TCAP PROJECT 2014 SUMMARY

Executive summary

***Objective 1: Identification of new valuable alleles in diverse barley and wheat germplasm*:** Extensive phenotyping was performed in controlled environments and in field conditions for disease resistance, water use efficiency (WUE) and nitrogen use efficiency (NUE). Significant improvements were made in canopy spectral reflectance (CSR) protocols and analysis. Genotypic and phenotypic data sets from multiple environments were integrated into genome wide association studies (GWAS) that yielded valuable marker-trait associations for the different target traits.

Disease resistance: The barley core collection and dedicated association mapping (AM) panels were evaluated for resistance to current races of major barley pathogens. Resistance alleles for stripe rust, stem rust, leaf scald, spot blotch, spot-form net blotch and cereal yellow dwarf virus were identified. Highly significant QTL for stripe rust resistance were identified by GWAS in the NSGC wheat core collections. GWAS were also performed for stem rust and leaf rust in both spring and winter wheat panels. Several GWAS studies were published (Appendix I1). Resistance genes were also identified in biparental populations for resistance to stem rust race Ug99, leaf spot diseases, stem sawfly, Hessian fly, and orange wheat blossom midge. Many of these resistance genes have been incorporated into commercial varieties.

***Water use efficiency (WUE)*:** Barley WUE phenotyping activities were completed and all data was uploaded to T3. Facultative barley varieties with improved low temperature tolerance (LTT) are being developed to take better advantage of winter precipitation. Using a large germplasm collection novel QTL were identified and known QTL were validated. The spring and winter wheat AM panels were evaluated in multiple locations in the US, Canada, and Mexico for WUE. GWAS identified several highly significant QTL for normalized water index NWI3 and related WUE traits, some consistent across locations. Valuable associations were detected in a tetraploid x hexaploid wheat population and in recombinant lines of the rye 1RS introgression. Studies of root characteristics and physiological traits associated with WUE and heat tolerance were completed and published in 2014 (Appendix I1).

Nitrogen use efficiency (NUE): In barley, all scheduled NUE field trials for the AM panels (spring six-row, spring two-row, and winter six-row) in low (70%) and normal (100%) nitrogen treatments have been completed and data have been uploaded to T3. Preliminary association analyses of NUE-related traits and NUE indices in the spring six-row panel have identified a major QTL at the *Gpc-1* locus and two other minor QTL. In wheat, the favorable *Gpc-B1* allele has been deployed in several commercial varieties. This allele increases grain protein content resulting in concrete improvements in NUE. The hard and soft winter wheat panels have been genotyped and phenotyped for yield, yield stability, CSR and NUE at multiple locations under different N levels. Variation in NUE was detected among accessions.

***Population development*:** Development of the barley six- and two-row nested association mapping (NAM) populations was completed. A seed increase and preliminary trait evaluation of the six-row NAM was conducted in MN. The seed increase for the two-row NAM is being conducted in greenhouses in ND. The spring wheat NAM population was

completed, the seed was increased and the first four sub-populations were phenotyped. The rest of the populations were planted for evaluation in 2015. The winter wheat NAM populations were advanced one generation as planned.

Objective 2: Accelerate breeding through marker technologies: Two approaches have been followed to accelerate breeding cycles: marker-assisted selection (MAS) and genomic selection (GS). Using the 9K and 90K iSelect chips, AM panels and mapping populations are genotyped in a few months, greatly accelerating the pace of marker development and gene discovery. More than 7,000 barley and wheat lines were genotyped with these chips. In addition, high-throughput marker assays have been developed for valuable genes and have been shared with public and private breeders. Genotyping of the barley and wheat GS populations and advancement of the planned cycles of GS is on target. Preliminary results from the barley GS showed that two cycles of GS were equivalent to one cycle of phenotypic selection.

Objective 3: Implement sequence-based genotyping methodologies: The two main technologies evaluated this year were exome capture and genotyping-by-sequencing (GBS). Barley and wheat exome capture assays were used for re-sequencing the parents of the NAM populations. A subset of 62 diverse wheat lines was genotyped by exome capture and GBS and 1.57 million SNPs were identified. These data revealed several selective sweeps that likely resulted from human-driven selection. These selective sweeps showed little overlap among genomes suggesting the importance of polyploidy in broadening adaptive variation. A second generation exon capture assay was used to re-sequence 1,000 tetraploid wheat TILLING mutant lines and to identify ~2,500,000 mutations. GBS pipelines have been developed and tested in barley and wheat and GBS has been adopted as the main genotyping tool for the GS, AM and NAM populations.

Objective 4. Implement web-based tools to integrate genotypic and phenotypic information. Phenotype and genotype data in the Triticeae Tool Box (T3) has been greatly expanded. In November 2014, T3 included 17,600 lines with phenotypic data (768,000 data points) and 24,000 lines with genotypic data (131 million data points). The database now accommodates individual plot data and provides new analysis tools including the automatic calculation of indexes for CSR data. The ability of T3 to work with the Android Field Book was improved.

Objective 5: Develop and implement a Plant Breeding Training Network (PBTN): A total of 136 graduate students participated in PBTN. Ninety-five graduate students were directly mentored by TCAP PIs, and of those, 54 were at least partially funded by TCAP, with two of those at minority serving institutions (the remaining 41 participated in online classes). Eight students graduated. TCAP has trained 108 undergraduates, with 67 mentored by TCAP faculty and graduate students, and 41 by faculty from Minority Serving Institutions (MSIs). TCAP has trained 25 postdocs and 27 visiting scientists (Appendix I3 – I6).

The online *PBTN* environment was used to deliver webinars and courses. Graduate students participated in a face-to-face workshop in collaboration with industry, and in a poster session at PAG. Eighteen TCAP students were supported to visit CIMMYT. Undergraduate students were supported through eleven online meetings. TCAP supported attendance of 98 students at the National Association of Plant Breeders Meeting, where

15 TCAP graduate students presented posters and participated in a professional support workshop. PBTN has provided an excellent communication tool for the project.

TCAP PIs and students gave 35 stakeholder presentations increasing interest and awareness in plant breeding. Funding of MSIs continued and new funding sources to increase diversity were explored through a collaborative grant writing group. Information about research and education was shared both internally and externally through TCAP seminar series, quarterly newsletters and meetings at PAG. Evaluation tools were refined; surveys and interviews were performed; and evaluation reports were created. Evaluation information was used to produce talks, posters and papers.

Publications and germplasm releases: Since the last report, TCAP participants published 66 new peer reviewed scientific articles (Appendix I1). Publications from the first three years of the project have been cross-referenced 1,839 times (~15 references per article) documenting the impact of TCAP research. In addition, 18 new cultivars (13 with PVP) and 32 new germplasm were released (Appendix I2).

TCAP PROJECT NARRATIVE

The TCAP project progressed as planned during the fourth year of the project without major pitfalls. Publications and data generation increased from previous years. This report is organized by objective, and within each of the five objectives into outputs, milestones and deliverables, outcomes and impact, and planned work for the fifth year. The broad impacts are summarized all together at the end of the Objective reports. In objective 1, the work for the three different traits is further divided into barley and wheat activities. The page numbers of each section is described in the Table of Contents below.

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A. OBJECTIVE 1

Discover and deploy beneficial alleles from diverse wheat and barley germplasm.

This objective stated that a diverse set of barley and wheat germplasm would be genotyped with high-throughput genotyping platforms and phenotyped for climate change-related traits to identify and deploy valuable alleles that help mitigate negative impacts of climate change. These traits include disease resistance (A1), WUE (A2) and NUE (A3). Additional mapping and association mapping populations are being developed to support future discoveries (A4).

A1. Disease resistance

A1.1. Outputs disease resistance

A1.1.1 Barley disease resistance genes

A1.1.1.1. ***Barley stripe rust, leaf rust and scald resistance***: Over 1,000 NSGC core accessions were rated for adult plant field resistance to stripe rust and a subset of these were also rated for leaf rust and scald reaction. Currently, over 700 NSGC accessions have at least two years of stripe rust field data. Assessments for seedling resistance to specific stripe rust races were also generated. All data have been uploaded to T3. Six QTL were detected for stripe rust, five of which are novel. Eight QTL were detected for seedling resistance to specific stripe rust races, six of them are novel. Natural epidemics provided the opportunity to map four QTL associated with leaf rust and one QTL associated adult plant resistance to scald. The leaf rust and scald resistance QTL were not novel; thus, the search for additional resistance will need to be broadened.

A1.1.1.2. ***Barley stem rust resistance***: The informative Core (iCore) subset of the NSGC barley core collection was evaluated for seedling reaction to race TTKSK in the BSL-3 facility in St. Paul. Approximately 14% (277 accessions) of this collection showed moderate to high resistance levels. The spring subset of the iCore was planted in Njoro, Kenya in both the 2014 off-season and 2014 main season. In the 2014 main and off-season nurseries, about 13% of the collection (~250 accessions) had moderate to high levels of resistance. The winter subset of the iCore was evaluated in the 2014 main season in Kenya, and only 7% (25 accessions) showed any resistance. The entire spring iCore collection was also evaluated in St. Paul to the virulent domestic race QCCJB. About 10% (203 accessions) of the collection had moderate to high levels of stem rust resistance.

GWAS analysis was conducted on the spring subset of the iCore collection for the 2014 Njoro, off-season, the 2013 St. Paul QCCJB nursery, and the TTKSK greenhouse seedling evaluation. Significant marker associations were detected for adult plant resistance in Njoro on the short arm of chromosome 5H, which were distinct from the *rpg4*/*Rpg5* gene complex. Other significant marker associations were found for seedling resistance to race TTKSK on chromosome 2H and 4H. Complete adult plant field data for the iCore collection to virulent African and domestic stem rust races as well as seedling

data to the same rusts from the greenhouse have been collected. Data upload to T3 will be completed by the end of 2014.

A1.1.1.3. ***Barley spot blotch resistance***: Among 2,062 barley accessions from the NSGC that have been screened, 44 were resistant at the seedling stage to the new and highly virulent spot blotch (*Cochliobolus sativus*) isolate ND4008. The 44 barley accessions resistant to isolate ND4008 were tested using two other isolates ND85F (pathotype 1) and ND90Pr (pathotype 2) in the greenhouse. The results showed that all but four accessions were resistant to ND90Pr, while only seven accessions were resistant to ND85F. The seven accessions resistant to ND85F were also resistant to ND90Pr. Therefore, we have identified barley accessions with resistance to all major pathotypes of *C. sativus*. Significantly, three barley lines developed in North Dakota are among these resistant barley accessions and can be used directly in the barley breeding programs. All spot blotch phenotypic data have been uploaded into T3.

The AM panels for six-rowed spring barley (256 entries), two-rowed spring barley (256 entries), and six-rowed winter barley (300 entries) have been phenotyped with the spot blotch pathogen ND4008. GWAS analysis identified one QTL for resistance to ND4008 on chromosome 6H in both two- and six-rowed barley AM panels, and another QTL for resistance to ND4008 on chromosome 2H only in the six-rowed barley panel. QTL associated with resistance to ND90Pr were located on chromosome 1H in the two-rowed barley panel, and on chromosomes 6H and 2H in the six-rowed barley panel. Crosses were made between a ND4008-resistant accession (PI 235186) and two susceptible parents (PI 356741 and PI 356746). Evaluation of the F_2 populations with isolate ND4008 showed that a single major dominant gene controlled spot blotch resistance to ND4008. Data are being uploaded into T3.

A1.1.1.4. ***Barley spot-form net blotch (SFNB) resistance****:* The two-row and six-row elite association mapping (AM) panels were evaluated using three isolates of *Pyrenophora teres* f. *maculata*. Only one line was classified as resistant and none as highly resistant. We developed six populations with two of the most resistant NSGC lines identified from the spot-form net blotch (SFNB) phenotypic data collected in 2012-2013 crossed with two- and six-rowed malting barley cultivars. We simultaneously advanced six other crosses to the F_6 generation utilizing PI67381 and the second most resistant NSGC line (PI84314) to develop six recombinant inbred line (RIL) populations. GWAS identified 25, 19, 65, and 42 markers significantly associated with SFNB resistance against isolates FGOB10ptm-1 (North Dakota isolate), SG1 (Australian isolate), NZKF2 (New Zealand isolate) and DEN2.6 (Denmark isolate), respectively. GBS-based genetic maps of the Celebration/ PI67381 and the Pinnacle/PI PI84314 RIL populations are being developed.

A *Pyrenophora teres* f. *maculata* mapping population was developed using the North Dakota and Australian pathogen isolates. Phenotyping was completed for 118 progeny isolates on 12 barley lines including local varieties and lines chosen from the initial TCAP phenotyping project. GBS-derived SNPs were mapped and QTL analysis is underway. Preliminary data shows several different genomic regions that harbor virulence, with some virulence QTL being barley-line specific.

Two additional populations for resistance to *P. teres* f. *maculata* have been developed and are being phenotyped and genotyped. This project will lead to the characterization of the virulence loci associated with spot-form net blotch of barley.

A1.1.1.5. *Cereal Yellow Dwarf Virus (CYDV)*: Two major QTL inherited from the tolerant parent, Madre Selva were identified on chromosomes 2H (Qcyd.MaBu-1) and 7H (Qcyd.MaBu-2), and 4 minor QTL from the moderately susceptible parent (Butta 12) were identified in chromosomes 3H, 4H, and 2H. Two lines from this population showed good malting quality and are in large field trials for evaluation for potential release.

A1.1.2. Wheat disease resistance genes

A1.1.2.1 *Wheat stripe rust:* To facilitate the characterization of the NSGC spring and winter wheat core collections for stripe rust resistance, each collection was divided in two subsets (1st subset= 1,000 lines and 2nd subset= 2,040 lines) that were evaluated in multiple environments and in greenhouse seedling tests with specific stripe rust races. These lines were genotyped with the iSelect 9K-assay.

NSGC 1st set of spring wheat: 1,000 lines from this worldwide collection were evaluated in six field environments under high and uniform stripe rust pressure for adult plant resistance. In addition, seedlings were evaluated under controlled conditions with four standard races of stripe rust that are frequent in the western USA. Ten QTL were identified that were consistent across three or more environments and that showed experiment-wise significance. Several of the ten significant QTL are likely novel sources of resistance. Results have been published in G3 (Appendix I1).

NSGC 2nd set of spring wheat: To complete the characterization of the spring wheat core collection, we evaluated 2040 accessions in two locations in Washington in 2012, 2013 and 2014. The field data were complemented with greenhouse seedling tests using the standard stripe rust races prevalent in the Pacific Northwest. The field data were submitted to the T3 database.

NSGC 1st set of winter wheat: The winter wheat core collection (1,414 accessions) was evaluated for stripe rust resistance in field experiments at two locations in Washington. The data were submitted to the T3 database. Over 90 loci associated with resistance to stripe rust were detected and statistical analyses to prioritize these loci are underway.

NSGC 2nd set of winter wheat: The second set includes 664 winter accessions from the core collection. We generated a second year of field data from two locations in Washington and submitted the data to the T3 database. The nursery is already planted in two locations this fall to generate the final 2015 field data. Single-race seedling tests are being carried out in the greenhouse using the standard races of stripe rust.

Stripe rust resistance data was obtained from additional AM panels that were originally developed for leaf rust resistance. These panels include the elite southeastern US soft red winter wheat, the elite hard spring wheat, and the global leaf rust resistance panel. These panels were successfully phenotyped for stripe rust at multiple locations in Washington. Preliminary analysis has identified numerous resistance loci that are being compared with the resistance loci identified in the NSGC spring and winter wheat core collections.

A1.1.2.2. *Wheat stem rust*: Two GWAS studies were performed to identify novel resistance loci. The first GWAS, including 137 lines from cooperative U.S. winter wheat nurseries, confirmed the presence of six known *Sr* genes and identified seven potentially new resistance loci. Results were published in PLoS One (Appendix I1). The second GWAS study is in progress and includes 2,040 spring wheat lines from the NSGC core collection. We completed the third year of evaluations of adult plant stem rust response and severity for this panel in St. Paul, MN. The three years of data are being combined for GWAS.

In addition to the two GWAS, we mapped seventeen Ug99 resistance genes and QTL. For two of them, high-density maps were developed for positional cloning. We also initiated the combination of pyramids of four Ug99 resistance genes in adapted US wheat lines. We developed a WEB tool to integrate information about Ug99 resistance genes http://maswheat.ucdavis.edu/protocols/StemRust/index.htm. Progress by gene is described below.

Sr13: A high-density map of *Sr13* based on 12,162 gametes was developed that delimited the candidate gene region to a 0.03 cM interval. A 950 kb physical map of the *Sr13* region from tetraploid wheat Langdon was completed and sequenced. Two CC-NBS-LRR genes completely linked to *Sr13* were identified and are strong candidate genes. We identified mutations that generate premature stop codons in each gene and seeds are being increased for testing against Ug99 in the Cereal Disease Laboratory.

Sr21: We completed a high-density map and characterization of the Ug99 resistance genes *Sr21* from *Triticum monococcum*. We showed that *Sr21* resistance is modulated by temperature. We mapped *Sr21* ~50 cM from the centromere on the long arm of chromosome $2A^m$. A high-density map based on 7,500 gametes was used to precisely map *Sr21* within a 0.2 cM interval. Results were published in Theor. Appl. Genet (Appendix I1).

SrTm4: This gene from *T. monococcum* confers resistance to Ug99 and related races, but the resistance is recessive, which differentiates *SrTm4* from previously cloned stem rust resistance genes. We mapped *SrTm4* on chromosome arm $2A^mL$ within a 2.1 cM interval and initiated the transfer of *SrTm4* to hexaploid wheat.

Sr35: The *T. monococcum* chromosome segment carrying *Sr35*, previously transferred to hexaploid wheat, is relatively long and may carry undesirable linked traits. We used two rounds of homoeologous recombination induced by the *ph1b* mutation to reduce the introgressed segment. Using a perfect marker for *Sr35* and flanking markers *cfa2170* and *wmc169*, two independent lines with a short *T. monococcum* segment carrying *Sr35* were identified. After elimination of the *ph1b* mutation, seeds will be ready for distribution.

Sr12: Four QTL for adult plant resistance to Ug99 were detected in the cross *Thatcher x Mc Neal*. Three QTL were inherited from Thatcher and one, *Sr57*, was inherited from McNeal. The QTL on 3BS explained 27-35 % of the variation and was at the same location as *Sr12* seedling resistance (effective to race SCCSC). The data suggest that *Sr12* or a linked gene confers APR when combined with other resistance loci. Results were published in Theor. Appl. Genet. (Appendix I1).

Sr595667 (Sr42): Wheat accession PI595667 is resistant to Ug99. $F_{2:3}$ families derived from PI595667 x LMPG-6 segregate for a single gene controlling resistance to Ug99. The

lines were genotyped by GBS and the resistance locus (tentative designation *Sr595667*) was mapped to chromosome 6DS linked to 9 GBS markers. Based on linkage and pedigree data, we propose that *Sr595667* is *Sr42*. We further assayed the population with 90K SNP chips and identified 12 SNP markers that are linked to the resistance locus. A total of 23 markers (nine GBS, one SSR, one SCAR, and 12 SNP) were identified. We converted three GBS markers and five SNP into breeder-friendly KASP assays.

SrZelma (Sr15b): Hungarian winter wheat cultivar 'MV Zelma' is resistant to a number of *Pgt* races including TTKSK (Ug99). In the cross 'MV Zelma/LMPG-6' resistance to TTKSK fits a single gene segregation (tentative designation *SrZelma*). Our 9K SNP chip genotyping and linkage analysis indicates that *SrZelma* is located on chromosome 7AL. We identified a total of seven molecular markers linked to the gene and converted four of them into KASP markers for marker-assisted breeding. An allelism test suggested that *SrZelma* could be a novel allele of *Sr15* with resistance to Ug99. Further experiments are being conducted to allow a firm designation of *Sr15b*.

SrPI508385, SrPI574250, SrPI520282: Three additional F_2 populations were phenotyped and genotyped (PI508385, PI574250, PI520282). Bulked segregant analysis using the 9K SNP chip narrowed down the candidate genes to specific chromosomes (6AS for PI508385 against TRTT, 1B for PI574250 against TTTTF). KASP conversions based on BSA and 9K SNP chip data are underway.

Qsr.umn-2B.2: The Minnesota spring wheat lines 'RB07' and 'MN06113-8' exhibit medium to high levels of adult plant resistance (APR) against Ug99. An F_6-derived population composed of 141 RILs was evaluated for APR to Ug99 in Kenya in 2012 and 2013; and in Ethiopia in 2013. The population was also evaluated in St Paul, MN for APR to North American stem rust races. The population was genotyped using genotyping by sequencing (GBS) and 10 QTL distributed on seven chromosomes were detected in the population but only one on chromosome 2BS was detected in all environments. The QTL, designated as *Qsr.umn-2B.2*, could be a novel source of resistance to Ug99.

*KS05HW14*2/Kingbird*: 380 BC_1F_5 derived RILs were genotyped with the 90K SNP chip and the population was evaluated for stem rust resistance in Kansas for two seasons and the third will be scored in May, 2015. The main target is stem rust resistance, but the population is also being phenotyped for leaf rust and stripe rust in the field. We also have data on half of the population for Ug99 in Kenya for two seasons. Selections have been made from this population in the KS stem rust nursery. One line was selected in 2014 (U6380R2) with useful levels of resistance against Ug99.

Nine old Kenyan varieties and a Minnesota cultivar with high adult plant resistance to Ug99 were crossed with the susceptible line 'LMPG-6' to obtain a NAM population comprising of 852 RILs. The population was evaluated for APR to stem rust races in South Africa in 2012, Kenya in 2013, and St. Paul, MN in 2012 and 2013. The NAM population was genotyped using GBS and a combined linkage map was developed with 930 SNP markers. Iterative QTL mapping (iQTLm) detected 27 QTL distributed on 11 chromosomes with six QTL represented multiple times in more than one environment. Eleven QTL detected by iQTLm were also detected in individual populations. We are evaluating if these QTL represent new sources of resistance.

The KS group is pyramiding *Sr22* (reduced segment), *Sr26* (reduced segment), *Sr35* (non-reduced segment), and *Lr34/Yr18/Sr57* in adapted backgrounds. BC_1F_3 and BC_2F_2 families carrying the four gene combination were developed in a 'Duster' background. It may take one or two more generations to achieve homozygosity for the desired pyramid. The KSU group also made crosses and a few backcrosses of pyramided lines to elite cultivars 'Byrd', 'Everest', 'Iba', 'KanMark', 'T158', and 'Winterhawk'.

A1.1.2.3. ***Wheat leaf rust:*** New resistance genes and QTL for leaf rust were identified in the NSGC core spring and winter wheat collections, AM panels and populations described below. The spring and winter wheat core collections were genotyped with the iSelect 9,000 wheat SNP-chip.

NSGC spring wheat core collection: 1,000 hard red spring wheat lines from the NSGC core collection were selected for association mapping. These lines have been evaluated in St. Paul and Crookston in 2011, 2012, 2013, and 2014. In addition to the field experiments, seedling resistance was tested in greenhouse. Preliminary GWAS identified several loci associated with leaf rust resistance. We are testing approaches to account for spatial variation within the field and make adjustments to the phenotypic data.

NSGC winter wheat core collection: 2,078 lines were selected from the NSGC winter wheat core and evaluated for adult stem rust response and severity in St. Paul, MN. Data are being combined for GWAS.

Hard red spring wheat association mapping panel: An association mapping panel of 381 HRS wheat lines was evaluated for field resistance to leaf rust in St. Paul in 2012, 2013, and 2014. Seedling resistance was tested in greenhouse trials in 2012 and 2013. The panel was genotyped with the iSelect 9K wheat chip. GWAS analysis was completed and several leaf rust resistance loci were identified.

Ulen x Thatcher RIL mapping population: An F_5 population with ~120 lines was evaluated for leaf rust resistance in Crookston and St. Paul, MN (2012-2014) and in the greenhouse. We postulated three resistance genes in Ulen: *Lr14b* (chromosome 7B), *Lr23* (2B), and an additional adult plant resistance gene. Bulk segregant analysis with genotypes from the iSelect 9K wheat chip identified tentative resistance loci on chromosomes 7B, 2D, 1B, and 4A. Allelism tests confirmed the presence of *Lr14b* and *Lr23*. KASP markers were developed for *Lr23*.

RB07 x Faller RIL mapping population: An F_6 population of 160 lines derived from MN line RB07 x Faller was evaluated in Crookston and St. Paul, MN for leaf rust resistance (2012-2014). This population was genotyped using GBS and a leaf rust resistance QTL on chromosome 3BS was detected in all environments.

*Lakin*2/Roelfs F2007*: 380 BC_1F_6-derived RILs were genotyped by GBS. Phenotypic leaf rust data are available from 3 location years and two more will be obtained in May, 2015. One greenhouse screen is planned for winter 2015. The population is also being phenotyped for stem rust and stripe rust in the field. Data are being analyzed.

Germplasm screen: Nine leaf rust races were used to screen a collection of 296 durum lines and 570 common wheat lines that showed some resistance in previous screening. Both selected resistant durum and common lines were also tested with two stem rust races from the Ug99 race group.

Evaluation and characterization of resistance gene Lr67: Adult plant resistance gene *Lr67* was shown to provide stable and effective stripe rust resistance in diverse environments. A nearly diagnostic DNA marker that allows routine detection of this gene in diverse germplasm was developed and published in Molecular Breeding (Appendix I1).

A1.1.2.4. ***Wheat septoria tritici blotch (STB)***: We completed the mapping of the STB resistance gene *Stb3* on the short arm of wheat chromosome 7A completely linked to SSR locus *Xwmc83* and published the results in Crop Science (Appendix I1). In addition, a panel of elite spring wheat was phenotyped for STB at multiple locations in Ethiopia. A GWAS is in progress.

A1.1.2.5. ***Wheat bacteria leaf streak and leaf spot diseases***: A subset of the NSGC core was used for association mapping of bacterial leaf streak disease and multiple loci have been detected. GWAS revealed novel QTL associated with resistance to multiple leaf spot diseases of spring wheat. Results were published in PLoS One (Appendix I1).

A1.1.2.6. ***Wheat Insect resistance***: Resistance to wheat stem sawfly was mapped in a RIL population of wheat derived from two resistance sources and was published in Plant Breeding (Appendix I1). Resistance to Hessian fly (*Mayetiola destructor*) in the spring wheat cultivar 'Louise' was mapped and linked molecular markers were identified to accelerate the transfer of this gene.

Marker-assisted selection was used to introduce a gene for resistance to orange wheat blossom midge (OWBM) into wheat variety Egan adapted to northwestern MT. Egan will be the first OWBM-resistant line for this area. OWBM-resistance was pyramided with the *Gpc-B1* allele for high grain protein, *Yr36* for stripe rust resistance, and a high gluten strength allele at *Gli-B1/Glu-B3*.

A1.2. Milestones and deliverables disease resistance

A1.2.1. ***Barley diseases milestones****:* We completed the 2014 proposed milestones in the barley disease resistance area which included the completion of the screening of the barley NSGC and of dedicated AM panels. We also completed the GWAS of resistance to stripe rust, stem rust (both domestic and African races), spot blotch, and spot-form net blotch in the core collection. As planned, we initiated the development of bi-parental populations for validation of the resistance loci identified in the GWAS studies, and the deployment of resistance genes in advanced breeding lines.

Deliverables include the identification of 14 QTL for stripe rust resistance (11 of which are novel loci), four QTL for leaf rust resistance, three QTL for resistance to stem rust races of the Ug99 complex and one scald resistance QTL. GWAS also identified two QTL for resistance to the new and highly virulent spot blotch isolate ND4008 and three for isolate ND90Pr. Preliminary analysis also identified several QTL for resistance to the spot-form of net blotch. The markers linked with these QTL provide useful tools to barley breeders to rapidly deploy and combine resistance genes against the major barley pathogens.

A1.2.2. ***Wheat diseases milestones****:* We completed the proposed milestones for disease resistance in wheat and identified multiple genes for resistance to stripe, leaf and stem rust. In the area of resistance to Ug99 we completed the mapping of several genes conferring resistance to Ug99 including *Sr21, SrTm4, Sr12, Sr595667*(*Sr42*), *SrZelma* (*~Sr15b*), *Sr28, SrND643,* and several unnamed QTL. During this year we also developed high-density maps for *Sr13* and *Sr21,* a required step for the positional cloning of these genes. The markers linked with Ug99 resistance genes and QTL are valuable tools for wheat breeders interested in pyramiding different resistance genes against the major rust pathogens. The increase in marker-tagged genes will help to diversify the set of deployed resistance genes.

An important delivery from this area was the conversion of several SNP markers into user-friendly KASP markers for Ug99 resistance genes *SrZelma*/*Sr15* and *Sr595667*/*Sr42.* We demonstrated that KASP markers can be efficiently developed from GBS and SNP markers. KASP technology is being used in other mapping populations (PI508385, PI574250, and PI520282) to identify additional resistance genes.

A1.3. Outcomes/Impacts disease resistance

The large number of resistance genes identified in this project by GWAS is enabling novel approaches for breeding for resistance. In the past, breeders were forced to use a very limited number of resistance genes, which increased the risks of major epidemics. Today barley and wheat breeders can select from a wide inventory of resistance genes that facilitates the diversification of the sources of resistance. This new information-rich environment is forcing breeders to develop new strategies to deploy and combine larger numbers of resistance genes (e.g. genomic selection). The deployment of resistant barley and wheat cultivars is reducing the negative impact of fungicide applications, benefiting the environment and reducing production costs.

The research on disease resistance is one of the most successful areas of the project as documented by 25 publications in 2014 and by the release of several cultivars with pyramids of resistance genes. As an example, wheat varieties in which different stripe rust resistance genes were combined using marker assisted selection and TCAP support, covered 23% of the wheat acreage in California in 2014 (127,000 ac.).

A1.3.1. ***Barley diseases outcomes/impact***: This research has led to the identification of important new sources of resistance to stripe rust, stem rust, spot blotch, and spot form net blotch in the barley core collection of the NSGC. SNP markers found associated with these favorable alleles are being used to facilitate the transfer of multiple disease resistance into elite barley germplasm by marker-assisted or GS.

A1.3.1. ***Wheat diseases outcomes/impact***: GWAS analyses for stripe, leaf and stem rust have provided a large number of markers for resistance genes that are being deployed in the breeding programs. In the area of stripe rust, the analysis of a global collection was essential for the identification of the Punjab region in India and Pakistan as a valuable source or resistance alleles for stripe rust. This new information will likely reshape future germplasm collections and GWAS analyses from this region.

A1.4. Plan-of-Work 2015 diseases

A1.4.1. ***Barley diseases plans for 2015***: Selected wild barley introgression line populations (in cv. Rasmusson background) segregating for disease reaction will be evaluated at the seedling or adult plant stage as appropriate at the respective institutions.

Selected NAM populations segregating for disease reaction will be evaluated at the seedling or adult plant stage at the respective institutions.

The most resistant accessions from the barley core collection (n=~120) will be evaluated with a set of the most widely virulent African and domestic stem rust races at the seedling stage in the BSL-3 facility in St. Paul, MN.

Mapping of adult plant stem rust resistance in the PI383313/Hiproly (311 RILs + controls) and Heitpas-5/Hiproly (316 RILs + controls) populations will be conducted in Kenya and MN. The Oregon Promise bi-parental population (n = 200 + checks) will be evaluated for stripe rust in OR at the adult plant stage.

For a second year, the winter Low Temperature Tolerant (LTT) panel (n = 941 entries + checks) will be evaluated for stripe rust in OR (adult plant stage). The winter/facultative naked food barley genomic selection training population (~ LTT panel (n = 700 entries + checks) and composite population will be evaluated for stripe rust in OR (adult plant).

New sets of biparental-derived two-rowed facultative/winter malting doubled haploids (n ~ 400 entries + checks) will be evaluated for stripe rust in OR (adult plant stage). All data from these trials will be uploaded to T3 and analysis to identify favorable alleles will be conducted and manuscripts prepared.

A1.4.2. ***Wheat diseases plans for 2015***: For the stripe rust studies we will initiate the validation of the 10 major QTL identified in the 1st set of the spring wheat NSGC. Sources of the different resistance alleles will be crossed with Avocet for validation and high-density mapping. For the NSGC 2nd spring set and the two winter sets we will complete the GWAS analysis and prepare the results for publication.

For the Ug99 resistance gene *Sr21*, we will complete the physical map and map the susceptible mutants identified in the LMPG+*Sr21* hexaploid wheat line. For *Sr13*, we will use two available truncation mutants from our TILLING populations and transgenic complementation to determine which of the two identified candidate NBS-LRR genes is *Sr13*. For *SrTm4* and *SrTm5* we will complete high density maps in *T. monococcum*. For *Sr35*, we will self-pollinate the current F_1 hybrids and generate an F_2 population and select plants homozygous for wild type *Ph1b* gene and for the presence of the reduced introgression of *Sr35*. The resulting materials will be published and distributed widely.

We will also continue the GWAS and mapping studies for leaf and stem rust and publish the results. For the breeding applications, we will emphasize the development of KASP markers for the multiple resistance genes identified in the previous years of the project. Development of KASP and TaqMan assays, validation and high-resolution mapping, population development, dissemination of results, and deployment of new resistance genes in commercial varieties are our primary goals for 2015.

A2. Water use efficiency (WUE)

The objective of the WUE group is to develop and use specialized genetic populations to identify genes impacting drought and heat tolerance, and to determine the relationship between physiological measures (e.g. CSR) and improvements in WUE.

A2.1. Outputs WUE

A2.1.1. *Barley WUE*: All WUE trials are completed for the spring six-row, spring two-row, and facultative/winter six-row AM panels. All data have been submitted to T3. *Barley LTT*: We assembled a very large GWAS panel (n = 941), which was evaluated at 14 locations around the world in 2013/2014. The panel includes 300 lines from the TCAP facultative-winter six-row set derived from elite lines at OSU and UMN breeding programs. Additionally, the panel contains the USDA winter core accessions and accessions from the AGOUEB and ExBarDiv Projects led by the James Hutton Institute and the University of Dundee in the UK.

Genotyping was done with the 9K iSelect chip. In 2013-2014, differential winter survival was observed in four of the test environments (ID, MN, OH (USA), and Alberta, Canada). The GWAS validated the effects of known LTT-related genes (*FR-H1*, *FR-H2*, *FR-H3*, *PPD-H2*) and identified novel significant QTL on all other chromosomes. Interestingly, *VRN-H2* was not a significant determinant of LTT, even though the panel included facultative and winter accessions. Facultative accessions were among the accessions with highest LTT. We have initiated the validation and physiological characterization of the novel QTL for LTT.

NSGC evaluation for yield and WUE: The NSGC AM panel including 480 two-row spring barley accessions was phenotyped in 2014. A total of 480 plots were evaluated under three field conditions including irrigated/normal nitrogen, terminal drought/normal nitrogen, and terminal drought/low nitrogen. Yield, plant height, and heading dates were measured for all plots. CSR readings were recorded at three growth stages (booting, heading, and grain filling). GWAS data analyses are in progress.

A2.1.2. *Wheat WUE*: Activities in this area include the characterization of the AM and NAM for WUE and the dissection and precise mapping of known sources of WUE and yield under water stress. The spring and winter wheat elite AM panels (~250-300 lines each) and the NAM populations have provided common research resources and focus for these research activities. Throughout the project, the WUE AM panels have been grown in 36 environments throughout the wheat producing areas of the US, Mexico, and Canada (2012-2014). All lines were genotyped using the 90K iSelect SNP array.

Spring wheat AM panel (AM): This panel, containing 239 elite spring lines, was evaluated for WUE in 16 different environments since 2012. This year we conducted preliminary analysis of the WA data from 2012 and 2013, which included experiments under drought and fully irrigated conditions. Normalized water index (NWI) was measured from heading to late grain fill, and least square mean across growth stages was used in the preliminary GWAS based on 11,609 SNP markers. A total of 38 significant QTL were identified. These QTL will be compared with QTL identified in other

environments to prioritize 3-6 QTL for validation experiments. The spring AM panel was also evaluated for root traits under controlled environment. Genetic variability for root traits was detected in this panel and contrasting genotypes for root traits were identified. These results were published (Appendix I1).

Normalized water index (NWI) showed consistent associations with grain yield in multi-year, multi-location trials. Additional stress adaptive traits such as normalized chlorophyll pigment ratio index (NCPI) and photochemical reflectance index (PRI) showed a highly-significant association with grain yield under stressed environments. Multiple measurements of NDVI over growth stages were used to estimate stay green properties, crop cover/stand and pigment abundance. It was possible to categorize the spring wheat AM population into groups with low, medium and high stress tolerance levels and identify the most tolerant genotypes. CSR data from this AM panel were used to improve the efficiency of handheld reflectance measurements. We identified the most informative growth stages to obtain data and developed statistical techniques to adjust for confounding factors.

Photoperiod insensitive spring wheat panel: Since photoperiod sensitive materials are not adapted to the Mediterranean climate of California, the photoperiod sensitive lines from the previous panel were replaced. This panel has 146 lines in common with the original panel and 117 new photoperiod insensitive spring wheat accessions from the USA and CIMMYT. This panel was evaluated in CA and Mexico for WUE in 2013 and 2014. Preliminary GWAS analyses of the normalized water index NWI3 data across four environments (Davis 2013, Imperial 2013 and 2014, Obregon 2013) resulted in the identification of 17 significant QTL. Two of the significant QTL overlapped with those detected in the original spring wheat AM panel.

Spring wheat NAM Panel: The spring wheat NAM panel consists of 75 lines for each of 30 crosses of diverse landraces crossed to a common parent Berkut. The first year of yield testing was conducted in 2014. Lines were tested in augmented designs in three environments. Each testing environment (MT, SD, and WA) grew 25 lines per cross (total 75). Data collected includes yield and its components. High-throughput phenotyping data including CSR and canopy temperature depression (CTD) was collected on the lines. Identification of favorable alleles for breeding will be accomplished using genotyping data from the 90K iSelect SNP chip and GBS. An initial analysis of the NAM experiment from WA (Table 1) showed that many of the CSR-derived indices provide the ability to predict yield in low water environments, which provides a potential selection tool to wheat breeders. Preliminary GWAS revealed a potentially important QTL for NDVI on chromosome 1A.

Table A2.1.2.1. Pearson correlation of reflectance indices with agronomic traits for the spring wheat NAM population.

	VI	**NDVI**	**SR**	**CI**	**SRPI**	**WI**	**NWI_1**	**NWI_4**
YIELD	0.316	0.303	0.277	0.303	0.300	0.364	-0.365	-0.392
P	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

VI= Vegetation Index; NDVI=Normalized difference vegetation index; SR =Simple ratio; CI=Canopy index; SRPI=Simple ratio pigment index; WI=Water index; NWI-1=Normalized water index-1; NWI-4=Normalized water index-4

Photoperiod sensitive spring wheat NAM Panel: Eight NAM populations well adapted to Mediterranean environments are being evaluated in California. Four of these populations (Berkut x PBW343, Berkut x DHARWAR_DRY, Berkut x LR23, Berkut x LR3) were evaluated for the first time in Davis, CA in 2014. A total of 332 plots were evaluated under terminal drought treatment in an augmented design. Data have been collected for heading date, plant height, physiological maturity date, flag leaf chlorophyll content, yield and yield components. CSR data was also collected and data analysis is underway.

Winter wheat AM panel: The hard red winter wheat AM panel (n=300) has been evaluated in 16 locations since 2012. In 2014, this panel was evaluated for grain yield and forage quality traits in OK. Subsets of 45-50 lines showing contrasting responses to soil moisture based on 2012 and 2013 results were evaluated under a range of moisture conditions in Fort Collins and Greeley, CO and Manhattan, KS in 2014. Data on yield components, phenology, morphology, CSR, and NDVI were obtained. Preliminary analysis indicates a significant correlation (R= 0.6-0.7) between grain yield and water indexes calculated from CSR measurements during early grain filling, indicating that CSR could be a useful secondary trait for evaluating crop performance under moisture stress. Roots were collected from 1-meter deep soil cores and digitally analyzed to obtain information on root length and diameter at different depths. We were not able to establish a relationship between root traits from soil cores and grain yield, reflecting the difficulty of obtaining repeatable below-ground data.

A subset of the AM winter wheat genotypes previously evaluated for WUE were selected for more in-depth analysis during the growing season. Using data for yield differences between dryland and irrigated experiments, five genotypes were selected from each of three categories; most sensitive, least sensitive, and intermediate. Finally, five checks were randomly chosen. Replicated experiments were planted under rain-out shelters in KS to impose different levels of drought. Yield, and yield components along with canopy spectral reflectance (CSR) were measured. Plots have been harvested and are being processed and analyzed.

Tetraploid x hexaploid wheat population: Two sets of tetraploid and hexaploid RILs were developed from a cross between Mountrail durum wheat and Choteau spring wheat. The lines within each ploidy are recombinant for genes on the A and B genome. The lines were tested in four environments and genotyped using the 90 K iSelect SNP chip. Key findings from data analysis in 2014 were that the presence of the D genome resulted in greater yield potential and larger seed. However, alleles at QTL for seed size and other favorable traits resided in the A and B genome chromosomes from durum wheat. The favorable alleles at these QTL were present in hexaploid wheat at extremely low levels. Favorable QTL from durum wheat that are not present in hexaploid wheat are candidates for introgression into hexaploid wheat to improve grain yield under dry conditions.

Effect of the 1RS.1BL rye translocation on WUE: We confirmed that an important gene(s) for yield and WUE is located in a small distal region of the 1RS chromosome. This 1RS region is associated with increased yield in both full- and limited-irrigation treatments, with larger effects in the latter. Plants carrying this distal 1RS segment have more access

to water resulting in improved water indexes, CID and stomatal conductance. These results have practical implications for breeding programs: the rye *Sec1* locus associated to sticky dough can be eliminated without compromising the positive effect of the 1RS chromosome on grain yield and WUE. Results were published in Theor. Appl. Genet. (Appendix I1).

We have recently completed the engineering of a 1B chromosome combining the 1RS distal region for drought tolerance, the *Yr15* gene for stripe rust resistance, and the high molecular weight glutenin allele *Glu-B1* 7Bx-over-expressor for strong gluten. This recombined chromosome is being deployed in our breeding program and is being distributed to other breeding programs.

Winter wheat NSGC: In 2014, 200 accessions were planted in a replicated trial with three replications, and were evaluated under irrigated condition. Yield, plant height, and heading dates were measured for all plots and CSR readings were recorded in three growth stages (booting, heading, and grain filling). This panel was previously evaluated in 2013 for WUE and NUE.

A2.2. Milestones and deliverables WUE

A2.2.1. Barley

Barley milestones WUE: The fourth year milestones for this objective (WUE experiments and submission of data to T3) were completed. The BARI, USDA-ARS (ID), and Montana State University programs evaluated the spring two-row AM panel using CSR and measured allied traits under dry and normal conditions. The BARI program evaluated the spring six-row panel under dry and normal conditions using CSR and measured allied traits. The OSU program evaluated the facultative six-row panel under normal and dry conditions. All data have been submitted to T3. The OSU group will conduct GWAS and prepare the resulting publication(s).

Barley milestones LTT: The fourth year milestones for this project included seed increase of 941 accessions at Corvallis, distribution of the resulting seed to 14 locations around the world, curating the LTT and associated data, and conducting GWAS. All activities were completed.

Barley NSGC evaluations: Yield, agronomic characteristics and other data for the 2013 NSGC six-row barley AM panels were uploaded to the T3 database.

A2.2.2. Wheat

Wheat milestones WUE: The milestones for WUE for year four have been completed both for the AM and NAM panels. AM panels have been evaluated over more than 15 environments in the US, Canada, and Mexico. Preliminary analysis of the data resulted in the identification of key QTL for WUE. Eight manuscripts related to WUE have been published in 2014. The milestones for the spring wheat NAM panel were also on schedule. The first yield trials were conducted in 2014 and will be repeated in 2015. The winter wheat NAM populations were advanced to the F_4 generation as planned.

The yield, agronomic characteristics and WUE data for the 2013 NSGC winter wheat association mapping panels were uploaded to the T3 database.

In addition to the analysis of the core AM and NAM populations, field trials including yield components and physiological measurements have been completed and published for the dissection of the 1RS rye translocation and for a gene causing stem solidness (Appendix I1). The drought tolerant B306 X PBW354 RIL population was grown under dryland and irrigated conditions in MT in 2014 to complete the phenotypic evaluation of this population. Genotyping using the 90K iSelect SNP chip was also completed. Data analysis will be conducted in 2015.

Physiological studies of high temperature stress defined the most sensitive stages and the thresholds for temperatures and duration of the stress. Effects of different treatment combinations on floret fertility and individual grain weight of wheat were determined and published (Appendix I1). This precise physiological characterization provides the basic information required for more precise genetic experiments to identify genes associated with high-temperature tolerance. Related studies identifying the impact of high night-time and high daytime temperature stress on winter wheat were also published in 2014 (Appendix I1).

As we continue experiments to identify new favorable alleles for heat and drought tolerance, marker-trait relationships identified in previous years are being validated and used to develop new varieties. This study has also provided empirical information regarding the value of high-throughput CSR data to estimate WUE.

A2.3. Outcomes/Impacts WUE

A2.3.1. ***Barley outcomes/impacts WUE and LTT***: The identification of genetic determinants of LTT is accelerating the development of winter barley varieties that can better utilize winter precipitation. GWAS has validated the effects of LTT-related genes discovered in biparental mapping populations and identified significant new QTL that can be used in MAS. These field studies have also identified varieties with high LTT, including many facultative accessions, which are being incorporated as parental lines in the breeding programs.

A2.2.2. ***Wheat outcomes/impacts WUE***: The characterization of wheat accessions from the NSGC and from elite germplasm in the AM panels has resulted in the identification of valuable QTL for WUE and also of superior lines that are being incorporated into the wheat breeding programs as parental lines. The characterization of the NSGC wheat panels in ID resulted in the identification of 39 spring wheat and 19 winter wheat accessions with improved drought tolerance and/or improved NUE. One QTL associated with grain yield and with potential for MAS was identified on chromosome 2B in the NSGC spring wheat panel and was validated in the NSGC winter wheat panel. These accessions are being incorporated into the breeding programs. The characterization of the elite AM spring wheat panel revealed several QTL conserved across locations, which can now be used in MAS.

The precise mapping of the 1RS chromosome region associated with improved WUE has resulted in an engineered 1BS chromosome combining increased WUE, stripe rust resistance and strong gluten. This chromosome is being deployed in breeding programs.

The characterization of the lines derived from the tetraploid x hexaploid cross has expanded the diversity of alleles for the improvement of WUE.

Given the high variability for precipitation in rain-fed regions, a high plasticity of responses to environmental variation in water availability is a valuable characteristic. Our analysis of the hard winter wheat AM population across 11 environments indicates that plasticity is positively correlated with yield under moisture-stressed conditions. This indicates the feasibility of developing cultivars that perform well across the wide range of moisture conditions encountered in rain-fed environments.

An additional outcome of the WUE field studies was the incorporation of canopy spectral reflectance (CSR) technologies in public barley and wheat breeding programs. This has fostered development of high-throughput phenotyping technology into breeding programs. Aerial spectrophotometric images are being used in NE and MN, and high-throughput tractor-mounted phenotyping systems developed specifically for wheat are in use in KS, WA and CO. These technological developments are now making possible the collection of important phenotypic data for thousands of lines.

A2.4. Plan-of-Work 2015 WUE

A2.4.1. ***Barley plan of work 2015 WUE and LTT:*** For a second year the winter LTT panel (n = 941+ checks) will be evaluated in OR, MN, ID, NE, OH, Spain, Canada, France, Germany, Hungary & Japan. A new location was added in Denmark. The LTT panel will also be assayed for vernalization sensitivity under greenhouse conditions at OSU. The accessions with the best LTT (n ~ 50 + checks) will be tested for LTT in controlled freeze tests. The large LTT germplasm collection will be used as a common resource for extensive phenotyping and GWAS. Various phenological, morphological, and biotic stress resistance traits will be measured in multiple environments. For all data sets, GWAS will be completed and manuscripts prepared.

A2.4.2. ***Wheat plan of work 2015 WUE:*** The major phenotyping objective for the spring wheat group in 2015 is to complete the second year of NAM evaluations. This trial consists of 2,400 entries, with a third of the trial being grown in three spring planting sites. In addition, 600 different lines will be evaluated in two locations in California under full- and limited-irrigation environments. Data will be collected for CSR and yield-related characters under water stress. GWAS analysis of WUE, CSR and yield components will be completed and published.

Winter wheat phenotyping will include analysis of a selected subset of 50 lines of the hard winter wheat AM panel for agronomic, phenological, physiological, and CSR traits. In Kansas, 45 selected genotypes of the HWWAMP panel (same as 2013-14) will be planted in two rainout shelters. Select lines from the SWAMP will be planted in the remaining two rainout shelters along with selected Chinese Spring substitution lines. The Chinese Spring substitution lines will also be planted in the field as irrigated and dryland

plots. All experiments will have CSR, phenology, canopy temperature, yield, and yield component data collected. These lines represent the phenotypic diversity discovered in 2012 and 2013 yield trials.

For the winter AM panel, we will collect phenotypic data for additional environments in 2015. These data will be combined with data from previous years for GWAS. Analysis of the hard red winter wheat AM panel, including CSR data will be completed within and across environments.

The NSGC will conduct a comprehensive field study in 2015 to understand better the genetic and physiological mechanisms underlying high yield and drought tolerance using winter and spring wheat accessions identified in the association studies. The winter wheat trial will compare 20 selected NSGC accessions and 10 adapted cultivars and the spring wheat trial will compare 40 NSGC accessions and 10 elite cultivars. The experiments will include 3 replications, will be grown under irrigated, terminal drought, and limited precipitation conditions, and will be evaluated for agronomic traits, yield components, leaf gas exchange, stomatal conductance, leaf transpiration rate, stomatal size and density, yield components, harvest index, and root system architecture.

A3. Nitrogen use efficiency (NUE)

A3.1. Outputs NUE

A3.1.1. *Barley outputs NUE*

All scheduled NUE trials are complete for the spring six-row (SP6), spring two-row (SP2), and facultative winter six-row (WN6) association mapping panels in a low (70%) nitrogen and normal (100%) nitrogen environment. Nearly all of the phenotypic data from these trials has been uploaded to T3.

A comprehensive data set was obtained for the WN6 germplasm across two years (2012 and 2013 harvests) and two locations (Corvallis, OR and Logan UT). Small plots were used in UT in both years and in OR in 2012. In 2013, the OR program used full size yield trial plots (7 m^2). Agronomic and CSR data were obtained and have been uploaded to T3. In addition, malting quality data were obtained from OR in both years.

NUE indices showed significant genetic variation. GWAS of these indices is in progress. GWAS for 14 agronomic and nine malting quality traits for two years (OR) and two locations (UT, agronomic traits only) is completed. Thirty QTL were detected for 11 agronomic traits (0-6 QTL/trait), including three yield component traits. Thirty QTL were detected for nine malting quality traits (0-9 QTL/trait). Many QTL were consistent across years and locations (despite contrasting management systems). Analyses of the agronomic and malting quality QTL are in progress, and include (i) alignment with prior reports, (ii) evaluation of correlated traits, and (iii) identification of candidate genes.

For the SP6 panel, association mapping using data from three trials (MN 2011, 2012 and ND 2012) has identified a large effect QTL for grain protein concentration and NUE indices on chromosome 6H in the *Gpc-1* region and three additional loci. Three dominant

haplotypes were identified at the 6H QTL that correspond to the Karl, Lacey, and Chevron genotypes and low, intermediate, and high grain protein concentrations, respectively. A significant association was detected in the same region for grain hardness in a GWAS study of a much larger set of barley genotypes.

Analysis of the SP2 panel was the responsibility of the MT group. With the retirement of Tom Blake, the Smith lab will analyze these data and incorporate it with the SP6 analysis for publication. Jamie Sherman was hired as the new barley breeder in MT and will continue this work.

NSGC evaluation for NUE: an AM panel of 480 two-row spring barley accessions from the NSGC was phenotyped in 2014. A total of 480 Plots were evaluated under three field conditions including irrigated/normal nitrogen, terminal drought/normal nitrogen, and terminal drought/low nitrogen. Yield, plant height, and heading dates were measured for all plots. CSR readings were recorded at three growth stages (booting, heading, and grain filling). Protein content was measured after harvesting. GWAS for NUE of WUE data from this panel is in progress.

A3.1.2. *Wheat outputs NUE*

Introgression of the high-grain protein content Gpc-B1 gene into commercial varieties: The functional *Gpc-B1* allele from *T. dicoccoides* increases N remobilization and grain protein content. This allele was incorporated into several new wheat varieties and breeding lines including Egan in Montana, UC1745 and Patwin 515-HP in California, and Farnum and WA8184 in WA. The WSU winter wheat breeding program is incorporating the *Gpc-B1* gene into both hard red and white winter wheat germplasm and the California wheat breeding program is incorporating it into durum and common wheat programs.

Functional characterization of the Gpc1 gene: The high grain-protein content gene *Gpc1* was characterized using mutants for this gene (and its paralog *Gpc2*) in both hexaploid wheat and tetraploid wheat. These two studies were published in 2014 (Appendix I1). RNA-seq of these mutants at different time points showed that *Gpc1* is a key regulator of nutrient remobilization during the early stages of senescence. We demonstrated that loss-of function mutations in these two genes alters the expression of ~21% of the senescence-regulated genes, and that most of these changes occur by 12 days after anthesis. Interestingly, this group of upregulated genes includes transporters from the *ZIP* and *YSL* gene families, and genes involved in the biosynthesis of chelators that facilitate phloem-based transport of nutrients to the grains. Results were published in BMC Plant Biology (Appendix I1).

Cooperative, multi-state studies: The 2013-14 field trials focused on 1) validating results from the past two years of field trials (2011-12, 2012-13) and initiating evaluation of the Allele Based Breeding (ABB) project. The 2014 trials for the hard red winter wheat programs (HRW) and the soft red winter programs (SRW) evaluated 2,196 lines in 18 environments and are described below. These field trials were focused on the following objectives:

- Yield QTL Validation Trials (YQV).
- Canopy Spectral Reflectance Validation trial (CSRV).

- Nitrogen Use Efficiency Validation trial (NUEV).
- Yield and NUE Validation Panel (YNVP).
- Allele based-breeding trials (ABB).

Table A3.1.2. Description of 2014 field trials for winter wheat yield and NUE projects

Wheat Class	Test	States	No. Loc. planted / harvested 2014	No. of Lines	No. Loc. planned 2015	90K SNPs	GBS SNPS
HRW	YQV	NE	4/4	220	3	Submitted	Submitted
HRW	CSRV	NE	1/1	120	1	Submitted	Submitted
HRW	NUEV	NE	2/2	12*	2	Submitted	Submitted
HRW	ABB	NE, KE, TX, CO	10/8	283	10		Submitted
		Total	17/15	635	16		
SRW	YNVP	OH, VA ,KY, MO	5/4	300	6	Submitted	Submitted
SRW	NUEV	VA, OH	4/3	12*	3	Done	Submitted
SRW	ABB	OH, NY, MI	5/4	413	5	Submitted	Submitted
SRW	ABB	VA, KY, IL	6/6	366	4		Submitted
SRW	ABB	AR, GA, LA, NC	4/4	480	4		Submitted
		Total	15/14	1571	22		

*6 best and 6 worst NUE lines tested at 5 N rates for yield response to N.

Canopy spectral reflectance (CSR) evaluations of multi-state studies: CSR data for the HRW was collected weekly over a 5-6 week interval from booting to physiological maturity. These data were used to calculate the following indexes in the YQV, NUEV and CSRV trials (Table A3.1.2):

- Enhanced Vegetation Index (EVI).
- Chlorophyll Index (CI).
- Normalized Difference Vegetation Index (NDVI).

The SRW data was collected from the YNVP trial (VA, KY, MO, and OH), the NUEV trial (VA and OH) and the ABB trial (VA). The CSR data from these trials were used to calculate:

- Normalized Difference Vegetation Index (NDVI).
- Red Edge.
- NIR.
- Red Band.
- NDRE (Normalized Difference Red Edge).
- Relative index of chlorophyll concentrations.

Studies at the University of Kentucky: Three additional studies on NUE were performed at the University of Kentucky:

- A genotype x environment x management at three locations to evaluate NUE.
- The response to N rates using a subset of TCAP elite mapping panel.
- The effect of higher temperatures in the roots on yield and NUE. The same lines from the previous two studies were grown in headrows in which the rhizosphere was warmed + 5 ° C with heating cables.

Studies at the University of Missouri: CSR data was used to calculate 17 different indices at four stages of wheat development to determine which would be most significant for indirect yield selection in soft red winter wheat. Results across years suggest that the R^2 between these different indices and yield range from 0.45 to 0.70. Virginia Tech has also analyzed their CSR-based vegetative indices for relationship to yield. Over two seasons, the best models explained 84% and 83% of total variation in grain yield and N uptake respectively. Models further accounted for 85% and 77% of total variation for yield, and 85%, and 81% of total variation for grain protein under low and normal N conditions, respectively.

A3.2. Milestones and deliverables NUE

A.3.2.1. ***Barley NUE***: Fourth year milestones in this objective, including data curation, uploading of trials and conducting GWAS are on target. A major effect in grain protein content was identified in the *Gpc-H1* locus and different alleles have been identified in the barley germplasm collection facilitating its use in breeding programs. GWAS of the winter six-row AM panel is in preparation. Publications, including a germplasm release, an agronomic trait/NUE GWAS paper, and a malting quality QTL paper are in preparation.

A.3.2.2. ***Wheat milestones NUE*****:**

Soft winter wheat yield and trait stability: The SRWW elite panel has been evaluated for yield at 12 environments. The main findings are:

- Yield, heading time, height, and test weight showed high heritability values ranging from 0.75-0.95.
- Despite high heritability we were unable to find large effect QTL for yield and NUE in this specific panel.
- The accuracy of GS in this panel ranged from 0.33 to 0.65 over all traits.
- We used phenotypic data from one year to estimate GEBV and then correlated to the phenotypes obtained in the second year. The correlations ranged from 0.46 to 0.82 and were very similar to the correlation of phenotypes between years.
- GS for trait stability for yield and test weight was similar to that reported for the traits themselves.
- Yield and yield stability were quite independent.

Soft red winter wheat NUE: The SRWW elite panel has been also evaluated for response to N at four environments. NUE was calculated as yield under low N as a percent of yield under high N. Analysis of the complete panel showed very low heritability, but these values improved to 0.7 when the analyses were restricted to the lines showing consistently high or low NUE values. An experiment using the best/worst lines and five N rates was performed in 2014 to validate the previous results (data not analyzed yet). We also initiated crosses between the lines with the best and worst NUE values with the intent of creating DH populations to map the genes controlling NUE.

Hard winter wheat NUE: The HRW elite panel has been evaluated for response to N at four environments. The main findings were:

- Some NUE traits had within-trial heritability comparable to or greater than grain yield and the genotypic correlation of some NUE traits across the two NE trials was greater than for grain yield.
- NUE was evaluated as N uptake efficiency (NUpE) and N utilization efficiency (NUtE). Within years, NUtE was the most heritable of the NUE traits (H^2 = 0.56 to 0.76). Between years, the genotypic correlation for NUtE was 0.37, compared with 0.26 for grain yield. Post-anthesis N uptake had the highest between-year genotypic correlation (r = 0.43) among the NUE traits.
- Some lines had consistently good NUE characteristics. Some of the best and worst NUE genotypes are being grown in a study with five N rates to confirm their NUE.
- Substantial genotype x year interactions were observed for most traits.
- The relationship of grain protein concentration with grain yield was consistent with grain protein concentration decreasing by 10 g kg^{-1} for each kg ha^{-1} increase in yield.
- Grain concentrations of iron and zinc were strongly positively correlated with grain protein concentration, as also shown in previous *Gpc-B1* studies.
- Results from the 2012 HWWAMP panel showed that Enhanced Vegetation Index has a higher correlation with anthesis and maturity biomass, grain N yield, and grain yield (R= 0.68, 0.72, 0.67, and 0.77 respectively) than Normalized Difference Vegetation Index (NDVI) or Chlorophyll Index.

Introgression of the Gpc-B1 allele into commercial varieties: The functional *Gpc-B1* allele was incorporated by marker-assisted selection into multiple wheat varieties in CA, MT and WA resulting in concrete increases in NUE. The *Gpc-B1* germplasm has been shared with international colleagues and the favorable *Gpc-B1* allele is being introgressed in wheat lines from several countries (e.g. India, China, Canada, Argentina, etc.)

Functional characterization of the Gpc-B1 gene and downstream targets: A large RNAseq study identified several nutrient transporters that are upregulated by *Gpc1* during the early stages of senescence. These transporters are now targets of new allelic variation studies and biotechnological approaches to improve NUE.

A3.3. Outcomes/Impacts NUE

A3.3.1. ***Barley NUE outcomes/impact***: We confirmed the importance of the *Gpc-H1* region on chromosome 6H for grain protein concentration using the SP6 AM panel. Genetic recombinants and near-isogenic lines have been identified for N-response studies and for introgression into elite breeding germplasm. We will also use marker haplotype information from this region in the NAM parents to identify and characterize additional

functional haplotypes using the NAM populations. For the facultative six-row panel, the OR and UT sites provided data with significant variation for NUE indices. GWAS of agronomic and malting quality traits across the two locations and two years is complete.

A3.3.2. ***Wheat NUE outcomes/impact***: Trait stability importance increases as environment variability increases with climate change. Our studies in soft winter wheats showed that GS for yield stability and test weight stability was as accurate as GS for yield and test weight *per se*. These studies also showed that for yield and test weight, trait *per se* and trait stability were independent and, therefore that it would be possible to select for both simultaneously. Trait stability is very hard to estimate as it requires extensive phenotyping so GS will be a very useful tool to improve yield stability.

We established that NUE heritability varied by wheat class with components of NUE showing good heritability in hard winter wheat but not in soft winter wheat. In both classes of wheat, we identified some lines with consistently superior or inferior NUE. The results to date indicate that our elite winter wheat populations have variability for NUE but breeding for improved NUE will be difficult without marker-assisted breeding.

An interesting outcome of our CSR studies in soft and hard winter wheats was the discovery that the Enhanced Vegetation Index (EVI) was highly correlated with biomass, grain N yield, and grain yield which are traits that are components of NUE and/or NUE. These results indicate that CSR, and particularly EVI, can be a useful tool for selecting for these traits.

The high grain protein allele *Gpc-B1* from *T. dicoccoides* is now routinely used in several wheat breeding programs resulting in varieties with reduced N fertilization requirement to achieve similar levels of grain protein content.

The RNASeq study of the *gpc* mutants provided an overview of the transport mechanisms activated in the wheat flag leaf during the early stages of monocarpic senescence. It also identified promising gene targets to improve nutrient remobilization to the wheat grain including several iron and zinc transporters.

A3.4. Plan-of-Work 2015 NUE

A3.4.1. ***Barley NUE plans for 2015****:* In the next year we will complete the GWAS analysis in all three barley AM panels and submit publications. Using haplotype information for the 6H QTL discovered in the SP6 panel, we will identify an informative set of lines for the six-row NAM population that contain the existing haplotypes at that locus. These lines will be evaluated in a yield trial and characterized for yield, grain protein concentration, CSR, and other traits to determine if there is variation beyond the three haplotypes we have identified.

A3.4.2. ***Wheat NUE plan for 2015***

Winter wheat validation trials (YQV, CSRV, NUEV, and YNVP): In 2015, the HRW validation trials will be evaluated in six locations and the SRW in nine locations. The number of lines in each validation trial will be similar to the numbers evaluated in the

2013-14 season and described in Table A3.1.2. These validation panels will be genotyped with the 90K SNP chip and GBS.

Allele-based breeding (ABB): The ABB trials will be repeated in the 2014-15 season with mostly new lines in 10 locations for the HRW and 13 locations for the SRW.

Winter wheat NAM for NUE: Both the hard and soft wheat projects anticipate having F_5 plants of their respective NAMs by September of 2015. F_5 plants will be genotyped with GBS before the end of the grant.

Winter wheat AM panels: The HRW and SRW elite panels will be genotyped by GBS. Since GS for winter wheat will use GBS it is important that the training populations are genotyped with compatible markers.

A4. Population development

Objective: Develop Nested Association Mapping (NAM) populations for barley and wheat to increase the power of association studies for WUE and NUE and yield.

A4.1. Outputs population development

A4.1.1. Barley

A4.1.1.1. *Six-row NAM population development and characterization*: Kevin Smith (UMN) is developing the six-row NAM population. A total of 93 crosses were made between the NSGC parents and Rasmusson. The 93 F_2 populations are comprised of approximately 100 individuals and the 93 BC_1 populations are comprised of 1-40 individuals. The populations were advanced by single seed descent beginning in the fall of 2012. The BC_1F_4 and F_5 generations were grown in the greenhouse in the winter of 2014. In the summer of 2014 each line was grown in a single row 1 m plot in an augmented design with repeated checks. Data collected included heading date, height, and bacterial leaf streak rating (naturally occurring). Two intact spikes were harvested from each plot and the remaining plot was harvested and threshed in the field. The Minnesota barley program collaborated with Dr. Ian McCray to get a single time point of aerial imaging of the six-row NAM population trial grown out on over 8,000 1 m single row plots. We are learning how to work with these images to get spectral reflectance measurements at the plot level that can be correlated with ground-based measurements. GBS is being conducted on the population and will be completed in 2015.

A4.1.1.2. *Two-row NAM population development and characterization*: Rich Horsley (NDSU) is developing the two-row NAM population. A total of 128 crosses were made between the NSGC parents and Conlon. The F_1's from these crosses were planted in January 2012 and 115 backcrosses were made to Conlon. Each BC_1 population is comprised of approximately 100 individuals. The BC_1 populations were advanced by single seed decent beginning in the fall of 2012. The BC_1F_4 generation was in 2014. A seed increase is being conducted in the greenhouse in the fall of 2014. GBS is being conducted on the population and will be completed in 2015.

A4.1.1.3. *Wild barley introgressions*: Twenty-five wild barley accessions were used to develop a wild barley introgression population in the cultivar Rasmusson. The wild barley introgression population consists of 792 BC_2F_4 derived lines (approximately 30 lines derived from each wild barley accession). All trials (3 MN, 1 ND and 1 MT) have been completed for this population. Phenotype data from the St. Paul, MN 2011, St. Paul, MN 2012, Crookston, MN 2012, Crookston, MN 2013, Fargo, ND 2013, and Bozeman, MT 2013 trials has been uploaded to T3. Exome capture has been conducted on the 25 wild barley parents and Rasmusson. The population was genotyped with a custome 384 SNP chip and the parents were genotyped with the 9K iSelect chip and exome capture sequenced. SNPs from the 9K iSelect chip and exome capture were imputed on the population. GWAS has been conducted and QTL for heading date, height, productive tiller number, yield, protein content, and wax content on the spike and sheath have been detected.

A4.1.2.Wheat

A4.1.2.1. *Spring wheat NAM population development and characterization*: We completed the development of 43 spring wheat NAM populations (F_6) from crosses between Berkut and 38 land-race accessions from around the world and 15 elite spring wheat lines from Montana, California, CIMMYT and Australia. Sets of 75 lines for each of 30 crosses of diverse landraces crossed to a common parent Berkut were phenotyped in CA, MT, WA and SD in 2014. Seed increases were performed in 2014 for the lines that will be evaluated in 2015. A total of 2400 lines from 32 families have been genotyped using the 90K SNP Illumina array. On average ~23,000 polymorphic markers were placed on genetic maps in each of the 32 NAM families.

A4.1.2.2. *Hard winter wheat NAM populations*: This population was initiated with 31 crosses between winter accessions from the NSGC Core collection and 'Overland'. These 31 populations were advanced through F_3 in the field and greenhouse. $F_{3:4}$ families are now being planted for the 2014-15 greenhouse and field season. Two populations were discarded in 2014 due to segregation for sterility. Therefore 29 populations are being advanced and we are retaining >100 lines per family. We anticipate having $F_{4:5}$ families (or F_5 individuals) available for genotyping in August 2015.

A4.1.2.3. *Soft winter wheat NAM populations*: Thirty-six $F_{2:3}$ families from crosses between 'Branson' and diverse accessions from the NSGC Core collection were planted in the fall of 2013. Some populations were lost to winter kill. We have identified 25 populations that have a decent portion of $F_{2:3}$ winter survival and appropriate agronomic characteristics (not excessively tall, late maturing, or lodging prone). We harvested $F_{3:4}$ seeds and will plant these in the fall of 2014 and harvest $F_{4:5}$ in July of 2015. We also generated F_3 seed of each of the 25 populations and planted them in the greenhouse. After an additional generation in the greenhouse we expect to have $F_{4:5}$ seeds by August 2015. Between the field and greenhouse we expect 120 $F_{4:5}$ from each cross, that will be genotyped by GBS in 2015.

A4.1.2.4. *NUE male-sterile facilitated Hard Winter Wheat recurrent selection population:* Utilizing the NAM donor parents and a dominant male sterile we have generated this population that has been subjected to two cycles of random mating in the field.

A4.1.2.5. *Race-specific leaf rust resistance mapping populations*: Six $F_{2:3}$ RIL populations with 120-150 lines were selected from the 10 initial populations developed from selected leaf rust resistant NSGC core collection lines. Bulk resistant and susceptible samples were genotyped using the iSelect 9,000 wheat chip for bulk segregant analysis. $F_{2:3}$ populations were phenotyped at the seedling stage with two leaf rust races. Tentative results from bulk segregant analysis identify one or two resistance loci in each population. Use of seedling tests and molecular markers are currently being conducted to determine whether characterized genes near loci identified through bulk segregant analysis contribute to observed resistance.

A4.1.2.6. *Race non-specific leaf rust resistance mapping***:** Four $F_{4:5}$ RIL populations with 160-240 lines were selected from the five populations developed specifically for race non-specific leaf rust resistance from selected NSGC lines. These populations were evaluated at two field sites in Minnesota in 2013 and in 2014. Two populations have been genotyped using the iSelect 90 K wheat chip with the goal of mapping new race non-specific leaf rust resistance genes.

A4.1.2.7. *Small NAM population for stripe rust resistance gene validation*: One hundred and forty eight accessions with unique combinations of stripe rust resistance loci identified in the published AM study (Appendix I1) were crossed and backcrossed to the stripe rust-susceptible Avocet S. The objectives are to develop a small NAM population segregating for stripe rust, to validate loci identified in the AM panel and, in the long term, develop single gene isogenic lines in the Avocet background.

A4.1.2.8. *Stripe rust KSU:* We are mapping putative major genes for resistance to the stripe rust pathogen in these two populations:

1) *Fuller/Lakin* RIL population. We have field data for two years. This population still needs to be genotyped by GBS.
2) *Tiger/Danby* DH population. This population will be screened primarily in the greenhouse and one year in the field. It is being genotyped this winter by GBS.

A4.1.2.9. *Genomic selection for Soft Winter Wheat*: Crosses were made between the best lines of the elite soft winter wheat training/association mapping population. Selection was based on yield, yield stability, and NUE. We will have F_4 seed from these crosses by fall of 2015 and can begin GS based on the data from the training population.

A4.1.2.10. *Mapping population for NUE*: Several crosses were made between the soft winter wheat lines with the best and worst NUE. We will create a double-haploid mapping population from one of these crosses in 2015.

A4.2. Milestones population development

Both the barley and wheat spring NAM populations were completed and some of them were phenotyped for the first time in 2014. The winter wheat NAM population was advanced one generation as planned. Phenotype and genotype data for the wild barley introgression population have been obtained and uploaded to T3.

A4.3. Outcome /impact population development

The barley and wheat NAM populations will provide a very valuable resource for future genetic studies in wheat and barley. These populations provide more statistical power than the association mapping panels and, therefore, are particularly useful for traits with low heritability such as WUE, NUE and yield. These NAM populations are also becoming a hub for collaborative projects among different breeding programs.

The spring wheat NAM populations is being genotyped both with the 90K SNP iSelect ILLUMINA chip and by GBS, which will facilitate the integration of these two frequently used marker technologies into integrated genetic maps. These saturated integrated maps will accelerate positional cloning projects in wheat.

A4.4. Plan-of-Work 2015 population development

Barley NAM Populations: The six-row NAM will be evaluated in MN and ND and the two-row NAM will be evaluated in MT and UT. Data for heading date, height, aerial digital imaging data (some sites) and grain samples for protein analysis will be obtained. GBS of both populations will be completed. These will represent preliminary datasets for mapping. Data from the wild barley introgression population will be analyzed and manuscripts prepared.

Wheat NAM Populations: For the spring wheat NAM populations, we will complete the genotyping of all the lines with the iSelect 90K chip (almost completed in 2014) and GBS. These spring populations are being evaluated for WUE in multiple environments. For the winter wheat NAM populations, we will advance them to F_5 and genotype them by GBS.

B. Objective 2

Accelerate breeding through marker-assisted selection and genomic selection.

Two approaches have been followed to accelerate breeding cycles: marker-assisted selection (MAS, based on known marker-trait associations) and genomic selection (GS, based on whole genome markers and data from training populations). MAS approaches are actively used in all barley and wheat breeding programs and have already resulted in multiple improved varieties and germplasm (Appendix I2). Genomic selection is underway in barley and is being evaluated in the winter wheat program, where longer phenotypic breeding cycles make GS very attractive.

B1. Outputs objective 2

B1.1. ***Barley GS for low temperature tolerance (LTT)***: We have completed five cycles of GS using an index based on predictions for yield and winter survival. Parental lines and subsets of random and selected lines from cycles 1 and 2 were combined in an experiment to evaluate gain from selection for 4 traits: winter survival, heading date, yield, and Fusarium head blight severity. Of these traits, only winter survival showed significant improvement after the two cycles of selection. Genotypic data for cycles 1 and

2 were compared, and markers known to be linked to winter hardiness moved to (or near) fixation after cycle 2. Subsequent cycles (3-5) have been advanced by inbreeding, seed increases, and spring and fall planted trials. Cycle 3 was planted in spring 2014 and fall 2014 yield trials. Cycle 4 was planted in fall 2014 yield trial. Cycle 5 was planted as F3's and genotyped this fall in the greenhouse.

Cycles 3 and 4 were genotyped with the 384-VeraCode SNP array during 2014 (1,824 samples). To shift marker technology to GBS for cycle 5, a new training population was created consisting of 288 lines derived from cycle 1 and cycle 2. These lines were genotyped using GBS first with 96 samples pooled in each Illumina HiSeq lane and later cycle 5-lines were genotyped by pooling 192 plants in each lane. SNP genotyping was done after aligning reads against the barley reference genome using the TASSEL GBS discovery pipeline. HapMap files with genotypic data were delivered to collaborators and new predictions were generated with the cycle 1 and 2 genotype and trait data. One hundred lines were selected from cycle 5 based on an index combining yield, LTT, deoxynivalenol concentration and three malting quality parameters (malt extract, beta-glucan, and free amino nitrogen).

A new barley GS project was initiated to utilize new alleles in the wild barley introgression lines for yield improvement. Marker and phenotypic data were used to design crosses among the top yielding introgression lines to maximize transgressive segregation for yield. Remnant DNA of 960 wild barley introgression lines that had been genotyped with the 384 SNP arrays was used to perform GBS for model training. Genotypic data were delivered to collaborators in HapMap files after analyzing data as described above. Genotypes for 3,072 cycle 1 F_3 plants for GS were determined using the TASSEL GBS production pipeline that allows rapid allele calling of SNPs identified in the original training population. Selected $F_{3:4}$ lines were planted in an off-season nursery in New Zealand to increase seed for 2015 yield trials.

B1.2. ***Wheat GS****:* The results of the association analysis for yield and NUE in soft wheat did not detect any large effect QTL. This indicates that GS is likely to be more effective than MAS in improving yield and NUE in the soft wheat germplasm. The soft red winter panel (SRWW) was evaluated at 12 environments and these data were used to estimate GS accuracy, which ranged from 0.33 to 0.65 over all traits (yield, test weight, heading date, height, quality parameters, and trait stability). These values are sufficiently high to justify GS in winter wheat. The vernalization requirement of winter wheat results in long cycles of phenotypic selection: we estimate a cycle of phenotypic selection to take 5 – 7 years for complex traits. A cycle of GS can be completed in one year for winter wheat so even moderate prediction accuracies can produce an advantage in terms of gain per year.

Trait stability will be important as environment variability increases with climate change and can be a selection criterion if variation for stability is heritable. If GS can effectively model trait stability then it would prove stability is under genetic control, something that is very difficult to show using other analyses. We estimated trait stability using regression and principal component approaches. The regression approach produces a stability parameter that can also be viewed as a response to stress. In the soft winter wheats, GS for yield and test weight stability was as accurate as GS for yield and test weight *per se*. This indicates trait stability is under genetic control. For yield and test weight, trait *per se*

and trait stability were independent from one another indicating that it would be possible to select for and combine high trait values and stability. The estimates of regression stability for yield indicated that there was variation for yield response to stress. Trait stability is very hard to estimate as it requires extensive phenotyping. Thus, the use of GS has the potential to accelerate selection for improved stability and yield response to stress. The Ohio State University program made crosses among the best lines from the training population and is generating an inbred population that will be used in GS.

GS is being also tested in the hard winter wheat elite panel. GS accuracy (r) for multiple NUE traits in the HWW elite panel in 2012 was < 0.4, except for anthesis biomass, anthesis biomass N concentration, and anthesis biomass N quantity (r = 0.46 – 0.60). In 2013 accuracy for NUE traits also was < 0.4, except for N harvest index, N utilization efficiency, and grain yield (r ~ 0.7), spike density (r = 0.66), kernel weight (r = 0.51), and plant biomass at maturity (r = 0.46). GS models predicted CSR phenotypes within the same trial with accuracies from 0.4-0.57. The prediction accuracy of most CSR phenotypes was higher than prediction accuracy of yield *per se* (0.4). GS models will be validated in the Yield QTL Validation panel trials and CSR Validation panel trials as soon as marker data become available. These validations are underway and are a valuable evaluation of the utility of GS models for applied breeding. If validated, GS could be very useful for breeding for yield and NUE for the Great Plains.

Wheat breeding programs at WSU are also developing capacities for GS. They are now routinely genotyping all fixed experimental lines (~3000/y) by GBS. Current emphasis is on development and analysis of training populations of spring and winter wheat and assessment of prediction accuracy for the most important traits, along with identification of diagnostic markers for specific loci to inform GS models. Genomic prediction will be implemented as a selection tool prior to yield testing over the next 2-3 years.

B1.3. *Cooperative allele-based breeding (ABB) strategy*: The winter wheat breeders are using an allele-based breeding strategy that includes shared genotyping resources to enhance cooperation among breeding programs. The premise is that replicating alleles over environments is important to efficient molecular breeding. Alleles and GS models are being evaluated within and among programs, genetic backgrounds, environments, and years. This strategy is helping breeders to determine the value of existing and introduced alleles in a relevant breeding context.

The 2013-14 field trials for the hard red winter included 283 breeding lines that were planted in 10 locations. Traits evaluated in the HRW ABB trials included yield (9 env.), agronomic score (1 env.), leaf rust (1 env.), stripe rust (1 env.), powdery mildew (1 env.), height (6 env.), heading date (4 env.), test weight (5 env.), and bacterial streak (3 env.). There were three SRWW ABB trials involving a total of 1,259 breeding lines that were evaluated in 3-4 locations each. The SRWW ABB lines were evaluated for different traits in different trials. All trials included evaluations for yield, heading date, height, and test weight while harvest index, yield components, NDVI, CTD, grain protein, winter survival, and pre-harvest sprouting were rated only at some locations. Samples of the HRWW and SRWW ABB sets have been submitted for genotyping by GBS. The ABB tests will be of similar size in 2015, but will consist of mostly new entries.

GBS libraries of 1,200 samples from the allele-based breeding (ABB) hard winter wheat region, Mid-Atlantic and Southern soft winter wheat regions were prepared using *PstI* and *MseI* restriction enzymes. Pooled libraries were sent to KSU for sequencing and SNP discovery. Libraries will be prepared for an additional 400 samples from the Northern soft winter wheat region prior to 12/2014.

B1.4. *iSelect SNP chips*: During 2014, the improved wheat iSelect 90K SNP platform was used to genotype 4,224 wheat samples including bi-parental and association mapping populations, a collection of 96 accessions from different *T. aestivum* subspecies, the hard winter wheat validation panel for NUE and yield, and 2,544 samples of the spring wheat NAM populations. Genotypes were delivered to collaborators. Samples to be genotyped with the 92K array prior to December 2014 include 304 lines in the soft winter wheat validation panel, and 600 additional spring wheat lines from the NAM populations.

A total of 384 lines from University of Minnesota and Oregon State University were genotyped with barley 9K array. The data files were released to each lab in April 2014.

B1.5. *Improved genotyping methodology*: Illumina is no longer supporting the 384-SNP Vera-Code platform that was being used to genotype barley lines for GS. Two NGS approaches were investigated to replace this platform: genotyping by multiplex amplicon sequencing (GBMAS) and GBS of reduced representation libraries. The GBS protocol was determined to be cost-effective and straightforward for generating genome-wide marker data for both barley and wheat. GBS was used to genotype new and updated training populations in both crops.

The GBMAS protocol continues to be investigated as a method of using next generation sequencing for targeted genotyping in both wheat and barley. After modifying the adaptor sequences in the PCR primers used to amplify the targeted regions, the Fargo ND genotyping lab has improved the protocol in barley and observed data agreement among control samples of 95% or better. This approach is also being investigated for use in wheat at Manhattan, KS where GBMAS primers were developed for 32 markers for wheat genes that are routinely screened. Preliminary results indicated that 26 markers generated genotypic data consistent with KASP results. After further optimization and adding more markers to the set, the set of SNP will be used in routine screenings. The combination of GBMAS and GBS is an alternative being explored.

B1.6. *Genotyping of barley and wheat populations using 48-SNP- Sequenom, KASP assays and other markers*: Thus far, 8,934 wheat and 864 barley samples have been evaluated with panels of SNP using either the Sequenom or KASP platforms. The smaller SNP platforms were used to characterize advanced breeding lines and to perform selection among three-way F_1s, as well as validating markers identified in the AM panels and in segregating populations. Additional, 1,680 wheat samples in the elite panels, validation panels, and ABB projects are currently being evaluated with the small SNP panels to provide information about the presence of major genes and alien-translocations that may influence GWAS or GS models for traits under evaluation. Part of the original barley samples planned for the 48-SNP platform were replaced by GBS (5,088 samples in the second half of 2014).

In addition to the different SNP chips, the genotyping labs still provide markers for individual traits to the breeding programs (SSR, STS, CAPs, etc.). As an example, the WSU genotyping lab provided 4,000 marker assays for the STS marker for low cadmium gene *Cdu1* to the CA durum wheat breeding program.

B1.7. *Improved markers for high-throughput MAS developed*. New KASP assays for several rust resistance genes in wheat were developed, including *Sr24/Lr24, Sr25/Lr19, Sr26, Sr22, Sr40, Sr42* and *SrZelma*. These KASP markers simplify the evaluation of breeding lines and germplasm for these resistance genes. A KASP assay specific for a mutation in the *PSY-E1* gene was also developed that can identify white endosperm lines carrying the *Sr25/Lr19* translocation. A new KASP assay for the *Tsn1* gene for sensitivity to toxins produced by the pathogens causing the diseases tan spot and *Staganospora nodorum* blotch is being used to identify wheat lines that may have increased susceptibility to these pathogens. Diagnostic KASP assays were developed for a *Ppd-A1* insensitive allele having a large deletion in the promoter and for the *vrn-B1* winter allele for short vernalization duration requirement. These important genes affecting adaptation in wheat were found to be widely distributed in southeastern soft winter wheat and were also detected in the winter wheat NAM parents.

B2. Milestones and deliverables objective 2

B2.1 *Barley GS milestones*: An important milestone of the fourth and fifth cycles of barley GS for LTT was the prediction of genomic estimated breeding values (GEBV), which were used to select the top 100 lines in each cycle. Field trials are being conducted to assess gain from selection from cycles 1 and 2. Cycles 3 – 5 are being advanced to yield trials which will be used to identify variety candidates for industry testing.

B2.2 *Wheat GS milestones*: We confirmed the potential of GS to improve both traits and trait stability. Our studies showed that GS for yield and test weight stability was very similar to GS for yield and test weight. Our studies also showed that yield and yield stability are independent from one another and therefore, that selection can be applied to both the trait and its stability.

B2.2 *Genotyping milestones*: Genotypes for thousands of SNP tags distributed throughout the genomes of wheat and barley are now available for GWAS, development of GS models and calculation of GEBV in selection populations.

- Since 2011, the genotyping labs have processed more than 68,000 wheat and barley samples with the iSelect SNP platforms, 15,000 of which were TCAP populations and panels.
- Dense genome-wide marker data sets are available for more than 1500 hard and soft winter wheat lines for identification and validation of marker-trait associations for NUE and yield. Genome wide barley SNP data are available for identifying genes important for LTT and disease resistance.
- Genotypes are available for construction of high-density linkage maps of the spring wheat NAM populations, integrating 90K iSelect and GBS data.

- Genotypes for genes affecting important traits are available for MAS, parental selection in breeding programs and germplasm evaluation. Breeder-friendly KASP assays have been developed for important adaptation and resistance genes.

B3. Outcomes/Impacts objective 2

B3.1. ***Barley GS outcomes/impact***: The preliminary results from the gain from selection experiments in winter barley show that two cycles of GS, consisting of a multi-trait index with LTT weighted about 50%, was equivalent to one cycle of phenotypic selection.

In barley, we have implemented a GS breeding program that has successfully improved LTT. This trait is difficult to improve by phenotypic selection because of the highly variable winter conditions. Two breeding lines from cycle 1 were entered into a national winter malting barley trial planted at 20 locations. This breeding program established routine protocols and logistics to conduct multiple breeding cycles per year. In the past year, we have transitioned from fixed array-based marker screening to genotyping by sequencing. This will serve as a model for other programs to implement GS.

B3.2. ***Wheat GS outcomes/impacts***: In a more theoretical study of GS, we proposed new solutions to integrate environmental data and crop modeling into the GS framework to predict G*E. Using a crop model to derive stress covariates from daily weather data for predicted crop development stages, we proposed an extension of the factorial regression model to genomic selection. The method was tested using a large winter wheat dataset and showed an 11.1 % increase in the accuracy in predicting genotype performance in unobserved environments for which weather data were available, and 10.8% decrease in variability in prediction accuracy. These results showed the potential value of this approach. This study was published in Theor. Appl. Genet. (Appendix I1).

A separate theoretical study was performed to optimize GS training set populations using wheat and rice examples. This study compared five different training set sampling algorithms for prediction accuracy in the presence of different levels of population structure. This study showed that maximizing the phenotypic variance captured by the training set is important for optimal performance. It also showed that the best selection criterion to optimize the training set populations depends on the interaction of trait architecture and population structure. This study was also published in Theor. Appl. Genet. (Appendix I1).

B3.3. ***Impact of allele-based breeding****:* In the past, public breeding programs did not exchange lines at such early stages of testing. The ABB project has changed this philosophy and now these breeding lines are shared earlier, facilitating the testing in multiple locations and a wide expansion of the germplasm evaluated in each breeding program. Using this cooperative approach breeders cooperatively test a greater number of alleles in multiple environments.

B3.4. ***Impact of iSelect marker platforms***

- Using the 9K and 90K iSelect chips, the genotyping of AM panels and mapping populations are now completed in a few months compared with years in the past, greatly accelerating the pace of marker development and gene discovery.
- These SNP data have been also used for population genetics and crop evolution studies, and have contributed to the anchoring and ordering of contigs of the draft genome sequences for wheat and barley.
- The genotyping of the NSGC core collections and the NAM populations has increased the value of this germplasm and renewed interest in its utilization.
- Since 2011, more than 100 high-throughput marker assays have been developed for important genes affecting plant adaptation, disease and insect resistance and quality traits.
- KASP and other assays developed by TCAP researchers have been used extensively to perform MAS in segregating populations, to characterize elite breeding lines and cultivars and to characterize lines from the NSGC both by public and private breeders.

B4. Plan of work 2015 for objective 2

B4.1. ***Barley GS plan 2015***

In 2015, we will compare gains from random, phenotypic selection and GS in barley in three locations: MN, NE, OR. Yield trials will be grown from the first 2 cycles of selection. Data will be collected for winter survival, yield, heading date, plant height, FHB, and lodging. Data from these experiments will be combined with 2014 data to evaluate prediction accuracy and estimate gain from the first 2 cycles of GS.

We will evaluate selected lines from cycles 1, 2 and 3 in advanced yield trials; evaluate cycle 4 in preliminary yield trials; and conduct a seed increase for cycle 5 lines for future experiments. We will obtain additional agronomic performance data from all the previous lines and genomic estimated breeding values (GEBVs) for cycle 5 lines.

B4.2. ***Wheat GS and ABB plan 2015:*** The HRW and SRW elite winter wheat panels were phenotyped in 2012 and 2013 and genotyped with the 90K chip in 2013. We have decided to add GBS genotyping data to these panels to increase their value for future GS studies that will also use GBS. The allele-based breeding tests in 2015 will be of similar size as in 2014, but will consist of mostly new entries.

B4.3. ***GBS genotyping plans 2015***: These GBS genotyped populations, AM panels and NAM populations will be used to identify favorable alleles for disease resistance, WUE and NUE. The genotyped lines will be also used to advance the GS programs and to develop high-density maps integrating GBS data and 90K iSelect chips. The specific lines that will be genotyped by GBS in 2015 are listed in sections C4.1 and C4.2.

C. Objective 3

Implement sequence-based genotyping methodologies to discover new allelic diversity.

C1. Outputs objective 3

First wheat exome capture: The whole genome exome capture (WGEC) assay targeting 107 Mb of coding and low copy number sequence in the wheat genome was designed by our group in collaboration with the Nimblegen company (manuscript is under review). The assay was used to re-sequence 62 diverse wheat lines resulting in 1.57 million high-quality variant calls. Thirty-two of these re-sequenced lines were the founders of the nested-association mapping (NAM) population. The WGEC assay was used to characterize the patterns of genetic variation in wheat. A publicly available bioinformatics pipeline to identify mutations in polyploid wheat using WGEC was developed in collaboration with Dr. L. Comai and was published in Plant Cell (Appendix I1).

Second wheat exome capture: A second generation exon capture focused only on the coding regions of ~82,000 non-redundant genes (~286,800 exons) was developed in collaboration with Nimblegen. This second design includes exons padded by 30 bp of flanking sequence. In total, we have targeted 286,799 exons from all three sub-genomes. This capture was used to re-sequence 1000 tetraploid TILLING lines resulting in the identification of ~2.5 million SNPs. An additional 500 lines are being sequenced.

Barley exome capture: The WGEC assay for barley was designed and used to re-sequence 150 barley lines that served as founders of the barley NAM population. The remaining 44 founder lines of the NAM population are currently being re-sequenced. Once all NAM founders are re-sequenced, SNPs will be called and all detected SNPs will be imputed on the NAM population. The 26 founders of the wild barley introgression population were re-sequenced and the resulting ~500K SNPs were imputed on the population. The barley WGEC assay is also being used in an international collaboration to sequence a large collection of landraces and wild barleys.

Genotyping-by-sequencing (GBS): The number of samples genotyped using next generation sequencing methodology in each of the four USDA-ARS genotyping labs increased greatly this year. A pipeline for variant calling based on TASSEL-GBS and UNEAK was set up and used to perform variant calling. The two-enzyme (*PstI* and *MseI*) complexity reduction protocol was used to generate GBS data for:

- 2400 RILS of the spring wheat NAM population (also genotyped by 90K iSelect). On average, ~23,000 polymorphic markers were mapped in each of the 32 NAM families. Data is being integrated with exome capture SNPs.
- 288 elite soft winter wheat lines.
- 300 lines in the soft winter wheat validation panel.
- 300 lines in the hard winter wheat validation panels.
- 1,536 lines from TCAP breeding populations.
- 4,946 samples from 19 bi-parental mapping populations of wheat, including landrace populations segregating for resistance to stem and stripe rust as well as

populations for identification of new GA sensitive genes for reduced height.

Sequencing was done using in-house Ion Torrent platforms in the Manhattan, KS and Pullman, WA genotyping laboratories, and using the Illumina HiSeq platform at the NCSU Genome Science Lab in Raleigh.

GBS of barley NAM population: Tissue was harvested from 19,200 samples of the barley NAM populations and parents and DNA was isolated and normalized. GBS libraries were prepared using *PstI* and *MspI* restriction enzymes, and were sequenced using Illumina HiSeq. The publicly available draft of the barley genome (Morex assembly3) was used to prepare pseudo-chromosomes for aligning reads from the low coverage sequencing in the TASSEL-GBS discovery pipeline. Analysis of sequence data from the parents of the barley NAM identified 251,454 SNP having homozygous genotypes in the recurrent parents and MAF greater than 0.01. Comparisons of the genotypes of the NSCG accession parent with either the Conlon or Rasmusson parent identified between 8,000 and 30,000 SNPs segregating in each NAM family.

GBS libraries were also prepared from a sample of 190 RILs and were sequenced in a single lane on Illumina HiSeq to evaluate the amount of missing data observed with this level of coverage. At this pooling level, an average of 10% or less missing data was observed for 23% of SNPs segregating in each of the five six-row barley NAM populations evaluated. On average, 50% or less missing data was observed for 51% of SNP. Based on these results, GBS of the remaining NAM lines is being done at the 192-plex level and is expected to be completed early in 2015.

C2. Milestones and deliverables objective 3

Whole genome exome capture assays (WGEC), GBS and 90K iSelect were used to build high-density genetic maps of NAM populations in wheat and barley that interconnect these genotyping platforms.

The compilation of map locations of both the GBS and iSelect SNP in the many bi-parental and NAM mapping populations will allow further ordering of contigs and refinement of orders in the barley and wheat reference genomes.

Reference genome based pipelines for identifying SNPs connect GBS tags with information available for draft sequences of both wheat and barley. The SNPs from the wheat and barley iSelect arrays are also connected with the draft genome sequences, allowing researchers and breeders to rapidly focus in the genomic regions of interest.

The diversity maps generated and the NAM populations developed by TCAP researchers are providing the foundation for fine-scale dissection of complex traits and gene discovery in wheat and barley in the future.

The level of SNP diversity observed in the barley NAM parents indicates that it will be feasible to provide much more dense coverage of the barley genome than would have been possible with the originally proposed 384-SNP genotyping platform.

C3. Outcomes/Impacts objective 3

Increased marker outputs: An important outcome of the increased utilization of next generation sequencing for genotyping is a dramatic increase in the capacity of the genotyping centers to generate sequence based marker data for mapping populations in different wheat market classes.

Characterization of genetic diversity: The integration of GBS and exome capture SNPs resulted in 1.57 million SNPs that allowed us to characterize diversity in the functional portion of the wheat genome providing insights into the nature and patterns of variation in coding sequences. The study showed that regions showing evidence of selection were different in the different genomes. The study has been submitted for publication.

Generating new genetic diversity: The second exon capture is delivering on average 2500 mutations per line at 97% confidence. Extrapolating to the 1500 lines that will be sequenced and analyzed by February 2015, this experiment will provide 3.3 million mutations in the coding regions of wheat (>30 mutations/kb). This mutation density is sufficient to find loss-of-function mutations in most wheat genes. This resource will greatly enhance the ability of wheat researchers to study gene function. These TILLING mutants have already been used to engineer new wheat germplasm with a 7-10 fold increase in resistant starch that are publicly available (Appendices I1 and I2).

Validation of wheat genomic reference: The chromosome based survey sequence of wheat released in July, 2014 has been used to construct pseudo-molecules for reference-based SNP discovery in wheat using TASSEL-GBS. Good agreement of contig orders (R = 0.92) was observed between a de novo linkage map constructed in the Massey x MPV57 RIL population and the previously published POPSEQ ordering of contigs. In this population, 98% of SNP tags in each linkage group aligned to contigs originating from a single chromosome. These data indicate that application of reference based SNP calling pipelines in wheat should allow ordering of GBS tags in diverse germplasm, similar to what has been reported in barley.

Training in marker technologies: In response to request from TCAP participants interested in utilizing GBS, a webinar was presented by Dr. Brown-Guedira on the TASSEL-GBS Discovery and Production pipelines. Google Drive is being used for sharing of the pseudo-molecules of the reference genome sequences in wheat and barley as well as scripts for SNP calling, locating SNPs on contigs and naming SNPs using the format specified for wheat and barley that includes the name of the assembly, name of the contig and position of the SNP on the contig. An additional webinar was presented for the whole genome exome capture assays (WGEC) and their utility for genome analyses (http://xtalks.com/Exome-Sequencing-for-Crops.ashx). These training sessions are accelerating the incorporation of these high-throughput genotyping platforms.

C4. Plan of work 2015 for objective 3

The lines that we plan to genotype by GBS in 2015 are indicated by species below.

C4.1. *GBS plans for barley*

- 19,200 barley NAM lines.
- 800 accessions of the barley iCore.
- 318 lines from the wild barley diversity collections.

C4.2. *GBS plans for wheat*

- 5000 winter wheat NAM lines.
- 400 HRWW lines from the ABB projects.
- 1200 SRWW lines from the ABB projects.

The GBS data from the NAM populations will be used to construct sequence-based genetic maps of wheat and barley. In wheat these maps will be integrated with the 90K iSelect chip that was already used to genotype the spring wheat NAM population.

We will develop a T3 module to handle sequence-based genotyping data. We plan to use the NAM populations to construct sequence-based genetic maps of wheat and barley.

D. Objective 4

Implement web-based tools to integrate marker-assisted selection and genomic selection strategies in breeding programs.

D1. Outputs objective 4

New data released: Open access to increasing amounts of data through T3 continues. Combining wheat and barley, seven new genotyping trials were uploaded, accounting for over 25 million data points. Phenotyping trials were more numerous: PIs contributed 192 TCAP trials containing over 220 thousand phenotypes. An additional 93 non-TCAP trials were contributed containing 30 thousand phenotypes. Significant increase of non-TCAP trials submitted indicates that users view the T3 model of open-access data favorably.

Table D1. Summary of TCAP current data

Trials	**Barley**	**Wheat**
Phenotype Trials	603	265
Genotype Trials	109	41
CAP data programs	27	15
Lines		
Line records	33,499	11,562
Breeding programs	24	42
Lines with genotypes	15,822	8,138
Lines with phenotypes	9,359	8,205
Phenotype Data		
Traits	110	128
Total phenotype data	415,789	352,146
Genotype Data		
Total genotype data	46,876,220	84,582,589

New functionality: The ability of T3 to store and manipulate CSR data was expanded to a new device (Crop Scan) and to an additional CSR index (Enhanced Vegetation Index). The ability of T3 to work with the Android Field Book improved. T3 can now design field trials according to a number of different designs (RCBD, augmented, modified augmented) and can make field maps for some designs. Map and trait files can be created ready for use on Field Book. In turn, data collected using the Field Book can be uploaded directly back to T3 without going through upload templates. T3 can now handle genotyping by sequencing (GBS) marker data. These data are much more complicated than Illumina SNP chip data. All experiments using Illumina produce data on the same markers. All GBS experiments produce somewhat different markers. We have developed BLAST tools to synonymize marker data across experiments. Additionally, GBS experiments may produce many more markers. New functionality can now accommodate experiments with in excess of a million markers. T3 can now provide experiment-specific genotype data rather than consensus data from multiple experiments.

New training: A further video tutorial has been produced on the T3 YouTube channel.

Coordination with other database groups: The T3 team coordinated a second annual PAG workshop on “Managing Crop Phenotype Data”. This workshop is helping us gear up to improving T3 interfacing, through application programming interfaces, with other databases.

TCAP researcher Jean-Luc Jannink is also a PI in the "CGIAR Genomics Back Office" (CGBO). T3 programmers funded by TCAP are ensuring that T3 will be able to access analyses offered by the CGBO. The "Application Program Interface" effort tries to standardize queries across different breeding database platforms to allow platforms to access each other's data and analysis tools. We expect to benefit synergistically from CGBO efforts to improve the "Big Data" performance of T3. This is an additional example of how the TCAP funding has enabled US researchers to be important players in large international projects.

D2. Milestones and deliverables objective 4

As in the previous year, the large increase in the amount of data now openly accessible through T3 represents the most important contribution made by T3. We believe that T3 is facilitating sharing of data earlier in the research cycle than previously, making the data more useful globally.

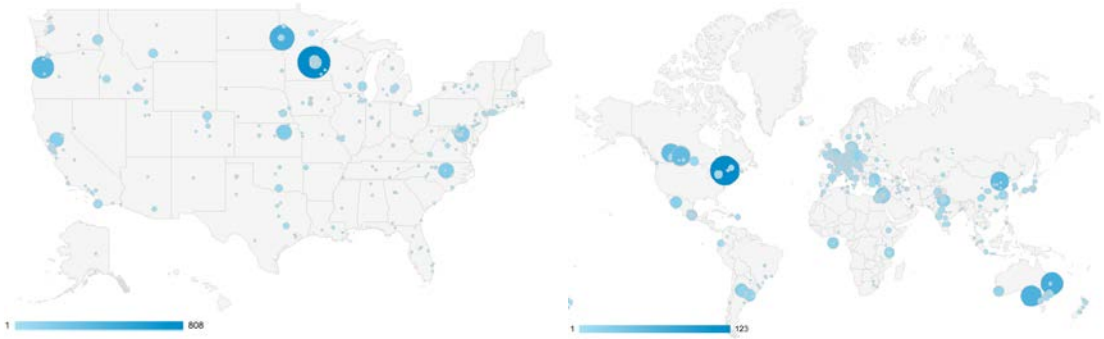
Improved handling of GBS markers represents a second important expansion. GBS markers are an important new option in research and breeding. The data are clearly more complex to work with than SNP chip data because of problems such as higher missing rates and more potential errors in heterozygous loci. The data, however, come at a lower cost and require little up front development.

D3. Outcomes/Impacts objective 4

The most far-reaching impact T3 is having is changing the scientific culture surrounding

data. As is common in genomics, this change of culture is coming first from human genetics where sharing of data is becoming the norm and where cleverness in exploiting data is becoming as important as ability to collect it. In that context, the observation has been that, for example, meta-analyses with 200,000 subjects still deliver valuable results over and above those with 100,000 subjects. We suspect the same will be true in crop genomics and we are pleased that T3 may help catalyze greater collaboration.

The figures below show T3 usage for the past year. Visitors to T3 came from 46 of the 50 states, with 31% more visitors over 2014 than 2013. International visits represented 30% of the total traffic to T3, with heaviest traffic from Canada, Australia, China, and across the European Union. Increases in international traffic over the previous year (72%) were greater than that of national traffic.



D4. Plan of work 2015 for objective 4

Planned new analyses tools*:* A training population design tool will be developed. This tool will allow the user to specify a set of N_c lines that are candidates to be in a training population of size N_t ($N_c > N_t$) and a set of lines that are the target of prediction. The analysis selects the N_t such that the prediction accuracy on the target set is expected to be maximized.

A definition of core germplasm sets tool will also be developed. Similar to training population design tool described above, the user defines a set of N_c lines that are candidates to be in a core set of size N_t ($N_c > N_t$). The criterion the analysis maximizes in choosing the N_t lines, however, is the variation present in the core set, rather than its ability to predict other lines.

Multivariate analysis will also be developed. Initially, this will be limited to multivariate prediction methods, rather than GWAS. The user specifies a set of N_t training lines, a set of traits, and a set of lines that are the target of prediction. The analysis predicts values for all traits for the targets.

Planned integration with other networked genomics resources

Integration with other networked genomics resources: Increased collaboration with GrainGenes will be developed. We view GG as a “knowledge base” that contains information from peer reviewed studies, whereas T3 is a “diversity base” that contains

the raw information used in such studies. The two databases should not contain redundant information but should communicate with each other when one database needs information that the other stores. T3 uses genetic maps that summarize an analysis of segregation data. These maps should be stored in GG but made accessible to T3. Likewise, we aim to facilitate linking from T3 to GG. When a T3 analysis identifies a genomic segment of interest, it should be possible to query GG about that segment.

Integration with reference sequence resources: progress has been made in developing reference sequences for both barley and wheat. Such references provide powerful indexes to knowledge at all levels of the genome. Most T3 markers are annotated with context sequence that allows direct alignment to reference sequence and therefore to position-indexed knowledge. Minimally, T3 will provide a JBrowse genome visualization.

Application program interfaces to other breeding-related database systems: A number of other groups are creating tools for breeding data management. Different tools have features that focus them in different ways. T3 at the origin has been a tool to consolidate and make available data from many partners in a large coalition. The decision to run T3 on a centralized server with a web interface makes T3 a strong option for this purpose. Other tools are more adept at interfacing with the breeders' local program, e.g., by providing seed inventory management, trial design, and crossing design tools. We will collaborate to develop standards and protocols to allow different systems to communicate such that breeders can leverage the strengths of each system.

Planned features

Improved handling of GBS markers and big data: T3 currently uploads and can retrieve and process GBS data. Nevertheless, with the size of the datasets, manipulations are slow and need to be improved. We will continue to focus on this feature.

Experimental designs with field maps: T3 currently allows researchers to design trials with a modified augmented design that is spatially explicit. Such designs can be uploaded to the Android Field Book to facilitate data collection and can also be analyzed with spatial statistical models. We will expand the list of experimental designs to randomized complete block and augmented designs.

Deposit of defined datasets used, for example, in a publication: T3 can provide a permanent repository for datasets that are at the origin of analyses reported in publications. Having these datasets available facilitates independent replication of research findings. The repository would serve specifically for the case where a researcher initiates analyses on data downloaded from T3. In that case a dataset identical to that used for analysis can be stored with a permanent link provided to the data.

E. Objective 5

Develop and implement a Plant Breeding Training Network

E1. Output objective 5

E1.1. *Integrated education and research programs.*

Annual meeting at PAG: The 2014 TCAP annual meeting was held in San Diego, CA. Approximately 100 people attended the meeting including TCAP participants (PIs and students), stakeholders and the scientific advisory board. The meeting was split into four parts including: (1) a reporting session for the stakeholders; (2) a reporting session for the scientific advisory board; (3) a breakout session to coordinate the year 4 activities and; (4) a student poster session. Two short sessions were devoted to short talks by the students where they provided a short synopsis of their projects. The scientific reporting session was followed by breakout groups focused on discussing future efforts related to T3, barley phenotyping and breeding, wheat phenotyping and breeding, genotyping, and data analysis. Ed Buckler (USDA-ARS, Ithaca, NY), a member of the scientific advisory board, provided a very positive assessment of the progress of the project and suggestions for improvements. The meeting ended with a reception and a well-attended student poster session. The breadth and depth of the project was highlighted in the poster session as the students presented results from all aspects of the project.

TCAP seminar series: To encourage communication about the TCAP project and to support innovative research a seminar series was held online. Table E1 provides a complete list of seminars and attendance. Seminars have continued to be useful as evidenced by archival views (http://passel.unl.edu/communities/pbtn).

Table E1. Seminar series

Presenter	Title	Date	Participants	Views**
Shuyu Liu	Detection of epistasis and QTL by environment interaction in QTL Network	9/25/2013	47	88
Rex Bernardo	Strategies for the routine use of GS in an inbred development program	10/23/2013	80	228
Craig Morris	Wheat grain composition: what it is, how we measure it and why	11/6/2013	12	88
Michael Gore	Connecting genotype to phenotype: Progress with a NGS platform in maize & cotton	12/4/2013	13	118
Brian Arnall	NUE: Agronomic Perspective	9/24/2014	37	3
S. Baenzinger	NUE from a Genetics Perspective	10/8/2014	31	2
Natalia de Leon	Maize GWAS Trait Discovery	10/22/2014	15	3
C. Lawrence	Model Organism Database and Maize GDB	11/5/2014	15	2
Pat Byrne	Breeding for Drought Tolerance	11/12/2014	25	NA
G. Brown-Guedira	Genotyping for Breeding	11/19/2014	55	7

*Participants – Attendees of the live webinar presentation
**Views- Number of asynchronous views of webinar recording.

Use of online environment: A total of 1,557 visitors accessed the Plant Breeding Training Network (http://passel.unl.edu/communities/pbtn) in 2014, representing 50 states and 133 countries. The online environment is being used as a communication tool for project management as well as to provide educational opportunities through classes and seminars (see Table E2). Other groups continue to use the site for communication (e.g. NAPB Student committee meets monthly to plan student activities).

Successful plant breeding stories were gathered by the Plant Breeding Coordinating Committee and hosted at PBTN to showcase the positive impact of USDA funded plant breeding.
PBTN continues to link to the Plant Breeding Coordinating Committee and advertise their webinars to PBTN members.

Additional Related Online Activities:

- 32 Archived Webinars, 55 Plant Breeding Class Lectures and 19 undergrad webinars/presentations are currently archived on PBTN.
- All recordings are archived on Vimeo channel, where videos are less likely to be blocked by user firewalls.

Table E2. Use of the online environment.

PAGE	Dates	Total Visits	Pages/Visit
PBTN (entry into all offerings)	8/1/13-7/31/14	9259	2
PBTN Plant Breeding Class Lectures (all archived recordings from TCAP grad courses available from this page)	8/1/13-7/31/14	947	2
PBTN Archived Webinar Tab (all TCAP Seminar Series webinars available from this page)	8/1/13-7/31/14	136	3
PB Grad Student community	8/1/13-7/31/14	30	5
Entering mentoring (Spring 2012 class)	8/1/13-7/31/14	87	8
Plant Breeding Strategies (Last offered fall 2011)	8/1/13-7/31/14	15	21
Quantitative genetics	8/1/13-7/31/14	46	12
Association Genetics (Fall 2012 Class)	8/1/13-7/31/14	36	13
TCAP Graduate courses (offerings)	8/1/13-7/31/14	202	4
TCAP Undergrad Community	8/1/13-7/31/14	197	4
Plant Breeding Coordinating Community (launched Summer 2013)	8/1/13-7/31/14	36	13

Supported International plant breeder education online through CGIAR creation of educational materials for the Integrated Breeding Platform: Draft content that was written by subject matter experts for use in online learning was reviewed. Power-point lectures were transformed into word documents and additional text and quiz questions,

were added and reformatted where needed. The work has been focused in three main areas. The lessons included:

- *Molecular Breeding*: Selection of markers for molecular breeding, marker assisted breeding (MAB), genetic diversity and germplasm selection, phenotypes in MAB, genetic linkage and mapping, quantitative trait analysis, qualitative analysis and quality control.
- *Agricultural Statistics*: Introduction to agricultural statistics, experimental design.
- *Research Management*: Budget norms for research stations-projections and assumptions for well-structured experiment station, research station management, well organized stations of national agricultural research. International education is also supported by PBTN online materials being globally available. People from 133 countries are registered and participate in the PBTN.

E1.2. *Communication with stakeholders*

Newsletters: The fall 2013, winter 2014, summer 2014, and fall 2014 eNewsletters were produced and distributed among TCAP participants, stakeholders and members of the scientific advisory board.

Barley Blog: Information was shared with stakeholders through 33 posts on the barley blog on eXtension at http://blogs.extension.org/barleynews/page/4/.

Face to face stakeholder presentations: TCAP PIs and students made 35 stakeholder presentations.

E1.3. *Recruit a diverse group of undergraduates to plant breeding*

MSI / TCAP bridge: Seven MSI faculty supported in mentoring students reported on collaborative research projects. All projects were renewed at the same level in 2014. Marceline Egnin, a participant in the TCAP/MSI collaborations from Tuskegee University, wrote a successful grant proposal using the TCAP model for recruiting diverse undergraduates to plant breeding. During 2014, TCAP has hosted five meetings with interested MSI faculty, working to write a large grant to implement the TCAP model more broadly.

MSI student visits TCAP facilities: Jessica Bess, a student from Prairie View A&M University, interned at Colorado State University working with Dr. Pat Byrne and Sarah Grogan. The objective of her research was to characterize 50 hard winter wheat varieties for drought tolerance and yield-related traits. She gained experience with several types of measurements including leaf area index with a ceptometer, canopy spectral reflectance with Jaz and Crop Circle instruments, and canopy temperature with an infrared thermometer.

Undergraduate students mentored in research: A total of 108 undergraduates have participated in research internships through the TCAP with 67 being mentored by TCAP faculty and graduate students, and 41 by MSI PIs. Thirteen graduate students have completed Entering Mentoring to support their undergraduate mentoring efforts. A complete list of undergraduate students is provided in Appendix I5.

E1.4. ***Plant breeding students trained***

Graduate student training: A total of 136 graduate students (Appendix I3 & I4) have participated in the plant breeding training network. Ninety-five are directly mentored by a TCAP PI, while 54 students are at least partially funded by TCAP with two of those at minority serving institutions. 41 students were unaffiliated with TCAP, but have participated in the online courses.

Graduate courses: Ashu Guru, a professor at the Jeffrey S. Raikes School of Computer Science and Management at UNL, created a cross disciplinary course for plant breeding and computer science students. The cross disciplinary and collaborative nature is highly supported by industry. To begin, Dr. Guru worked closely with TCAP students to determine common R analyses. He then created learning modules for his computer science students using these real life examples. TCAP students participating thus far are from CSU, KSU, WSU, MSU and UNL. Sixty-seven students registered for this course in spring 2014. For five weeks participants were able to interact with each other and course instructors, Ashu Guru and Deana Namuth-Covert. Students continue to complete the course at their own pace. Early feedback has been that the video modules are very easy to follow and meet the needs of both those new to R and those individuals who have used R in the past (survey results available at http://www.triticeaecap.org/education-reports/). Plans are to offer a synchronous session again in 2015, joining with computer science students. The course is at: http://passel.unl.edu/communities/computational

Objectives of R collaborative course:

- Bring real world data (especially large datasets) from research into classroom to be used during the instruction of various modeling and statistical analysis techniques.
- Increase use and fluency in using modeling techniques and statistical environments such as R among UNL students and Plant Breeding graduate students.
- Create a collaboration roadmap between Raikes students and Plant Breeding students so both groups enhance their communication and collaboration skills while working in cross-functional teams.

A self-paced quantitative genetics course was created with 31 registrants thus far. This course utilizes presentations developed by Jamie Sherman and Clay Sneller, packaged in a manner that creates an interactive learning experience for participants. Upon successfully completing quizzes, participants earn an electronic badge. Four have received a badge in 2014. The course is: http://passel.unl.edu/communities/tcapquant.

Entering mentoring was offered in spring 2014 to improve mentoring skills by providing an intellectual frame-work, an opportunity to experiment with different mentoring techniques, a forum to discuss mentoring dilemmas, and second-hand exposure to more students and situations through group sharing. TCAP has offered *Entering mentoring* three times on the PBTN and a total of 13 students have completed the course.

TCAP undergraduate student offerings: There are currently 44 undergraduate students supported by TCAP research internships. Fifteen of these are minority students and are involved in research collaborations between faculty at six minority student institutions

and six TCAP PIs. An online community of practice for undergraduate research interns was developed with the goals of helping students to connect to the broader TCAP research community, build communication skills as a scientist and learn about graduate school and research opportunities in industry. The fall 2014 schedule of meetings includes discussions with scientists from Syngenta, DuPont Pioneer, faculty researchers, undergraduate students and TCAP educators (schedule below). Thus far, 17 students have participated in online meetings. In all, 19 recorded meetings are posted on the Plant Breeder Training Network website.

In 2014, the TCAP Undergraduate Research Academy was created to promote student participation in the TCAP undergraduate research community and provide exceptional undergraduate research interns with additional experiences to prepare them for graduate studies. Students are required to conduct independent research, participate in the online community of practice and present their research at a national research conference. Students are nominated by their faculty advisors and recipients receive support to cover expenses associated with presenting research at a national conference. Three students were identified and are currently being supported by the TCAP Research Academy. They are Nathan Wyatt, North Dakota State University, Nikayla Strauss, Colorado State University and Ge Cheng, Fayetteville State University. Nathan Wyatt will present his research at both the TCAP Annual Meeting and also the Plant and Animal Genome Conference in January 2015.

Table E3. Fall 2014 TCAP Undergrad Online Meeting Schedule

Date	Topic	Discussion lead
15/9/2014	Internship opportunities with DuPont Pioneer	Dr. T. Abadie, DuPont Pioneer
29/9/2014	A day in the life of a Syngenta intern	Michael White, APS Undergraduate, Univ. of Minnesota
6/10/2014	How to make your internship work for you!	Dr. Mary Brakke Univ. of Minnesota
8/10/2014	The role of a field scientist: From discovery to commercialization.	Dr. Deane Jorgenson, Syngenta
13/10/2014	Genetic basis of wheat cell wall biosynthesis	Dr. Christopher Botanga Chicago State Univ.
23/10/2014	Hessian fly resistance in wheat	Dr. Lieceng Zhu Fayetteville State Univ.
27/10/2014	Mechanisms of cadmium transport in wheat	Dr. Renuka Sankaran Lehman College, CUNY
3/11/2014	Barley resistance to SPNB	Nathan Wyatt, NDSU TCAP Res. Academy Recipient
13/11/2014	Wheat breeding with CAPS markers	Nikayla Strauss, CSU TCAP Res. Academy Recipient
19/11/2014	Biochemistry of wheat seeds	Dr. Joseph Onyilagha Univ. of Arkansas
12/12/2014	Presenting your TCAP Research	Dr. Mary Brakke Univ. of Minnesota

Workshops and symposium: TCAP supported student attendance at the Plant and Animal Genome meetings and at the National Association of Plant Breeders meeting. At PAG, both technical skill development and scientific communication were supported through the poster session. A soft-skills workshop was also offered on January 10, 2014 by the TCAP Education team. The workshop discussed a recent study describing the skills needed by the plant breeding industry (Miller *et al.*, JNRLSE, 2011). The 35 students in attendance formed small groups to develop questions for a panel of plant breeding employers in order to clarify experiences and skills they value. Panel members included Donn Cummings, Fred Bliss, Allen Van Deynze, Ed Souza, Sally Clayshulte, Stephen Baenizer, and Bob Dietrich. Each panel member was interviewed by a single student group for 30 minutes and then switched to a new group. Each student group was able to visit with three panel members. The students then shared the insight they had gained as well as the questions they still had. The workshop ended with a brainstorming session of potential actions to better prepare students for future jobs

Both the students and the professional panel have indicated the success of this workshop. Fifty-nine percent of students indicated an improvement of both their awareness of skills valued by plant breeding employers and knowledge of tools and strategies useful in obtaining skills valued by plant breeding employers. Forty-seven percent of students were interested in developing and improving skills valued by employers of plant breeders. Complete Survey results are available at http://www.triticeaecap.org/education-reports/.

NAPB: TCAP supported attendance of 98 students at the National Association of Plant Breeders Meeting, where 15 TCAP graduate students presented posters. Duke Pauli, a TCAP student, organized a student workshop where career opportunities were discussed.

2014 CIMMYT: Eighteen TCAP students from eight different universities traveled to the CIMMYT Conference in Obregon Mexico for nine days. Students learned about global plant breeding and were able to network with scientists from around the world (complete student comments are available at http://www.triticeaecap.org/education-reports/).

E1.5. *Evaluation*

Evaluation of educational tools: Participants of courses, workshops and activities were surveyed (complete surveys are available at http://www.triticeaecap.org/education-reports/).

Independent evaluation of the education activities: Evaluation instruments (surveys, interviews, and observations of online courses and webinars) have been developed through joint efforts of the education team and an external evaluation team. Information obtained with these tools is being used to 1) identify and address factors that affect realization of TCAP education goals, 2) characterize baseline perceptions, confidence levels and professional networks of participants and 3) measure changes in perceptions, confidence levels and professional networks. Information gathered through evaluation is helping us to assess the effectiveness of educational activities in relation to project goals:

- Train plant breeders with technical and professional skills that enhance performance in industry and academia.

- Promote interactions among faculty, graduate students and members of the plant breeding industry.
- Increase the number and diversity of undergraduate students interested in careers in plant breeding.

Independent evaluators interviewed a subset of participants. A total of six TCAP PIs were interviewed after selection based on their geographic location, gender, ethnicity, and school size. All PIs have been previously interviewed. All seven MSI PIs were interviewed. Eight graduate students were interviewed, with 3 students being fully funded by the TCAP, while the remaining five students received partial TCAP funding. Five of the students have been involved in the TCAP since its inception, while the remaining three students have been in the TCAP between one and two years. Four of the eight students have been interviewed in previous years. Interviews lasted between 15 to 30 minutes and were recorded. All interviews were completed between November and December of 2013 over the telephone. Notes taken during the interviews were used to identify quotes for each section, which were later transcribed using the recordings.

Surveys were administered online to undergraduate students (TCAP and MSI), graduate students (TCAP and non-TCAP) and PIs (TCAP and MSI) in spring and summer of 2014. Thirty-two TCAP graduate students, 25 non-TCAP graduate students, 10 TCAP undergrad students, 10 MSI students and 32 TCAP PIs completed the survey either partially or completely. Interview and survey reports were written by independent evaluators and shared with education team. Results were not only used for program development, but were also compiled for presentations and papers still to be submitted in 2014. Complete survey data and report summaries are available at http://www.triticeaecap.org/education-reports/. Table E4 below reports the results of the plan of work for 2014.

Table E4. Results of Plan of work for 2014

Planned Activity	Completed	Next step
Create a T3 Tutorial =T4	3 tutorials completed	Update current tutorials to reflect changes in T3 and create more
Create a short online training in R to tie in with T4.	Pilot run spring 2014	Offer again in 2015
Create an online course for plant breeding for drought tolerance.	Course offered online through CSU	Offered again in 2015
Online course in plant breeding strategies.	Organization of modules completed in 2014	Open to students in 2015
Student organized TCAP seminar series.	Seminar series ran in fall 2013 and 2014	Education team had to organize as no students volunteered
Group meeting at PAG.	Jan-14	Repeat in 2015
Conduct human capital workshop.	Jan-14	Obj. 2015 – sci. communication and program development

Graduate student poster session.	Jan-14	Repeat in 2015
Interact with Turkish delegation at PAG.	Delegation not able to attend	Support of international breeders through the PBTN and collaborations with IBP
Support attendance of 50 students at the National Association of Plant Breeders (NAPB) meeting.	Aug-14	Support in 2015
Support 10 students to visit CIMMYT.	Spring 2014	Support travel in 2015 to communicate TCAP results and further student development
Support research collaborations with faculty and students at MSIs.	Supported in 2014	Continue in 2015
Recruit students from underrepresented groups to the plant breeding profession.	Supported development of 1 successful grant proposal	Support the development of a broader grant proposal
Support undergraduate research internships at TCAP institutions.	Supported in 2014	Continue in 2015
Support mentors of undergrads through entering mentoring.	Entering Mentoring offered in Spring of 2014	Offer again in 2015.
Undergraduate online synchronous and asynchronous support.	Offerings in 2014	Students often can't attend, in 2015 continue to record and post
Undergraduate research presentations.	Supported in 2014	Continue to support in 2015
Ag*Idea Plant Breeding certificate.	Submitted proposal to AgIdea	Proposal rejected. Ohio State administrators have offered to help with AgIdea proposal
Pilot partnerships to expand TCAP/PBTN.	Created 3 modules about global transfer of material with Syngenta. Supported course development with CGIAR for IBP.	Continue to support CGIAR educational development
PBTN maintenance and expansion.	Completed	Ensure long-term sustainability of PBTN
In depth interview of PIs, students and MSI faculty.	Completed	Exit interviews to document success of grant and identify important next steps
Survey PIs, students and MSI faculty.	Completed	Repeat in 2015 to document success of grant and identify important next steps
Evaluation report.	Completed	Report findings in publications to broad audience.
Advisory panel meeting.	Completed	Repeat to guide new proposal
Dissemination of project knowledge.	Three manuscripts currently being drafted	2015 Manuscript on R course, ID other manuscripts

E2. Milestones and deliverables objective 5

Goal 1: Train plant breeders with technical and professional skills that enhance performance in industry and academia

We have surpassed the original goal of the number of graduate students that would be trained by TCAP (30) by leveraging of TCAP funds with other sources of funding. Besides those directly funded by TCAP, other students are also benefiting by participating in online educational activities. This year alone a total of 1,557 visited PBTN (http://passel.unl.edu/communities/pbtn), representing 50 states and 133 countries.

An advantage of the online environment is the long-term availability of materials. Therefore, we have focused on transforming current courses into a self-paced format. In 2015, we will pilot course delivery and a badging system with a small fee. The fee will support long-term availability, staff to trouble-shoot and support students and development of new courses. We will provide scholarships in 2015 to pilot. Although the Ag*Idea certificate program is still being pursued, we have been unsuccessful so far. Through surveys (complete surveys available at http://www.triticeaecap.org/education-reports/) of those participating in online courses, we have learned that participants have a variety of reasons for taking the courses with more than 40% primarily motivated by wanting to learn what they currently need, while only about 20% were motivated to complete the course. While completion rates are low in comparison to accredited course completions, they are actually high when compared to other non-credited courses. From the surveys we also learned that more than 70% of students' goals for participating in the courses were met. An important factor is that more than 50% of the participants had no access to a similar course either because they are not affiliated with a University or because their University does not offer it.

Interactions with industry and educational representatives have encouraged us to not only develop student technical skills, but also support the development of professional skills. People are more likely to adopt a new behavior if it is supported by either someone in direct authority or by peers. Therefore, PIs and coPIs can impact students' decision to take and complete courses. The education team has been developing interventions (including face-to-face workshops with industry representatives) to improve faculty and student perceptions of the value of developing plant breeder human capital. Ideally professional skills will be integrated into courses as we have done with the R course. However, full integration continues to be a challenge.

Goal 2: Promote interactions among faculty and graduate students in plant breeding and members of the plant breeding industry

Based on the number of individuals participating in courses and webinars offered through the online platform, PBTN appears to be succeeding in the dissemination of important information for plant breeding researchers. Members of the PBTN continue to increase, with an increase in global participation. Collaboration between graduate students with similar research projects has been facilitated through PBTN and can be broadened to

facilitate global collaboration. We have been approached by industry and non-profits to collaborate in educational activities world-wide.

Goal 3: Increased number and diversity of undergraduate students who are interested in careers in plant breeding

The TCAP has mentored over 100 undergraduates in research with almost 40% of those at MSIs. Based on student responses, it appears that TCAP funded undergraduate research experiences had positive impacts on students' awareness of, and interest in, plant breeding, as well as student interest in pursuing graduate studies in plant breeding. Student participation in research had at least some positive impacts on student confidence in their ability to succeed in graduate school. Collaborative research provides opportunities to participate in plant breeding research, which students at MSIs otherwise may not have. Online presentations have the potential to expose students to a wider variety of research and career opportunities. However, synchronous attendance has been low possibly due to scheduling conflicts. Presentations will continue to be recorded and made publically available.

From surveys of undergraduate students, barriers to graduate school in plant sciences and plant breeding include financial support, admission, location of programs, interest, fear of commitment to the length of graduate studies, and students lack of identification with those currently in the field. Although TCAP had a positive impact for some students, many reported a much earlier interest in plant sciences that was sparked by family or earlier life experiences.

An important milestone was the successful grant proposal written by Marceline Egnin of Tuskegee based on the TCAP model of building bridges for minorities between undergraduate and graduate school. A focus of this work has been to test the support necessary to encourage undergrad research at MSI teaching institutions. A barrier for entering science is often the necessity of graduate education. Students at teaching MSIs are often not exposed to graduate school or graduate students. Summer internships can provide a bridge to graduate school. The collaborative research and opportunities for students to interact with research institutions might make them aware of and more successful in graduate school. A challenge is a need for broader support so that the model could be tested with more students across more institutions. Finding a funding opportunity that would allow us to test this model more broadly has been difficult. In 2015, we will try to identify funding sources, but our focus will be on encouraging MSI partners to seek support for collaborative research to attract minorities to plant sciences.

E3. Outcomes/Impacts objective 5

The results of surveys and interviews enable the TCAP education team to identify changes in knowledge, changes in action, and changes in conditions.

E3.1. ***Change in availability of plant breeding courses through PBTN***

The courses created and posted on the PBTN are otherwise unavailable for many participants. The collaborative cross-disciplinary R course is unique. It is also unique for mentoring development to be delivered online.

E3.2. ***Change in graduate student knowledge, actions and skills***

As part of the evaluation of the TCAP, an annual online survey is administered to fully and partially funded TCAP graduate students each year. The survey assesses students' graduate educational experiences and is administered online by a member of the evaluation group. This year, a group of graduate students not funded by the TCAP were also surveyed to serve as a comparison group to the TCAP students. Non-TCAP students consisted of students who are members of the National Association of Plant Breeders (NAPB). The survey was administered online using an anonymous link and sent through email by a member of the educational committee. Thirty-two TCAP students and twenty-five non-TCAP students responded to the survey. Students were compared on their confidence in 10 plant breeding knowledge areas, confidence in 19 plant breeding skills, exposure level to 42 educational experiences, importance of 13 educational processes, perceptions of 5 interests and experiences in the plant breeding field, and networking experiences with 13 different groups. The differences between the two groups include:

- TCAP students participated independently more often in determining genotypes, making marker trait associations, identifying new alleles for improvement, utilizing SNPs, utilizing a database to manage data, collaborating on multi-disciplinary teams, using on-line technology to collaborate and problem solve, and developing a budget for a research project than non-TCAP students.
- TCAP students were more confident than non-TCAP students in the following areas: plant breeding strategies, genetics, management of large data sets, identification of new alleles for improvement, MAS, and resource management.
- TCAP students interacted more than non-TCAP students with the following groups: undergraduate mentees, students at other institutions, researchers at other institutions.

One of the evaluation components is a yearly survey to assess perceptions of the principal investigators (PIs) involved in the project. A comparison of the 2013 and 2014 survey responses from PIs support the changes in graduate student experiences (complete evaluation results available at http://www.triticeaecap.org/education-reports/). Comparisons of the surveys indicate that student independent participation increased by at least 10% between 2013 and 2014 in the following areas:

- Choose parents and make crosses
- Make phenotypic selections
- Make genotypic selections
- Implement markers in breeding
- Collect genotyping data
- Troubleshoot and resolve a research problem
- Perform statistical analysis of field data
- Perform statistical analysis of lab data
- Use of SNPs
- Use of GBS
- Write a paper
- Make a scientific presentation to a lay audience
- Leadership role in some aspect of a research project

- Collaborate with faculty and students outside the group
- Collaborate with faculty and students on a multidisciplinary team

E3.3 ***Change in graduate students' awareness of skills required by the plant breeding industry and willingness of students' to change behaviors in order to acquire skills required by the plant breeding industry***

The TCAP has provided many opportunities to inform and develop professional skills including a workshop with industry representatives in 2014. Survey results from that workshop indicate that students were more aware of the skills required by the plant breeding industry after the workshop (35% before 93% after). The students also acquired greater knowledge of tools or strategies to obtain skills valued by employers of plant breeders through the workshop (29.4% before 88% after) and 70% of the students were interested in changing their behavior to improve skills valued by employers.

E3.4. ***Change undergraduate students' views of plant breeding and allied fields***

Undergraduate research experience changed 86% of student's plans to attend graduate school with 78% of students surveyed being extremely or moderately motivated to attend graduate school. Participation in TCAP changed 61% of the student's knowledge or perception of careers in plant sciences and changed 50% of the student's knowledge or perception of careers in plant breeding with 41% being interested in pursuing careers in plant sciences and 28% being interested in pursuing careers in plant breeding.

E4. Education plans for year 5

Plan-of-Work 2015: Develop and implement a Plant Breeding Training Network.

Table E4. Educational activities plans including expected outputs, outcomes and impact.

Activity	Outputs	Outcomes	Impacts
Continue to create a T3 Tutorial = T4.	Archived tutorial for T3 - 30 graduate students test and give feedback, utilizing data from T3.	Students trained in using a database for the analysis of large data sets, develop analytical and problem solving skills of students.	Plant breeders trained in collaborative work.
Rerun R course that was created in 2014.	15 TCAP grad students and 15 undergrad computer science students attend.	Plant breeding and computer science students work together to analyze TCAP research data.	Cross-discipline collaboration between plant breeding students and computer scientists.
Rerun online course for plant breeding for drought tolerance.	Course offered online through CSU (eventually through Ag*Idea).	Students trained in plant breeding for abiotic stress.	Plant breeders trained in collaborative work and abiotic stress breeding.
Online self-paced plant breeding strategies.	Online course self-paced course with detailing	Students exposed to divergent plant breeding issues.	Plant breeders around the world introduced to plant breeding

	plant breeding strategies.		strategies.
Student organized TCAP seminar series.	12 live and then archived seminars.	Students learn about current research topics from experts and have leadership opportunity.	Students gain leadership experience and PBTN helps sharing information.
Group meeting at PAG.	Enhanced communication within TCAP and with stakeholders.	Increased integration of TCAP participants and stakeholders. Long-term community building.	Stakeholders have a better understanding of the project. Improved collaboration among TCAP participants.
Conduct human capital workshop (Student presentations and Grant design)	30 students attend human capital training workshop.	Continue human capital training, support development of student communication, collaboration & leadership skills, team building.	Plant breeders equipped with technical & professional skills. Better trained plant breeders.
Graduate student poster session.	30 students communicate research projects supported by TCAP to participants and stakeholders.	Support development of student communication skills, increased integration of project. Provide networking opportunity.	Better trained and more employable plant breeders.
Support attendance of 50 students at the National Association of Plant Breeders (NAPB) meeting.	50 students attend the NAPB meeting.	Provide exposure of students to other plant breeders and students working on a variety of crops in both private and public sector.	Better trained and more employable plant breeders.
Support Graduate Student travel.	Travel Grants to support student's presentation of TCAP results, learning new technology or technique, or developing new collaboration	Provide experience in scientific communication. Spread TCAP findings. Broaden student exposure and awareness.	Better trained and more employable plant breeders.
Support research collaborations with faculty and students at Minority Serving Institutions (MSIs).	Seven TCAP faculty and seven MSI faculty and their students collaborate on research.	Faculty develop deeper collaborations with students from MSIs. Students attracted to internships at TCAP institution or industry.	Recruit more diverse students into plant breeding careers.
Attracting diverse students to plant sciences grant	Submit a collaborative grant building and testing the model we have created with TCAP	Engage more institutions in collaborative research model, more broadly test components of model	Recruit more diverse students into plant breeding careers.
Support undergraduate research internships at TCAP institutions.	30 undergraduates mentored by TCAP PIs/Graduate students.	Increase undergraduate student confidence in research and interest in graduate studies.	Recruit more diverse students into plant breeding careers.
Support mentors of undergrads through entering mentoring program.	Provide supportive information and training opportunities for mentors.	Improved undergraduate research experience.	Improve plant breeders as mentors.
Undergraduate online synchronous and	Students interact with each other and	Support undergraduate research experience and	Help recruit students into plant breeding

asynchronous support.	professionals in the field, students are provided with career information.	expose undergrads to other students and others working in the field.	careers.
Undergraduate research presentations.	Support undergraduate research presentation through travel funds	Enhance undergraduate research experience.	Better prepare undergrads for future scientific careers
Pilot offering self-paced short courses with badging system	4 self-sustaining short courses	Enhance continuing education opportunities	Better trained workforce
Continue to explore long-term means of offering online courses	Permanent means of offering online plant breeding courses	Long-term access to plant breeding training around the globe	Better trained workforce
Pilot partnerships to expand TCAP/PBTN.	Support and host a course with CGIAR.	Expand educational training opportunities for TCAP students and beyond, while strengthening new PBTN partnerships.	International expansion of online plant breeding community and sustainability beyond TCAP funding.
PBTN maintenance and expansion.	Survey current users for feedback on effectiveness of current tools and for new ideas.	Prioritize items identified and implement updates.	Expansion of PBTN and international community building among plant breeding professionals.
In depth interview of PIs, students and MSI faculty.	Further explore survey results.	Gain a better understanding of expectations of PIs and students, development of publications.	Improvement of project.
Survey PIs, students and MSI faculty.	Compile surveys.	Compare and contrast between groups and years.	Improvement of project.
Evaluation report.	Summarize evaluation findings.	Report findings in publications to broad audience.	Improve TCAP education impact.
Advisory panel meeting.	Solicit advice from advisory panel.	Improve education component.	Improve TCAP education impact.
Dissemination of project knowledge.	Three manuscripts on graduate training and recruitment of minority students.	Increased understanding of successful strategies for graduate training and student recruitment.	Better prepared and more diverse plant breeders.

F. Broad Impacts

***Scientific publications*:** Studies supported by the TCAP project have resulted in the publications of 186 peer-reviewed scientific articles in the first four years of the project, including 66 publications in 2014 (Appendix I1). To document the academic impact of these publications, we determined the impact of each publication using Google Scholar Citation Metrics and calculated the total number of cross-references of TCAP publication per year for the first three years of the project (2011 to 2013). The results are summarized

in Table F1, which shows a total of 1,839 citations of the TCAP publications, resulting in an average of 15.3 references per publications. This number documents the high impact of the scientific studies generated in the TCAP project.

Table F1. Number of publications and cross-citations of TCAP peer reviewed publications. Complete list in Appendix I1.

TCAP	Year	Published	Citations
Year 1	2011	27	494
Year 2	2012	38	671
Year 3	2013	55	674
Year 4	2014	66	NA
Year 5	2015		
Totals		186	1,839

Varieties and germplasm releases: TCAP breeders continued to be responsible for a large proportion of the barley and wheat varieties and germplasm released in the US. TCAP releases in 2014 included 18 new cultivars (13 with PVP) and 32 new germplasm (complete list in Appendix I2). According to a 2012 study, the value of the barley and wheat production generated from public varieties is approximately $12 billion dollars per year (http://www.nifa.usda.gov/nea/plants/pdfs/t_cap_econ_final.pdf).

Table F2. Number of released germplasm. Complete list in Appendix I2

	Varieties	Germplasm	Populations
WheatCAP [a]	5		
2011	7	12	2
2012	12	2	
2013	13	16	13
2014	13	32	6
Pending PVP application	7		
Released with no PVPV	5		
Total with PVP	50 (+7 pending)		
Total	62	62	21

[a] These 5 varieties were completed by TCAP researchers during the WheatCAP but were not reported before

New marker platforms and T3 database: The exome capture and genotyping by sequencing platforms developed in this project have provided more than 1.5 million SNPs and extremely dense genetic maps. These high-resolution maps have provided a detailed vision of the distribution of diversity across the genome and of the regions targeted by selection. These tools are accelerating the positional cloning of genes controlling important agronomic traits. These improvements in marker technologies are also helping the USDA-ARS genotyping laboratories to increase rapidly the number of

marker data-points provided to the breeding programs for marker assisted selection.

All these marker information and phenotypic data are well organized and safely stored in the T3 database. The T3 database is also providing the tools to retrieve and analyze this valuable information and has accelerated the time from data collection to data availability in a public form. In summary, these new marker platforms and database tools are accelerating the identification and deployment of beneficial alleles.

Enhanced value of the NSGC: An important outcome of the TCAP activities is the renewed interest in the wheat and barley core germplasm collections. The detailed genotyping and phenotyping for WUE, NUE and disease resistance has increased the value of the US wheat and barley NSGC core germplasm collections. This added value is attracting breeders to utilize this germplasm in their breeding programs. The landrace accessions found to outperform current cultivars are especially interesting as they may contain novel alleles not found in current small grains breeding programs.

Disease resistance Research in disease resistance has resulted in 25 peer reviewed publications and in the releases of barley and wheat varieties and germplasm with improved disease resistance. In both wheat and barley, the screening of large and diverse germplasm collections genotyped with thousands of SNPs, has improved our knowledge of the genomics of disease resistance genes in barley and wheat and has provided a global vision of the available resistance resources. These studies have also yielded a bounty of novel resistance genes against the main pathogens of these two important commercial species. These new resistance genes are now being introgressed into advanced breeding lines by marker-assisted and genomic selection. The evaluation of these germplasm collections has also revealed vulnerabilities for some diseases, which will require the expansion of the screened germplasm collections.

WUE and NUE: Wheat and barley accession from the NSGC core collections were evaluated for grain yield, WUE and NUE under irrigated and water-stressed environments. These data, together with data from the elite AM panels was used in GWAS studies to identify new loci associated with WUE and NUE. After validation, beneficial alleles can be incorporated into commercial varieties to improve these traits. Canopy spectral reflectance (CSR) techniques provided valuable information for WUE and NUE studies, and in some breeding programs CSR indexes are being incorporated as an indirect selection tool. The TCAP project was instrumental in the incorporation of CSR technology into breeding programs. The winter wheat panels revealed useful variation for NUE, yield, and yield stability.

In the area of WUE, the precise mapping of a region of the 1RS chromosome associated with WUE has resulted in the engineering of a wheat chromosome combining improved WUE, stripe rust resistance and improved bread-making quality. In barley, the LTT panel identified novel alleles for LTT and accessions with high LTT. LTT barley varieties can be planted in the fall, maximizing the use of winter precipitation. In the area of NUE, the deployment of the high grain protein content allele *Gpc-B1* into commercial wheat varieties is providing concrete improvements in grain protein content. A GWAS study showed that the same *Gpc1* locus is important for NUE in barley, with three different haplotypes associated with low, medium and high protein.

Population development: An important legacy of this project is the development of publicly-available AM panels, NAM populations, a wild introgression population, and biparental populations. The availability of fully genotyped AM and NAM populations will have a long-term impact in wheat and barley research by providing a valuable public resource for the rapid identification of linkage between traits and genotypes.

High-density maps integrating GBS and iSelect SNP chips have been generated from the spring NAM populations. These NAM populations have higher statistical power than previous AM panels and therefore will be useful to detect QTL with smaller effects for traits with lower heritability. The genotyping and phenotyping of the wild barley introgression lines have expanded the genetic diversity available to barley breeders.

Extension activities: The TCAP project has provided excellent opportunities for extension activities that showcased the benefits of new biotechnological developments in wheat and barley improvement. These activities have included field days and presentations to growers and growers' associations, seed handlers, and industry representatives across the US. Industry representatives have participated in TCAP annual meetings. TCAP outreach efforts have been critical to increase stakeholders' confidence in marker technologies and have resulted in strong support from the barley and wheat industries.

Education: Changes in student recruitment and training are creating more diverse and better-trained plant breeding professionals. As TCAP students graduate, they are being rapidly hired by a growing plant breeding industry. The online communication tools have increased collaboration of project participants. Training tools have been utilized by plant breeders around the globe. Participants have a broader perspective of plant breeding and are trained from the beginning of their careers in the collaborative and modern approaches required for successful breeding projects. The educational activities have accelerated the adoption of modern phenotyping and genotyping technologies in wheat and barley breeding programs nationwide. The education component of the grant has served as an integrative force for the TCAP and has helped coordinate research and education activities. In summary, a large cohort of plant breeders are being trained in traditional and modern breeding strategies and a new, and more diverse generation is being attracted to plant breeding. These actions will provide the continuity required for sustainable cereal breeding activities in the US.

G. Training

Graduate student training: A total of 136 graduate students (Appendix I3) have participated in the plant breeding training network. Ninety-five are directly mentored by a TCAP PI, while 54 students are at least partially funded by TCAP with two of those at minority serving institutions. 41 are unaffiliated with TCAP (Appendix I4).

Undergraduate students: A total of 108 undergraduates have participated in TCAP with being mentored by TCAP faculty and graduate students, 41 by MSI PIs (Appendix I5).

Postdocs and visiting scientists: TCAP has trained a total of 25 Post-Doc’s and 26 visiting scientists (Appendix A6)

H. Concluding statement (year 4, 2014)

The TCAP has made very good progress during the fourth year on all of the objectives of the grant, exceeding the original milestones in several objectives. TCAP researchers have phenotyped and genotyped large collections of wheat and barley germplasm and association mapping panels. GWAS studies have revealed multiple beneficial alleles for disease resistance, WUE and NUE. These alleles are being deployed into commercial varieties. High-throughput marker-based breeding approaches are being implemented nation-wide and commercial varieties and improved germplasm using MAS are being released. MAS and GS strategies are accelerating breeding cycles helping breeders to ameliorate the negative impacts of climate change. Nation-wide MAS approaches have been implemented for both wheat and barley, which together with GS are accelerating the release of improved varieties. GBS and gene capture technologies have yielded millions of polymorphic markers. An expanded T3 database is integrating all the genotypic and phenotypic data from the project and is improving the tools to retrieve and analyze this information. This database will serve current and future generations of barley and wheat breeders. The Plant Breeding Training Network (PBTN) is functioning well as a central hub for the education activities and is helping faculty to attract new students into plant breeding. The high level of integration generated among research and breeding programs, genotyping laboratories, germplasm collections and disease laboratories would not have been possible without the TCAP project. TCAP has also had a very positive impact in fostering international collaborations, a contribution that was recognized in 2014 by the NIFA “Partnership Award for Program Improvement through Global Engagement”.

I. Appendices (year 4, 2014)

I1. Publications since the previous 2014 TCAP report.

I2. Germplasm Release 2014.

I3. Trained Graduate students.

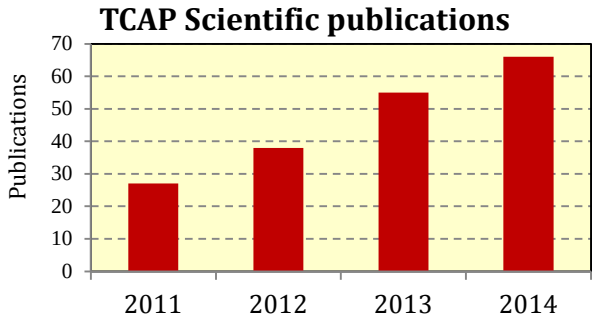
I4. Non-TCAP graduate students that attended TCAP training.

I5. Trained Undergraduate students.

I6. Trained postdocs and visiting scientists.

Appendix I1. Peer reviewed publications year 4 (since last report)

Since the last report TCAP researchers published **66** peer reviewed scientific publications. This list includes several articles in high impact journals (PNAS, Plant Cell, Science, Plant Biotechnology J, etc.). The figure to the right summarizes the number of publications per year since the start of the grant.



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Appendix I2. Varieties, germplasm and population development 2014

Since the last report we released 13 cultivars with PVP plus 5 without PVP, 32 germplasm and six mapping populations. The cumulative total over the last 4 years is 62 varieties, 62 germplasm and 21 populations.

2014 Cultivar releases with PVP

1. 'Egan' (PVP= 201400394) is a hard red spring wheat released by MT State University. Egan was developed by MAS to contain a gene for resistance to the orange wheat blossom midge, a major yield and quality-reducing pest for western valleys of Montana.
2. 'Linkert' (PVP= 201400242) is an hard red spring variety released by the MN wheat breeding program. Linkert is a strong straw, high protein variety. Linkert contains *Lr34* and has shown near-immune adult-plant resistance to stem rust, including race TTKSK.
3. 'NE06545' (PVP= 201300398) is a hard red winter variety released by the University of Nebraska wheat breeding program. 'NE06545' was released for its exceptionally broad adaptation in the northern Great Plains. NE06545 will marketed as Husker Genetics Brand 'Freeman'. NE06545 is resistant to wheat soilborne mosaic virus and has good quality. Molecular markers were used to determine the presence of the allele for wheat soilborne mosaic virus resistance and the glutenin alleles associated with better end-use quality characteristics.
4. 'Sprinter' (PVP= 201400223) is a hard red winter wheat selected for the *Gpc-B1* gene for high grain protein content (PVP= 201400223) released by Washington State University.
5. 'Yorktown' (PVP= 201400306) is a SRW released by Virginia Tech wheat breeding program that was selected with markers for the 1A.1R translocation.
6. 'Southern Harvest 3200' (PVP= 201400389), 'Featherstone 73' (PVP= 201400388), '072014415' (PVP= 201400305), MCIA Venus (SWWW VA09W-188WS, PVP= 201400307) and LCS Wizard (HRW VA08HRW-80, PVP= 201400349) are five varieties released by Virginia Tech. Molecular markers for *Lr9*, *Lr37/Yr17*, *H13*, *FHB5AS*, *1A.1R*, and high molecular weight glutenins were used to select these lines.
7. UI Platinum (IDO694C, PVP= 201400419) is a hard white spring wheat cultivar released for irrigated production by Jianli Chen from the University of Idaho wheat breeding program. This cultivar has industry preferred end-use quality for whole grain products. This line was selected using markers for *GluD1* and *GluA3* and *GluB3* strong gluten alleles and for a QTL associated with baking volume.
8. NE05548 (PVP = 201400410) is a hard red winter variety released by the University of Nebraska wheat breeding program. 'NE05548' was released for its very tall plant height for a semi-dwarf cultivar and its adaptation to western Nebraska where conventional tall wheat cultivars are needed due to drought, but do not have the grain yield of modern semi-dwarf cultivars. NE05548 has the yield and shorter coleoptile length of a semi-dwarf wheat, but the height of a conventional tall wheat. It will be marketed as Husker Genetics Brand 'Panhandle'. Molecular markers were used to characterize the line (especially the dwarfing genes) and the genes that it contains confirming our phenotypic data.
9. 'Doublestop CL Plus' (PVP= 201400228) is two-gene Clearfield HRW variety released the OAES. Owing to its partial resistance to leaf rust and tolerance to barley yellow dwarf, gene *Lr34* was shown to be present in some plants of Doublestop CL Plus, as confirmed by a resistant haplotype for three polymorphic marker sites.

2014 Cultivar releases without PVP

10. 'Alba' (released without PVP) is a six-row winter barley released by Oregon State University and developed with support of TCAP markers. This variety is resistant to stripe rust and scald and has good kernel plumpness. High yields in the Pacific Northwest make it a first choice for feed and craft malting. In press at Journal of Plant Registrations.
11. 'Full Pint' (released without PVP) is a spring two-row barley released by Oregon State University and developed with support of TCAP markers. This semi-dwarf variety is resistant to stripe rust, leaf rust, and lodging. It has excellent quality for craft brewing and distilling and brings unique barley flavors to beers and spirits made with its malt.
12. 'Amaze 10' (VA07H-31WS) is a high yielding hulless barley with good grain quality released by Virginia Tech Intellectual Properties, Inc. Molecular markers were used to characterize the final line.
13. Pembroke 2014 is a soft red winter developed by the University of Kentucky. Molecular markers were used to confirm the presence of 3 FHB-resistance QTL.

2014 Germplasm releases

1. Three lines with combined mutations in the starch branching enzymes *SBEIIa* and *SBEIIB* in the A genome (PI 670159), the B genome (PI 670171), and both A and B genomes (PI 670160). The last line shows a 600% increase in resistant starch (Journal of Plant Registration).
2. Eleven lines including isogenic pairs of tetraploid and hard spring hexaploid wheat lines with and without the Hope *FT-B1* early flowering allele (PI 671995 to PI 672005). On average lines with the Hope *FT-B1* allele show 2.6 days acceleration of flowering ($P<0.0001$) and 4.1% increase in spike weight ($P=0.0093$), although differences were detected among varieties (Journal of Heredity).
3. Four near-isogenic lines winter wheat germplasm with good agronomic characteristics and contrasting in *Fhb1* for Fusarium head blight resistance: PI 668559, PI 668560, PI 668561, and PI 668562.
4. #STRKR is a three-component blend of six-row hull-less winter barley lines. It is a resource for selection of varieties with soft kernel texture and resistance to stripe rust. Submitted to Journal of Plant Registrations.
5. 'OK05312' is a HRW wheat developed cooperatively by the Oklahoma Agricultural Experiment Station (OAES), the Kansas Agricultural Experiment Station (KAES), and the USDA-ARS. OK05312 provides a contemporary source of resistance to multiple biotypes of wheat curl mite in a high-yielding background adapted to areas where the curl mite is most active. Marker analysis confirmed the presence of *Cmc4* in OK05312 and the absence of any wheat-rye translocated chromosome and henceforth the absence of *Cmc3*, carried on the TIAL·IRS translocation.
6. Three near-isogenic lines of the 1RS translocation from Petkus in the Hahn , carrying a distal wheat introgression of the *Glu-B3/Gli-B1* locus for strong gluten (PI 672839), a proximal introgression eliminating the *Sec1* locus associated with sticky dough (PI 672838), and both wheat segments combined (PI 672837).
7. Three durum lines with the introgression of the *Glu-D1* 2+12 allele from *T. aestivum* replacing the *Glu-A1* allele from *T. turgidum*. UC 1113-*Gpc-B1*-2+12 (PI 672996), UC 1171-2+12 (PI 672997) and UC 1308-2+12 (PI 672998).

8. Mutants of the high-grain protein content genes *GPC-A1*, *GPCD1* and *GPC-B2*. Tetraploid wheat mutants: PI 673413 (*GPC-A1* WT: *gpc-B2* MT), PI 673414 (*gpc-A1* MT: *GPC-B2* WT) and PI 673415 (*gpc-A1* MT: *gpc-B2* MT). Hexaploid wheat mutants PI 673410 (GPC-A1 WT: *gpc-D1* MT), PI 673411 (*gpc-A1* MT: *GPC-D1* WT), and PI 673412 (*gpc-A1* MT: *gpc-D1* MT). All lines have a non-functional copy of *gpc-B1*.

2014 Mapping populations

1. Barley recombinant inbred line population (161 lines) Innovation x PI67381 to map resistance/susceptibility to spot form net blotch. Innovation is a susceptible six-rowed malting variety released by Busch and PI67381 is the most resistant SFNB line identified from the core collection.
2. Barley recombinant inbred line population (138 lines) Quest x PI67381 to map resistance/susceptibility to spot form net blotch. Quest is a SFNB susceptible six-rowed malting variety released by UM and PI67381 is the most resistant SFNB line identified from the core collection.
3. Barley recombinant inbred line population (130 lines) AC Metcalfe x PI67381 to map resistance/susceptibility to spot form net blotch. AC Metcalfe is a susceptible two-rowed malting variety released by Ag Canada and PI67381 is the most resistant SFNB line identified from the core collection.
4. Barley recombinant inbred line population (160 lines) Celebration x PI67381 to map resistance/susceptibility to spot form net blotch. Celebration is a susceptible six-rowed malting variety released by Busch and PI67381 is the most resistant SFNB line identified from the core collection.
5. Barley recombinant inbred line population (101 lines) Conrad x PI67381 to map resistance/susceptibility to spot form net blotch. Conrad is a susceptible two-rowed malting variety released by Busch and PI67381 is the most resistant SFNB line identified from the core collection.
6. Barley recombinant inbred line population (160 lines) Pinnacle x PI 84314 to map resistance/susceptibility to spot form net blotch. Pinnacle is a susceptible two-rowed malting variety released by NDSU and PI84314 is the second most resistant SFNB line identified from the core collection.

Appendix I3. Trained Graduate students

A total of 136 graduate students (Appendix H3) have participated in the plant breeding training network. Ninety-five are directly mentored by a TCAP PI, while 54 students are at least partially funded by TCAP with two of those at minority serving institutions. Eight students completed their PhD (indicated by an * before the name). The remaining 41 are unaffiliated with TCAP.

Graduate Student	Mentor	Funding TCAP	Institution
Acharya, Roshan	Jamie Sherman	Full	Montana State Univ.
Ahlquist, Kaileigh	Mark Sorrells	-	Cornell
Ando, Kaori	Mike Pumphrey	-	Washington State Univ.
Assanga, Silvano	Shuyu Liu	-	Texas A&M
Awad, Wahid	Pat Byrne	-	Colorado State Univ.
Bajgain, Prabin	James Anderson	Full	Univ. of Minnesota
Barnes, Ryan	Anne McKendry	Partial	Missouri State Univ.
Becker, Steve	Pat Byrne	-	Colorado State Univ.
Belcher, Araby	Patri.ck Hayes	Full	Oregon State Univ.
Bowman, Brian	Jianli Chen	Full	Univ. of Idaho
Brasier, Kyle	Carl Griffey	-	Virginia State Univ.
Cai, Jin	Guihua Bai	-	Kansas State Univ.
Carlsen, Steven	Robert Brueggman	Full	North Dakota State Univ.
Case, Austin	Brian Steffenson	Partial	Univ. of Minnesota
Chappell, David	Anne McKendry	Partial	Missouri State Univ.
Cobo, Nicolas	Jorge Dubcovsky	Partial	UC Davis
Cooper , Jessica	Scott Haley	-	Colorado State Univ.
Dwoney, Samantha	Mike Pumphrey	-	Washington State Univ.
Edae, Erena	Pat Byrne/Scott Haley	-	Colorado State Univ.
Elmore, Elizabeth	Robert Bowden	TCAP	Kansas State Univ.
Falcon, Celeste	Kevin Smith	Full	Univ. of Minnesota
*Fang, Tilin	Liuling Yan	Partial	Oklahoma State Univ.
Fatima, Nosheen	Guihua Bai	-	Kansas State Univ.
Frels, Katherine	P. Stephen Baenziger	Full	Univ. of Nebraska, Lincoln
Gizaw, Shiferaw	Arron Carter	Full	Washington State Univ.
Godoy, Jayfred Gaham	Michael O. Pumphrey	Full	Washington State Univ.
Gonzales, Ana	Peter L. Morrell	Full	Univ. of Minnesota
Graebner, Ryan	Pat Hayes	-	Oregon State Univ.
Green, Andrew	Carl Griffey	-	Virginia State
Grogan, Sarah	Pat Byrne	Full	Colorado State Univ.
Guttieri, Mary	B. Waters/S. Baenziger	Partial	Univ. of Nebraska, Lincoln
Haaning, Alison	Gary Muehlbauer	Full	Univ. of Minnesota
Haas, Matthew	Brian Steffenson	-	Univ. of Minnesota
*Hale, Iago	Jorge Dubcovsky	-	UC Davis
Harrington, Judy	Pat Byrne	-	Colorado State Univ.

Hazard, Brittany	Jorge Dubcovsky	Partial	UC Davis
Hegarty, Josh	Jorge Dubcovsky	Partial	UC Davis
Herb, Dustin	Pat Hayes	Full	Oregon State Univ.
Hitz, Katlyn	David A. Van Sanford	Full	Univ. of Kentucky
Hoffstetter, Amber	Clay Sneller	Partial	Ohio State Univ.
Hofstad, Anna	Gary Muehlbauer	-	Univ. of Minnesota
Hohn, Christopher	Tyson Howell	-	Univ. of California, Riverside
Huang, Mao	Clay Sneller	Full	Ohio State Univ.
Howell, Tyson	Jorge Dubcovsky	Partial	UC Davis
Jernigan, Kendra	Arron Carter	-	Washington State Univ.
Kalous, Jay	Luther Talbert	Full	Montana State Univ.
Koladia, Vaidehi	Robert S. Brueggeman	-	North Dakota State Univ.
Larrea-Bueno, Alejandra	David VanSanford	Full	Univ. of Kentucky
Latshaw, Sue	Scott Haley	-	Colorado State Univ.
Lei, Lei	Liuling Yan	Full	Oklahoma State Univ.
Lin, Meng	Guihua Bai	-	Kansas State Univ.
*Liu, Shubing	Guihua Bai	-	Kansas State Univ.
Liu, Weizhen	Mike Pumphrey	-	Washington State Univ.
Lu, Yue	Guihua Bai	Partial	Kansas State Univ.
Mekonnen, Melaku	Pat Byrne	-	Colorado State Univ.
*Merrill, Keith	Gina Brown-Guedira	Full	North Carolina State Univ.
Mheni, Nafeti	Clay Sneller	-	Ohio State Univ.
Muleta, Kebede Tadesse	Mike Pumphrey	-	Washington State Univ.
Nair, Sindhu Gopalkrishnan	Mike Pumphrey	-	Washington State Univ.
Narayanan, Sruthi	P.V. Vara Prasad	Full	Kansas State Univ.
Nayak, Santosh	Jianli Chen	-	Univ. of Idaho
Nazarov, Taras	Deven See	Full	Washington State Univ.
Neupane, Anjan	TL Friesen, R Bruggaman	Full	North Dakota State
Neyhart, Jeffrey	Mark Sorrells	Full	Cornell
Nice, Liana	Gary Muehlbauer	Full	Univ. of Minnesota
*Nitcher, Rebecca	Jorge Dubcovsky	-	UC Davis
Nyori, Peter Bulli	Mike Pumphrey	-	Washington State Univ.
Ollhoff, Alexandrea	Kevin Smith	-	Univ. of Minnesota
Onweller, Kayse	Stephen Baenziger	-	Univ. of Nebraska
Osborne, Ruth	Chris Botanga	MSI	Chicago State Univ.
*Pauli, Duke	Tom Blake	Full	Montana State Univ.
Pavuluri, Kiran	Wade Thomason	Partial	Virginia State
Reid, Scott	Pat Byrne	Partial	Colorado State Univ.
Richards, Jonathon	Robert S. Brueggeman	Full	North Dakota State Univ.
Rife, Trevor	Jesse Poland	Full	Kansas State Univ.
Russell, Kathleen	David van Sanford		Univ. of Kentucky
Salcedo, Andres	Eduard Akhunov	-	Kansas State Univ.
Sharma Poudyal, Dipak	Xianming Chen	-	Washington State Univ.

Shroyer, Kyle	P.V.V. Prasad	-	Kansas State Univ.
Singh, Arti	Ron Knox	-	AAFC-SPARC Canada
Sthapit, Jinita	Deven See	Partial	Washington State Univ.
Tamang, Prabin	Robert Bruggeman	Full	North Dakota State Univ.
Tandukar, Zenith	Gary Muehlbauer	Full	University of Minnesota
Tavarez, Michael (MSI)	R. Sankaran/Waters	Partial /MSI	Lehman College
Tiede, Tyler	Kevin Smith	-	Univ. of Minnesota
Turner, M Kathryn	Jim Anderson	Full	Univ. of Minnesota
Varella, Andrea	Jamie Sherman	Partial	Montana State Univ.
Veenstra, Lynn	Jean-Luc, Mark Sorrells	Partial	Cornell
Wang, Rui	Shaobin Zhong	Full	North Dakota State Univ.
Ward, Brian	Carl Griffey	-	Virginia State Univ.
Xiong, Mai	Gina Brown-Guedira	Partial	North Carolina State Univ.
Yang, Xiping	Guihua Bai	-	Kansas State Univ.
Youngjun, Mo	Jorge Dubcovsky	Partial	UC Davis
*Zhang, Junli	Jianli Chen	Partial	Univ. of Idaho
Zia Ullah Zia	David Hole	-	Utah State Univ.

* Indicates students that successfully completed their PhD.

Appendix I4. Trained additional students

A total of 41 graduate students have participated in TCAP offering without other connection to the TCAP project

Student	Student	Student
Alegria ayala, Mario	Itle, Rachel	Ruff, Leah
Ali, Asjad	Jacobs, Ray	Sanchez, Julio
Alvord-Albanese, Stephanie	Jin, Feng	Schwieterman, Michael
Awad, Wahid	Kaur, Simarjit	Shjerve, Rachel
Blacker, Kendra	Keach, James	Smedo, John
Blissett, Elisabeth	Kennedy, Colleen	Su, Yuanjie
Call, Adam	Lei, Jian	Sykes, Virginia
Chai, Yuan	Lucas, Mitchell	Tee Tan, Chor
Chambers, Alan	Mabukaln, Pheonah	Yesudasan, Teddy
Cooper, Aaron	Mangandi, Jozer	Zambrano, Jose
Cortese, Laura	Mattia, Matthew	Zurn, Jason
Crain, Jared	Mingqin, Shao	
Gifford, Justin	Nasseer, Afaf	
Gilbert, Jessica	Nielsen, Ethan	
Huggins, Travis	Nunez, Gerardo	

Appendix I5. Trained undergraduate students

A total of 108 undergraduates have participated in the TCAP with 67 being mentored by TCAP faculty and graduate students, 41 by MSI PIs.

Undergrad Student	Mentor	Funding	Institution
Abourjeily, Benjamin	Dave Van Sanford	TCAP	Univ. of Kentucky
Adamu, Imran	Martin Matute	MSI	Univ. of Arkansass, Pine Bluff
Amole, Oyejare	Marceline Egnin	MSI	Tuskegee Univ.
Anderson, Angelle	Martin Matute	MSI	Univ. of Arkansas, Pine Bluff
Anderson, Troy	Martin Matute	MSI	Univ. of Arkansas, Pine Bluff
Apple, Deidre	Pat Hayes	TCAP	Oregon State Univ.
Bailey, Jessica	Joseph Onyilagha	MSI	Univ. of Arkansas, Pine Bluff
*Barnes, Ryan	Anne McKendry	TCAP	Missouri State Univ.
Barnes, Tanya	Chris Botanga	MSI	Chicago State Univ.
Bates, Andra	Joseph Onyilagha	MSI	Univ. of Arkansas, Pine Bluff
Bess, Jessica	Pat Byrne	TCAP	Colorado State Univ.
Bickford, Aidan	Tom Blake	TCAP	Montana State Univ.
Brown, Stephanie	JiaQian Zhu	MSI	Rust College
Burgess, Brandon	Brian Arnall	TCAP	Oklahoma State
Calister, Maddie	Baenzinger or Waters	TCAP	Univ. of Nebraska
Carlsen, Steve	Robert S. Brueggeman	TCAP	North Dakota State Univ.
Ceesay, Lolley	S. Grogan (Mentor) – P. Byrne - Zhu	MSI	Univ. of Nebraska
Cheng, Ge	Dr. Zhu	MSI	Fayetteville State Univ.
Chin, Alexander	Mark Sorrells	TCAP	Cornell Univ.
Clawson, Ryan	David Hole	TCAP	Utah State Univ.
Currie, Yaleaka	Zhu/Bai	MSI	Fayetteville St. Univ., NC
Dhakal, Smit	Shuyu Liu	MSI	West Texas A&M Univ.
Darboe, Fatoumata	Pat Byrne	MSI	Rust College
Dhakal, Smit	Shuyu Liu	MSI	Texas A&M
Dodd, Ashley	Joseph Onyilagha	MSI	Univ. of Arkansas, Pine Bluff
Ellis, Graham	Mike Pumphrey	TCAP	Washington State Univ.
*Elmore, Elizabeth	Robert Bowden	TCAP	Kansas State Univ.
England, Serina	Liu/Chen	MSI	Texas A&M
Gamble, Devona	Botanga/Anderson	MSI	Chicago State Univ.
Gaston, Jasmine	Matute/Arron Carter	MSI	Univ. of Arkansas, Pine Bluff
*Goldsby, Kaitlin	Guihua Bai	TCAP	Kansas State Univ.
*Grabbe, Reagan	Jamie Sherman	TCAP	Montana State Univ.
*Graebner, Ryan	Pat Hayes	TCAP	Oregon State
Graham, Anthony	Arron Carter	MSI	Univ. of Arkansas, Pine Bluff
Haring, Steven	Jim Anderson	TCAP	Univ. of Minnesota
Henkel, Kel	Gina Brown-Guedira	TCAP	North Carolina State Univ.
Hergenrader, Madison	Hergenrader, Madison	TCAP	Univ. of Nebraska
Hole, Chelsea	David Hole	TCAP	Utah State Univ.

Hughes, Austin	P.V.V. Prasad	TCAP	Kansas State Univ.
Hulbert, Bryn	Mike Pumphrey	TCAP	Washington State Univ.
Irvin, Leathen	Joseph Onyilagha	MSI	Univ. of Arkansas Pine Bluff
Jarrell, Criston	Gina Brown-Guedira	TCAP	North Carolina State Univ.
Johnson, Brittney	Jim Anderson	TCAP	Univ. of Minnesota
Johnson, Isiah	Jose Costa	TCAP	Univ. of Maryland
Johnson, Whitney	Onyilagha/Talbert	MSI	Univ. of Arkansas, Pine Bluff
Kellem, Mariam	Matute/Arron Carter	MSI	Univ. of Arkansas, Pine Bluff
Klarer, Emmi	Gary Muehlbauer	TCAP	Univ. of Minnesota
Krause, Margaret	Brian Steffenson	TCAP	Univ. of Minnesota
Lowry, Elizabeth	Eduard Akhunov	TCAP	Kansas State Univ.
Manning, Yvonne	Matute/Arron Carter	MSI	Univ. of Arkansas, Pine Bluff
Marquez Llosa, Luis	Pat Hayes	TCAP	Oregon State
McCabe, Matt	Luther Talbert	TCAP	Montana State Univ.
*McCauley, Cara	Mark Sorrells	TCAP	Cornell
McClendon, Amanda	Onyilagha/Talbert	MSI	Arkansas Pine Bluff
McShan, Shakuemie	Arron Carter	MSI	Arkansas Pine Bluff
Medlin, Jake	Liuling Yan	TCAP	Oklahoma State Univ.
Miller, Daniela	Jose Costa	TCAP	Univ. of Maryland
Moore, Angie	Patrick Byrne	TCAP	Colorado State Univ.
Moss, Delois	Martin Matute	MSI	Arkansas Pine Bluff
Nazar, Aneesh	Jorge Dubcovsky/Josh Hegarty	TCAP	UC Davis
Nelms, Eric 'Wes'	Jorge Dubcovsky/Marco Maccaferri	TCAP	UC Davis
Nesbary, Alicia	Chris Botanga	MSI	Chicago State Univ.
Nevarez, Martha	Botanga/Anderson	MSI	Chicago State Univ.
*Neyhart, Jeffrey	Mark Sorrells	TCAP	Cornell
Ngu, Ester	Shaobin Zhong	Partial	North Dakota State Univ.
Nguen, Jennifer	Gary Muehlbauer	TCAP	Univ. of Minnesota
Nutters, Vanessa	Jorge Dubcovsky	TCAP	UC Davis
Ocampo, Carla	Jorge Dubcovsky	TCAP	UC Davis
Oliver, Brian	David Van Sanford	TCAP	Univ. of Kentucky
Osborne, Taylor	Gina Brown-Guedira	Partial	North Carolina State Univ.
Palatino, Marielle	Jorge Dubcovsky	TCAP	UC Davis
Pfarr, Erin	Kevin Smith	TCAP	Univ. of Minnesota
Poe, Michelle	Joseph Onyilagha	MSI	Univ. of Arkansas, Pine Bluff
Prayer, Jeffrey	Gina Brown-Guedira	TCAP	North Carolina State Univ.
Raja, Cephra	Jorge Dubcovsky	TCAP	UC Davis
Rasmusson, Elijah	Keith Smith	TCAP	Univ. of Minnesota
Ray, Erin	Mary Guttieri	TCAP	Univ. of Nebraska
Reese, Angela	Botanga/Anderson	MSI	Chicago State Univ.
Roberson, Heather	Tim Close	TCAP/MSI	Univ. of California, Riverside
Rodriguez, Jose	Tim Close	TCAP/MSI	Univ. of California, Riverside
Roth-Krosnoski, Thomasina	Kevin Smith	TCAP	Univ. of Minnesota

Ryu, Victor	Eduard Akhunov	TCAP	Kansas State Univ.
Sallee, Katie	Liuling Yan	TCAP	Oklahoma State Univ.
Salvo, Kelsey	Pat Byrne	TCAP	Colorado State Univ.
Shatcher, Cody	Liu/Chen	MSI	Texas A&M
Shekleton, Joe	Jim Anderson	TCAP	Univ. of Minnesota
Shepherd, Robyn	Pat Hayes	TCAP	Oregon State Univ.
Skinner, Avarie	Arron Carter	TCAP	Washington State Univ.
*Sleper, Joshua	Gary Muehlbauer	Other	Univ. of Minnesota
Smith, Arianh	Martin Matute	MSI	Univ. of Arkansas, Pine Bluff
Smith, Gabe	Brian Steffenson	TCAP	Univ. of Minnesota
Stevens, Mary	P.V.V. Prasad	TCAP	Kansas State Univ.
Stringfield, Margie	Peter Morrell and Gary Muehlbauer	MSI	Fayetteville State Univ., NC
Strauss, Nikayla	Scott Haley	TCAP	Colorado State Univ.
Stuart, Nathan	Kevin Smith	TCAP	Univ. of Minnesota
Sun, Tianqi	Kevin Smith	TCAP	Univ. of Minnesota
Tavarez, Michael	R. Sankaran	MSI	Lehman College
Trice, Kerian	Joseph Onyilagha	MSI	Univ. of Arkansas, Pine Bluff
Underwood, Josh	Zhu/Bai	MSI	Fayetteville St.e Univ., NC
Van De Weghe, Michael	Gary Muehlbauer	TCAP	Univ. of Minnesota
*Varella, Andrea	Jamie Sherman	TCAP	Montana State Univ.
Vavra, Erik	Kevin Smith	TCAP	Univ. of Minnesota
Wagner, Secret	Marceline Egnin	MSI	Tuskegee Univ.
Walker, Paige	Martin Matute	MSI	Arkansas Pine Bluff
Wanza Ngao, Shiela	P.V. Vara Prasad	TCAP	Kansas State Univ.
Whitmore, Kenneth	Martin Matute	MSI	Univ. of Arkansas, Pine Bluff
Winters, Andrew	Jorge Duvcovsky	TCAP	UC Davis
Wyatt, Nathan	Robert S. Brueggeman	TCAP	North Dakota State Univ.

*moved on to graduate school

Appendix I6. Trained postdocs and visiting scientists

TCAP has trained a total of 25 Post-Doc's. Visiting scientists to TCAP include 1 visiting PhD Student and 26 visiting scientists.

Name	Postdoc/ Visiting Scientist	Trainer
Amy Bernardo,	Post-Doc	Guihua Bai
Antonio Cabrera	Post-Doc	Clay Sneller
Chor Tee Tan	Post-Doc	Shuyu Liu
Cyrille Saintenac	Post-Doc	Eduard Akhunov
Dadong Zhang	Post-Doc	Guihua Bai
Deniz Akdemir	Post-Doc	Jean-Luc Jannink
Djanaguiraman Maduraimuthu	Post-Doc	P.V. Vara Prasad
Eligio Bossolini	Post-Doc	Jorge Dubcovsky
Gautam Pradhan	Post-Doc	P.V. Vara Prasad
Jeness Scott	Post-Doc	Brian Steffenson
Liang Gao	Post-Doc	Jim Anderson
Marco Maccaferri	Post-Doc	Jorge Dubcovsky
Maria Munoz-Amatriain	Post-Doc	Gary Muehlbauer
Mohsen Mohammadi	Post-Doc	Kevin Smith
Maria Munoz-Amatriain	Post-Doc	Tim Close
Seifollah Kiani	Post-Doc	Eduard Akhunov
Shubing Liu	Post-Doc	Guihua Bai
Uttarasu Subramaniam	Post-Doc	P.V. Vara Prasad
Walid El-Feki	Post-Doc	Pat Byrne
Xiaofei Zhang	Post-Doc	Jim Anderson
Xiaojia Ji	Post-Doc	Guihua Bai
YueGang Wang	Post-Doc	Jianli Chen
Yueqiang Leng	Post-Doc	Shaobin Zhong
Yukiko Naruoka	Post-Doc	Arron Carter
Zhang, Junli	Post-Doc	Jorge Dubcovsky
Nora Honsdorf	Visiting PhD Student	Pat Hayes
Bernice Waweru	Visiting Scientist	Jim Anderson
Chuanlian Li	Visiting Scientist	Guihua Bai
Dhruba Bahadur Thapa	Visiting Scientist	Brian Steffenson
Elsayeed Mansour	Visiting Scientist	Pat Hayes
Feng Chen	Visiting Scientist	Jorge Dubcovsky
Feng Jin	Visiting Scientist	Guihua Bai
Ghulam Mustafa	Visiting Scientist	Guihua Bai
Huirong Mu	Visiting Scientist	Jianli Chen
Hwa Young Heo	Visiting Scientist	Luther Talbert/ Jamie Sherman

Jun Ji	Visiting Scientist	Guihua Bai
Jun Wu	Visiting Scientist	Jianli Chen
Kalaivani Nadarajah	Visiting Scientist	Brian Steffenson
Meng Yuan Wang	Visiting Scientist	Stephen Baenziger
Muzaffer Tosun	Visiting Scientist	Pat Byrne
Nader Abdelsalam	Visiting Scientist	Guihua Bai
Nora Honsdorf	Visiting Scientist	Pat Hayes
Sabina Asghar	Visiting Scientist	Brian Steffenson
Sangay Tschewang	Visiting Scientist	Guihua Bai
Satish Kumar	Visiting Scientist	Mark Sorrells
Shucai Ma	Visiting Scientist	Guihua Bai
Siroos Mahfoozi	Visiting Scientist	Arron Carter
Thi Hoa Tran	Visiting Scientist	Guihua Bai
Tianrong Huang	Visiting Scientist	Yan, Liuling
Valentina Spanic	Visiting Scientist	Jim Anderson
Xianghui Zhang	Visiting Scientist	Guihua Bai
Zhengqi Su	Visiting Scientist	Guihua Bai