Grant Code: AP3679

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

Personnel: Drs. Jianli Chen (PI) and Rui Wang (Co-PI)

Collaborators (non-funded): Mr. Tyler Gordon, Dr. David Hole, Dr. Michael Bonman, 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 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 received 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 is 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 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 two major QTL on chromosome 6DL and 7AL associated with dwarf bunt resistance in other populations (Table 1). The proposed project will be based on 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 host-pathogen interaction, the proposed research will also assign each QTL to specific bunt resistance gene using newly established greenhouse protocols.

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. 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 assign each QTL to a specific dwarf bunt gene.

Procedures:

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

The 7DS QTL was previously identified in the IRRIL population. 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 four KASP markers were developed. In FY21, we will use a large near-isogenic line population to fine map the region.

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)

Two major QTL on chromosome 6DL and 7AL were identified using Illumina 90K SNP assay in IDO835 x Moreland derived doubled haploid (DH) population. The 6DL QTL was also identified in UI Silver x Shaan89150 DH population. The genetic map of the two QTL were better saturated than the 7DS QTL, which were approximately flanked by a dozen markers in about 5 cM regions. To fine map the *QDB.ui-6DL*, three selected highly resistant DH lines were crossed to two highly susceptible DH lines and six sets of F_1 seeds were obtained. In FY2021, we will develop several large F_2 populations that segregate for *QDB.ui-6DL* and plant them in Logan's DB nursery to do phenotyping. We will genotype the F_2 population that shows a 3:1 segregation ratio and run a statistical analysis to refine the QTL region.

Objective 3. 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 chromosomes. This set of materials and dwarf bunt differential lines will be inoculated by a specific race of dwarf bunt in a greenhouse. Based on the gene-for-gene system, we will be able to assign each QTL to a specific gene and propose potential genes in each resistant line.

Duration: 3 years (FY2021 falls in year 3 of the 3-year project)

Cooperation: Dr. Chen is the supervisor and will serve as a PI for this project. Dr. Rui Wang will serve as a co-PI to coordinate and implement this 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 dwarf bunt nursery and plants our materials each year. We will work together on assessing disease at the 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 a race-specific inoculation study and the development of F_2 population segregating for *QDB.ui-6DL* (*Bt9*) in the greenhouse at Aberdeen, ID. Molecular markers developed in this project will be used to genotype materials being planted in variety trials lead by Dr. Marshall.

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 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*, refereed 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 have only been 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.

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

Dumalasová V., Simmonds J., Bartoš P., Snape J. 2012. Location of genes for common bunt resistance in the European winter wheat cv. Trintella. Euphytica 186:257–264

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.

Knox R.E., Campbell H.L., DePauw R.M., Gaudet D., Puchalski B., Clarke F.C. 2013. DNA markers for resistance to common bunt in 'McKenzie' wheat. Can. J. Plant. Pathol. 35:328–337

Martens, J., Seaman W.W.L., Atkinson T.H. 1984. Diseases of Field Crops in Canada. Canadian Phytopath. Soc., Ottawa, Canada

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., Huss, H., Hole, D., Buerstmayr. H. 2018. Comparative mapping of bunt resistance genes in winter wheat. Proceedings of the XX international workshop on smuts and bunts. Logan, Utah, USA.

Steffan P.M., Torp A.M., Borgen A., Backes G., Rasmussen S.K. 2017. Mapping of common bunt resistance gene Bt9 in wheat. Theor. Appl. Genet. 130:1031–1040.

Steuernagel B., Periyannan S.K., Hernandez-Pinzon I., Witek K., Rouse M.N., Yu G., Hatta A., Ayliffe M., Bariana H., Jones J.D.G., Lagudah E.S., Wulff B.B.H. 2016. Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture. Nat. Biotechnol. 34:652–655

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.

Witek, K., Jupe, F., Witek, A. I., Baker, D., Clark, M.D., and Jones, J.D.G. (2016). Accelerated cloning of a potato late blight-resistance gene using RenSeq and SMRT sequencing. Nat. Biotechnol. 34:656–660.

IDAHO WHEAT COMMISSION - BUDGET FORM

Principal Investigator: Jianli Chen

If applicable,	Allocated by	Idaho Wheat Commission	during FY 2019	$ 53,259
If applicable,	Allocated by	Idaho Wheat Commission	during FY 2020	$ 53,329

REQUESTED FY2021 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 Post Doc (50%)	$ 24,959	$ -	$ 10,108	$ 3,000	$ 8,000	$ -	$ 46,067

TOTAL BUDGET REQUEST FOR FY 2021: $ 46,067

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

Budget Categories	Jianli Chen	*(Insert CO-PI Name)*	*(Insert CO-PI Name)*	*(Insert CO-PI Name)*
(10) Salaries	$ 24,959		$ -	$ -
(12) Temp Help	$ -		$ -	$ -
(11) Fringe Benefits	$ 10,108		$ -	$ -
(20) Travel	$ 3,000		$ -	$ -
(30) Other Expenses	$ 8,000		$ -	$ -
(70) Graduate Student Tuition/Fees	$ -		$ -	$ -
TOTALS	$ 46,067		$ -	$ -
			Total Sub-budgets	$ 46,067

Brief Explanatory Comments: (see FY2021 RFP for guidance)
$24959 is requested for Rui Wang's 50% salary to oversee and implement the project.
$10108 is requested to cover 50% of benefits for Rui Wang.
$3000 is requested for travels to IWC meeting, off-station nurseries, and one professional meeting.
$8000 is requested to cover lab chemicals and supplies as well as the field nursery operation fees and supplies.

Fall 2019 Version

ANNUAL REPORT

Grant Code: AP3679

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

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

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

Accomplishments:

We assessed dwarf bunt resistance in the nursery in the 2018-2019 season at Logan, UT for approximately 600 rows (headrows and spaced-planting lines), including 1500 near-isogenic lines (NILs) derived from a cross of IDO444 and Rio Blanco, 15 dwarf bunt differentia lines, 42 Moreland mutant lines, 320 NSGC accessions, and around 300 breeding lines. QTL analysis results using two DH populations (IMDH and SSDH) were published in a refereed journal. In additions, more KASP markers (in total 18) 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

Four 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 2.4 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, UT in the fall of 2018 and phenotyped in the summer of 2019. The data analysis is still ongoing. In addition, the updated peak KASP marker and all other 13 flanking markers 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).

Using the advanced sequencing technologies, including target capture, exosome capture, and promoter region capture, more KASP markers have been designed to saturate the candidate region of the resistance gene, which facilitates the development of the diagnostic markers for breeding purposes and the gene cloning.

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.

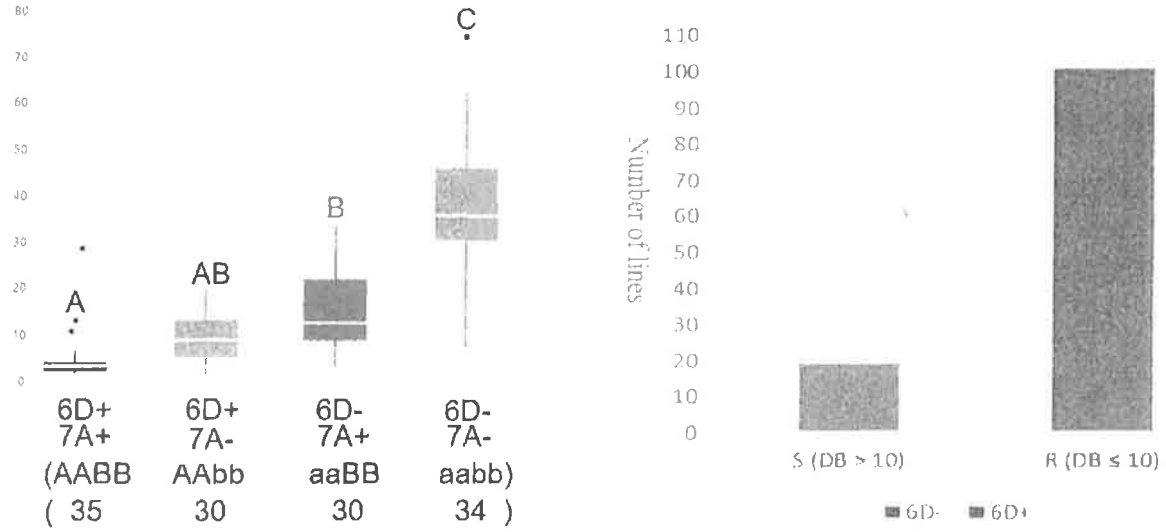

Fig. 1. A). Allele effects of the two QTL based on the BLUE data in IMDH population. B). Stacked column chart showing the allele effect of *QDB.ui-6DL* in SSDH population.

To fine map the *QDB.ui-6DL*, three selected highly resistant DH lines were crossed to two highly susceptible DH lines and six sets of F_1 seeds were obtained, which will be used to develop F_2 populations that segregate for *QDB.ui-6DL*.

Projections:

Based on multiple years' phenotyping on DB disease screening 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. In addition, the progress on the fine mapping of two major QTLs will enable us to further develop diagnostic molecular markers that can be used in molecular marker-assisted selection, which will significantly promote the dwarf bunt resistance breeding by reducing the time and labor cost. Furthermore, the fine mapping work is the foundation to 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) . In the long term, growers, especially the organic farming growers, will benefit from growing the durable and high-level resistance cultivars and increase their profit.

Publications:

Two papers were published or accepted in the journal of *Theoretical and Applied Genetics*:

Wang R, Gordon T, Hole D, Zhao W, Isham K, Bonman JM, Goates B, Chen J. 2019. Identification and assessment of two major QTLs for dwarf bunt resistance in winter wheat line 'IDO835'. Theoretical and Applied Genetics. 25:1-2.

Gordon T, Wang R, Hole D, Bockelman H, Bonman JM, Chen J. 2019. Genetic characterization and genome-wide association mapping for dwarf bunt resistance in bread wheat accessions from the USDA National Small Grains Collection. Theoretical and Applied Genetics. Accepted in Dec. 2019.

Table 1. Phenotypes and 7DS KASP marker haplotypes of the investigation panel.

Line	Genes	DB	1.26	1.94	1.97	2.24	2.50-1	2.50-2	2.56	3.82	5.38	5.58	7.59	10.72	10.84	11.43
RB-659			R	R	R	R	S	S	S	S	S	S	S	S	S	S
RB-839			R	R	R	R	S	S	S	S	S	S	S	S	S	S
Rio Blanco	Unknown	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
IDO444	Unknown	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Blizzard (PI 512302)	Unknown	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
CI 14106	*Bt12*	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S
CI 14107	*Bt12*	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S
Promontory (PI 55545	*Bt-3, Bt-9, Bt-10*	R	R	R	S	S	S	S	S	R	R	R	R	S	S	S
Manning (CItr 17846)	*Bt-3, Bt-9, Bt-10*	R	S	S	R	R	R	R	R	R	S	S	R	R	R	R
Deloris (PI 631447)	*Bt-3, Bt-9, Bt-10*	R	S	S	R	R	R	R	R	R	S	S	R	R	R	R
Lewjain (CItr 17909)	*Bt-8, Bt-9, Bt-10*	R	R	R	R	R	S		R	R	S	R	R	R	R	R
Winridge (CItr 17902)	*Bt-8, Bt-9, Bt-10*	R	R	R	R	R	S		R	R	S	R	R	R	R	R
PI 178383 (M69-19)	*Bt8, Bt9, Bt10 +*	R	R	R	R	R	S		R	R	S	R	R	R	R	R
Stava	*Bt-8, Bt-9, Bt-10*	R	R	R	R	R	S		R	R	R	R	R	R	R	R
Utah-100 (PI 594920)	*Bt-3, Bt-9, Bt-10*	R	S	S	R	R	R	R	R	R	S	S	R	R	R	R
Cheyenne (CItr 8885)	S	S	R	R	R	R	R	R	R	R	S	S	R	R	R	S
M85-4 (PI 554101)	*Bt1*	S	R	R	R	R	R	R	S	R	S	S	H	R	R	R
M85-6 (PI 554097)	*Bt2*	S	R	R	R	R	S		R	R	S	S	R	R	R	R
M81-2008 (CI 6703)	*Bt3*	S	R	R	R	R	S		R	R	S	S	R	R	R	S
CI 1558	*Bt4*	S	R	R	R	R	R	R	R	R	S	S	R	R	R	S
M82-2052 (CI 11458)	*Bt5*	R	R	R	R	R	S		R	R	R	R	R	R	R	R
Rio (CI 10061)	*Bt6*	S	R	R	R	R	S	S	R	R	S	S	R	R	R	S
Sel. 50077 (PI 554100)	*Bt7*	S	R	R	R	R	R	R	S	R	S	S	R	S	S	S
PI 173438/Eg (M82-21	*Bt8*	R	R	R	R	R	R	R	R	R	R	R	H	R	H	R
Elgin/PI 178383 (M90-	*Bt9*	R	R	R	R	R	S		R	R	S	R	R	R	R	R
Elgin/PI 178383 (M82-	*Bt10*	R	R	R	R	R	S	S	R	R	S	R	R	R	H	R
Elgin/PI 166910 (M82-	*Bt11*	R	R	R	R	R	S	S	N	R	S	S	R	R	R	R
PI 119333	*Bt12*	R	R	S	R	R	R	R	R	R	R	R	R	S	S	S
PI 181463 (Thule III)	*Bt13*	R	R	R	S	S	R	R	R	R	R	R	R	S	S	S
CItr 13711 (Doubbl)	*Bt14*	R	H	R	R	R	S		R	N	N	R	R	H	N	H
CItr 12064 (Carlton)	*Bt15*	R	N	S	S	R	S		R	H	N	R	R	N	H	H
SM22	Unknown	Unknov	S	S	R	R	R	R	R	R	S	S	R	R	R	R
UI Silver	IDO498*2/UT9441	0	S	S	R	R	S	R	R	R	S	S	R	R	R	R
IDO1101	DW*2/IDO444	0	R	R	R	R	R	R	R	R	S	S	R	R	R	R
A10601WDH103	IDO835/Moreland		R	R	R	R	S		R	R	S	S	R	R	R	S
DB 44	Dongxuan3/DW R	10	R	R	R	R	R	R	R	R	S	S	R	R	R	R
Moreland	Moreland		R	R	R	R	S		R	R	S	S	R	R	R	S
IDO1108DH	IDO658 x Simon	0	R	R	R	R	S		R	R	R	R	R	R	R	R
Simon	Haven/Lambert//	30	R	R	R	R	S		R	R	R	R	R	R	R	R
IDO816	DW/Utah 100		S	S	U	R	R	R	R	R	S	S	R	R	R	R
IDO1101	Use F412 Breeder seed		R	R	R	R	R	R	R	R	S	S	R	R	R	R
IDO1607W (A10601WDH061)		0	S	S	R	R	S		R	R	S	S	R	R	R	R
A10244W-4 (IDO1906)		0	R	R	R	R	S		R	R	S	S	R	R	R	S
Moreland			R	R	R	R	S		R	R	S	S	R	R	R	S
IDO835			R	R	R	R	S		R	R	S	S	R	R	R	S
UI Silver			S	S	R	R	U	R	R	R	S	S	R	R	R	R
Shann89150			R	R	S	S	S	S	S	R	R	R	R	S	S	S
Shann89150			R	R	S	S	S	S	S	N	R	R	R	S	S	S