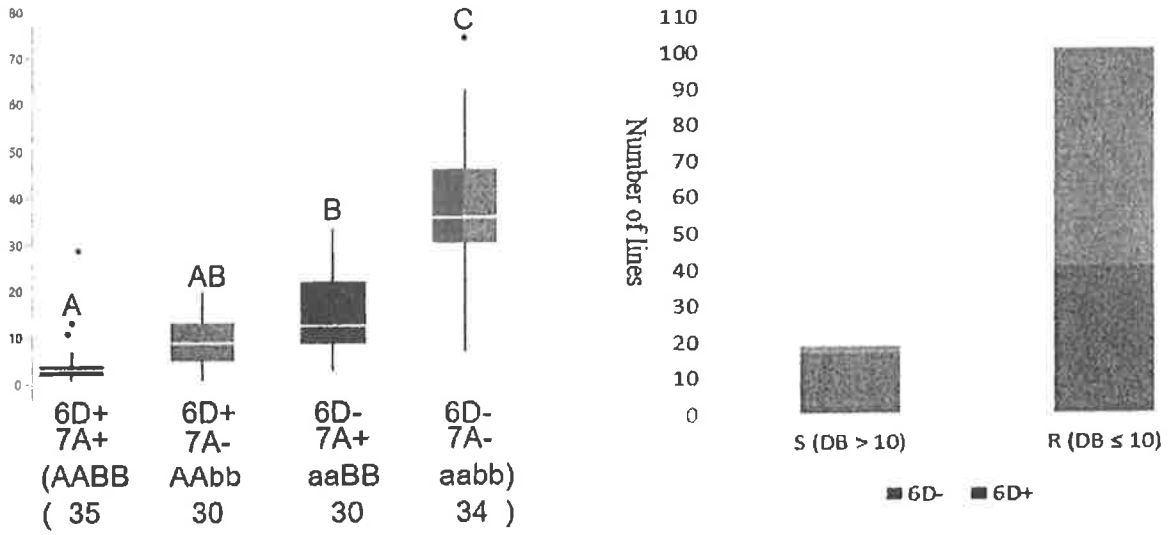

Fig. 1. A). Allele effects of the two QTL based on the BLUE data in IMDH population. B). Stacked column chart showing the allele effect of *QDB.ui-6DL* in SSDH population.

Based on the haplotype for the two QTLs, ten DH lines, including 6 resistant and 4 susceptible lines, from two DH populations were selected and planted in cold room for vernalization. The resistant lines will be crossed with susceptible lines to develop a couple of F_2 populations segregating for *QDB.ui-6DL*.

Projections:

At first, based on multiple years' phenotyping on DB disease screen for the three DH populations, we are able to select some high resistant DHLs that can be released as germplasm or cultivars based on their agronomic performance. Furthermore, the five developed KASP markers (Table 1) for three major QTLs can be used in molecular marker-assisted selection, which will significantly promote the dwarf bunt resistance breeding by reducing the time and labor cost. In long term, growers, especially the organic farming growers, will benefit from growing the durable and high-level resistance cultivars and increase their profit. At last, the progress on the fine mapping of two major QTLs will enable us to further identify and pyramid multiple resistance genes using the most advanced technology, as reported in IWC Press (*New gene-detecting technology could lead to 'super wheat'*; http://www.idahowheat.org/media/press.aspx?id=177) .

Publications:

Two abstracts were presented in the "Proceedings of the XX international workshop on smuts and bunts":

Wang, R., Chen, J., Hole, D., Zhao, W., Isham, K., Gordon, T., Bonman, J.M. Guttieri, M., Souza, E.J., Goates, B. 2018. Prospective and Challenges for Breeding Bunt Resistant Wheat Using Molecular Marker Assisted Selection. 2018. Proceedings of the XX international workshop on smuts and bunts. Logan, Utah, USA.

Gordon, T., Wang, R., Hole, D., Goates, B., Bockelman, H., Klos, K.E., Chen, J., Bonman, J.M. 2018. Genome-Wide Association Mapping for Dwarf Bunt Resistance in the National

Small Grains Collection. Proceedings of the XX international workshop on smuts and bunts. Logan, Utah, USA.

Table 1. Phenotypes and KASP marker haplotypes of the investigation panel.

Line	Genes	DB	6DL-J-2	6DL-J-5	7AL-J-1	7AL-J-3	*Bt10* (6DS)	7DSS-K13
Cultivars								
Blizzard (PI 512302)	Unknown	R	-	-	+	+	-	+
Bonneville (PI 557015)	Unknown	R	-	+	+	+	-	-
Golden Spike (PI 614813)	Unknown	R	+	+	+	+	+	-
Gary (PI 620632)	Unknown	R	+	+	+	+	+	-
Promontory (PI 555458)	*Bt-3, Bt-9, Bt-10*	R	+	+	+	+	-	+
Manning (CItr 17846)	*Bt-3, Bt-9, Bt-10*	R	+	+	+	+	+	-
Deloris (PI 631447)	*Bt-3, Bt-9, Bt-10*	R	+	+	-	-	+	-
Lewjain (CItr 17909)	*Bt-8, Bt-9, Bt-10*	R	-	-	+	+	-	+
Winridge (CItr 17902)	*Bt-8, Bt-9, Bt-10*	R	-	-	+	+	-	+
Stava	*Bt-8, Bt-9, Bt-10*	R	-	+	+	+	-	+
Utah-100 (PI 594920)	*Bt-3, Bt-9, Bt-10*	R	+	+	+	+	+	-
Resistance sources								
PI 476212 (SM4)	Unknown	R	-	-	+	+	-	+
PI 178383 (M69-19)	*Bt8, Bt9, Bt10* +	R	+	+	+	+	+	+
CI 14106	*Bt12*	R	-	-	+	-	-	+
CI 14107	*Bt12*	R	-	-	+	-	-	+
Differentials								
M85-4 (PI 554101)	*Bt1*	S	+	+	-	-	N	-
M85-6 (PI 554097)	*Bt2*	S	+	+	+	+	N	-
M81-2008 (CI 6703)	*Bt3*	S	-	+	+	+	N	-
CI 1558	*Bt4*	S	-	-	+	+	N	-
M82-2052 (CI 11458)	*Bt5*	R	-	+	-	-	-	+
Rio (CI 10061)	*Bt6*	S	+	+	+	+	+	-
Sel. 50077 (PI 554100)	*Bt7*	S	-	-	-	-	-	-
PI 173438/Eg (M82-2161)	*Bt8*	R	+	+	+	-	-	+
Elgin/PI 178383	*Bt9*	R	+	+	-	-	-	+
Elgin/PI 178383	*Bt10*	R	+	+	-	-	+	+
Elgin/PI 166910	*Bt11*	R	-	+	-	-	-	-
PI 119333	*Bt12*	R	-	-	-	-	+	+
PI 181463 (Thule III)	*Bt13*	R	-	-	-	-	-	+
CItr 13711 (Doubbi)	*Bt14*	R	-	-	+	+	-	+
CItr 12064 (Carlton)	*Bt15*	R	-	-	+	+	-	-