PROJECT NO.: New

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

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JUSTIFICATION/RATIONALE:
Dwarf bunt (DB) caused by *T. contraversa* J.G. Kühn can severely reduce wheat yield and quality when epidemics occur because the grains in the infected spikes become bunt balls filled with brown-black, unpleasant smelling spores. Dwarf bunt resistance has been neglected in the past two decades due to the availability effective fungicide seed treatment. However, recent interest on expanding organic wheat production makes it essential to develop durable bunt resistance since the fungicide seed treatment is not allowed in an organic cropping system. The 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 B.J., 1996) and the disease can only be assessed when plants are mature. Molecular marker-assisted selection (MAS) is an alternative selection method and an efficient way of pyramiding multiple genes to achieve durable and high level of resistance. Currently, fifteen resistance genes (*Bt1* to *Bt15*) were proposed based on phenotypic evaluations in the differential lines (Goates B.J., 2012), but none of them have been well characterized at the molecular level. 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 three additional major QTLs on chromosomes 6DL, 7BS, and 7DL associated with dwarf bunt resistance in other two populations (Table 1). The proposed project will be based on these solid preliminary results and focus on the development of breeder friendly molecular markers (e.g. KASP markers) that can be directly used to select each QTL (gene) and pyramid multiple QTLs (genes) to develop durable and high levels of bunt resistance. To better understand the relationship among different QTLs (genes) and the molecular basis for host-pathogen interactions, the proposed research will also assign each QTL to specific bunt resistance genes using newly established greenhouse protocols.

Table 1. QTL identification in the three bi-parental populations.

Population	QTL	Chromosome	LOD	R^2	Possible *Bt* gene
IRRIL	*QDbt.ido-7D.1*	7DS	35.2	53.4	*Bt12*
	QDbt.ido-1A	1AS	9.8	9.9	Minor QTL
	QDbt.ido-2B	2BS	3.7	3.7	Minor QTL
IMDH	*QDbt.ido-6D*	6DL	13.8	38.8	*Bt9*
	QDbt.ido-7D.2	7DL	18.3	48	Major QTL, *Bt?*
SSDH	*QDbt.ido-7B*	7BS	7.8	25.8	Major QTL, *Bt?*
	QDbt.ido-6D	6DL	3	10.9	*Bt9?* Minor QTL?

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 breeder friendly molecular markers and use them in MAS to breed durable and high levels of dwarf bunt resistance. The objectives of this study are: 1) fine mapping of the 7DS QTL with targeted capture sequencing technology and development of KASP markers for MAS; 2) to develop KASP markers for QTLs on chromosome 6DL, 7BS, and 7DL for MAS; 3) to assign each QTL to specific dwarf bunt gene. The ultimate goal is to develop molecular markers that are associated with major *Bt* genes and use these markers to develop durable and high level resistance in wheat cultivars.

Preliminary Results

We have developed three bi-parental mapping populations: Population 1 (IRRIL), a recombination inbred line (RIL) population (160 lines) derived from the cross IDO444 x Rio Blanco; Population 2 (IMDH), a double haploid (DH) population (130 lines) derived from the cross IDO835 x Moreland; Population 3 (SSDH), a DH population (130 lines) derived from the cross UI Silver x Shaan89150. For P1, the disease screen had been done for 3 years at two locations and the QTL mapping results were published in 2016; for P2, the population was phenotyped for three years and the manuscript for QTL analysis is in preparation; for P3, the population was phenotyped for two years with seed planted for one more year of disease evaluation. Based on the genotyping and phenotyping data for the three bi-parental mapping population, four major QTL (genes) and two minor QTL have been identified (Table 1).

PROCEDURES/PLAN OF WORK:

Objective 1. Fine mapping the 7DS QTL and develop KASP markers for MAS (Year 1)

The 7DS QTL was identified in 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-used markers, we propose to use a new sequencing technology (Targeted Capture Sequencing). For this purpose, the two parents and selected 20 resistant and 10 susceptible RILs will be used in DNA library construction, capture, and sequencing. Resulting sequenced reads will be mapped to the reference sequence containing the 7DS QTL, and variants segregating with the phenotype will be identified. Sequencing and variant analysis will be

conducted by the Institute for Bioinformatics and Evolutionary Studies (IBEST) Genomics Resources Core (Sam Hunter).

Objective 2. Develop KASP markers for QTL on chromosome 6DL, 7BS, and 7DL (Year 1 and 2)

The major QTL on chromosome 6DL, 7DL, and 7BS were identified using Illumina 90K SNP assay in two DH populations derived from the crosses IDO835 x Moreland and UI Silver x Shaan89150, respectively. The map of the three 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. The second step, if necessary, is to develop better markers using the proposed approach in objective 1 (year 2).

Objective 3. Assign identified QTL to specific dwarf bunt genes (Year 2 and 3)

Upon the completion of objective 1 and 2, we will be able to select a set of lines that contain individual QTL or multiple QTL among the 6DL, 7BS, 7DS, and 7DL. This set of materials and dwarf bunt differential lines will be inoculated by specific race of dwarf bunt in greenhouse trials. Based on gene-for-gene system, we will be able to assign each QTL to specific genes and propose potential genes in each resistant line. This set of materials will also be planted in field nursery and inoculated with composite races of dwarf bunt pathogen in Logan, UT. We will determine whether any epistasis interactions among these QTL/genes occurs based on both greenhouse and field data. The epistasis interactions, if any, will be studied in future research.

DURATION: 3 years

COOPERATION/COLLABORATION: Dr. Chen is a supervisor of this project. Dr. David Hole, a wheat breeder at Utah State University, has been our collaborator on dwarf bunt research for the past ten years. His program will establish a 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 studies in a greenhouse at Aberdeen, ID. Rui will conduct genomics work and do phenotyping with assistance from Weidong. Dr. Sam Hunter at IBEST, University of Idaho, Moscow is a co-PI of this proposal and will conduct sequencing related work.

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 the long term, growers, especially organic growers, will benefit from growing durable and high-level resistance cultivars to 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, refereed journals, and presented at different growers and professional meetings. One Postdoctoral Fellow will be 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 (Cherewick 1953; 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 resistances (Fofana et al., 2008; Wang et al., 2009; Dumalasová et al. 2012, Knox et al. 2013; Singh et al., 2016) and none of their usefulness in MAS was evaluated. We recently identified four major QTLs (genes) conferring resistance to dwarf bunt. They will be well studied in the development of molecular markers and understanding of the relationship between different resistance genes as well as molecular host-pathogen interaction, which will ultimately enable us to breed super wheat.

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IDAHO WHEAT COMMISSION - BUDGET FORM

Principal Investigator: Jianli Chen

Allocated by	**Idaho Wheat Commission**	**during FY 2017**	**$**	**-**
Allocated by	**Idaho Wheat Commission**	**during FY 2018**	**$**	**-**

REQUESTED FY2019 SUPPORT:

	Salary (*staff, post-docs, etc.*)	Temporary Help	Fringe	Travel	OE	Graduate Tuition/Fees	TOTALS
Idaho Wheat Commission	**$ 23,754**		**$ 7,792**	**$ 3,000**	**$ 18,713**	**$ -**	**$ 53,259**

TOTAL BUDGET REQUEST FOR FY 2019: **$ 53,259**

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

	Jianli Chen	*Sam Hunter*	*(Insert CO-PI Name)*	*(Insert CO-PI Name)*
Salary (50% Rui)	**$ 23,754**	**$ -**	**$ -**	**$ -**
Temporary Help		**$ -**	**$ -**	**$ -**
Fringe Benefits	**$ 7,792**	**$ -**	**$ -**	**$ -**
Travel	**$ 3,000**	**$ -**	**$ -**	**$ -**
Operating Expenses	**$ 8,000**	**$ 10,713**	**$ -**	**$ -**
Graduate Student Tuition/Fees		**$ -**	**$ -**	**$ -**
TOTALS	**$ 42,546**	**$ 10,713**	**$ -**	**$ -**

Total Sub-budgets **$ 53,259**

Explanatory Comments: (see FY2019 RFP for definition)

Fall 2017 Version