Response to Previous Review

In 2012 we submitted a proposal to the **AFRI Integrated Approaches to Climate Adaptation and Mitigation in Agroecosystems** program. Our proposal number was 2012-00996 and the proposal title was "Bringing barley to the table: addressing the challenges of climate change and human nutrition". It was an integrated proposal with a significant (and funded) Extension component. *The proposal was ranked "high priority" but was not funded.*

Panel Synthesis *"A strong, integrated project with a high probability of success based on the project personnel.*" For the purposes of response to review we will address only the negatives and weaknesses identified by the panel and the reviewers.
Panel comment - negative: "*The panel recognized that this research group has existing funding for this type of research, and the overlap (or lack thereof) between this proposal and the currently funded research project should have been justified."*
Response: Members of our group are indeed funded to perform genome wide association mapping and genomic selection in barley, but these activities do not involve food barley. Our efforts are focused on water use efficiency, nitrogen use efficiency, and low temperature tolerance in very distinct germplasm that is bred for superior malting quality. There are many distinctions that preclude the same variety from being optimum for malting and food. Our experience with genome wide association mapping and genomic selection for other traits positions us to efficiently and productively direct our efforts at food quality. We emphasize in the current proposal that the Federal investment in the CAP projects allows us to leverage value for food barley improvement. If we were to start this food research de novo, the cost would be prohibitive.

Reviewer 1 ranked the proposal Very good/Excellent in all categories and had no negative comments.

Reviewer 2: *"I'm still not sure how this proposal ties in to climate change, as the authors haven't really hit me over the head with it, but I can see that if barley is genetically engineered to be high-yielding and has a low temperature tolerance, and is facultative in terms of a vernalization period, then there might be some advantages to growing this plant in a climate that is warmer and has precipitation events that are shifted to earlier season periods."*
Response: This review is rather puzzling, since we did not (and do not) propose to develop genetically engineered barley. Rather our focus is on understanding and exploiting the native genetic variation in the crop. While climate change remains a framework for our work, it is not a central theme.

Reviewer 3: This reviewer had issues with our management plan, logic model and the nature of the (funded) Extension component. The one page limit for this Response to Review precludes addressing these in detail. However, there is no need to do so: these are not required components in 2013. We recognize the need for Outreach and Extension. The grower organization and industry letters of support for the 2013 proposal indicate that we have engaged growers and the industry. We appreciate that our Extension colleagues face budget constraints and are in no position to take on new and un-funded endeavors. Nonetheless they are engaged and willing to help as the can with the 2013 project, as witnessed by their letters of support.

Project Narrative

Introduction

Long-term goals and supporting objectives: Our long-term goal is to integrate contemporary plant breeding and cereal chemistry in order to develop innovative breeding methodologies and novel varieties. Our model is barley and our focus is healthy grain that will assist the nation in addressing the challenges of serious health issues aggravated by diets high in processed foods, sugars, and fats. Our research is conducted with a general framework of climate change: our focus is on winter germplasm that will make the most efficient use of precipitation patterns and shifting temperature regimes. We recognize the critical importance of Extension in efficiently translating breeding and chemistry results for growers and industry. Funding constraints limit formal incorporation of Extension into this project. However, all participants in this project have close ties with Extension and also participate directly in communication with growers and industry. The attached letters of support from farmer organizations and food processing industries attest to the value and effectiveness of these linkages.

Our supporting objectives are integrated but can be classified as "Plant Breeding" and "Cereal Chemistry".

Breeding Objectives: 1) Initiate doubled haploid genomic selection: a breeding method that will allow for the accelerated and sustained development of new food barley varieties; 2) Discover new genes and novel alleles at known genes, using a genome wide association mapping panel. This panel will also serve as a training population for genomic selection; 3) Develop diverse doubled haploid lines with low temperature tolerance, resistance to diseases, and a menu of food quality characteristics.
Cereal Chemistry Objectives: 1) Define basic parameters for barley food quality in a range of germplasm; 2) Contribute to a fundamental understanding of the genetics of food quality traits and their environmental plasticity; 3) Develop and extend a menu of barley food products.

The body of knowledge: For the purposes of exposition, the following summary is divided into four separate sections that address (1) barley foods, (2) barley as a crop, (3) winter and facultative growth habit, and (4) contemporary plant breeding.

1. Barley for food and health: Barley has a long history as a viable crop and its nutritional properties are well documented. The need is to move from research to application: barley is the fourth most important cereal crop in the world (FAO-STAT, 2011) and most familiar as the principal grain used for malting and brewing, and as an animal feed. However, barley is also one of the world's oldest food crops. Unfortunately in modern day America, barley has all but disappeared as a food raw material despite its virtues as a fiber-rich and versatile grain. Creating a food barley market in the 21st century has proven as challenging as creating a market for entirely novel grains, such as teff or quinoa. However, there is renewed enthusiasm for food barley and it comes from increasing public awareness of the value of healthy eating. To support the value of barley as part of mainstream American diets we cite the AARP-NIH cohort study (Park et al., 2011). In this study of 388,000 participants, dietary fiber was linked to decreased risk of death from cardiovascular disease, cancer, and infectious and respiratory diseases. Notably, Park et al. (2011) concluded that "Dietary fiber from grains, but not from other sources, was significantly inversely related to total and cause-specific death in both men and women".

This conclusion emphasizes the need to get food products rich in cereal fiber into the mainstream diets of Americans. Barley, as a rich source of cereal fiber, is part of the solution and can help to address one of the USA's most pressing dietary problems: the grossly inadequate fiber intake of most Americans (Slavin, 2005). The principle fiber found in barley is "beta-glucan", a soluble fiber. Barley has the advantage for consumers that it provides its fiber and other healthful components in a package that has half or less of the fat content of the other main cereal beta-glucan source, oats (Svihus and Gullord, 2002), and with a greater total dietary fiber content than wheat, oats, or rye (Cho et al., 1999; Izydorczyk, 2010). Barley beta-glucan is effective in reducing the incidence and severity of "metabolic syndrome" (PubMed Health, 2011) through increased satiety, slowed macronutrient absorption, reduced post-prandial glucose response, lowered blood cholesterol levels, reduced insulin resistance, and reduced abdominal fat (AbuMweis et al., 2010; Arndt, 2006; Bays et al., 2011; Behall et al., 2006; Casiraghi et al., 2006; Kim et al., 2009; King et al., 2008; Shimizu et al., 2008; Thondre and Henry, 2009; Tiwari and Cummins, 2011;Vitaglione et al., 2010). The capacity of barley foods to reduce cholesterol was the key factor in the successful approval of the FDA health claim for barley (21 CFR 101.81) (Ames and Rhymer, 2008; National Barley Foods Council, 2003). Barley also supplies other bioactive nutrients (phenolics, phytate, and tocols) that are potent antioxidants (reviewed by Baik et al., 2010; Holtekjolen et al., 2011). Barley can enrich foods that are otherwise lacking in these valuable components (Verardo et al., 2011).

Barley starches vary in their amylose content (Lagassé et al., 2006). Amongst starch variants, *high amylose starches* are favored for the creation of one form of resistant starch, which is formed by amylose retrogradation (also called recrystallization)(reviewed by Ross, 2011; Ross, 2013). However, even normal barley starches tend to have a higher amylose to amylopectin ratio than wheat and accordingly retrograde more readily (van Amelswoort and Westrate, 1992; Sullivan et al., 2012). Resistant starch is not digested in the human digestive tract but is fermented in the colon (Topping and Clifton, 2001; Nugent, 2005). All colonic fermentations produce short chain fatty acids but resistant starch is associated with higher levels of butyric acid. Butyric acid is believed to act as a cell growth regulator and has protective effects against the onset and proliferation of colo-rectal cancers (Fung et al., 2012). High amylose barley has been incorporated successfully into foods made with composite barley/wheat flours (Hatcher et al., 2005; Lagassé et al., 2006).

A notable advantage of food barley is that it can be produced, transported, stored, and processed with currently available infrastructure, thereby greatly reducing the need for additional investments throughout the value chain. For consumers, barley easily fits into familiar products. It can be used as whole (intact) or cracked grain, including its use as a high-fiber and tasty rice alternative (Edney et al., 2002; Gray et al., 2010). Barley flour or its fractions can be used as components in flatbreads (Izydorczyk et al., 2008), tortillas (Prasopsunwattana et al., 2009), and even in risen breads and sponge cakes where it can provide desirable textures and improved keeping quality (Gupta et al., 2008; Skendi et al., 2010). Breads have also been made from 100% barley flour without the admixture of wheat (Kim and Yokoyama, 2010; Kinner et al., 2011). Adding barley to mainstream U.S. diets will add a diversity of flavors, colors, and aromas as well as increasing the diversity of cereal fiber sources. The latter is important as humans need fiber from various sources for optimal functioning: e.g. beta-glucan from barley and oats, arabinoxylan from rye and wheat, and pectin from fruits.

One of the impediments to general acceptance of barley as a food raw material by processors is the lack of a classification system. The need stems from barley's wide genotypic

variation in processing and compositional traits: kernel hardness; hull-less/hulled character; starch amylose content; beta-glucan content; pericarp pigmentation; and total phenolic, phytate, and tocol contents (Baik and Ullrich, 2008). Despite the diversity of available traits at present, the commodity is generally sold only as "food barley". A classification system would let buyers know what they are getting and enable breeders to target specific classes, making breeding efforts more effective. Without a workable classification system, costly errors will occur: e.g., the inadvertent use of a proanthocyanidin-containing genotype, which may add color to products when it is not desirable (Quinde et al., 2004; Quinde-Axtell et al., 2005). Likewise, receiving a soft kernel type in a pearling operation for a rice substitute, where hard kernels are preferred, can also be costly (Baik and Ullrich, 2008). We propose to use the doubled haploid material to characterize quality and create a naming system that could be proposed to the grower and processor communities as a model for commercial classification of food barley. Within the 300 doubled haploids in the training population we anticipate finding most combinations of these categorizable factors. A key issue will to be to determine the feasibility of assessing traits that cannot be assessed visually (e.g. starch characters) in the marketing stream.

2. The sustainability and profitability of barley production: Barley is cultivated worldwide due to its adaptation to abiotic and biotic stresses. Climate change is expected to lead to more volatile climatic conditions and more frequent exposure to stresses of all types. However, even the most stress-tolerant crop must have economic value. Wheat is the usual alternative to barley, and in the U.S. returns from wheat have typically been higher than for barley. Summary price comparisons between wheat and barley are complicated given price fluctuations and the diversity of wheat market classes grown in Oregon, Idaho, Washington, and Minnesota (the four states that we are focusing on in the current project). By way of example, on February 9, 2013 soft white winter wheat delivered to Portland Oregon was quoted at 14.5 cents/lb and feed barley was 13.3 cents/lb. Quotes for food barley are not available, given the small market share currently occupied by food barley. However, assuming a premium structure for food barley and the improved position of barley in crop insurance programs, the wheat: barley gap is closing. In addition to direct price comparisons, barley brings to the farmer a shorter growing season and lower inputs than wheat. This reduces production costs, makes better use of expensive inputs, reduces risk, and increases the efficiency of machinery use. Finally, barley has nutritional and flavor properties that are simply not available in wheat.

3. Winter barley and climate change: The focus of this project is on winter food barley. Winter barley uses the fall period for establishment, thereby allowing the crop to take advantage of a longer growing season in the spring and early summer. The crop ripens prior to the summer heat and drought that robs spring-sown cereals of yield potential. Winter barley that can survive low temperature stresses offers solutions to many of the problems facing spring barley because it can be produced in areas where Fusarium head blight is not such a serious risk (e.g. Western states) and its early maturity may allow for production in Fusarium-prone regions such as the upper Midwest. In addition, due to its short growing season it may be double cropped, with soybeans for example, which would help to provide continuous cover, reduce erosion, and provide other ecosystem services. In regions that receive winter precipitation, dryland winter barley yields are typically 20% higher than those of spring barley. In production areas where winter precipitation is limiting but irrigation is available, winter and spring barley yields are similar but a winter crop can be produced with one to two fewer irrigation applications than a spring crop.

Winter barley consists of two growth habit types: obligate winter and facultative. Low temperature tolerance is essential for both types in our target environments. Obligate winter types require vernalization, a period of low temperature required for the transition from the vegetative to reproductive state. This limits winter types to fall-sowing. Facultative types have the capacity to cold-acclimate and achieve levels of low temperature tolerance equal to obligate winter types but they do not require vernalization. Therefore, they can give the grower absolute flexibility in planting date, which is a key consideration in the expansion of barley growing areas.

The OSU program has been an active contributor to the Triticeae genetics literature on low temperature tolerance, vernalization and photoperiod sensitivity and we continue this activity under the auspices of the Triticeae Coordinated Agricultural Project (TCAP). This research focus allows us to ensure that candidate genes for these target traits, or markers linked to QTLs determining these traits, are represented in the SNP genotyping platforms available for barley (see next section on contemporary plant breeding). Recent collaborations between the Oregon and Minnesota breeding programs and have led to significant strides in both breeding and genetics, including the discovery of a new low temperature tolerance QTL – *Fr-H3* (Fisk et al., 2013). The genetics of low temperature tolerance, vernalization sensitivity, and photoperiod sensitivity were reviewed by Fisk et al. (2013). For the purposes of this proposal and to benefit reviewers not familiar with this literature, key points are as follows. There are three principal QTLs determining low temperature tolerance *Fr-H1* (von Zitzewitz et al., 2005), *Fr-H2*, approximately 30 cM from *Fr-H1* on chromosome 5H (Francia et al., 2004; Skinner et al., 2005; Galiba et al., 2009) and *Fr-H3* located on chromosome 1H (Fisk et al., 2013). The epistatic interaction among alleles at three loci (*VRN1*, *VRN2* and *VRN3*) determines vernalization sensitivity (Yan et al., 2004; von Zitzewitz et al., 2005; Trevaskis et al., 2007). Candidate genes for all three loci are established. There are two loci determining photoperiod sensitivity: *PPD-H1* (Turner, 2005) and *PPD-H2* (Casao, 2011). Candidate genes are established for both loci. The winter allele at *PPD-H2* is essential in a fall-sown crop for delaying the vegetative to reproductive transition until daylength reaches a threshold that occurs after the major risk of late winter/early spring low temperature injury has passed. Facultative growth habit is defined by a spring (recessive) allele at *VRN2* coupled with a complete "winter" wiring system at all other low temperature tolerance, vernalization, and photoperiod loci. Facultative genotypes are, therefore, competent to acclimate and achieve maximum levels of cold tolerance if necessary. Since they do not require vernalization to flower, they can be planted at any time, giving growers tremendous flexibility in planting date.

4. Molecular Breeding: Breeding for new traits and types of plant products - such as food barley - is possible thanks to the tools of molecular breeding and the rapid generation advance via doubled haploids. Public sector cereal breeding programs have the opportunity to leverage significant Federal research investments to breed for new traits and attributes. The barley component of the current TCAP is focused on mapping and breeding nitrogen use efficiency and water use efficiency in spring barley and winter six-row malting barley. Improving barley for human nutrition is not supported by the TCAP, but we can leverage TCAP genotyping resources and analysis pipelines in our doubled haploid genomic selection food barley project. The USDA-NIFA Coordinated Agricultural Projects have generated marker resources, created a centralized trait and marker database with data analysis tools, developed mapping populations, conducted QTL mapping and genome wide association mapping, carried out extensive genotyping of core

collections and breeding germplasm, and initiated marker assisted selection and genomic selection approaches in breeding.

By capitalizing on the infrastructure and knowledge base created through these projects, we can realistically propose to develop new winter food barley varieties in a compressed time frame. We will use three principal tools of contemporary molecular plant breeding: genome wide association mapping, genomic selection, and doubled haploid technology. Jointly, these tools will allow us to characterize the genetic architecture of food quality traits and rapidly develop an array of winter food barley germplasm that will be the foundation for a nationally coordinated public plant breeding effort.

Genome wide association mapping: Any marker-based breeding effort will be better informed by a general understanding of the genetic architecture of the traits under selection. Of particular interest is the number of loci controlling the trait(s), the magnitude of the allelic effects, and repulsion phase linkages between unfavorably correlated traits. Genome wide association mapping allows investigators to assemble arrays of germplasm tailored to the question at hand. In barley, genome wide association mapping panels have been constructed from wild barley for the discovery of novel alleles as well as elite breeding material to characterize useful genetic variation that is more immediately useful in breeding (Roy et al., 2010; Cuesta-Marcos et al., 2010; Massman et al., 2011; Kraakman et al., 2004; Kraakman et al., 2006; von Zitzewitz et al., 2011). In addition, since germplasm arrays can be assembled from existing genotypes, it is possible to generate populations with sufficient size to overcome problems of lack of power to detect QTL and biased estimates of QTL effects (Vales et al., 2005; Beavis, 1994; Melchinger et al., 1998; Schön et al., 2004). The USDA Barley Coordinated Agricultural Project (CAP) was established to create large phenotypic and SNP marker data sets of elite breeding lines from ten U.S. breeding programs with which to conduct genome wide association mapping (Waugh et al., 2009). The SNP genotyping platform, trait and marker database (The Hordeum Toolbox; http://hordeumtoolbox.org), and wealth of QTL information generated from the CAP provide a rich framework and set of breeder's tools with which to initiate ambitious new breeding efforts such as winter food barley. We will use these tools on a panel of 300 doubled haploids constructed to capture broad genetic diversity for food barley traits and important agronomic traits necessary to deploy varieties in a range of target environments. This panel will serve as both the mapping population for genome wide association mapping and the training population to conduct genomic selection. As detailed in the Approach section, the panel will be genotyped with the Illumina Infinium 9K assay, optimizing QTL/gene discovery through marker density and providing an extensive catalog of markers for the Sequenom assays that will be used for genomic selection, as described in the next section. Although genomic selection is based on estimation of allele effects without prior knowledge of the underlying genes, the approach can benefit from the inclusion of markers based on genes known to be related to the target traits. In our case, we have an extensive catalog of markers in candidate genes for, or linked to QTLs associated with, low temperature tolerance, disease resistance, and multiple food quality traits (von Zitzewitz et al., 2011; Islamovic et al., 2013).

Genomic selection: Traditional marker assisted selection, while useful for simply inherited traits controlled by few loci, loses effectiveness as the number of loci increases. This is true for individual quantitative traits or when multiple traits are under selection. Genomic selection uses a training population that has been phenotyped and genotyped to estimate effects for a large set of markers distributed across the genome (Meuwissen et al., 2001). The marker effects are applied to an individual that has only been genotyped to estimate its breeding value

(GEBV). The primary benefit of genomic selection is that parents with superior breeding value for quantitative traits can be identified very early in the breeding process substantially reducing the breeding cycle time (Heffner et al., 2010). This allows for an accelerated recurrent selection program. In addition to rapid cycle selection of parents, genomic selection can be applied to segregating inbred or doubled haploid lines derived from early generation parents to predict line performance *per se*.

Promising assessment of genomic selection in animal systems has prompted a flurry of activity exploring the feasibility of genomic selection in plant breeding. Initial optimism was supported by simulation studies that demonstrated greater response to selection using genomic selection compared to conventional marker assisted selection or phenotypic selection (Bernardo, 2008; Iwata and Jannink, 2011). These were followed by empirical studies using cross-validation that further supported advantages of genomic selection (Lorenzana and Bernardo, 2009; Heffner et al., 2010, 2011). Subsequent studies have shown good prediction accuracy can be obtained with relatively small training populations (hundreds) compared to animals systems that use thousands of individuals (Heffner et al., 2011; Lorenz et al., 2012). Similarly, no significant increase in accuracy occurred when the number of markers increased beyond 384 in barley (Lorenz et al., 2012). In the same study, two closely related breeding programs were used as training and validation sets. Prediction accuracy was greater when the same program was used for the training population and selection candidates indicating that the composition of the training population is an important determinant of prediction accuracy. Comparison of various models to estimate marker effects have generally shown little difference among models and that the model with the simplest assumptions (ridge regression BLUP) can be used effectively (Lorenzana and Bernardo, 2009; Heslot et al., 2012; Zhong et al., 2009; Crossa et al., 2010; Lorenz et al., 2012). Taken together, these studies indicate that using ridge regression BLUP, a training population of 300 individuals that is closely related to the selection candidates, and ~400 markers will be the best approach to generate prediction accuracies that will substantially improve genetic gain per year in a facultative food barley breeding program.

Despite recent progress in assessing genomic selection in plant breeding, many questions remain unanswered with respect to the optimization of genomic selection and its application in various plant breeding scenarios. Most studies mentioned thus far have assessed prediction accuracy through cross-validation. A critical research need is to assess genomic prediction accuracy on progenies that result from crosses of parents that are members of the training population as would happen in breeding. Additionally, there are no empirical studies of the gain from genomic selection or comparison with marker assisted selection or phenotypic selection. Such studies are critical to determine whether the predictions based on simulation and cross-validation studies translate to useful breeding methods. While the importance of genotype x environment interaction has been noted by others, research directed toward evaluation of training population data sets comprised from different environments are needed to optimize the use of multi-environment data (Crossa et al., 2010). Theory predicts that trait and population genetic parameters such as number of QTL, effective population size, linkage disequilibrium, and minor allele frequency will affect prediction accuracy (Lorenz et al., 2012; Piepho et al., 2009). Investigating these parameters in a genomic selection program will provide useful insight into how these factors change through breeding and their influence on the effectiveness of the technique.

The University of Minnesota has implemented genomic selection in its spring six-row and winter six-row malting barley breeding programs. Both programs utilize the Illumina

BeadExpress Custom Array with 384 SNP markers. The spring and winter programs are carried out collaboratively with the USDA Small Grains Genotyping Centers at Fargo, ND and Raleigh, NC, respectively. For both programs tissue is harvested from 3-week old plants, dried, and shipped to the genotyping center for DNA extraction and SNP assay. We work with the genotyping centers to generate and validate SNP allele calls prior to generating GEBVs. The spring program was initiated in 2009 and conducts one cycle of selection per year on F2 or F3 plants. We select ~100 best lines based on GEBV for yield and Fusarium head blight resistance from populations of 1400-2000 per cycle. Prediction accuracy, corr (GEBV, phenotype)/sqrt(heritability), for disease resistance based on cross-validation within the training population is 0.78 (Lorenz et al., 2012). When we used the training population to predict the progenies in cycle 1, the accuracy was 0.58 (Smith et al., 2012). Experiments assessing gain from three cycles of selection will be conducted in 2013. In our winter program, initiated in 2010, we conduct two cycles of selection per year during the fall (F2) and winter greenhouse (F1) generations. We select the best 100 out of 768 lines using a selection index weighted for yield, FHB resistance, LTT, and malt extract. The correlation of field assessment and GEBV of LTT on 768 cycle 1 F3:4 lines was 0.60 while the cross-validation accuracy based on the training population was 0.72. We have just completed our third cycle of selection, and experiments to assess accuracy and gain from selection in cycle 1 for individual traits and the selection index are in progress.

Doubled haploids: Doubled haploid methods accelerate generation time by creating completely inbred lines from gametes sampled at any generation. In the most common application, F2 gametes are sampled from F1 plants and the resulting array of inbred lines are used for genetic mapping and breeding. In terms of the former, many bi-parental QTL mapping populations have been used effectively in barley and our research groups have been participants in many of these endeavors (reviewed by Cistue et al., 2011). Most recently, we used doubled haploid populations to identify a new QTL associated with low temperature tolerance (Fisk et al., 2013). In terms of breeding applications, doubled haploids are used extensively in maize for inbred development (Murovec and Bohanec, 2011) and in cereals for variety development (Cistue et al., 2011; Zheng et al., 2002). In barley, there are both gynogenetic (*Hordeum bulbosum*) and androgenetic (anther/microspore culture) available. Our lab has experience with both (Cistue et al., 2011) and within the past year the PI Hayes and co-PI Cuesta-Marcos have implemented anther culture, produced over 1,000 DH lines, and offered the service to the research community on a cost-recovery basis (http://barleyworld.org/doubled-haploid). Protocols have developed to the point that genotype specificity is not an issue, and as detailed in the Approach section, we are currently developing the DH lines for the training population - prior to the onset of this grant - with no issues related to genotype. Doubled haploid approaches are not a universal solution to plant breeding challenges: Li et al. (2013) reported that conventional advance via shuttle breeding was more advantageous than doubled haploid for the CIMMYT wheat program. While some have expressed doubt in the value and/or efficiency of doubled haploids compared to conventional line development, we are see tremendous opportunity in the context of genomic selection, as described in the next section. Furthermore, as described at several points in this proposal, doubled haploid genetic stocks provide an "immortal" resource for continual re-analysis, improvement, and launching new initiatives.

Doubled haploid genomic selection: We are proposing to couple doubled haploids and genomic selection in order to capitalize on the advantages of both techniques. Our methods are detailed in "Approach" – at this juncture it is important to stress that doubled haploid genomic

selection will simultaneously accelerate the development of variety candidates, maximize the accuracy of genome wide association mapping and estimation of GEBVs, and allow for reduction of linkage disequilibrium through successive cycles of intermating. Heffner et al. (2010), in a theoretical comparison of GS programs in maize and wheat, concluded that doubled haploids were useful in the former but not the latter. In our application, the doubled haploid technique is particularly useful for two reasons: (i) it allows us to rapidly develop a training population, which does not currently exist and (ii) it creates an array of genetic resources that can serve as a resource for continuing and future breeding/research projects involving a range of productivity and quality traits.

Rationale and Significance

Americans need healthy whole grain foods, barley is an underutilized source of whole grain nutrition, and the barley research community is fortunate to have at its disposal a host of molecular breeding tools and expertise provided by the USDA-AFRI-CAP projects. This rationale fits the intent of the Foundational Program, which *"focuses on building a foundation of knowledge in fundamental and applied food and agricultural sciences critical for solving current and future societal challenges."* Our proposed project is further aligned with the Foundational Program by:

- focusing on developing doubled haploid genomic selection as a breeding tool *("….conventional breeding, including cultivar and breed development, selection theory, applied quantitative genetics*")
- emphasizing barley for human food *("breeding for improved food quality"* and *"improved nutrient qualities of plant products"*)
- operating within a framework of low temperature tolerance and disease resistance ("*breeding for improved local adaptation to biotic stress and abiotic stress*"

Our doubled haploid genomic selection method represents a novel approach to accelerated breeding of self-pollinated crops in the context of an under-exploited suite of traits relating to climate change and human nutrition and meets the specific criteria of Plant Breeding for Agricultural Production Program Area Priority Code – A1141: "*Pre-breeding and germplasm enhancement, cultivar development, selection theory, applied quantitative genetics, participatory breeding, or development of novel approaches to phenotyping, especially focusing on public plant breeding programs"*; "*Plant genome structure and function to connect genotype to phenotype to reduce the breeding cycle time*."

The doubled haploid genetic resources we will develop are completely homozygous and homogeneous and will be archived (see letter of support from Dr. Harold Bockleman, USDA-ARS-NSGC), thus serving as a resource for long-term genetic analysis and breeding. Extensive SNP-based genetic data sets on the doubled haploids will establish a foundation for continued research and allele mining. The genotypic data, as well as deep phenotypic data on abiotic stress resistance, agronomic productivity, and human nutrition traits will also be archived (see letter of support from Dr. Jean-Luc Jannink, USDA-ARS). These data sets are a resource for assessment of new analysis tools and for instruction. We envision this USDA-AFRI project as establishing the foundation for a long-term, sustained program to develop barley varieties for human nutrition. Once established and operating, the doubled haploid genomic selection food barley breeding effort can be sustained by grower groups and a range of industry partners, as evidenced by the letters of support.

Approach

***a. Activities and sequence*:**

Overview The essence of our approach is shown in Figure 1 (page 16). We propose a five-year project that begins and concludes with thorough phenotypic and genotypic characterizations of doubled haploid barley food germplasm arrays. The project starts with development and assessment of a training population for genomic selection that will also be used for genome wide association mapping. The training population is also referred to as Cycle 0 (C0). There will be three cycles of genomic selection (C1, C2, and C3). Doubled haploids will be produced from selected plants at C1 and C3. At the conclusion (Year 5) there will be phenotypic and genotypic assessment of a validation population composed of doubled haploids from C0, C1, and C3. The five-year AFRI program is envisioned as a starting point for a sustained breeding program, as described in the supplementary "Description and budgeted plan for the release of research results."

Test environments: With resources from the AFRI project the training (n = 300) and validation (n = 200) populations will be grown at three locations: Corvallis, OR; St. Paul, MN; and Pullman, WA. These locations are representative of target mega-environments for food barley production and are distinct in terms of climate and production practices. Assessments at Aberdeen, Idaho will be conducted by Co-PI Hu with other resources (see letters of support from Dr. G. Hu and Ms. Kelly Olson, Idaho Barley Commission) as will trials at Mount Vernon, WA (see letter of support from Dr. Jones). At each location, a complete suite of agronomic, biotic stress resistance, and abiotic stress resistance traits will be recorded (see below – agronomic traits). Grain samples from each location will be used for assessment of a complete set of food quality characters (see below- quality traits). Budget constraints limit us to quality analyses from three locations for the training and validation populations at this time. The three locations submitting samples for AFRI-funded quality analyses will be selected based on the highest likelihood of generating meaningful data and if all locations had excellent trials, one will be selected at random to archive samples. We are confident that once we have generated data sets from three locations, the quality and value of the data will allow us to secure funding to analyze all samples. The procedures for quality analyses are clear and the necessary funding transparent: $10,500 per location. In all cases, archived samples of grain will be retained for future analysis and planting. This is a particular advantage of the doubled haploid system: since there is no heterogeneity or heterozygosity, all seed samples have a defined identity.

Doubled haploid production: The 300 doubled haploids that will form the basis of the training population will be selected from 1,000 doubled haploids produced prior to the initiation of this project and at no cost to the AFRI project. This represents an up-front investment of $35,000 by the OSU project in this endeavor. These resources were a one-time investment provided by salary savings and a one-year grant from the Oregon Agricultural Research Foundation. During the AFRI-funded component of the project, a total of 600 doubled haploids (300 at C1 and 300 at C2) will be produced at OSU.

Genotyping: The training population will be genotyped with the 9K Illumina chip at the laboratory of co-PI Dr. Chao (USDA-ARS, Fargo ND). Doubled haploids produced from C1 and C3, and the segregating populations that will be used for genomic selection, will be genotyped for 560 loci using Sequenom assays organized by the same facility.

Genome wide association mapping and genomic selection: The full phenotypic and genotypic characterization of the training population will allow for calculation of GEBVs for the three cycles of genomic selection for parents and two rounds of line selection in Cycles 1 and 2. These data will also allow for genome wide association mapping to identify genome coordinates of QTLs and candidate genes for all traits (more than 24 in total). The validation population will allow for empirical assessment of genetic gain and analysis of changes in allele frequencies and linkage disequilibrium resulting from selection. Both populations will allow for assessment of genotype x environment interaction for productivity, resistance to stresses, and food quality traits. The multi-location/multi-trait assessments will allow for comparison of alternative selection indices for each location and subsets of locations. Implementation of alternative indices at individual locations will not be funded by the AFRI project but is an attractive funding prospect for PIs at each location, or subsets of locations. Finally, the doubled haploids in the training population will be advanced to the elite trial stage within the timeframe of this project. It is very likely that variety candidates will be identified in this array. These elite trials will consist of selections made for across-location performance and selections made for environment-specific performance.

Agronomic traits: Agronomic trait assessment procedures will follow those of the USDA-AFRI-TCAP (http://www.triticeaecap.org/), which in turn are based on trait ontologies. There are two stages of agronomic assessment: head rows and yield trials. Head rows will be used for the first year after doubled haploid production. At the head row stage, data will be recorded for plant height, heading date, disease resistance (scald and stripe rust in Oregon; mildew and Fusarium head blight in Minnesota), and low temperature tolerance (Minnesota). These data will feed into the genomic selection training model and will be used, in conjunction with phenotypic selection, to reduce the number of doubled haploids that are advanced to yield trials. The head rows at Corvallis, OR will generate sufficient seed for yield trial assessment at participating locations. Traits measured at the yield trial stage at all locations will be grain yield, plant height, lodging, thresh-ability, and test weight. As biotic (stripe rust, scald, leaf rust, mildew, Fusarium head blight) and abiotic (low temperature, drought) stresses occur the symptoms will be recorded using standard scales/procedures.

Food quality traits: The food quality traits that will be measured on grain samples from three locations of the training and validation populations are: protein, beta glucan, grain amylose, grain color, kernel hardness, solvent retention capacity, gel hardness, gelatinization temperature, total anti-oxidant activity, steamed grain, tortillas, and risen bread. In addition, Dr. Wise (USDA-ARS, Madison, WI) has agreed to perform tocol analyses at no cost (see letter of support). Industry partners ConAgra, General Mills, Grain Millers, and Kellogg have agreed to perform additional trait assessments in their labs (see letters of support). The specific traits to be measured by industry collaborators will be determined once the project is underway and the first sets of phenotypic data are available for the training population.

Analysis: Intellectual investments for experiment design, analysis, and preparation of publications are budgeted as salary at OSU (co-PI Cuesta-Marcos) and at UMN for a post-doc. In addition, we have budgeted for mentored undergraduate student labor.

b. *Methods*

Germplasm: The program was initiated from crosses among parental lines belonging to three different germplasm pools (a list is provided as a supplemental file and posted online at http://barleyworld.org/food/AFRIprop. Briefly, the composition of the three germplasm pools is

as follows: 1) European, Asian, and US varieties and breeding lines with different food quality attributes: hulled/hull-less, colored/non-colored and waxy/non-waxy starch. This germplasm includes introgressions from Himalayan germplasm into adapted food barley genotypes; selected varieties from European food barley breeding programs; and food barley germplasm developed by the USDA-ARS program in Idaho; 2) US varieties and breeding lines with exceptional low temperature tolerance (LTT) (2 and 6 rowed). This germplasm was (i) developed and characterized for the Barley CAP project or (ii) assembled for an extensive LTT association mapping project supported by the Triticeae CAP; and 3) Varieties and breeding lines adapted to the environments that this project will focus on: Oregon, Washington, Idaho, and Minnesota. This germplasm was contributed by the four participating breeding programs and includes sources of resistance to diseases endemic to one or more of the target environments.

The crossing block was designed so that segregation for the traits of interest was maximized i.e., the two parents of each cross usually belong to different germplasm pools and have different attributes (e.g. hulled x hull-less, winter x spring or facultative, colored x non-colored). The training population (C0) will consist of doubled haploids derived from F1s of these crosses and from intermated F1s of these crosses. Remnant seed of the F1xF1 crosses will be used as segregating material in the first cycle of genomic selection. Thereafter, selected lines will be intermated. Through the cycles of genomic selection the frequency of favorable alleles for the traits that are under selection will increase and it is likely that some alleles may become fixed. The flexibility of our genotyping approach (described below) allows for updating marker panels to maximize genetic information at each cycle. Since the training population will be extensively characterized phenotypically and genotypically there is always the option to go back to the original F1s and create a new starting population based on completely different traits that may be of interest in the future. The prediction model is also continuously rejuvenated as genotypic and phenotypic information from elite lines derived from the participating breeding programs is incorporated into the prediction models. In this way, new germplasm can be infused into the system at any point. As lines derived from the newly infused germplasm advance in the breeding process, their genotypic and phenotypic information can also be incorporated into the prediction models.

Doubled haploid production: We are producing doubled haploids using anther culture, following the protocols described in Cistue et al. (2011).

Genotyping: The training population will be genotyped with the barley 9K SNP platform using the Infinium assay developed by Illumina. DNA will be extracted according to Bodo-Slotta et al. (2008). Genotype calling will be performed using Illumina's GenomeStudio software and manually evaluated for call accuracy. Co-PI Chao has extensive experience with these procedures. Her lab has performed more than 7,000 barley sample assays under the auspices of the Barley and Triticeae CAP projects. The genotyping of segregating progenies for the purposes of genomic selection will be done using the Sequenom assays: Chao and colleagues have extensive experience with the development and application of Sequenom assays based on the 9K Illumina chip, having performed more than 15,000 wheat and barley assays under the auspices of the CAP projects.

There are two notable advantages of using the Illumina 9K for the training population and the flexible Sequenom assay for genomic selection and characterization of the C1 and C3 doubled haploid lines: (i) with the Sequenom, specific loci can be targeted that have been identified in prior research to be determinants of, or associated with, target traits and (ii) if alleles at marker loci reach fixation during GS, other polymorphic loci can be substituted.

Genome wide association mapping and genomic selection: The PI and co-PIs have extensive experience with procedures for genome wide association mapping - from structure analysis to establishing false discovery rates to publication (Cuesta-Marcos et al., 2010; Massman et al., 2011; Roy et al., 2010; von Zitzewitz et al., 2011) and are engaged in continual training in new approaches via literature review and involvement in the T-CAP. Co-PI Smith has extensive experience with implementing genomic selection in barley (Lorenz et al., 2011; 2012); and the co-PIs are likewise engaged in genomic selection project design, implementation and refinement via the TCAP. Specifically for this project, the prediction model for cycle 1 will be based on phenotypic data (low temperature tolerance, growth habit, disease resistance, beta-glucan, protein content) from the 1000 doubled haploids fall planted in head-rows at Corvallis, Oregon and Saint Paul, Minnesota in 2013 and spring-planted at Corvallis and Saint Paul in 2014. The first cycle of genomic selection will be based on a segregating population of 480 individuals, whereas the cycle 2 and cycle 3 will have 384 individuals each. The prediction models for cycle 2 and cycle 3 will be based on the training population of 300 doubled haploids evaluated at four locations. At each cycle of genomic selection, we will genotype each plant with a Sequenom assay of 560 SNPs. We will select 10% of the plants based on their GEBVs. Selected lines will be intermated for the next cycle of genomic selection and used for doubled haploid production (at cycles 1 and 3). Budget resources are not sufficient for doubled haploid production at cycle 2.

In addition to applying modern plant breeding approaches to developing novel winter food barley germplasm, this project will also investigate key questions about genomic selection that have not yet been fully explored. Most research on application of genomic selection to date has focused on evaluating and optimizing marker density and distribution, training population size, training population composition, and model approaches to estimate marker effects (Heffner et al., 2011; Lorenz et al., 2012; Asoro et al., 2011). These studies have been conducted primarily through simulation and cross-validation. Our project will advance our understanding of genomic selection on several fronts. First, we will evaluate prediction accuracy based on progenies from a genomic selection breeding program for a wide range of traits and compare these accuracies to cross-validation estimates from the training population. Since the training population will also be used to conduct genome wide association mapping studies, we will examine the relationship between genetic architecture of traits with genomic selection performance. By conducting genome wide association mapping and estimating marker effects using lines developed in the training population (cycle 0), and cycles 1 and 3, we will measure changes in marker-trait associations resulting from population differentiation during the breeding process.

In addition to evaluating GS prediction accuracy we will assess gain from selection. To date, we are unaware of any published studies in plant breeding that have empirically measured gain from genomic selection. This project will conduct a total of three cycles of selection and develop doubled haploids from cycles 1 and 3. Within the timeframe of this project we will be able to quantify gain from selection in cycles 0, 1 and 3. Our project will conduct genomic selection both for parent selection and in segregating lines that will be advanced to yield trials. In the context of line selection we will directly compare genomic selection, phenotypic selection, and random selection in cycles 1 and 3.

In the validation experiment of year 5, we will directly and empirically compare GS prediction accuracy and assess genetic gains resulting from phenotypic, genomic, and random selection at four locations. To do that, we will create a validation population comprised of

approximately 200 entries. From each of the three cycles where DH lines are produced (Cycles 0, 1, and 3) we will select ~ 50 lines based on phenotype and ~ 50 lines based on their GEBV (the lines that would have been selected had the experiment not been phenotyped). We anticipate that many of the lines from both selections will coincide; therefore, including a sample of randomly selected lines, this validation population will have approximately 200 entries. The expected outcomes of the validation experiment of year five are: 1) assess the prediction accuracy of GS and compare genetic gains from genomic and phenotypic selected lines within each cycle, and 2) estimate the overall efficiency of GS since we will have a set of DH lines whose parents have undergone 0, 1 and 3 cycles of genomic selection.

This project will also offer the opportunity to assess the importance of genotype x environment interaction in genomic selection as training data sets will be generated from very different environments (Oregon, Washington, Idaho, and Minnesota). Germplasm developed from the genomic selection project will be evaluated in the same environments so that we can correlate GEBVs based on individual and combined environments to actual performance of breeding progenies at those same locations.

The implementation of genomic selection in breeding changes the context with respect to the feasibility and utility of selection indices. In many plant breeding programs, the availability of trait information is sequential such that only partial information is available at key points of selection. In this case, selection at each step is based on the data available where highly heritable and easy to assess phenotypes (e.g. plant height) are used early in selection and less heritable and more expensive traits (e.g. bread loaf volume) are used later. This certainly applies to our situation, where our first cycle of genomic selection will be based on data from head rows whereas the subsequent cycles will be based on yield trials. We will evaluate our training population for at least 12 resistance/productivity traits and 12 quality traits. The data sets generated from this project will allow us to explore various selection indices based on GEBVs for a wide array of traits. While we will not be able to empirically compare selection based on different indices, we will be able to compare prediction accuracies among different traits, among selection indices comprised of GEBV for sets of traits, and for predicted trait indices directly.

The breeding goals of this project are varieties with strong agronomic performance, abiotic/biotic resistances, and a range of quality traits suited to different food end-uses. Our baseline check is the variety Streaker (a hull-less food barley developed by OSU, with first commercial harvests in 2013). For agronomic and resistance traits, the goals are clear: higher yield and low temperature tolerance than the check. Agronomic variables contributing to yield are lodging resistance and grain test weight. For food quality, there are opportunities to develop a range of products, each with contrasting quality attributes. The baseline quality criterion is grain beta glucan higher than Streaker. Beyond that we will maintain genetic/phenotypic diversity for the other quality attributes with the framework of an overall goal of four principal germplasm types (all hull-less) that represent all possible combinations of starch type (waxy: non-waxy) and grain color (white; colored). At any point in the breeding process, our industry partners may provide us with additional specific selection criteria for any of the other nine quality traits (and if necessary the resources to apply these criteria). In the event of an inadvertent selection bottleneck (e.g. suppose high alpha tocopherol content emerges as a selection criteria after C3 but in C3 lines we have lower levels than in C1 or C0) we will have the remnant C1 and C0 doubled haploid seed and trained prediction models with which to implement a new genomic selection spin-off.

Agronomic traits: The Oregon, Idaho, Minnesota, and Washington barley breeding programs have extensive experience in agronomic assessment. They have the necessary equipment infrastructure for planting (head row and yield trial drills), harvest (plot binders, stationary threshers, and combines), and seed processing (de-awners and seed cleaners). They have experience with best agronomic practices appropriate for each location (e.g. seeding rate, fertility, weed control, and irrigation (Aberdeen, Idaho only)). We will use Type II Augmented designs for the head rows, the training populations, and the validation population. We will use Randomized Complete Blocks for the Advanced and Elite trials.

Food quality traits: The procedures used for quality assessment are described in alphabetical order.

- Beta-glucan content: measured using the AACC-International Approved Method 32-23.01 "Beta-Glucan Content of Barley and Oats—Rapid Enzymatic Procedure" (AACC-International Approved Methods 11th Ed) as modified by Hu and Burton (2008) where appropriate.
- Color of barley grain: measured using a tristimulus color meter according to Quinde et al. (2004) and Quinde-Axtell et al. (2005).
- Flour pasting analyses (starch type and gelatinization/pasting temperature): Determined in accordance with AACC-International Approved Method 76-21.01 "General Pasting Method for Wheat or Rye Flour or Starch Using the Rapid Visco Analyser", or Approved Method 22-08.01 "Measurement of alpha-Amylase Activity with the Rapid Visco Analyser" (AACC-International Approved Methods 11th Ed), or as modified by Crosbie et al. (2002), as appropriate. Guidance on any required modifications will be obtained from "The RVA Handbook" (Crosbie and Ross, 2007). RVA analyses will be used to provide *prima facie* evidence for starch amylose contents higher or lower than encountered in "normal" starch (Ross et al., 1997).
- Gel hardness: measured using the samples prepared for flour pasting analyses based on the method of Tan and Corke, (2002).
- Kernel hardness: measured using a Perten Single Kernel Characterization System according to AACC-International Approved Method 53-31.01 "Single-Kernel Characterization System for Wheat Kernel Texture" (AACC-International Approved Methods 11th Ed) as modified by Nair et al. (2010).
- Pearling of hulled barley: If required before further testing, barley will be pearled (dehulled) to a pearling degree of 10 to 15% (10 to 15% loss of mass: primarily only hull material removed) using a tangential abrasive dehulling device (Nair et al., 2010; Oomah et al., 1981).
- Product manufacture and assessment: Ross is an experienced artisan baker, has attended baking courses at the San Francisco Baking Institute, is a member of the Bread Baker's Guild of America, and has collaborated with world renowned bakers (e.g. Craig Ponsford, winner of the 1996 World Cup of Baking) on formulating barley into risen breads. From 1994 to 1998 Ross was the manager of the Asian products lab at the former Bread Research Institute of Australia where he gained expertise in the manufacture and assessment of a range of noodle products, Arabic (pita) breads, and Chinese steamed breads for the Australian "Interstate Wheat Variety Trials", which at that time assessed pre-release breeders' lines for end-use quality. Ross is experienced in Asian noodle manufacture and assessment (Crosbie and Ross, 2004; Crosbie et al., 1999; Ohm et al., 2006; Ross, 2006; Ross and Crosbie, 2010; Ross and Hatcher, 2005; Ross et al., 1997) as

well as in the general assessment of cereal quality (Ross and Bettge, 2009). Ross has demonstrated to bakers the use of barley in risen breads and soft pretzels at the "Kneading Conference West" at the WSU facility in Mt Vernon WA in September 2011 (Ross, 2011). Ross is also experienced in the quality assessment of cereal breeder's experimental lines. Risen breads made with composite barley/wheat flours will be produced and assessed based on the methods of Rieder et al. (2012) and Faergestad et al. (2000). Steamed or boiled grains will be processed and assessed using methods developed for rice cooking quality. Barley grain or grits will be cooked using a programmable rice cooker based on the method described by Meullenet et al. (2000). Cooked grains will be assessed for texture using a back-extrusion technique based on the methods described by Leelayuthsoontorn and Thipayarat (2006) and Meullenet et al. (2000). Color of cooked grains will be measured using a tristimulus color meter. If required, cooked grain elongation will be assessed based on methods described by Wang et al. (2007) and Ge et al. (2005). If required, degree of cook measurements will be based on adaptations of the methods of Ferrel and Pence (1964) and Whalen (2007).

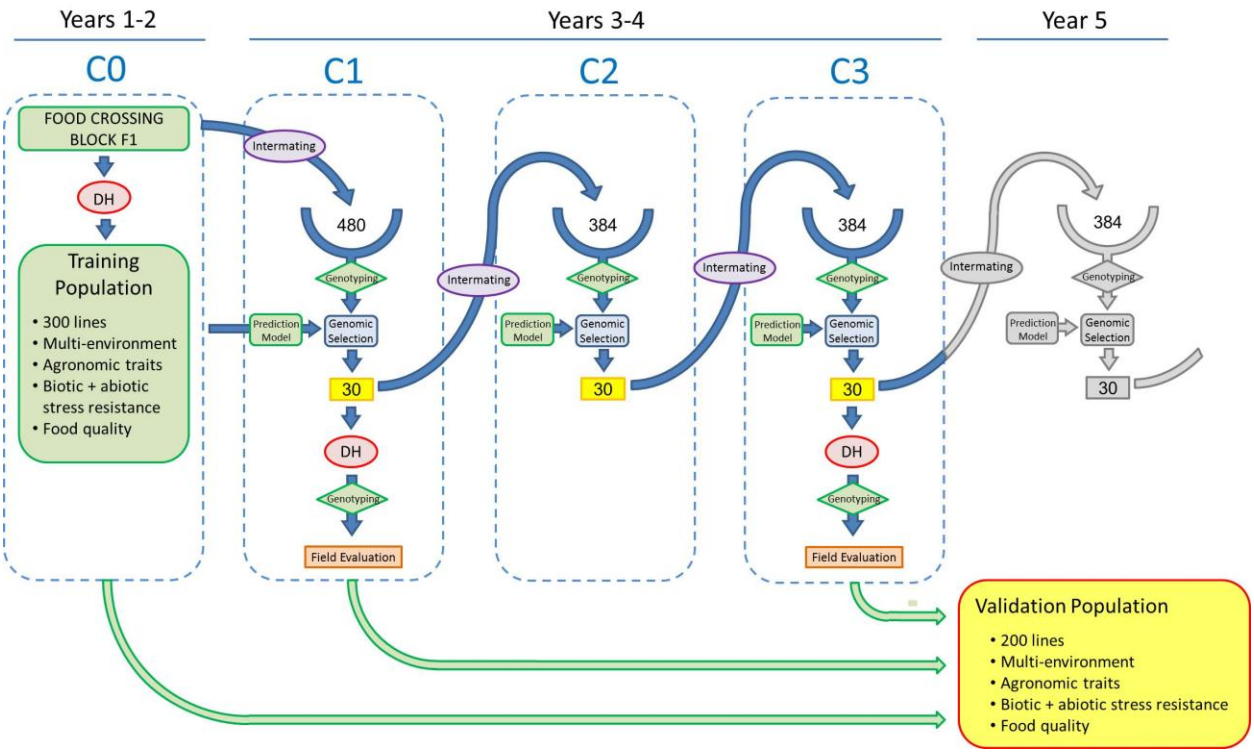
- Tortillas made with composite barley/wheat flours will be produced based on methods described by Guo et al. (2003), Toma et al. (2008), Prasopsunwattana et al. (2009), and Alviola and Awika (2010). Tortillas will be assessed for objective color and textural (mechanical) characteristics based on the methods described for color by Prasopsunwattana et al. (2009), and texture by Alviola and Awika (2010), and Prasopsunwattana et al. (2009).
- Protein Content will be determined by the AACC-International Approved Method 39-10.01 "Near-Infrared Reflectance Method for Protein Determination in Small Grains" (AACC-International Approved Methods 11th Ed). Methods applied by appropriate extension or breeding personnel during seed processing.
- Moisture Content is determined during the measurement of protein by the near-infrared reflectance method.
- Solvent retention capacity will be determined according to AACC-International Approved Method 56-11 "Solvent Retention Capacity Profile" (AACC-International Approved Methods 11th Ed) with modifications as needed to adapt them to barley.
- Starch apparent amylose determination: where required by a prima facie indication of high amylose content by paste viscosity measurements, apparent amylose content of the samples will be determined experimentally by the Con A method using the Megazyme amylose assay kit (Megazyme, 2011, Gibson et al., 1997) with modifications to our lab conditions.
- Total antioxidant activity of whole barley flour will be determined according to the methods of Serpen et al. (2008) and Gökmen et al. (2009).
- Total phenolic content will be determined using a modified method of Bendelow (1977) and Quinde et al. (2004).

c. Expected outcomes: The long-term and general outcomes will be greater farm profitability and sustainability; greater employment in food processing; and improved consumer health: in short, a healthier and more prosperous nation able to deal with climate change. Specific outcomes include: food barley varieties; food barley product standards and formulations; basic knowledge regarding the genetics and environmental plasticity of agronomic and quality traits; and a model breeding system that efficiently and cost-effectively provides varieties on a

sustained basis.

d. Analysis procedures: These are detailed in the Methods section.
e. How results and products will be used: We expect varieties to be used on-farm; processes and products to be used by food processors and producers; and products to be used by increasingly healthy consumers. Basic knowledge generated by this research will be peer reviewed and available to the community of science. Genetic stocks and data will be available through the NSGGC and the T3 database, respectively.
f. Pitfalls: None are envisioned beyond crop failures due to circumstances beyond our control. In such unfortunate events, the experiment will be repeated the following year.
g. Limitations: There are no limitations.
h. Hazards: There are no hazards.
i. Timelines, annual milestones and outcomes: Please see next 2 pages.

Figure 1. Schematic for food barley doubled haploid genomic selection. A larger version of this Figure is available online at http://barleyworld.org/food/AFRIprop and as a supplement to this proposal.



Bringing Barley to the Table – Timeline. CVO = Corvallis, OR; SPM= St. Paul, MN; AID = Aberdeen, ID; PWA = Pullman, WA; MWA (Mt. Vernon, WA). Activities in italics are not charged to the AFRI grant: these include all AID and MWA trials and most spring-sown trials. Options to leverage value are further described online at http://barleyworld.org/food/AFRIprop. Fall-sown experiments carry over until the following summer. Spring-sown experiments are concluded the same summer.

Season/Year	Phenotype	Genotype	Germplasm	Doubled haploid	Outcomes Analysis Reports
Winter 13			*Intermate F1*	*500 - F1*	
Spring 13				*500 - intermated*	
Summer 13					
Fall 13	500 DH –HR agro CVO SPM				
Winter 13/14					AFRI report
Spring 14	500 DH – HR agro CVO *SPM*				
Summer 14	Select 300 DH for TP				Deposit TP at NSGC
Fall 14	300 DH Training population agronomics CVO SPM PWA *AID MWA*	9K Illumina 300 DH Training population Sequenom 480 Intermated	Cycle 1 genomic selection 480	300 - Cycle 1	GS Model 1 Genotype data deposited at T3
Winter 14/15					AFRI report
Spring 15	*300 DH Training population – HR agronomics CVO SPM PWA AID MWA*	Sequenom 384 Intermated	Cycle 2 genomic selection 384		GS Model 2
Summer 15					GWAM Agronomics paper
Fall 15	50 DH ADV agronomics CVO SPM PWA *AID MWA*	Sequenom 384 Intermated + 300 DH C1	Cycle 3 genomic selection 384	300 - Cycle 2	GS Model 3; GS comparison paper Phenotype data deposited at T3
Winter 15/16	300 DH Training population quality traits				AFRI report
Spring 16					GWAM Quality

Summer 16	Cycle 1 300 DH agronomics HR CVO SPM				paper Phenotype data deposited at T3
Fall 16	25 DH ELITE agro CVO SPM PWA *AID MWA* Cycle 1 DH PYT agronomics CVO SPM PWA *AID MWA* Cycle 3 DH HR agronomics CVO SPM	Sequenom 300 DH C3			
Winter 16/17					AFRI report
Spring 17					
Summer 17					Phenotype data deposited at T3
Fall 17	200 DH Validation Agronomics CVO SPMN PWA *AID MWA* *200 DH Validation agronomics CVO SPMN PWA AID*				Deposit VP at NSGC
Winter 17/18					
Spring 18					
Summer 18	200 DH Validation Quality traits				GS agro and quality paper Deposit all data to T3