"Improving barley and wheat germplasm for changing environments"

Program Area Priorities Addressed: This project will use nationally coordinated high-throughput phenotyping and genotyping platforms, innovative marker-based breeding strategies, and an integrated plant breeding education network to mitigate the negative effects of climate change on crop production. Our research will contribute to the long-term objective of a 10% increase in nitrogen and water use efficiency in barley and wheat production through the development of cultivars that are better adapted to changing environments. This project addresses the specific objectives of Program Area Code A3121 of characterizing and improving relevant publicly-available germplasm, standardizing methods for high-throughput phenotyping under field conditions, accelerating breeding cycles, integrating data into public databases and generating informatics tools for breeders. Our project addresses the following educational priorities: expand outreach to minorities, increase interest of undergraduate students in agricultural sciences and climate change and train the next generation of modern plant breeders.

1. INTRODUCTION

Climate change increases the negative impact of abiotic and biotic stresses on wheat and barley production. Increasing CO_2 concentrations reduce the ability of wheat and barley to assimilate nitrates [1], high temperatures increase stress and change the geographic distribution of pathogens [2], and altered precipitation patterns increase the likelihood of short-term crop failures and long-term production declines. These constraints, compounded by increasing demand for food, and increasing costs for fertilizer, water and other inputs, require a national plan for innovative plant breeding and education. This project will change the paradigm of how we utilize germplasm resources for barley and wheat improvement from a view centered on the characterization of accessions to one centered on the discovery and deployment of valuable alleles. The overall goals of the project are to phenotype and genotype diverse barley and wheat germplasm pools to discover and deploy alleles that improve yield under biotic and abiotic stresses, and to use genetic markers to rapidly deploy favorable alleles and accelerate breeding cycles. This integrated research project will provide a problem-based learning environment to train a new generation of plant breeders and attract new students to agricultural sciences.

1.1. Specific objectives:

1) Discover and deploy beneficial alleles from diverse wheat and barley germplasm.
2) Accelerate breeding through marker-assisted selection and genomic selection.
3) Implement sequence-based genotyping methodologies to discover new allelic diversity.
4) Implement web-based tools to integrate marker-assisted selection and genomic selection strategies into breeding programs.
5) Develop and implement a Plant Breeding Education Network.

1.2. Background and literature review

1.2.1. Climate-change related traits. Wheat and barley production in the United States is conducted in environments where water deficits and heat stress are common, but climate change is increasing the frequency of these stresses [3]. Climate changes also alter the distribution of pathogen populations adding additional pressure on breeding programs [2]. These challenges will only be addressed through a concerted national effort to identify valuable alleles from diverse germplasm and develop efficient breeding strategies to deploy them. With input from most of the major US barley and wheat organizations (see attached supporting letters) we prioritized the

following climate change-related traits: yield, water use efficiency (WUE), nitrogen use efficiency (NUE), and resistance to emerging fungal diseases.

1.2.1.1. Yield. Estimated wheat and barley yield increases required to meet projected demand by the year 2050 (1.3 to 1.4% per year) exceed by 30-40% current average yield improvement rates (1% per year, Natl. Agr. Stat. Service 2010). Climate change will likely negatively impact the rates of gains. Therefore, a paradigm shift in breeding methods is required to meet future demands. Genomic Selection (GS) methods that estimate breeding value of particular lines based on marker and phenotypic data [4-6] provide a novel approach to accelerate the breeding cycles for yield improvement. We will take advantage of the extensive genotypic and phenotypic data generated in this grant to implement GS strategies for yield improvement. We also expect to increase yield stability by improving WUE, NUE and disease resistance as described below.

1.2.1.2. Water use efficiency. Drought tolerance is a complex trait that can be estimated as the ratio of yield under water stress vs. non-stressed conditions [7]. However, measuring yield is expensive and time consuming. Several parameters with high correlations with drought tolerance have been proposed as indirect indicators including carbon isotope discrimination (CID), canopy temperature depression (CT), and canopy spectral reflectance (CSR) [8-12]. CID integrates the response of the plant over the growing season and is negatively correlated with transpiration efficiency. CID has been used in wheat as a selection tool for improved yield in rainfed environments [13-14]. CT and CSR are non-destructive high-throughput measurements. CT is related to the plant's ability to cool leaves through transpiration and reflects the ability of the roots to access water [15]. Several CSR indices are highly correlated with biomass, CT and leaf water potential [11, 16-17]. Additional traits, such as rapid early season growth, deep root system, stay-green, and solid stems, have been suggested to affect drought tolerance in barley and wheat [18-21]. In this proposal we will use specialized populations to validate these relationships.

Climate change will likely produce warmer winters, thus increasing the potential to switch from spring to fall plantings to capitalize on winter precipitation and reduce irrigation. This is a particularly valuable opportunity for fall-sown barley (henceforth "winter barley"), but improved low temperature tolerance (LTT) is required to ensure stable production. Our strategy is to identify alleles necessary for maximum LTT and to configure them with short day photoperiod sensitivity and vernalization insensitivity ("facultative" growth habit) [22-24].

1.2.1.3. Nitrogen use efficiency. Nitrogen availability is a major constraint on grain production [13, 25-26] and the main component of the carbon footprint of cereal production [27]. Given the cost of N fertilizer and the negative environmental impact of high fertilizer use, increasing NUE is vital to sustainable cereal production. There are two major components of NUE: 1) uptake efficiency (UPE = N in plant/N applied), which is important in low to moderate N environments and 2) utilization efficiency (UTE = N in grain/N in plant), which is most important in high N environments. Environments with moderate N levels appear suited for selecting for both components [28]. Genetic variation for NUE has been noted in wheat and barley [29-31] but improvements in NUE have been limited by expensive and laborious phenotyping.

Various CSR indices show high correlations with barley and wheat grains yield, biomass and N concentration and are already used in N management strategies [11, 32-38]. The CSR estimates of biomass and N content can be combined with yield and grain protein to estimate UPE and UTE. The association of CSR with biomass is particularly important, as biomass is a key component of NUE and yield, it is expensive to measure, and harvest index is approaching its theoretical limit, restricting future yield gains to increases in biomass.

1.2.1.4. Fungal diseases. Climate change is also imposing novel biotic stresses. Higher temperatures result in changes in geographic distribution of pathogens [2], decrease the effectiveness of some resistance genes [39-41] and potentially increase pathogen fitness, *per se* [42]. Shifts in leaf, stripe, and stem rust races are of particular concern because of the devastating potential of these pathogens, which resulted in wheat and barley losses in the US of over $2.5 billion during the last ten years (http://www.ars.usda.gov/Main/docs.htm?docid=10123). In addition to the "Ug99" races of stem rust that threaten global barley and wheat production [43-46], leaf rust is causing increasing losses (14% loss in Kansas in 2007, the largest in the history of the disease survey), and stripe rust epidemics caused by more aggressive races [42] are affecting barley and wheat growing regions worldwide [47-49].

New forms of two additional pathogens threaten US barley production. A new isolate of spot form of net blotch (*Pyrenophora teres* f. *maculata*) threatens most cultivars in the largest barley-producing region in the US [50]. Moreover, a new race of spot blotch (*Cochliobolus sativus*) was detected in North Dakota in 2008 (S. Zhong et al., unpublished) and possesses high-virulence on all previously resistant malting cultivars in the Upper Midwest [51]. Spot blotch is usually more severe in warmer environments; thus, elevated temperatures will likely result in more frequent and severe epidemics. Yield losses of 30-40% can occur due to infection by these pathogens [52].

Genetic resistance is the most cost-effective and environmentally-friendly method for controlling these pathogens. Unfortunately, most of the race-specific resistance genes identified in the past [53], have been defeated by new races [42, 47]. Consequently, barley and wheat improvement programs need additional sources of resistance, particularly adult-plant resistance (APR), which has been more durable than race-specific resistance [54].

1.2.2. Genotyping and databases. MAS is vital to developing efficient breeding strategies for all these traits. Advances in genotyping are decreasing molecular marker costs and increasing genome coverage facilitating the implementation of new breeding strategies. The USDA-ARS has established four regional genotyping centers in KS, WA, ND and NC to accelerate the implementation of marker-based breeding in barley and wheat breeding programs. The integration of the genotyping laboratories in the previous CAP projects provides the foundation for this project to implement nation-wide marker-assisted selection (MAS) and genomic selection (GS) strategies to accelerate breeding cycles.

Barley and wheat researchers have developed Illumina-based 3072-SNP platforms for genotyping [55-56], which will be used in the initial phase of this project. One shortcoming of fixed SNP genotyping platforms is that SNPs must be initially identified in a discovery panel that includes a limited number of accessions. If this panel is not representative of all material that will be genotyped it can induce substantial ascertainment bias [57-58]. Some concerns about ascertainment bias for assessing genetic diversity in barley landraces have been raised for barley OPA1 SNPs [59]. However for barley OPA2 SNPs a deeper discovery panel was used, reducing the impact of ascertainment bias. The combined use of these OPAs has resulted in robust linkage maps [55] and association genetics analyses in cultivated barley, landraces and wild germplasm [60-61]. In wheat, the SNP discovery panel included cultivated and wild tetraploid to minimize ascertainment bias.

Genotyping by sequencing (henceforth GBS) reduces ascertainment bias [58, 62] as it uncovers the entire array of allelic variation increasing the power of the genetic analyses. We will explore two GBS approaches 1) complexity reduction based on restriction digestion of genomic DNA followed by gel-based size-selection [63], and 2) sequence capture using oligonucleotide baits for

the sequences of interest [64-65]. We will use the first approach to sequence all the parental lines for the wheat and barley nested association mapping (NAM) populations [66-67] and to genotype the wheat NAM populations. We will explore increased multiplexing in gene capture technologies to initiate a catalogue of gene variants in the core National Small Grains Collection (NSGC).

To manage the large amount of genotypic and phenotypic information generated in this project, we will take advantage of "The Hordeum Toolbox" (THT, www.hordeumtoolbox.org/), a database developed as part of the Barley CAP. Currently, THT stores SNP and trait data for all breeding lines in the project. These data can be queried, and downloaded files are compatible with TASSEL and the genotype viewer FLAPJACK. THT also stores pedigree and genetic map data. The GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml) hosts THT, which is linked with PLEXdb (www.plexdb.org/), HarvESTBarley (http://harvest.ucr.edu/) and Gramene (www.gramene.org/), providing integration with expression experiments, EST sequences in barley and access to grass comparative genomics information, respectively. THT will be renamed "The Triticeae Toolbox" (T3), and will be improved to include sequenced-based genotyping data, visualization tools, and used as the central database for this project.

1.2.3. Germplasm and populations. The first step of a breeding effort is the screening of germplasm for allelic variation. We will use four different sources of allelic variation: (1) elite breeding lines, (2) diverse barley and wheat accessions from the NSGC core collection and international collaborators, (3) wild introgression populations, and (4) genetic stocks and populations. A systematic genotypic and phenotypic characterization of these resources will be implemented to discover, and deploy favorable alleles in public cereal breeding programs.

Two basic strategies have been developed to evaluate alleles from unadapted germplasm in elite genetic backgrounds: (1) wild introgression or advanced backcross-QTL populations [68]; and (2) NAM populations [66-67]. Backcross populations derived from exotic donor parents and an elite recurrent parent result in breeding lines that are generally adapted, and identified favorable alleles can be directly integrated into breeding programs. NAM populations were first used in maize where 25 diverse founder parents were crossed to the standard inbred line B73 and 200 recombinant inbred lines (RILs) from each cross were derived and used to investigate the genetic architecture of flowering time [66]. The NAM experimental design provides improved statistical power for both standard linkage analysis and association mapping (AM) to detect QTL.

In addition, we will use AM panels of adapted germplasm to identify QTL for the targeted traits. AM is based on recombination accumulated throughout the evolutionary history of the population [69] and circumvents the need for genetic mapping populations. In wild barley, *Hordeum vulgare* ssp. *spontaneum*, intra-locus linkage disequilibrium (LD) decays rapidly within the first 300 bp [70] and is useful for intra-genic scans. In cultivated barley and wheat, LD is approximately 2-4 cM [71-72], which is appropriate for whole genome scans for marker-trait associations [73]. Population structure can lead to spurious associations in AM [74], which can be reduced through mixed-model analysis [75-76]. Significant associations with useful traits have been identified in elite barley and wheat AM panels [60-61, 77-78].

1.2.4. Marker-assisted (MAS) breeding and genomic selection (GS). MAS approaches have been used successfully in barley and wheat to introgress and combine genes with large effect [79-81]. However, this approach is less effective for complex traits regulated by many small effect loci. The era of inexpensive genotyping mandates that we re-think previous strategies based only on phenotypic selection for these complex traits. Genomic Selection (GS) is an allele-based strategy that estimates breeding value of individuals using marker and phenotypic data [4-6].

Marker data allows phenotypic data from all individuals to be used to determine value of alleles and thus to infer the value of individuals carrying those alleles. Although excellent phenotyping remains essential, it is now practical to select on marker-based estimates of the genetic value of alleles versus phenotype-based estimates of the value of individuals. We will use the extensive genotypic and phenotypic data from this grant to implement and evaluate multiple strategies to estimate allele values that can be used in MAS and GS.

1.2.5. Graduate education in plant breeding. With the loss of plant breeding positions in the public sector there has been a loss of infrastructure and expertise that supports plant breeding training, and a concomitant loss of a critical mass of students necessary to provide a stimulating learning environment [82-87]. The situation has been exacerbated as the demand for plant breeders in the private sector has increased [88]. A recent Delphi study [87] shows the complexity of training plant breeders, highlighting not only scientific content but also hands-on experience in scientific inquiry and the development of interpersonal skills. Shared training is an appropriate response to these challenges [85]. By combining the resources of the wheat and barley breeding communities we will cover most of the expertise required to efficiently train modern plant breeders [89]. With increasing urbanization, many students are unaware of job opportunities in agriculture. The integrated national network of students, educational experts, researchers, public and private breeders proposed here provides a rich learning environment, which together with targeted recruitment efforts, will ensure a continuous pipeline of students into agricultural sciences.

2. RATIONALE

Publicly-developed wheat cultivars cover ~78% of the US wheat area, which in 2008 represented a farm value of $10 billion. Barley production averages approximately 242 million bushels per year with an estimated annual value of $701 million as a raw agricultural commodity (2000-2009). Wheat and barley hold a unique place in the economy of rural America, often sustaining farms where no other crops survive. Barley and wheat breeding programs are still mainly in the public sector, providing a unique opportunity for training students in plant breeding.

This project brings together the barley and wheat communities to generate the research, tools, germplasm, and human capital required to mitigate the impact of climate change on barley and wheat production. This project leverages and builds on the successful USDA-funded barley and wheat Coordinated Agricultural Projects (CAPs) through synergy in numerous research and education areas. Wheat and barley public breeding programs are usually co-located and face similar challenges in the area of diseases (e.g. the new stem rust race Ug99 affect both crops), WUE, NUE and yield. SNP marker technologies have been developed for both species, and both scientific communities are exploring GBS marker systems and gene-capture technologies. The genetic data for both species is coordinated by the same database (GrainGenes) and the informatics tools are transferable between crops.

The increased throughput and reduced costs of marker systems has shifted the paradigm for exploiting national germplasm collections from ad hoc evaluations of accessions to systematic and comprehensive evaluation of alleles. This project will provide an expanded centralized marker and trait database coupled with extensive analysis tools to empower breeders to exploit allele information and improve selection models. We will implement and evaluate high-throughput phenotyping, MAS and GS to dramatically accelerate breeding cycles.

The attrition of public plant breeding programs has negatively impacted plant breeders' training. We will mitigate this problem by creating a national training network to share plant breeding

expertise, decrease student isolation, and improve collaboration, factors known to have a positive impact on learning [90-92]. High proficiency in all subjects important to plant breeding is rarely found in a single institution. However, our diverse group collectively has expertise in most of these areas, which can be shared through a learning community and centralized training sessions. The combined strengths of the barley and wheat research communities will be leveraged to implement shared training approaches to educate future plant breeders.

3. PRELIMINARY RESULTS

During the last four years the barley and wheat CAPs have helped public breeding programs to integrate MAS strategies with traditional field-based breeding. These two projects were also instrumental in the integration of the breeding programs with USDA-ARS genotyping laboratories and genomics research programs. Previous results are summarized below.

The **BarleyCAP** implemented a nation-wide association genetics mapping study tightly coupled with ten breeding programs. The goal was to identify QTL within breeding germplasm and use the marker-trait information for barley improvement. A 2,943 SNP marker map was developed [55] and these SNPs were used to genotype 3,840 breeding lines from 10 breeding programs. A subset of these lines represents the elite breeding lines to be used in the proposed project. The lines were phenotyped for over 40 traits and deposited in the NSGC. The Hordeum Toolbox (THT) database was developed to house and display the genetic and phenotypic data (www.hordeumtoolbox.org). Using AM approaches, QTL for agronomic traits, disease resistance, and malt and food quality were detected [60-61]. Educational opportunities for students, breeders and scientists included: symposia, 'combine-to-kitchen' tours; workshops on QTL mapping, AM and GS; an association genetics analysis session; and the publication of 30 peer-reviewed publications. The BarleyCAP supported training of 31 graduate students, 28 undergraduate students and 13 postdoctoral research associates. Extension efforts are focused on working with the SolCAP to develop an eXtension site that provides information on barley.

The **WheatCAP** project implemented MAS strategies to improve quality and disease resistance across the US public breeding programs. The project generated approximately 1,000,000 MAS datapoints that were used to develop 90 new germplasm lines and cultivars http://maswheat.ucdavis.edu/Achievements/cultivars.htm, and thousands of improved lines in breeding pipelines. Markers were developed for 47 Mendelian traits and 363 QTL [93-96], and two of these QTL were map-based cloned [97-98]. The WheatCAP published the first SNP screening of US cultivars [99] and developed a 1,536-plex Oligo Pool Assay (OPA) that was used to characterize 480 US wheat cultivars. Twenty-three mapping populations were genotyped, phenotyped and deposited in the NSGC and represent a valuable resource to map genes affecting yield, WUE and NUE in this project. The WheatCAP supported training of 117 undergraduates and 73 graduate students, many of which are being hired as breeders in companies and public institutions. Results were disseminated through personal contacts with over 21,000 stakeholders at field days and workshops and 93 peer-reviewed articles.

4. SCIENTIFIC APPROACHES

4.1. Approaches for Objective 1: Discover and deploy beneficial alleles from diverse wheat and barley germplasm.

A diverse set of barley and wheat germplasm and populations will be genotyped and phenotyped for climate change-related traits to identify and deploy valuable alleles that help mitigate negative impacts of climate change.

4.1.1. Germplasm.

The focus of our project is the discovery of valuable alleles for the targeted traits rather than the characterization of specific accessions. We will conduct AM and NAM studies to identify alleles within the core barley and wheat NSGC, elite barley and wheat breeding populations, and specialized populations including wild introgression populations.

4.1.1.1. National Small Grain Collection. A core collection of 2,571 barley and 5,490 wheat accessions was assembled by the NSGC to capture most of the genetic diversity. Single spikes were selected from each accession and are being increased to generate pure seed. Height and heading date data will be available by the beginning of the grant. DNA has been extracted from the purified accessions and has been already characterized for cloned genes for height, vernalization and photoperiod sensitivity (by Dr. Gina Brown-Guedira). The complete barley and wheat core collections will be genotyped the first year of the project and phenotyped throughout the grant for resistance to the new races of leaf, stem and stripe rust. The barley core collection will also be phenotyped for resistance to the spot form of net blotch and spot blotch. Evaluations at the seedling stage with multiple races will be carried out by specialists in these diseases (see management plan), whereas adult plant resistance will be evaluated in field nurseries.

The NSGC core collection is very diverse for plant height and growth habit, two traits that have large effect on WUE, NUE and yield. Height and heading time data currently being collected will be used to assemble AM panels (4 barley and 6 wheat) as uniform as possible for these two traits. Results will be analyzed both separately and combined within species using height and heading time as covariates in the analysis. All lines will be evaluated in three main treatments: irrigated - normal N, drought – normal N, and drought -low N. Water stress will be applied with drip irrigation and determined by crop water use and rainfall information (http://www.usbr.gov/pn/agrimet/). N stress will be applied based on soil tests before planting and monitored during several growth stages. A total of 1200 barley and 1800 wheat lines will be evaluated in these three treatments in Aberdeen, ID (<10 inches of rain per year). Each year 600 lines will be evaluated in the different treatments (total 1,800 plots, 5 feet x 10 feet each) for grain yield, WUE (see 4.1.3.1) and NUE (see 4.1.3.3). The objective is to identify valuable alleles within the NSGC collection rather than to characterize accessions. Replication of alleles will occur across accessions, and individual genotypes will be included once within each of the three treatments, as has been shown to be more efficient for QTL identification [136-137].

4.1.1.2. Nested Association Mapping (NAM): The second strategy uses a small and diverse set of core NSGC accessions and incorporates the allelic diversity from the un-adapted accessions into a common adapted parent using NAM populations [66]. NAM populations will be developed by single seed descent (SSD) to facilitate selection for growth habit and height. The NSGC parental lines will be selected based on the genotyping data generated during the first year to maximize diversity. Each SSD population will be reduced to ~100 lines of uniform height and heading date. Larger NAM populations will be developed for barley than for wheat because barley lines are simpler to genotype. To expand the number of NSGC wheat lines characterized in this project, 300 additional NSGC wheat lines will be selected based on genotype and phenotype and used in crosses with top yielding lines and advanced through the normal breeding cycles.

The following NAM populations will be developed in this project.

- Six-row spring barley: 9,600 lines derived from the cross of the six-row barley cultivar 'Rasmusson' with 96 diverse accessions.
- Two-row spring barley: 9,600 lines derived from the cross of the two-row barley cultivar 'Baronesse' with 96 diverse accessions mostly different from the six-row NAM.
- Spring wheat: 2,500 lines derived from crosses between the drought-tolerant semi-dwarf cultivar 'Berkut' from CIMMYT with 25 diverse spring lines from the wheat core NSGC.
- Hard winter wheat: 2,500 lines derived from crosses between the high-yielding cultivar 'Jagger' with 25 diverse hard winter lines from the wheat core NSGC.
- Soft winter wheat 2,500 lines derived from crosses between the cultivar 'Shirley' with 25 diverse soft winter lines from the wheat core NSGC.

Seeds from the NAM lines will be deposited in the NSGC as a long-term resource for QTL mapping, allele mining and gene discovery. Subsets of the spring barley and wheat NAM populations will be phenotyped for NUE and WUE during the second half of this grant. For the winter wheat NAM, three Jagger populations developed during the wheat CAP are ready for phenotypic evaluations. However, the rest of the winter wheat NAM populations will require the entire grant for development, and will be transferred to the winter wheat breeding programs that have committed to future evaluations.

4.1.1.3. Elite AM panels. We will also explore the diversity currently present in elite barley and wheat breeding populations by assembling elite AM panels to identify marker associations with biotic and abiotic stress tolerance and to train GS models. Because these alleles are already present in the breeding programs, this effort will facilitate the immediate selection and deployment of valuable alleles. We propose the following AM panels:

- Spring six-row barley: 256 advanced breeding lines and cultivars for NUE, WUE and yield.
- Spring two-row barley: 256 advanced breeding lines and cultivars for NUE, WUE and yield.
- Winter six-row barley: 256 advanced breeding lines and cultivars for NUE, WUE and yield.
- Barley LTT panel: 384 lines from international collections for winter hardiness improvement.
- Spring wheat: 288 lines for drought tolerance (collaboration with CIMMYT).
- Wheat diseases: 384 lines for leaf rust and 384 lines for stripe rust.
- Hard winter wheat: 300 hard wheat lines for WUE, NUE and yield evaluation.
- Soft winter wheat: 300 soft wheat lines for NUE and yield evaluation.

The barley AM panels were developed and genotyped in the BarleyCAP. The wheat (and barley LTT) AM panels will be genotyped in this grant (3,072 SNP platforms). The elite winter wheat AM panels (hard and soft) will include entries from regional uniform trials so that yield data from those trials can supplement the data from this grant. All wheat AM panels will also include parental lines of the 23 RIL populations developed in the previous WheatCAP. Collaborative-breeding winter wheat panels (4.2.3.) will be used for validation.

4.1.1.4. Special mapping populations. We will use available NILs, wild chromosome backcross introgressions, and targeted RIL populations to study specific aspects of WUE and/or NUE.

- Wild barley introgression population: 900 BC_2 lines derived from 25 wild barleys [100] crossed with 'Rasmusson' are available. The population will be genotyped with U.S. Barley Genome Project funding. Seed from each line (BC_2S_4) will be available in the 2nd year of the grant for field phenotyping for WUE, NUE and yield.

- Wild Triticeae introgressions in wheat: 300 alien *Triticeae* translocation and substitution lines into CS wheat will be genotyped and phenotyped for WUE. Superior lines will be crossed to *ph1b* mutants to produce shorter translocation stocks and map the beneficial QTL.
- Isogenic wheat lines for stem solidness: Solid stems are highly correlated with water soluble carbohydrates in the stem, which are hypothesized to be remobilized to the grain during drought stress [18]. We have developed six sets of NILs to test this hypothesis [101].
- Isogenic wheat lines for long grain fill period: Increased grain fill period has been shown to be important in environments with terminal drought stress [102]. A major QTL for delayed senescence has been identified [102] and used to develop 60 NILs in several genetic backgrounds for QTL validation.
- Isogenic wheat lines for *Rht*8 height alleles: The *Rht8* allele reduces final plant height without impacting coleoptile length, allowing deeper sowing in drought-stressed areas [103]. Sixteen pairs of *Rht-B1b*, *Rht-D1b*, and *Rht8* NILs in four short standard height cultivars will be evaluated for WUE.
- Isogenic wheat lines for the 1RS rye translocation: The 1RS.1BL translocation increases drought tolerance, root biomass, and grain yield under reduced moisture conditions [104-105]. Thirty lines with different 1RS translocated segments [106] will be used to map the drought resistance locus and to separate it from 1RS loci associated with poor quality.
- Wheat backcross RILs for drought tolerant C306 (drought tolerant)/2*PBW534: 400 BC_2 RILs will be genotyped with 3,072 SNPs and phenotyped for WUE.
- Wheat Hexaploid and Tetraploid RIL from a Spring Wheat x Durum Cross: 200 RILs from the cross Choteau (6x) x Mountrail (4x) will be genotyped and phenotyped for WUE to identify favorable alleles for transfer between species.
- Barley RIL populations for drought tolerance. 200 RILs derived from the cross Otis (drought tolerant) x Garnet (drought susceptible) and 200 RILs from Otis x Golden Promise (drought susceptible) will be genotyped and phenotyped for WUE.

4. 1. 2. Genotyping and data analyses. Genotyping is changing dramatically and it is not possible to predict when GBS will replace current SNP platforms. Therefore, the genotyping platforms that will be used for a specific objective in the last years of this project will be selected according to these criteria: 1) low cost; 2) high density; 3) robustness; 4) compatibility with historical data; and 5) minimize ascertainment bias.

In the first year of the grant, the complete barley and wheat core NSGC collections will be genotyped with the available Illumina SNP platform including 3,072 SNPs in a single OPA for each species at the USDA-ARS Genotyping Lab at Fargo, ND [99]. The founders of the barley and wheat NAM populations will be sequenced using high-throughput sequencing and complexity reduction [63] and will be simultaneously genotyped with a dedicated 1,536-plex OPA enriched in common-parent-specific SNPs [107] to act as bridges among platforms and to facilitate the implementation of marker imputation methods for joint analysis of panels that have been scored with different marker platforms [108-110].

For barley, we plan to genotype the LTT-AM panel with the 3,072 barley OPA (other panels have been genotyped as part of the BarleyCAP) and the NAM and wild introgression populations using the 384 SNP platforms, unless GBS technology becomes more competitively priced. Wheat has a more complex genome and is less polymorphic than barley so it requires more markers for adequate genome coverage. Thus, in wheat we will use 1,536-plex OPAs for GS studies and 3,072-plex OPAs for elite AM panels. The wheat spring NAM populations will be

genotyped with GBS and 1,536 SNP platforms enriched in the common parent SNPs, to integrate markers into a single map. Winter wheat NAM populations will be genotyped only by GBS.

We will generate separate winter and spring (barley and wheat), hard and soft (wheat), and two- and six-row (barley) AM panels to take into account the subpopulation structure that exists within the US germplasm [71-72, 99]. We will use mixed-model analyses [75-76] and multi-locus analyses to overcome the problem of additional subpopulation structure and to reliably identify loci of smaller effect [111-113]. Validation of marker-trait associations will be accomplished in several ways: 1) comparison to previous QTL studies, 2) validation in NAM and RIL populations, and 3) development of NILS from heterozygous breeding lines.

4.1.3. Phenotyping.

The germplasm and populations described above will be evaluated for WUE, NUE, yield and fungal pathogens as described below. The WUE, NUE, yield evaluations will be performed in locations with no disease pressure or will be treated with fungicides to avoid the confounding effects of differences in disease resistance. High-throughput phenotyping methods will be tested and implemented to evaluate WUE and NUE (CT, CSR and CID). Disease resistance will be evaluated in controlled environments and field locations with high diseases pressure.

4.1.3.1. WUE parameters: Experiments will be performed at field sites where drought is observed almost every year, and water availability will be regulated by irrigation. We will assess drought tolerance using the ratio of productivity (grain size, weight and yield) under water stress vs. non-stressed conditions. The following additional parameters will be determined to characterize WUE of the tested lines: heading, height, flag leaf senescence, canopy temperature (at three growth stages) [15], CID (grain $\Delta C^{13}/C^{12}$) [14], and normalized water CSR indices, NWI-1 and NWI-3, which have shown the best correlations with grain yield in environments with limited soil moisture [12, 16]. We will explore correlations among the different parameters and perform association and linkage studies to identify markers associated with valuable loci.

4.1.3.2. Barley LTT. As an additional strategy to improve barley WUE, we will select lines with improved LTT that can be planted in the fall to take advantage of winter precipitation and avoid drought. We will use high-throughput machine-planted head rows and repeated checks combined with visual survival assessments. In addition to the field assays, we will use available perfect markers to determine the alleles for vernalization and photoperiod sensitivity [22-24, 114-116].

4.1.3.3. NUE parameters: We will assess NUE using the ratio of productivity (grain size, weight and yield) under N limiting vs. non-limiting field conditions. N will be measured from flag leaves at anthesis and in mature grains. These parameters will be correlated with different CSR indexes that are highly associated (R^2= 0.83-0.87) with leaf N concentration (REIPle and λo) and leaf N accumulation (MSS-SARVI and FD742) [36]. Additional indices will be used to estimate biomass and predict yield. N and CSR phenotypes will be used in AM studies to identify loci responsible for genotype differences in NUE. We will record test weight, kernel plumpness, height, heading date, yield and grain protein content. In the hard wheat, we will continue with the incorporation of the high grain protein content gene *Gpc-B1* cloned during the previous CAP grant [97]. The functional *Gpc-B1* allele increases N remobilization from the leaves to the grain, resulting in significant gains in total grain N [117] and quality [118]. We will deploy the high grain-protein content alleles in hard wheat [97, 117] and barley lines [119] to rapidly increase NUE.

4. 1.3.4. Yield. Yield *per se* and yield ratios between stressed and optimum environments will be used as selection criteria to improve productivity in water and N stressed environments. Yield data will be collected from replicated trials conducted over multiple years and locations. Multivariate statistics will be used to determine G by E interactions and yield stability.

*4.1.3.5 Biotic stresses***:** We plan to evaluate all 8,060 accessions in the barley and wheat core NSGC collections for resistance to new races of stem, leaf and stripe rust. Multi-pathotype seedling resistance screens under controlled environments will be completed in years 1 and 2 (see management plan). Resistance to the new race of spot blotch will be tested under controlled environments to avoid field escapes. In addition to the NSGC core lines, two dedicated 384-AM wheat panels will be evaluated for stripe and leaf rust.

Field evaluations for APR will be distributed over the five years of the grant. All barley accessions from the core collection will be evaluated for stem rust (domestic races) in MN, APR to Ug99 in Kenya, stripe rust in CA and OR, and to the spot form of net blotch and spot blotch in ND (see management plan). Wheat field evaluations for APR to stripe rust will be conducted at CA, OR, WA, and KS. Leaf rust and stem rust will be evaluated in TX, OK, KS, and MN (see management plan). The combination of seedling and APR data with genotypic data will allow postulation of known genes and identification of novel sources of resistance. Novel sources of leaf and stripe rust APR will be targeted during years 4 and 5 of the project. High-density mapping and validation of APR genes will be performed using NILs derived from the breeding program or available RILs from previous barley and wheat CAPs and other projects. New APR genes will be deployed in the breeding programs using MAS.

4.2. Approaches for Objective 2: *Accelerate breeding through MAS and GS*.

Our goal is to facilitate the implementation of barley and wheat molecular breeding programs by developing 48, 384, and 1536 SNP platforms. The 48-SNP chips will be used for MAS efforts targeted to known genes and QTL, whereas the 384- and 1,536 chips will be targeted mainly to implement and evaluate GS approaches for yield and NUE in barley and wheat, respectively.

4.2.1. Wheat and Barley MAS: SNPs for known genes and linked QTLs will be incorporated by the Genotyping Centers into 48-SNP platforms. In barley, additional SNP-trait associations are already known from the BarleyCAP. As new SNP-trait associations are identified within this project, they will be incorporated into additional 48-SNP platforms.

In barley, we will develop 48-SNP platforms for the two-row programs, the Midwestern six row programs, and the winter program. In wheat, the initial 48-SNP chips will include the main agronomically important cloned genes (e.g. photoperiod [120], vernalization [114, 121], height [122], gluten strength [123], grain texture [124-125], grain protein [97]; durable resistance [98, 126]) as well as diagnostic markers for disease resistance genes identified during the previous WheatCAP. The 48-SNP chips will be designed and evaluated in year 1 and 8,000 assays per year (Y2-Y5) will be divided among the public breeding programs to accelerate selection of favorable alleles.

4.2.2. Genomic Selection (GS) – GS complements MAS by using all marker information to calculate genomic estimated breeding values (GEBVs) enabling marker-based selection for complex traits. Selection on GEBV without phenotyping can significantly shorten breeding cycles. We will use ridge regression and a stochastic search variable selection method to train GS models [6]. The best model will be chosen on the basis of cross-validation accuracy in each panel. In both barley and wheat, we will use a genotyping strategy of high-density markers on parents coupled with low-density markers on progeny selection candidates (barley 384-SNP chips, wheat

1,536-plex OPA). Using progeny markers, inherited parental alleles can be accurately imputed to allow high-density predictions of GEBV [127].

In barley, we plan to do two cycles of selection per year for the LTT facultative barleys, which compares favorably to a typical three to four year cycle using phenotypic selection. Two barley breeding programs will participate selecting initially for LTT and yield. Genotyped facultative barleys will have been tested for those traits by the start of the grant, and a GS prediction model trained using that data. In total, seven cycles of selection will be possible prior to the end of the grant. Additions to the GS training population will be made each year by fall planting of head rows of all plants genotyped, and rating the head rows for winter survival.

In wheat, GS will be implemented in six winter breeding programs. Because of the longer growing cycle of winter wheat, we developed a breeding scheme that will enable one cycle of GS for yield per year, which favorably compares to a typical five-year cycle using phenotypic selection. By July 2013, we will have phenotypes for traits impacted by climate change on elite panels to train global and program-specific GS prediction models. These models will be used to complete two cycles of GS in winter wheat.

4.2.3. Cooperative allele-based breeding strategy: Since the winter wheat NAM populations will not be available for phenotyping until the end of the grant, the winter wheat breeders developed an allele-based breeding strategy that will use equivalent genotyping resources and enhance cooperation among breeding programs within three winter wheat regions. Each of the 13 participating breeders will select ~160 lines per year that will be entering the first year of replicated, multi-environment yield evaluations (YR1 trials). Half of these will enter that breeder's YR1 trials, the other half will be evenly split among other breeders' YR1 trials within the region. Breeding programs will provide yield data (years 2-4) and the project will provide genotyping for 5,000 lines. Alleles and GS models will be evaluated within and among programs, genetic backgrounds, environments, and years. This strategy will determine the value of existing and introduced alleles in a relevant breeding context, leverage public breeding resources, and develop a long-term yield improvement strategy.

4.3. Approaches for Objective 3: Genotyping by sequencing (GBS). The dramatic reductions in costs make GBS methods competitive with current SNP platforms. We will test and implement two genome complexity-reduction methods for GBS: sequence capture using oligonucleotide baits [64-65] and restriction digestion of genomic DNA [63]. Initially, we will anchor GBS polymorphisms to bi-parental populations in both wheat and barley, and sequence the 192 barley and 75 wheat founders of the NAM populations. GBS is budgeted for the 7,500 wheat NAM lines and will be extended to other barley and wheat populations as it becomes cost-effective. Scoring of NAM progeny will greatly enhance accuracy of sequence-read anchoring on the genetic map. Single sequencing reaction costs can be spread across many genotypes by multiplexing using bar-coding [128-130]. We will construct 12- to 96-plex pools to determine optimal plex levels to minimize costs while ensuring robust allele calls.

4.3.1. Sequence capture. Resequencing will be used to assess the haplotypic structure of 20,000 genes and will provide an unbiased estimation of the distribution of gene diversity within the NSGC core collections. Genes for re-sequencing will be selected from the UniGene section of the NCBI database to be uniformly distributed across the genetic maps. In-solution sequence capture reactions will be performed using the SureSelect kit (Agilent). We have tested this approach by re-sequencing 3,500 gene fragments (Akhunov et al., unpublished data) and

achieved a 1,200-fold enrichment level for targeted sequences. We will validate this approach in barley in year 1. Sequence capture technologies are still too expensive to be used for large-scale genotyping. Multiplexing will be done prior to capture to reduce per-genotype cost. Multiplexed libraries will be combined into a single capture reaction, sequenced in a single lane of Illumina HiSeq2000 flow-cell and the best level of multiplexing will be selected for further analyses. These data will provide a first picture of gene diversity in the core NSGC. This information, together with functional annotation of the SNPs will be incorporated into the T3 database.

4.3.2. Whole genome genotyping by sequencing (GBS). Sequence tags of DNA fragment ends generated by digesting with a restriction enzyme are a rich source of SNPs and have attractive features for mapping [63]. The number of tags can be regulated using different restriction enzymes allowing optimal reduction of complexity and high-density genome coverage. Preliminary experiments will be performed with previously mapped RILs. Libraries will be developed using both 6-base and 8-base restriction enzymes. Sample pools will be sequenced on a single lane of Illumina HiSeq2000 to determine suitable levels of multiplexing and the best restriction enzymes for maximum genome coverage with tagged sites.

4.3.3. SNP detection and data analysis. Sequence alignments for SNP discovery will be performed using the SOAP software (soap.genomics.org.cn), which includes programs for both *de novo* assembly and identification of SNPs in sequence alignments. Reference sequences will be created by assembly of sequence data into contigs using the SOAP *de novo* assembler and used for mapping sequence reads and discovering SNPs with SOAPsnp [131]. Genetic map positions for new SNPs will be determined by analyzing the genotypic data collected for mapping populations. Analysis of restriction based sequence reads will be also conducted using TASSEL (http://sourceforge.net/projects/tassel/). Mapped SNP markers will be used to anchor the sequence reads and to compare de-novo map construction using only GBS. For barley, sequence reads will be anchored to the physical map as sequences become available. Sequence data will be used for studying haplotype variation and patterns of LD across genomes and selecting tagSNPs for genome-wide association mapping [132-133]. Sequence data from founders of NAM populations will be transferred to RILs using common-parent-specific SNPs [107].

4.4. Approaches for Objective 4: Databases and informatics tools

We will expand "The Hordeum Toolbox" to wheat and develop protocols for depositing and analyzing genotype, phenotype and pedigree data to integrate genomics information with plant improvement. We will also develop the theoretical framework and the web-based tools to help breeders implement MAS and GS in breeding programs and integrate data across programs.

4.4.1. Database. The Hordeum Toolbox is the database that holds phenotype and genotype data collected by the BarleyCAP. With limited changes the current design will be adequate for the storage requirements associated with this proposal. Most of the THT schema will be duplicated in the project T3 database and used to store wheat data, with a few tables shared by both the barley and wheat schemas. The current design will be modified to accommodate data generated by next-generation sequencing by adding fields to the existing schema. Sequence data will be stored as binary large objects (BLOBs) in a compressed binary format. The format and scripts for writing and reading data of that type have been developed by the GDPDM database team. The creation, hosting, and administration of T3 will be handled by GrainGenes.

4.4.2. Data Curation. This project will provide an unprecedented data set for investigating the genetic basis of variation for WUE, NUE, yield and disease resistance in wheat and barley. To

ensure maximum use of this information all the germplasm, mapping and allelic information will be made accessible through T3. The database website will allow participants to upload data, run basic data quality checks, and incorporate data that passes those checks to the database. Automated quality control of marker data will be implemented in the database using data imputation methods [109]. Personnel from laboratories participating in the project will be trained to use these web-based tools to submit data in a format ready to be deposited in the project database, which will in turn provide data to collaborating databases maintained by GRIN, Gramene, and GrainGenes. A project curator will monitor data quality, provide participants with help in using the upload tools, and ensure that participants submit data in a timely manner.

4.4.3. Data Management, Access and Analysis. Software development efforts will be needed to load the data and to make it useful to project participants and the broader research community. In addition to basic upload and query tools, the project will develop data analysis and visualization tools. In particular, analyses, text and graphic output will be developed to provide breeders with the ability to apply data collected by the project to cultivar improvement. Those tools will include AM analysis using a mixed model to correct for population structure, a GS toolset that builds models from training sets and uses those models to predict phenotype from genotype, and methods for mapping QTL in NAM populations. Because the list of potential applications will greatly exceed the resources available to develop them, a user group will be established at the beginning of the project to propose, describe, and prioritize data access, analysis, and visualization tools and to test new applications and provide feedback. Work on analysis tools in the first two years will focus on implementing existing analyses. In the final three years, the effort will shift to algorithm and analysis method development for GS and QTL identification.

An obstacle to widespread use of MAS and GS by plant breeders is the need for more sophisticated data management to coordinate genotype data and phenotype data with genomic data from external sources then to combine and format these data for further analysis. This project will provide participants with the ability to upload non-project data from their programs and make use of data management and analysis tools for their own breeding programs and to share data and analyses with collaborators. Thus, these project resources will be leveraged to provide value to the breeding community outside of specific project goals.

4.4.4. Relationships to external databases. Data will need to be moved between T3 and GRIN. T3 will store all phenotype and genotype data collected by the project. GRIN will store data for all accessions in the NSGC. Data transfer will be handled by periodic batch updates, with details to be worked out in the first year of the project. Data from T3 will be made available to other GrainGenes databases and to the Gramene database with formatting issues to be handled by T3 staff. Links to appropriate GrainGenes and Gramene data will be built into the T3 website.

4.5. Approaches for Objective 5: Plant Breeding Education Network

To insure well-trained plant breeders we will: 1) actively recruit students to agricultural sciences, especially under-represented minority students, and 2) create and implement a Plant Breeder Training Network (PBTN), a collaborative learning community for training graduate and undergraduate students in plant breeding. This project will support 29 PhD students for four years and one undergraduate student per year per PhD student (~100 undergraduates), and will provide travel support for them to attend centralized training and student symposia.

4.5.1 Recruitment and support of minority students

Minority students represent less than 15% percent of those who receive degrees in plant sciences (FAEIS, June 2010). Our efforts will be aimed at understanding factors that discourage minority students from entering plant science and developing messages, materials and programs that address these factors. We will present plant breeding as a life science career and promote interest among underrepresented students.

The first year we will build bridges with Minority Serving Institutions (MSI) and other undergraduate institutions by working with faculty to create curriculum support, and joint research projects. We will visit institutions and give talks in classes, seminars and/or club meetings utilizing materials created in collaboration with Montana State Univ. film school (see Management Plan) to enhance student and faculty understanding of, and interest in, programs and careers in plant breeding. Interested faculty will be hosted at UMN, UNL and MSU, to help design the recruitment and training program and to create curriculum that features plant breeding science using a Problem Based Learning (PBL) approach (see below). Thirty students from MSIs will be hosted in years 2 to 5 for a 7-day plant breeding exploration trip to UMN, UNL and MSU. These students will experience plant breeding research in the laboratory and field. The trip will include visits to production fields, grain handlers, millers, bakers, and brewers to better understand the work involved in maintaining our food chain. It is our hope that outreach to MSIs will enrich the diversity of future undergraduate and graduate students in agricultural sciences.

4.5.2 Plant Breeder Training Network (PBTN)

The focus of this objective is to develop and implement an interconnected network of undergraduate and graduate students, private and public breeders, geneticists, computer scientists, and international partners. The PBTN will be used to train students and project participants in the multiple subject areas required for modern plant breeding [84, 87, 134]. This network will include both online and face-to-face interactions modeled after the "Plant breeding education in a university without walls" program [89].

4.5.2.1. PBTN on line interactions: The objectives of this online environment are to decrease student isolation, improve collaboration, and provide access to technical expertise in multiple areas of plant breeding. Dr. D. Namuth-Covert will administer the online environment that will be housed on UNL servers and will leverage resources invested by NSF to expand the eLibrary to include social networking tools, text-based blogging tools, and the ability for instructors to create individualized web environments. The eLibrary is a growing electronic learning environment of peer-reviewed learning objects that distributes content to ~129 countries (half million visits last 12 months). Funds from this grant will support a post doctoral computer scientist to develop additional features including audio and video communication tools. Dr. Namuth-Covert is a co-leader in the Plant Breeding and Genomics Community of Practice in eXtension and will facilitate communication and coordination of activities with eXtension (see letter of collaboration from Dan Cotton). Linking educational resources within the eLibrary, eXtension and PBTN will bring together important partners in the plant breeding education field.

4.5.2.1.1. Problem based learning (PBL): Both synchronous and asynchronous PBTN online environments will focus on PBL to improve problem-solving skills, increase collaboration, and provide students a participative perspective on approaches to plant breeding. Real-world breeding challenges for food production will be introduced in the bi-weekly, on-line meetings to provide compelling problems that students will be asked to address in teams. Teams will meet and work together (online) to identify questions, locate data and resources, and discuss results

that will be shared at large-group meetings. Through collaborative efforts and collective discussions the group will propose different approaches to the problem. Students will rotate among teams and research different problems gaining a broad understanding of plant breeding and building new networks. Undergraduate PBL materials will connect biology concepts that are foundational to plant breeding goals. Online courses will also rely heavily on PBL strategies.

4.5.2.1.2. Asynchronous courses and materials: PBL curriculum, including written and video descriptions of problem scenarios, online resources and instructor guides, will be developed for use in undergraduate courses in collaboration with teachers from MSIs and other programs. These materials will be created with open source software and will be housed at "The Plant and Soil Sciences eLibrary" (http://plantandsoil.unl.edu) (see Management Plan).

4.5.2.1.3. Synchronous meetings: PhD and undergraduate students participating in the project will use this on line environment to meet bi-weekly during the nine-month academic year. The educational coordinators with input from the students and the professional education evaluators (see management plan) will design content and activities and coordinate meetings. Project researchers and invited speakers from industry and international research centers will participate in the meetings (see industry letters of support).

Each of the breeders and researchers with PhD student funded by this project has agreed to present at least two lectures per year in their area of expertise, guaranteeing students' access to the best specialists in different areas of plant breeding. The goals are to share expertise across institutions and to provide students a venue to share their research experiences and seek input from breeders and researchers at collaborating institutions. This online environment also will be used to train students in the different tools generated by the project research teams.

4.5.2.2. PBTN face-to-face interactions. The online training will be complemented by hands-on and face-to-face training in plant breeding. The PhD students and their matched undergraduate students will work directly with the PDs and the breeding programs at their home institution to obtain research experience in all aspects of plant breeding. This traditional training will be complemented by a variety of activities and resources implemented through the PBTN and aimed at broadening knowledge and building critical skills:

- Each graduate student will mentor one undergraduate student per year, resulting in the training of ~100 undergraduate students. Students will be trained to be good mentors through "Entering Mentoring" an eight-session seminar led by Sherman, Brakke, and Lee [135].
- Students will attend centralized training workshops on phenotyping (yield, WUE, NUE, CSR, diseases), genomics (MAS and GS), and bioinformatics.
- Students will attend the annual PAG meetings and a symposium in Plant Breeding in Response to Climate Change in collaboration with Canadian breeders (see collaboration letter) to learn about current topics in genomics and climate change.
- We will organize graduate and undergraduate symposia at PAG each year where students will present their research to their peers, to develop their communication skills, and to establish a well-interconnected cohort of young plant breeders.
- We will provide students opportunities to conduct short internships in industry (see attached letters from Pioneer and Monsanto). Additionally, members of industry will be invited to join online meetings to provide information and perspectives.
- To provide graduate students with insights on global aspects of plant breeding and to provide them the opportunity to build international collaborations we will organize a one-week visit

to CIMMYT (see attached letter from CIMMYT). CIMMYT will provide hands on phenotyping experience and an introduction to international plant breeding.

5. OVERSIGHT AND EVALUATION

5. 1 Oversight research activities. Each participant will be required to submit a yearly progress report and work plan that will be reviewed by the project directors and the executive committee. The Scientific Advisory Board and the Industry Liaison Committee (see management plan) will receive the reports detailing progress achieved towards each milestone. The PD will provide USDA a written report including board recommendations and actions taken by the project Examples of evaluation include but are not limited to: 1) Utility of various approaches to identify beneficial alleles for climate change related traits. 2) Utility of genotyping-by-sequencing approaches. 3) Number of breeders that utilize SNPs and T3 database and tools. 4) Number of SNP datapoints generated by the project. 5) Number of marker associations with valuable traits discovered. 6) Number of breeders initiating MAS and GS for climate change-related traits. 7) Number of cultivars, germplasm stocks and populations released. 8) Number of publications.

5. 1 Oversight educational activities: Oversight will be provided by an advisory committee composed of nationally recognized education and plant breeding experts, two professional educational evaluators (see management plan) and two education PhD students that will perform research projects on the evaluation of the educational activities from this project. Evaluators and advisors will receive annual reports and provide recommendation to the educational coordinators and coPDs. Educational activities will be evaluated through monitoring and controlled studies, tracking both quantity and quality. Design experiments will be used to improve educational tools. Surveys and focus sessions will assess student interest and attitude. Network analysis will be used to assess professional networks as a result of PBTN. Comparison studies will be conducted to assess recruitment, networks, collaboration and problem-solving skills.

6. EXPECTED OUTCOMES AND DISSEMINATION PLAN

6.1. Research outcomes for barley and wheat: 1) Elite lines genotyped and characterized for climate change related traits. 2) New germplasm and cultivars better adapted to changing environments. 3) QTL identified for climate change related traits. 4) Large NAM and AM populations developed and characterized. 5) Beneficial alleles for biotic and abiotic resistance identified in the core NSGC. 6) New technologies to genotype and phenotype large numbers of accessions. 7) Genotypic and phenotypic information for a large number of breeding lines. 8) GS models developed for climate change-related traits. 9) MAS and GS approaches to reduce the length of breeding cycles. 10) Development of allele-based breeding strategies. 11) Utilization of the breeding database. 12) Website tools for uploading, downloading and analyzing data.

6.2. Education outcomes: 1) 29 well-trained plant breeders using novel methods. 2) Increased exposure to, and interest in plant sciences among undergraduate students 3) A plant breeding community of learning, the Plant Breeder Training Network. 4) Research experiences for approximately 100 undergraduate students. 5) Undergraduate students better prepared for graduate school. 6) Extended bridges with MSIs and more opportunities for MSI students. 7) Novel materials to support students' education freely available on line. 8) Broader educational experiences for undergraduate and graduate students. 9) Improved content delivery. 10) Better mentors. 11) Implementation of Problem Based Learning in undergraduate and graduate courses.

6.3. Dissemination plan: Knowledge generated from this project will be disseminated through peer reviewed publications, presentations in scientific and stakeholder meetings, workshops, symposia, presentations to stakeholders and growers in field days, and demonstration plots in collaboration with extension specialists and farm advisors. Data will be publicly available on T3. Education materials will be disseminated via the project website and the eLibrary. Online components of the PBTN will be made available to interested institutions.

7. STAKEHOLDER INVOLVEMENT

Support and collaborations from stakeholders is documented by attached letters from: 1) Barley and wheat growers represented by the National Association of Wheat Growers, Wheat Associates, the National Barley Improvement Committee, and 27 state commissions and associations from all US barley and wheat growing states. 2) Barley malting and brewing industry (Anheuser-Busch, Rahr, Millers Coors). 4) North America Millers' Association. 5) American Bakers Association 6) Seed companies (Monsanto and Pioneer). 7) International collaborators (CIMMYT and Canada). 8) Educational partners including the Plant Breeding Coordinating Committee, 9) eXtension, and 10) minority serving institutions.

8. TIMELINE

Obj.	Tasks and milestones*	Y1	Y2	Y3	Y4	Y5
1a	NSGC characterization (wheat Y1, 3 & 5, barley 2 & 4)	x	x	x	x	x
1b	NSGC genotyping	x				
1c	AM panel genotyping and phenotyping		x	x		
1c	NUE and yield phenotyping		x	x	x	x
1d	WUE phenotyping (NRILs, NILs, AM Y1- 3, NAM 4-5)	x	x	x	x	x
1e	Disease phenotyping	x	x	x	x	x
1f	NAM population development	x	x	x		
1g	NAM population genotyping and phenotyping			x	x	x
1h	Specialized pops. & genetic stocks characterization	x	x	x		
2	MAS and GS in breeding programs		x	x	x	x
3	Re-sequencing by sequence capture	x	x	x		
3	GBS development and sequencing of parental lines	x	x	x		
3	GBS of NAM populations			x	x	x
4a	T3 database development	x	x			
4b	Analysis tools		x	x	x	x
5	MSI recruitment tools created & teachers trained	x	x			
5	MSI student experiential trips		x	x	x	x
5	Development of PBTN (online and face-to-face)	x	x	x	x	x
5	Curriculum created to support plant breeding training	x	x	x	x	x
5	PBL modules created and delivered	x	x	x	x	
5	Graduate and undergraduate student training	x	x	x	x	x
5	Workshops for phenotyping (WUE, NUE), MAS, GS	x	x	x	x	x
5	Student symposia	x	x	x	x	x
5	Mentor training and support	x	x	x	x	x
5	Climate change symposia with PBCC	x	x	x	x	x
5	Trip to CIMMYT			x		x
5	Internships in industry		x	x	x	

* Personnel responsible for the different tasks are listed in 'Key Personnel'

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