

PROPOSAL TO THE AGRICULTURAL RESEARCH FOUNDATION OREGON WHEAT
COMMISSION

Title: Using Genomics to Enhance Traits and Decrease Allergy-Inducing Proteins from Wheat
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Abstract:

An important component of our research is based on the utilization of wheat genomics technologies and manipulation of targeted genetic variation. Since wheat cultivar development is dependent on a continued supply of genetic variability, our project is focused on the use of molecular marker-facilitated manipulation of factors for cereal yellow dwarf virus (CYDV) and Cephalosporium stripe resistance, grain protein, iron, and zinc content (Gpc-B1), and adult plant resistance genes against stripe rust (Yr18, Yr29, Yr30, Yr36, and htap-1). The transfer of some of these genes to Oregon wheat germplasm has been achieved but transfers of others are still in progress. So far, we have finished the development of wheat germplasm based on Stephens, OR943576, Weatherford, and Winsome that now carry Gpc-B1 and Yr36. This finished material is being evaluated and characterized under field conditions. End use quality in wheat is primarily determined by grain hardness or texture. The 'super-soft' grain characteristic positively affects flour yield and end-use quality. Since this characteristic has resulted in the development of a novel class of soft white wheat with the potential to widen export markets, there is now an interest in understanding the genetic basis of this trait and to identify markers for its manipulation. Thus, we are using map-based methods to better understand the "super-soft" characteristic. Another component of our research effort addresses celiac disease, an allergic reaction to some seed storage proteins in wheat flour. Currently, the only treatment for celiac disease is the lifelong avoidance of food products that contain wheat and other cereal grains. Seed storage proteins, known as gliadins, have been identified as the principal allergy-eliciting agents. In addition, studies have shown that removal of gliadins from flour or dough yield food that are tolerated by celiac disease patients. Thus, we have a project to use transgenic technology to engineer gliadin-free wheat. We have produced over 200 transgenics and we are now analyzing the pattern of gliadin accumulation in these materials. Wheat devoid of gliadins would be a much needed food alternative to manage this important human ailment.

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), Cephalosporium stripe, and stripe rust; 2. develop germplasm with increased grain protein, iron, and zinc concentration; 3. identify DNA markers associated with the "super-soft" trait; and 4.

determine the pattern of gliadin accumulation in transgenics engineered to eliminate the production of gliadins

Procedures:

Objectives 1 and 2: Development of germplasm with resistance to CYDV, Ceph. stripe, stripe rust, and increased grain protein, iron, and zinc content

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 three generations. After the 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. In this proposal we seek support to continue with the marker-assisted transfers that are described below.

Transfer of Th. intermedium chromatin in P29, TC14, and TA5551 to Oregon wheat germplasm: Yellow dwarf [caused by the cereal yellow dwarf virus (CYDV) and the barley yellow dwarf virus (BYDV)] is the most important viral disease of wheat worldwide. High levels of resistance to this disease is limited in winter wheat. On the other hand, complete resistance to BYDV and CYDV have been documented in some wheat relatives such as Th. intermedium. Consequently, we have been transferring this source of resistance to Oregon wheat germplasm. TC14 and TA5551 (Banks et al. 1995) 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. The movement of the pertinent Th. intermedium chromosome (and CYDV resistance) in subsequent generations is being monitored using Thinopyrum-specific DNA markers (3P3/3P4, gwm37, Bdv2, and BYAgi). The third and fourth backcross generations (BC3, and BC4) were produced in the 2005-2006 and 2006-2007 seasons. During our backcrossing, we discovered that our advanced derivatives were male-sterile. Because the CYDV donors were used as the original female parents, we suspect that the lack of male fertility is due to nuclear-cytoplasmic incompatibility. Thus, we crossed our materials to male fertility restorers during the spring and summer of 2007. These materials will be grown next season and crossed to move them into a more receptive cytoplasm to permanently restore self fertility.

Transfer of a Th. elongatum chromosome in WA7437 and CI13113 to Oregon wheat germplasm: Cephalosporium stripe (caused by *C. gramineum*) is an important vascular disease of wheat in the U.S. Pacific Northwest and worldwide. Resistance to Ceph. stripe in wheat germplasm is limited but resistance has been found in a wheatgrass, Th. elongatum. Consequently, we have been transferring this resistance to Oregon wheat germplasm. CI13113 and WA7437 are Ceph. stripe-resistant lines (Cai et al. 1996) where the Th. elongatum (or Th. ponticum) 6Ae#2 chromosome has replaced the 6A wheat chromosome. The movement of the 6Ae#2 Th. elongatum chromosome (and Cephalosporium stripe resistance) is being monitored using a Thinopyrum-specific DNA marker (3P3/3P4). In 2007, we screened BC3S1 materials to identify those that still carried the factor of interest. Because the marker we used (3P3/3P4) for selection is dominant, we will perform progeny

tests next season (2008) to identify families where the resistance factor is now fixed.

Transfer of Gpc-B1 and Yr36 to Oregon wheat germplasm: Grain protein concentration (GPC) and composition affect the nutritional value of wheat as well as its end-use quality. Improvement of GPC by conventional breeding methods has been effective but difficult due to limited genetic variation and the negative correlation of GPC with grain yield. One promising source of high-GPC is the wild tetraploid *T. dicoccoides* (Joppa and Cantrell 1990). Recent research has shown that a stripe rust (caused by *Puccinia striiformis*) high-temperature adult plant (HTAP) resistance gene (Yr36) is closely linked to the GPC locus (Gpc-B1) (Uauy et al. 2005). In addition, materials that carry Gpc-B1 have been shown to contain higher levels of iron and zinc in their seeds (Distelfeld et al. 2007). Consequently, we have been transferring these cluster of genes into Oregon wheat germplasm. Tetraploid and hexaploid wheat stocks LDN(DIC-6B), and Glupro contain a *T. dicoccoides* chromosome 6B segment (Mesfin et al. 1999) that harbors Gpc-B1 and Yr36. The movement of the Gpc-B1 and Yr36 is being monitored with three to four DNA-based markers (gwm508, ASA, uhw89, and gwm193). In 2006, we finished lines based on Stephens and OR943576. In 2007, we finished lines based on Weatherford and Winsome. In 2008, we will finish lines based on Tubbs. All of the finished lines are under field evaluation.

Transfer of durable resistance against stripe rust: Stripe rust (caused by *Puccinia striiformis*) is a devastating disease of wheat. A number of race-specific genes conferring resistance to this disease have been identified in wheat germplasm. Because the majority of these major genes are currently not effective against current races of stripe rust, there is a need to concentrate on resistance genes that are non-race-specific and more durable. Oregon wheat germplasm carry some non-race-specific high temperature adult plant (HTAP) resistance to stripe rust. At least one determinant of HTAP in the variety Stephens (htap-1) resides on chromosome 6B and DNA markers linked to this resistance is known (Santra et al. 2005). Similarly, wheat germplasm from the International Maize and Wheat Improvement Center (CIMMYT) in Mexico carry adult plant resistance (APR) genes to stripe rust that are different from those used in the U.S. Pacific Northwest. Parula, a wheat variety developed at CIMMYT, carries three stripe-rust adult plant resistance genes (Yr29, Yr18, and Yr30). Gene mapping activities at CIMMYT and elsewhere have localized Yr29, Yr18, and Yr30 on chromosomes 1B, 7D, and 3B, respectively (Suenaga et al. 2003, William et al. 2003). Thus, the introduction of Yr29, Yr18, and Yr30 in addition to the hta1-1 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 Foote, Tubbs, OR980757, and OR010920. In 2007, we generated lines that are at the first and second backcross level (BC1 and BC2). In 2008, we plan to perform another round of marker-assisted backcrossing.

Objective 3: Identify DNA markers associated with the "super-soft" trait End use quality in wheat is primarily determined by grain hardness or texture. The 'super-soft' grain characteristic positively affects flour yield and end-use quality. Thus, there is considerable interest in knowing the location, number, and effect of genetic loci that control this trait. In order to understand the genetic basis of this trait, we have constructed a genetic map (Wang et al. 2007) using a mapping population derived from a cross between Stephens (hardness index ~37) and OR9900553 (hardness index ~20), a breeding line with the 'super-soft' grain characteristic. In a preliminary analysis, four quantitative trait loci (QTL)

were detected on chromosomes 4B, 4D, 5A, and 5B. Present results indicate that the 'super-soft' characteristic is controlled by several factors that are different from the hardness (Ha) locus on chromosome 5D. A more thorough assessment with a larger phenotypic data set and a denser map is ongoing. This activity is part of my research project's involvement with a Coordinated Agricultural Project (CAP) grant from USDA's National Research Initiative. The CAP project was awarded to the wheat breeding community (~17 breeding programs) and its major thrust is the identification of genes of agronomic interest and the implementation of DNA-based markers for crop improvement. We had specifically targeted grain hardness as the area that our group would emphasize. Due to the large scale of the CAP project and the number of research groups involved, funding from USDA-NRI will not be sufficient to fully staff a research project. Consequently, each group is expected to secure additional extramural funds to permit the successful completion of this project. In this regard, a portion of the funding from this proposal will be used to complement our involvement in this national wheat CAP project. The aim of this component of our proposal is to identify markers that are associated with the "super-soft" grain trait.

Objective 4: Determine the pattern of gliadin accumulation in transgenics engineered to eliminate the production of gliadins. Celiac disease is an allergic reaction to gliadins in wheat flour that affects ~1% of the U.S. population (Fasano et al. 2003). 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 to use transgenic technology to engineer gliadin-free wheat. There are a number of strategies that have been proposed to produce wheat with reduced levels of gliadins. Given the number of gliadin genes (25 to 100) and chromosomes (1A, 1B, 1D, 6A, 6B, and 6D) that harbor them, the one-gene-at-a-time approach of mutagenesis, cytogenetic manipulation, and selective breeding will not be as efficient as an approach that eliminates all gliadins simultaneously. For this reason, we have opted for an approach whereby a transgene engineered to stop the expression of multiple gliadins is introduced into wheat through genetic transformation. Since we have already produced the transgenics, we seek support to continue our first level of analysis of these materials. This analysis entails an assessment of gliadin accumulation in the grain of transgenics and their progeny.

We have already produced ~224 independent transgenic lines of Bobwhite wheat expressing a gliadin silencing transgene using biolistics transformation protocols (Klein et al. 1988, Barcelo and Lazzeri 1995). Last year, we performed a preliminary screening of first-generation (T0) transgenics to check for insertion of the pertinent transgene and to assess its expression. This year, we performed additional assays to identify lines that contain the gliadin-silencing construct in the correct configuration and also showed strong expression for a linked glufosinate (herbicide) resistance gene. These screenings identified ~99 lines that we have started to analyze with respect to gliadin production. Gliadin composition of seeds of transgenics and the untransformed Bobwhite control will be principally performed using acid polyacrylamide gel electrophoresis (A-PAGE) (Ng et al. 1988). Subsequently, reverse-phase high pressure liquid chromatography (RP-HPLC) (Wieser et al. 1998) will be used to validate results from A-PAGE. Lines that have a characterized reduction or loss of gliadins will be increased and prepared for the second level of analysis. In the second level of analysis, we would be interested in determining the impact of reduced levels or a lack of

gliadins on end-use quality of the flour as well as the allergenicity of wheat products from these materials. This second level of analysis is beyond the scope of this proposal.

Timeline:

All introgressions of Gpc-B1 and Yr36 will be completed by summer 2008. Finished materials will then be moved to the field for the pertinent evaluations. The introgression of CYDV will require one to two more seasons of crossing and selection to restore male-fertility. The transfer of *Cephalosporium* stripe resistance is basically completed, only requiring one season of progeny testing. The introgression of various adult plant stripe rust resistance genes (Yr18, Yr29, Yr30, Yr36, and hta^p-1) will require two to three generations of backcrossing and selection. The genetic dissection of the "super-soft" trait will be completed by the end of the fall 2008. The characterization of gliadin accumulation in transgenic material and the seed increase of selected materials should be completed by fall 2008.

Justification:

The aim of the proposed research is to contribute to the profitability and sustainability of wheat production in Oregon based on research and utilization of 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 genetic variability in *Th. intermedium*, *Th. elongatum*, and *T. dicoccoides*. Similarly, the identification of genetic factors that control the "super-soft" trait and incorporation of more durable non-race-specific stripe rust resistance genes is consistent with this aim and it is complementary to our current activities. The use of genomics tools complements other wheat breeding and improvement efforts at Oregon State University. Wheat is widely used in a number of food products. However, a significant fraction of the American population (1% or 3 million individuals) cannot consume wheat products because they suffer from celiac disease, an allergic reaction to gliadin proteins in wheat flour. Currently, the only treatment for celiac disease is the lifelong avoidance of food products that contain wheat and other cereal grains which is not only an unpalatable proposition but also quite expensive. Thus, our project to produce gliadin-free wheat would result in a welcome food alternative for celiac disease patients. Gliadin-free wheat also has the potential to penetrate the gluten-free and health foods market.

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. We have recently developed DNA-based markers to track Pch1, a major gene that confers resistance against strawbreaker foot rot (eyespot) (Leonard et al. 2007). The subject of this proposal represents a leading-edge non-funded component of our research effort on wheat that complements our current efforts.

References

Banks et al. 1995. The use of cell culture for subchromosomal introgression of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. *Genome* 38:395-405.

Barcelo et al. 1995. Transformation of cereals by microprojectile bombardment of immature inflorescence and scutellum tissues. p113-123. In H. Jones (ed.) *Methods in Molecular Biology*. Vol 49: Plant gene transfer and expression protocols. Humana Press Inc., Totowa, NJ.

Cai et al. 1996. Characterization of an *Agropyron elongatum* chromosome conferring resistance to *Cephalosporium* stripe in common wheat. *Genome* 39:56-62.

Distelfeld et al. 2007. Multiple QTL-effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. *Physiol. Plantarum* 129:635-643.

Fasano et al. 2003. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States. *Arch. Intern. Med.* 163:286-292.

Joppa and Cantrell. 1990. Chromosomal location of genes for grain protein content of wild tetraploid wheat. *Crop Science* 30:1059-1064.

Klein et al. 1988. Transfer of foreign genes into intact maize cells with high-velocity microprojectiles. *Proc. Nat. Acad. Sci. USA.* 85:4305-4309.

Leonard et al. 2007. Identification of a candidate gene for the wheat endopeptidase Ep-D1 locus and two other STS markers linked to the eyespot resistance gene Pch1. *Theor. Appl. Genet.* (in press)

Mesfin et al. 1999. RFLP markers associated with high grain protein from *Triticum turgidum* L. var. *dicoccoides* introgressed into hard red spring wheat. *Crop Sci* 39:508-513.

Ng et al. 1988. A catalog of biochemical fingerprints of registered Canadian wheat cultivars by electrophoresis and high performance liquid chromatography. Publication no. 139 of the Food Sci. Dept. university of Manitoba, Winnipeg, Canada, pp.1-83.

Santra et al. 2005. Mapping QTL for high temperature adult plant resistance to stripe rust in wheat (*Triticum aestivum* L.). *Agronomy Abstracts*. (<http://crops.confex.com/crops/2005am/techprogram/P4381.HTM>)

Suenaga et al. 2003. Microsatellite markers for genes Lr34/Yr18 and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. *Phytopathology* 93:881-890.

Uauy et al. 2005. High-temperature adult-plant (HTAP) stripe rust resistance gene Yr36 from *Triticum turgidum* ssp. *dicoccoides* is closely linked to the grain protein content locus Gpc-B1. *Theor. Appl. Genet.* 112: 97-105.

Wang et al. 2007. Identifying QTLs for the 'extra-soft' characteristic in wheat. In: *Abstracts of Plant & Animal Genome XV*, January 13-17, San Diego, CA. P283

Wieser et al. 1998. Quantitative determination of gluten protein types in wheat flour by reversed-phase high-performance liquid chromatography. *Cereal Chem.* 75:644-650.

William et al. 2003. Molecular marker mapping of leaf rust resistance gene Lr46 and its association with stripe rust resistance gene Yr29 in wheat. *Phytopathology* 93:153-159