PROJECT TITLE: Using Genomics Tools to Enhance Wheat Traits

SUBMITTED TO: Agricultural Research Foundation for the Oregon Wheat Commission

SUBMITTED BY:

____

Dr. Oscar Riera-Lizarazu Date
Associate Professor
(541) 737-5879

APPROVED BY:

____

Dr. Russell S. Karow Date
Head
Department of Crop and Soil Science
(541) 737-5857

____

Dr. Sonny Ramaswamy Date
Dean, College of Agricultural Sciences
(541) 737-2331

____

Agricultural Research Foundation Date

PROPOSAL TO THE AGRICULTURAL RESEARCH FOUNDATION
OREGON WHEAT COMMISSION

Title: Using Genomics to Enhance Wheat Traits
Investigator: Oscar Riera-Lizarazu – Dept. of Crop and Soil Science, Oregon State University
Cooperators: C.J. Peterson & A. Ross – Dept. of Crop and Soil Science, Oregon State University

Funding for FY 2007-2008: $US 25,000
Funding for FY 2008-2009: $US 40,985
Funding for FY 2009-2010: $US 43,037
Proposed Funding for FY 2010-2011: $US 46,410

Abstract: Developments in genomics and genetics research in wheat are yielding useful information on the genetic basis of traits of agronomic importance. Thus, the opportunity now exists to design and construct wheat lines with defined genetic architectures. This concept of 'breeding by design' is exemplified by the activities of this research project where we utilize wheat genomics technologies to produce wheat lines containing specific combinations of genetic factors to cope with limitations that either affect wheat productivity or product quality. For example, we have developed wheat lines based on Stephens, OR943576, Weatherford, Winsome, and Tubbs that now carry a gene that boosts grain protein, iron, and zinc content (*Gpc-B1*) and an adult-plant resistance gene against stripe rust (*Yr36*). We have also produced material based on Winsome that carry combinations of three adult-plant stripe rust resistance genes (*Yr18*, *Yr29*, and *Yr30*). Finally, we have material based on Tubbs and Weatherford that carry a chromosome that provides resistance against Cephalosporium stripe. These finished materials are being evaluated and characterized under field conditions. Our project is also in the final stages of producing material with resistance or tolerance against the cereal yellow dwarf virus (CYDV). Our project is also focused in constructing genetic architectures for durable stripe rust resistance by combining adult plant stripe rust resistance genes found in germplasm from the International Maize and Wheat Improvement Center (CIMMYT) and the U.S. Pacific Northwest (PNW) (*Yr18*, *Yr29*, *Yr30*, *Yr36,* and various QTL). In the area of end-use quality, we have identified genetic factors that affect the 'super-soft' grain characteristic that positively affects flour yield and end-use quality. This work will yield molecular markers that may be used for its manipulation. In addition, we are using marker-assisted backcrossing to produce hard white winter germplasm with *Gpc-B1* (a factor that boosts grain protein content) and a combination of high molecular weight (HMW) glutenin loci known to improve the visco-elastic properties of the dough. We are also exploring the use of genetic factors from barley and other relatives to increase the accumulation grain β-glucan, a natural cholesterol-lowering compound. Finally, we are also exploring various strategies to eliminate allergenic seed storage proteins (gliadins) in wheat flour that cause celiac disease.

Objectives: The specific objectives of this proposal are to: 1. develop germplasm with marker-characterized genes for resistance to the cereal yellow dwarf virus (CYDV) and stripe rust; 2. develop germplasm with enhanced quality and nutritional value; and 3. test strategies to eliminate the production of gliadins in the wheat grain.

Procedures:

Marker-assisted backcrossing: The introgression of a given genetic factor is initiated by crossing the donor of the factor of interest with the recurrent recipient line. The movement of the pertinent gene or factor in subsequent generations of backcrossing is monitored using a tightly associated DNA-based marker. Marker-assisted backcrossing will be performed for two to three generations. After the second or third backcross, marker-selected lines will be self-pollinated. Subsequently, progeny from self-pollination will be screened with the pertinent DNA marker to identify lines carrying the factor of interest in the homozygous condition (fixed for the trait). Lines that have been produced through this marker assisted backcrossing process will resemble the adapted elite recurrent parent but will also carry the factor of interest.

Objectives 1: Development of germplasm with resistance to CYDV and stripe rust

Transfer of CYDV resistance in TC14 and TA5551 to Oregon wheat germplasm: Yellow dwarf [caused by the cereal yellow dwarf (CYDV) and the barley yellow dwarf (BYDV) viruses] is the most important viral disease of wheat. Resistance to BYDV and CYDV has been documented in a wheat relative, *Thinopyrum intermedium.* TC14 and TA5551 are CYDV-resistant wheat lines that possess a chromosome translocation where the distal end of the long arm of chromosome 7D was replaced by a syntenic 7Ai-1 *Th. intermedium* chromosome segment[1]. The movement of the pertinent *Th. intermedium* chromosome (and CYDV resistance) in our backcrossing program is being monitored using DNA markers (3P3/3P4, gwm37, Bdv2, and BYAgi). During our backcrossing, we discovered that our advanced derivatives (third and fourth backcross generations) were male-sterile. In 2007, we crossed our materials to male fertility restorers and, in 2008, fertility-restored lines were crossed as males to move the CYDV resistance into a more receptive background with permanent self-fertility. In 2009, we selfed lines known to carry the resistance factor but were also completely fertile. In 2010, we will grow fertility-restored material and select lines carrying CYDV resistance in a homozygous condition.

Transfer of CIMMYT-based durable resistance against stripe rust: Stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) is a devastating disease of wheat. A number of race-specific major genes conferring resistance to this disease have been used in wheat breeding. Because major genes are not effective against current races of stripe rust, there is a need to work on resistance genes that are non-race-specific and more durable. Parula, a wheat variety developed at CIMMYT, carries at least three stripe-rust adult plant resistance genes (*Yr29, Yr18,* and *Yr30*)[2]. Gene mapping activities at CIMMYT and elsewhere have localized *Yr29, Yr18*, and *Yr30* on chromosomes 1B, 7D, and 3B, respectively[3]. Thus, the introduction of *Yr29*, *Yr18*, and *Yr30* can be pursued using the marker-assisted backcrossing approach described earlier. The aim of this portion of the project is to transfer *Yr29, Yr18,* and *Yr30* to the wheat lines based on ORCF101, ORCF102, Goetze, Tubbs, Weatherford, Stephens, Foote, and Winsome. In 2010, we will identify materials that are fixed for various combinations of these stripe rust resistance genes. These materials will then be evaluated in the field in the next growing season.

Objectives 2: Development of germplasm with enhanced quality and nutritional value

Enhancing protein quality and quantity in hard white wheat germplasm: We used marker-assisted backcrossing to generate germplasm based on the elite hard white winter line OR943576 that now carry *Gpc-B1*, a factor that boosts grain protein content. In order to improve the quality of protein of these materials, we will use marker-assisted backcrossing to introduce a combination of high

molecular weight (HMW) glutenin loci known to improve visco-elastic properties of the dough. A strong association between the over-expression of the Bx7 HMW glutenin subunit encode by the *Glu-B1al* allele and high dough strength has been shown[4]. Furthermore, an additional increase in dough strength has been documented in lines carrying the over-expressed Bx7 ($Bx7^{OE}$) subunit and the Dx5+Dy10 HMW glutenin subunits encoded by the *Glu-D1d* allele[4]. Since perfect markers for $Bx7^{OE}$ and Dx5+Dy10 have been developed[5,6], the introduction of these HMW glutenin subunits into material based on OR943576 carrying the high-GPC gene, *Gpc-B1*, can be accomplished using marker-assisted backcrossing. To do effect, we have already made crosses with Red River 68 a cultivar known to carry the $Bx7^{OE}$ and Dx5+Dy10 HMW glutenin subunits[5]. These crosses were used in 2009 as starting points for the first round of marker-assisted backcrossing. In 2010, we plan to perform another round of marker-assisted backcrossing.

Grain β-glucan concentration: Dietary fiber has been found to be highly beneficial in the prevention and treatment of human health conditions including colorectal cancer, high serum cholesterol, cardiovascular disease, obesity, and non-insulin-dependent diabetes[7]. Unfortunately, wheat has a notoriously low level of grain β-glucan[8] and the wheat gene pool appears to be devoid of genetic variation for this trait. The opposite is true for barley where grain β-glucan levels can range from 3 to 6%. The synthesis of β-glucan in barley has been actively researched leading to the discovery of cellulose synthase-like genes (known as *HvCslF*) that appear to be responsible for β-glucan synthesis[9]. Since variability for grain β-glucan content is absent in wheat, we are exploring the use of barley as a source of high grain β-glucan. Luckily, wheat and barley can be intercrossed[10] and barley chromosomes can and have been added to wheat[11]. So, one can assess the effect of individual barley chromosomes or chromosome segments on any trait in a wheat background. A preliminary inspection of data on the expression of barley genes in wheat using a GeneChip platform[12], suggested that a β-glucan synthesis gene, *HvCslF6*, on barley chromosome 7H was expressed in a wheat background at levels comparable to the expression of this gene in normal barley. Thus, we reasoned that grain β-glucan levels may also be affected in these carrying various barley chromosomes. We tested this possibility in various experiments were we tested β-glucan levels of various wheat lines carrying different barley chromosomes[11]. These analyses showed that wheat carrying barley chromosome 7H had β-glucan levels that were 50% greater (nearing 1%) than the normal values in wheat (0.5%). This confirmed the notion that β-glucan levels in wheat may be increased by introgressing genes from barley and that group 7 chromosomes may be of interest. We are currently testing combinations of barley chromosomes added to wheat and testing to see if there are synergistic effects on wheat grain β-glucan levels. In 2010, we would like to test more material carrying various group 7 chromosomes from other wheat relatives to see if these may yield additional boosts in grain β-glucan.

In barley, there is a significant positive correlation between grain β-glucan and waxy starch. Consequently, waxy barley varieties on average have 50% greater levels of grain β-glucan than their non-waxy counterparts [13]. We have already observed that the addition of barley chromosome 7H results in a 47% boost in grain β-glucan whereby wheat lines contain ~1% grain β-glucan. We suspect that combining barley chromosome 7H and the waxy starch characteristic could result in an additional boost in β-glucan content that could yield wheat lines with ~2% grain β-glucan. Thus, we would like to assess the level of grain β-glucan that may be achievable by combining waxy endosperm and barley chromosome 7H. In 2010, we plan to initiate crosses between stocks carrying barley chromosome 7H and stock with waxy endosperm. The waxy endosperm stocks will be used as

the recurrent parents in two cycles of backcrossing and barley chromosome 7H will be tracked with DNA-based markers as we have done on other projects.

Objective 3: Test strategies to eliminate the production of gliadins in the wheat grain

Celiac disease is an allergic reaction to gliadins in wheat flour that affects ~1% of the U.S. population[14]. Because research has shown that removal of gliadins from flour or dough yield food that are tolerated by celiac disease patients, we have a project with the goal of producing gliadin-free wheat. There are a number of strategies that have been proposed to produce wheat with reduced levels of gliadins. We have opted for an approach whereby a transgene engineered to stop the expression of multiple gliadins is introduced into wheat through genetic transformation. We have already produced ~224 independent transgenic lines of Bobwhite wheat expressing a gliadin silencing transgene using biolistics transformation protocols[15]. Analyses of these materials have revealed quantitative variation for gliadin production but lines with a clear cut silencing of gliadins have not been identified. Our results are in line with other research on the silencing of multigene families in plants[16] where the majority of trangenics will show quantitative variation and only a fraction (~10%) will produce a knockout phenotype. Faced with these results, we have changed our strategy whereby lines with deletions (knockouts) of key genes in the gliadin regulatory network are being produced.

Timeline: All introgressions of *Gpc-B1* and *Yr36* were completed in 2008. Finished materials have been moved to the field for the pertinent evaluations. The introgression of CYDV will be finished in the next few months. The transfer of Cephalosporium stripe resistance has also been completed. Material has been planted in the field and will be evaluated in the upcoming growing season. The introgression of various adult plant stripe rust resistance genes (*Yr18*, *Yr29*, *Yr30*, *Yr36*, and various QTL) will require one generation of marker-assisted selection. The genetic dissection of PNW-based stripe rust resistance has been completed and we are in the process of identifying breeder-friendly markers for MAS. The genetic dissection of the “super-soft” trait has been completed also and we are in the process of identifying markers for indirect selection. The pyramiding of the *Gpc-B1* locus and various HMW glutenin loci in adapted hard white winter germplasm will require one to two rounds of marker-assisted backcrossing. The assessment of barley chromosome combinations on grain β-glucan content will be assessed this year. The effort to combine waxy endosperm and barley chromosome 7H will commence this year and will require two cycles of backcrossing. The characterization of gliadin accumulation in material with key gene deletions will start this season.

Justification: The aim of the proposed research is to enhance wheat germplasm for our region using genomics tools and targeted genetic variation. Wheat cultivar development is dependent on a continued supply of genetic variability. Thus, our research aims at widening the genetic base by tapping marker-tagged genetic variability. Similarly, the production of gliadin-free wheat and identification and use of new genetic factors that control the “super-soft” trait, adult-plant stripe rust resistance, protein quality, and grain β-glucan content is consistent with this aim and it is complementary to other wheat breeding and improvement efforts at Oregon State University.

Budget:

Salaries

Research Associate (0.25 FTE x 43,000)	$US 10,750

Other Payroll Expenses (OPE 57%)	$US 6,128
Two hourly workers	$US 20,400
Other Payroll Expenses (OPE 8%)	$US 1,632
Salaries Subtotal	$US 38,910
Services/Supplies	$US 7,000
Travel	$US 500
Overall Total	**$US 46,410**

The salary for the research associate was determined for 3 work-months assuming a 12-month full time yearly salary of $43,000 and a fringe benefit (OPE) rate is 57%. The research associate will have the responsibility of coordinating and planning experiments, summarizing, and analyzing data and performing other technical tasks germane to this project. The salary for two hourly student workers was determined for a 15hr/wk labor during the academic year and 40 hr/wk labor in the summer (~1,020 work-hr) each at $10.00/hr and a fringe benefit (OPE) rate of 8%. The hourly wage worker, under the supervision of the research associate, will have the responsibility of planting, growing, maintaining, harvesting plant material, inventorying seed, and performing assays germane to this project. Services and supplies dollars will be used to pay for laboratory and greenhouse supplies and fees, and biochemical reagents needed for the project. Travel dollars will be used to pay for travel expenses for the PI and other personnel associated with the execution of this research including in-state or out-of-state trips to annual review meetings and off-site meetings with collaborators on this project.

Relation to Other Research: Other research projects that are underway in my laboratory include a wheat applied genomics (USDA-NRI) project with the aim of developing a genetic map of wheat and the identification of genetic factors that control traits of agronomic importance. We also have an active project aimed at research and utilization of wheat genomics technologies for the manipulation of targeted genetic variation. The subject of this proposal represents a leading-edge non-funded component of our research effort on wheat that complements our current efforts.

References

1. Banks, P. M. et al., The use of cell culture for subchromasomal introgressions of barley yellow dwarf virus resisitance from *Thinopyrum intermedium* to wheat. *Genome* **38**, 395 (1995).
2. Singh, R. P., Huerta-Espino, J., and William, H. M., Genetics and breeding for durable resistance to leaf and stripe rusts in wheat. *Turk J Agric For* **29**, 121 (2005).
3. Suenaga, K., Singh, R. P., Huerta-Espino, J., and William, H. M, Microsatellite markers for genes *Lr34/Yr18* and othr quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. *Phytopathology* **93** (7), 881 (2003); William, M. et al., Molecular marker mapping of leaf rust resistance gene *Lr46* and its association with stripe rust resistance gene *Yr29* in wheat. *Phytopathology* **93** (2), 153 (2003).
4. Butow, B. J. et al., Molecular discrimination of Bx7 alleles demonstrates that a highly expressed high-molecular-weight glutenin allele has a major impact on wheat flour dough strength. *Theor Appl Genet* **107** (8), 1524 (2003).

[5] Butow, B. J. et al., Dissemination of the highly expressed Bx7 glutenin subunit (*Glu-B1al* allele) in wheat as revealed by novel PCR markers and RP-HPLC. *Theor Appl Genet* **109** (7), 1525 (2004).

[6] Ma, W., Zhang, W., and Gale, K. R., Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. *Euphytica* **134**, 51 (2003); Ragupathy, R. et al., Evolutionary origin of the segmental duplication encompassing the wheat *GLU-B1* locus encoding the overexpressed Bx7 ($Bx7^{OE}$) high molecular weight glutenin subunit. *Theor Appl Genet* **116** (2), 283 (2008).

[7] Brennan, C. and Cleary, L., The potential use of cereal (1→3,1→4)-β--glucans as functional food ingredients. *J Cereal Sci* **42**, 1 (2005); Braaten, J. T. et al., Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *Eur J Clin Nutr* **48** (7), 465 (1994).

[8] Sundberg, B. et al., Mixed-linked beta-glucan from breads of different cereals is partly degraded in the human ileostomy model. *Am J Clin Nutr* **64** (6), 878 (1996).

[9] Burton, R. A. et al., Cellulose synthase-like CslF genes mediate the synthesis of cell wall (1,3;1,4)-beta-D-glucans. *Science* **311** (5769), 1940 (2006).

[10] Riera-Lizarazu, O., Dewey, W.G., and Carman, J.G., Gibberellic acid and 2,4-D treatments for wheat x barley hybridization using detached spikes. *Crop Sci* **32**, 108 (1992).

[11] Islam, A.K.M.R., Shepherd, K.W. , and Sparrow, D.H.B., Isolation and characterization of euplasmic wheat-barley chromosome addition lines. *Heredity* **46**, 161 (1975); Taketa, S. and Takeda, K., Production and characterization of a complete set of wheat-wild barley (*Hordeum vulgare* ssp. *spontaneum*) chromosome addition Lines. *Breed Sci* **51**, 199 (2001).

[12] Cho, S., Garvin, D. F., and Muehlbauer, G. J., Transcriptome analysis and physical mapping of barley genes in wheat-barley chromosome addition lines. *Genetics* **172** (2), 1277 (2006).

[13] Rey, J. I. et al., Production of Dryland Barley for Human Food: Quality and Agronomic Performance. *Crop Sci* **49** (1), 347 (2009).

[14] Fasano, A. et al., Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch Intern Med* **163** (3), 286 (2003).

[15] Klein, T. et al., Transfer of foreign genes into intact maize cells with high-velocity microprojectiles. *Proc. Natl. Acad. Sci. USA* **85** (12), 4305 (1988); Barcelo, P. and Lazzeri, P. , in *Methods in molecular biology*, edited by H. Jones (Humana Press Inc., Totowa, 1995), Vol. 49, pp. 113.

[16] Wroblewski, Tadeusz et al., Silencing of the major family of NBS-LRR-encoding genes in lettuce results in the loss of multiple resistance specificities. *Plant J* **51** (5), 803 (2007).

CURRENT AND PENDING SUPPORT

NAME	SUPPORTING AGENCY	TOTAL $ AMOUNT	EFFECTIVE AND EXPIRATION DATES	% OF TIME COMMITTED	TITLE OF PROJECT
Current:					
O Riera-Lizarazu	NSF-PGRP via North Dakota St. Univ. subcontract	598,871	01/01/09 -12/30/12	4	Transformative research on the construction of high-resolution physical maps for the large plant genomes
O Riera-Lizarazu and CJ Peterson	USDA-NRI-CAP subcontract	182,750	11/1/05-10/31/10	2	Wheat Applied Genomics
O Riera-Lizarazu	ARF-OWC	43,037	7/1/09-6/30/10	2	Using Genomics to Enhance Wheat Traits by Design
O Riera-Lizarazu	ARF	11,707	7/1/09-6/30/11	2	Engineering a chromosome that confers resistance to a fungal disease of wheat
Pending:					
O Riera-Lizarazu	ARF	12,427	7/1/10-6/30/12	2	Enhancing the nutritional value of wheat by increasing its dietary fiber content