

Cover page

a. Award #: 2017-67007-25939

b. Project Title: “Validation, characterization and deployment of QTL for grain yield components in wheat”

c. Project Director: Jorge Dubcovsky

d. Project website: <https://www.triticeacap.org/>

- **Appendix 1.** Germplasm releases
- **Appendix 2.** Publications
- **Appendix 3.** Community resources
- **Appendix 4.** Graduate students.

e. Institution name: University of California, Davis

f. Requested amount: completed grant.

A. WheatCAP 2021 accomplishments

A. 1. WheatCAP overall productivity

The WheatCAP participants published a total of 237 peer-reviewed papers acknowledging the USDA-NIFA support (45 during this last year) and released 98 new commercial wheat varieties (19 during this last year) and 24 improved germplasms (6 during the last year). The complete lists of released varieties and publications are available in Appendices 1 and 2, respectively. Those lists are also available through the WheatCAP web site (<http://www.triticeaecap.org/>). Community resources are in Appendix 3 and students trained in Appendix 4. Students' personal profiles, workshops and links to the positional cloning projects are available at the WheatCAP web site. The Genotyping laboratories provided >1 billion genotyping datapoints to implement genomic selection and marker assisted selection projects in the public wheat breeding programs, and to support the map-based cloning efforts, which resulted in the identification of 16 genes affecting grain size, number of grains, grain yield and resistance to diseases. Progress in education, new genomic resources and T3/BreedBase are described below

A. 2. Education

Education: A central objective of the WheatCAP project was to educate 15 PhD students in molecular plant breeding. By leveraging funds from participating universities, 30 PhD students and 13 MS students have been supported by the WheatCAP grant. Twelve PhD students and eight MS students have graduated. During the WheatCAP project, a number of students were recognized for their leadership and dedication towards the field of plant breeding and genetics. In 2020, WheatCAP graduate students Saarah Kuzay (UC Davis), Brittney Brewer-Jones (Montana State University) and Ellie Taagen (Cornell University), received the "NAPB 2020 Borlaug Graduate Scholars" award. Additionally, in 2021, the Borlaug Global Rust Initiative awarded the Women in Triticum Early Career Award to Elina Adhikari (Kansas State University) and Ellie Taagen (Cornell University). Lastly, students were also successful in applying for internships with private companies including Priscilla Glenn (UC Davis) who was selected for a Wheat Breeding internship with BASF, and Ellie Taagan (Cornell University) who was selected for a Data Science & Quantitative Genetics internship with Bayer Crop Science.

During the course of the WheatCAP project, our students participated in seven in person and eight online education activities. When possible, the education material was posted on the WheatCAP website (<https://www.triticeaecap.org/educational-activities/>). The activities included technical training to help students complete their research projects and have the necessary skill set to be successful in their postgraduate careers. Additionally, students received soft skill training that was focused on developing their leadership skills by identifying and leveraging their strengths, learning how to manage a team, enhancing their interpersonal communication skills, and learning how to communicate with diverse audiences. Soft skill training included WheatCAP students hosting a Plant Sciences Symposia sponsored by Corteva Agriscience that was held in San Diego, CA in 2019. The Plant Sciences Symposia series is a global network of student-organized and student-driven conferences designed to give students an opportunity to harness their leadership and soft skills. Leading scientists and graduate students, most of whom were not

associated with the Wheat-CAP project, were invited to speak on the conference topic: “Bridging the Gap: Using Functional Genomics to Unlock Yield Potential.” Additionally, there was also a discussion panel comprised of individuals who work in academia and industry that answered questions from the audience regarding their career and work experiences. The symposia provided an opportunity for Wheat-CAP students to develop their leadership skills, work together as a team, network with leading scientists and learn the latest research focused on improving yield. This was the first Plant Science Symposium that was organized remotely, and served as a model for continuing the symposium series online during the COVID-19 pandemic.

Due to COVID-19, the travel to CIMMYT in Mexico and to public and private breeding programs located in Minnesota and North Dakota were cancelled. In lieu of our in-person workshops, the students participated in online education activities in 2020-2021. These activities included the formation of two writing groups where students met each week to discuss writing their manuscripts and dissertations. The students also organized an excellent 9-week webinar series titled “Wheat Breeding from plow to plate” that included invited guests and an opportunity for the WheatCAP students to present their own research. Because the pandemic forced all academic institutions to make their classes accessible online, this presented an opportunity for WheatCAP students to participate in online short courses covering topics of interest from leading experts that students would not have had access to at their home universities. To take advantage of this opportunity, WheatCAP supported student participation in short courses hosted by the UC Davis Plant Breeding Academy and the University of Minnesota Summer Institute in Plant Breeding. Courses attended by the students included Seed Production, Hybrid Breeding Strategies, Statistics and Experimental Design, Applied Genomics and Bioinformatics, Phenomics, Genomic Selection and Machine Learning.

WheatCAP educational coordinator assessed student progress using an annual survey to monitor student progress in learning concepts that are essential for completing their WheatCAP research project and preparing for future employment. The survey also serves as a guide to help plan future education activities. Over the past two years, the survey included questions about how the Covid-19 pandemic has affected student education and research progress, and offers to help students cope with the negative impacts of Covid-19. Overall, most of the students indicated the pandemic has caused delays in their research progress, and a few of the students said they had to drop projects. Additionally, students indicated they are suffering from isolation, because they are not able to interact with their peers on a regular basis. To help with the isolation issue, small groups were formed, which met online for a social hour that provided an outlet for social interaction. A summary of our survey results is posted online at the following link: (<https://www.triticeaecap.org/educational-activities/>).

A. 3. T3 database

Education and outreach: The T3 Breedbase website incorporated training videos that can be found on the website at About => Video Tutorials. As mentioned in the education section, the T3 group co-hosted the workshop: “Connecting Crop Phenotype and Genotype Data” at PAG. Presentations to the USWBSI on Breedbase Sept. 8th and 9th, 2020. These presentations have

been split into manageable-sized instructional video tutorials and are available on the Breedbase Youtube channel: <https://www.youtube.com/channel/UC3jrvvzGKKEHzOriDBgnj0A>.

Research: T3 completed the transfer to Breedbase backend: <https://wheat.triticeaetoolbox.org/> and is benefitting from the larger developer community that works on Breedbase and from its applied breeding features (both current and in development). This will provide a solid foundation for the next WheatCAP project. This report has three different components:

1. Curation and availability of wheat breeding datasets
2. Feature developments to enable integration of genomic sequence metadata (e.g., chromatin openness) with genomic prediction
3. Feature developments to improve T3/Wheat usefulness for breeders

1. New wheat datasets made Findable, Accessible, Interoperable, and Reusable (FAIR).

T3/Wheat data content status and additions

Data Category	Current Total	Added since Aug. 1 st , 2020
Accessions with phenotypes	26,612	5,973
Accessions with genotypes	16,375	4,902
Total Accessions	37,261	
Phenotyping Trials	3,087	469
Phenotype Observations	898,949	80,794
Genotyping Projects	40	2

Many of the datasets added include historical and current trials from the Northern Uniform Winter Wheat Scab Nursery. 65 of the trials added represent data from 2020 or 2021. Two substantial Genotyping Trials were added: full variants from Exome capture of the Watkins collection and from promoter capture of a global collection from Kansas State University.

2. Enabling integration of genomic sequence metadata with genomic prediction

Genomic prediction is an important applied breeding innovation, and requires advanced data management such as that provided by T3/Wheat. However, it is now clear that the next step to improve genomic prediction will be its integration with metadata on genomic sequence to leverage biological knowledge (e.g., knowledge of open chromatin or deleterious mutations) in genomic prediction models. An important component of the proposed extension of WheatCAP aims to push this integration at a rapid pace. To this end, T3 developed a new flexible storage mechanism for the efficient storage and retrieval of sequence annotations, consisting of any attributes associated with sequence ranges along a reference genome. Data is loaded using a standard gff3-formatted file. The data can be queried by position and attribute values using either a GUI or an API and visualized using a dynamically generated JBrowse track. Sequence metadata is cross-referenced with database marker information so that marker report pages display overlapping sequence metadata. Metadata accessible through these interfaces include:

- T3 Automated GWAS
- IWGSC Assembly Annotations
- Variant Effect Predictor
- MNase Open Chromatin
- Eduard Akhunov's eQTL Analysis (in prep)

Future sequence-referenced metadata proposed in the next WheatCAP will find a home here.

A challenge for a public service crop database is that many of the programs served by T3/BreedBase use different DNA marker platforms. T3 unified genotyping projects by genotyping protocol, but there are nevertheless many protocols. Furthermore, markers from different protocols that have different names often refer to the same underlying variant. To unify information coming from our many stakeholders, T3 developed a summary table allowing us to identify synonymous markers from different datasets. Marker report pages now include all synonymous markers, their genotype locations, reference and alternate alleles, flanking sequences, and, within the multi-species wheat complex, markers that share the same name but are on different genomes. Reports also link to Ensembl Plant variant detail pages that provide a wealth of information on the variant (e.g., the Variant Effect Prediction). As a by-product, T3 developed an efficient search page allowing users to filter markers based on name, reference genome, species, position, and/or protocol. Finally, though this feature is not fully in place yet, the table will be a key input to Practical Haplotype Graph imputation algorithms.

3. Improving the usefulness of T3/Wheat for applied breeding

T3/Wheat is currently working with three applied breeding program “guinea pigs” who are using it as their primary data management platform: Jessica Rutkoski at University of Illinois, Mary Guttieri at USDA-ARS in Kansas, and Nick Santantonio at Virginia Tech. T3 receives frequent feedback from these programs who are providing us with the tremendous service of beta-testing T3 performance live and without a safety net. The VTech program is using T3 mostly autonomously, installing Docker images of T3/Breedbase containing pre-built trait ontologies for wheat, barley, and oat. T3 provides regular update images that contain fixes from the growing community of Breedbase developers as well as our own feature additions. T3 maintains the systems administration for the UIUC and ARS/KS instances. Independently, however the users of those databases have added 67 phenotyping and one genotyping trials (UIUC) and 140 phenotyping and 1 genotyping trials (ARS/KS).

Responding to an important feature request coming from these users T3 developed improved seedlot management functionalities. A simple interface now exists for the quick data entry of seedlot events (e.g., seed removal for experiments, observation of germination), for flexible specification of where the lot is (e.g., shelving unit and level), and for the integration of barcodes to quickly identify the lot. Frequently, seedlots come from phenotyping experiments and now the seedlot data type can be created directly from the field plot data type. Representations of all of these data end points are being created in the BrAPI specification so that 3rd party mobile applications can interface directly with T3 in managing seedlots, and indeed, such an app is in the works from the group that has developed the Android Field Book.

Other relevant new features:

1. R Shiny interface to display eQTL data from the Akhunov Lab accessible from T3 menus Analyze => Wheat eQTL.
2. A “Dataset” specification feature. Until now, Breedbase could only manage lists of one data type (e.g., a list of accessions or a list of phenotyping trials). The Dataset specification enables the combination of multiple lists of different data types that go together as the appropriate inputs for a complete analysis. For example, lists of accessions, phenotyping trials, genotyping projects, and traits could go together to enter a genomic prediction analysis. Dataset specifications can be public or private.
3. Detailed Genotype Protocol Metadata. Protocols now contain links to original publications, to descriptions of the pipelines generating variant calls, to the raw data, and to specification of quality filtering.
4. Wheat. RefSeq v2.1 assembly genotype map and BLAST database.
5. WheatIS links have been updated to point to Breedbase report pages
6. T3 improved the mirror of our Ithaca, NY server on the Albany, CA server.
7. Further improved links to external resources: the GrainGenes JBrowse resource (T3 collaborated with Taner Sen’s group to populate that resource), UniProt, PlantCyc, and Plant Reactome are all linked to variants stored on T3 when feasible.

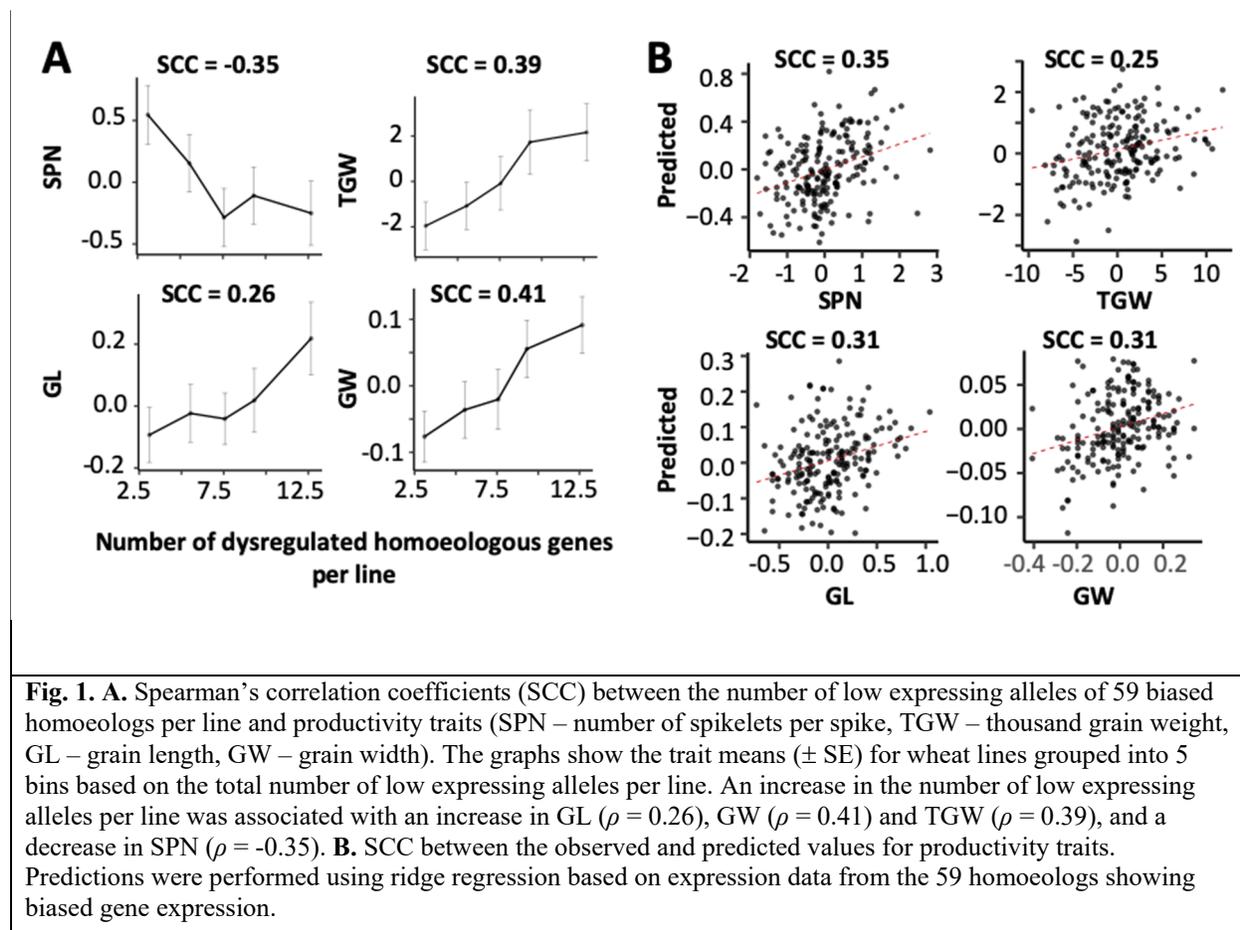
A. 4. Genomics resources

Regulatory Variants detected by Regulatory Sequence Capture: The KSU group used a previously developed regulatory capture assay (Gardiner et al. 2019) to identify 3,320,006 SNPs segregating in the regulatory and coding regions of the genome in 203 wheat accessions. The data were used to identify regulatory SNPs associated with gene expression variation in wheat (He et al., 2021). The raw data is deposited in NCBI, and the regulatory SNPs and associated genes are deposited to the GrainGenes and T3 databases (<https://graingenes.org/jb/?data=%2Fggds%2Fwhe-iwgsc2018>).

eQTL mapping identified homoeologous genes associated with variation in productivity traits:

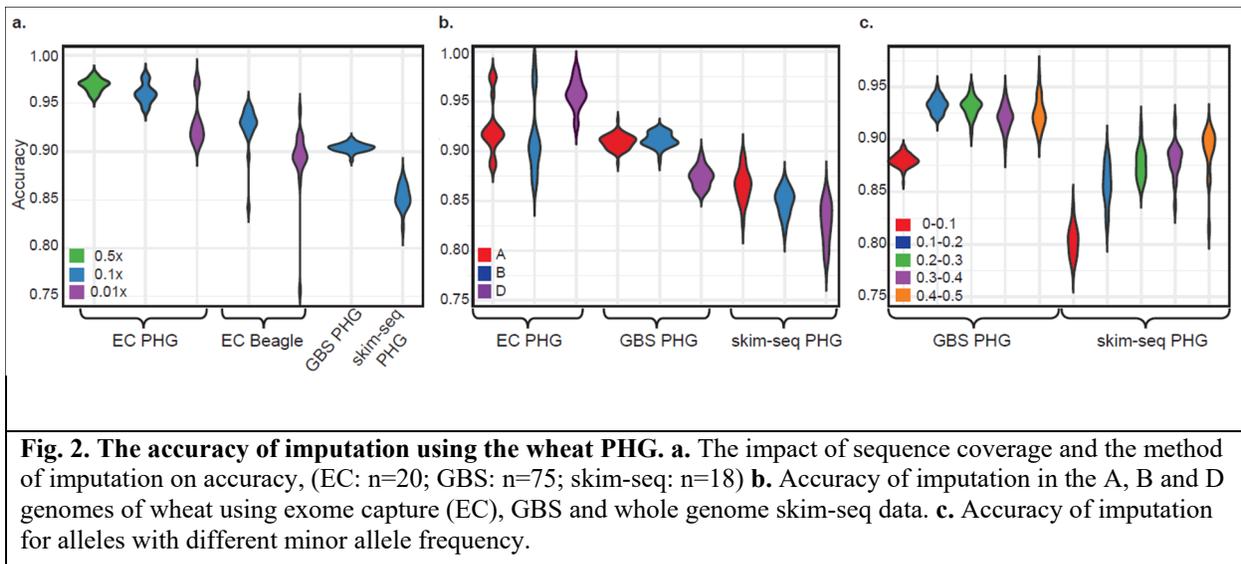
Using a panel of 198 diverse spring wheat cultivars and landraces genotyped using the regulatory capture assay, the KSU group identified 36,898 and 15,238 significant eQTL (FDR < 1E-5) in the seedlings and spikes, respectively. Using these data, they investigated the mechanisms of regulation of duplicated homoeologous genes and their role in controlling productivity traits. They showed that the presence of *cis*-regulatory variants in one of homoeologous genes in the wheat genome often leads to the biased relative expression of duplicated gene copies. They identified a set of 59 homoeologous genes that show biased expression due to the presence of low-expressing eQTL alleles in a large proportion of wheat lines, resulting in negative expression correlations ($\rho < -0.4$) between homoeologs in the panel. The total number of low-expressing alleles of homoeologs per line and the levels of expression of these 59 homoeologs

were highly predictive of variation in productivity traits (**Fig. 1a**). The correlations between predicted and observed trait values for major productivity traits ranged from 0.25 to 0.37 (**Fig. 1b**). Association between the biased homoeologs and phenotype was independently validated using a panel of lines from the WheatCAPs 1,000 wheat exomes project (He et al., 2019). By correlating the number of *cis*-eQTL alleles associated with the low-expressing copies of 59 biased homoeologs and traits the KSU group showed that an increase in the number of these alleles is linked with an increase in grain yield and decrease in heading date. These results indicate that the accumulation of eQTL variants associated with the relative changes in the homoeolog expression dosage could affect adaptive phenotypes affecting crop productivity. All eQTL and associated genes identified in this study were deposited to the GrainGenes (<https://graingenes.org/jb/?data=%2Fggds%2Fwhe-iwgs2018>) and T3 databases.



Developing the first-generation wheat Practical Haplotype Graph (PHG): The WheatCAP students and postdoctoral researchers participated in workshops organized in Cornell University by the T3 database team and Ed Buckler's group aimed at developing the wheat PHG. The Akhunov lab finalized the development and testing of the first-generation wheat PHG based on the exome capture data from wheat cultivars from US wheat breeding programs, including WheatCAP parents (Jordan et al., 2021). The wheat PHG was applied for imputing genotypes in wheat lines characterized using exome capture, genotyping-by-sequencing (GBS), or whole-

genome Skim-seq sequencing approaches. The imputation accuracy of 93% was achieved using low-coverage sequencing (0.01x), that was only slightly lower than the accuracy of imputation with 0.5x sequence coverage (96.9%) (Fig. 2). PHG imputation was shown to be ~4% more accurate than imputation performed using Beagle (p -value = 0.00027). The wheat PHG also imputed genotypes with 27% higher accuracy than Beagle in regions carrying a rare haplotype introgressed from a wild relative into wheat. The KSU group used the imputed genotypes to assess the genetic contribution to heading date variation in the nested association mapping (NAM) family. By applying stepwise regression to PHG-imputed genotypes, they identified 11 SNPs that together explain 90% of genetic variance. These results indicate that the increase in marker density after imputation helped to identify new loci with a broader range of effects together explaining a higher proportion of genetic variance compared to the previous studies. Our results indicate that the first-generation wheat PHG could be an effective tool for SNP data storage and retrieval in breeding programs and improve our ability to identify variants contributing to trait variation.



Haplotype analysis of wild-relative introgression in a winter wheat breeding population: The KSU group used whole-genome sequencing of parental lines and the sequence-based genotyping of introgression population derived from crosses between six hard red winter wheat cultivars and 21 diverse *Aegilops tauschii* accessions to investigate the impact of wild-relative introgressions on yield and yield component traits (Nyine et al., 2021). In the irrigated and non-irrigated field trials, 23% of the introgression lines produced more grain than the parents and check cultivars. The yield of twelve lines was stable across treatments, years and locations; five of these lines showed 9.8% higher yield than the average yield of check cultivars. The haplotype-trait associations were identified on chromosomes 2DS and 6DL for spikelet number per spike, on chromosomes 2DS, 3DS, 5DS and 7DS for grain length and on chromosomes 1DL, 2DS, 6DL and 7DS for grain width. They showed that introgressed *Ppd-D1* alleles identified by the whole-genome sequencing of the *Ae. tauschii* parents are associated with increased spikelet number and heading date (**Fig. 3**). The characterized repertoire of the introgressed haplotypes derived from

Ae. tauschii accessions provides a valuable source of alleles for improving the productivity of winter wheat by optimizing the contribution of component traits to yield.

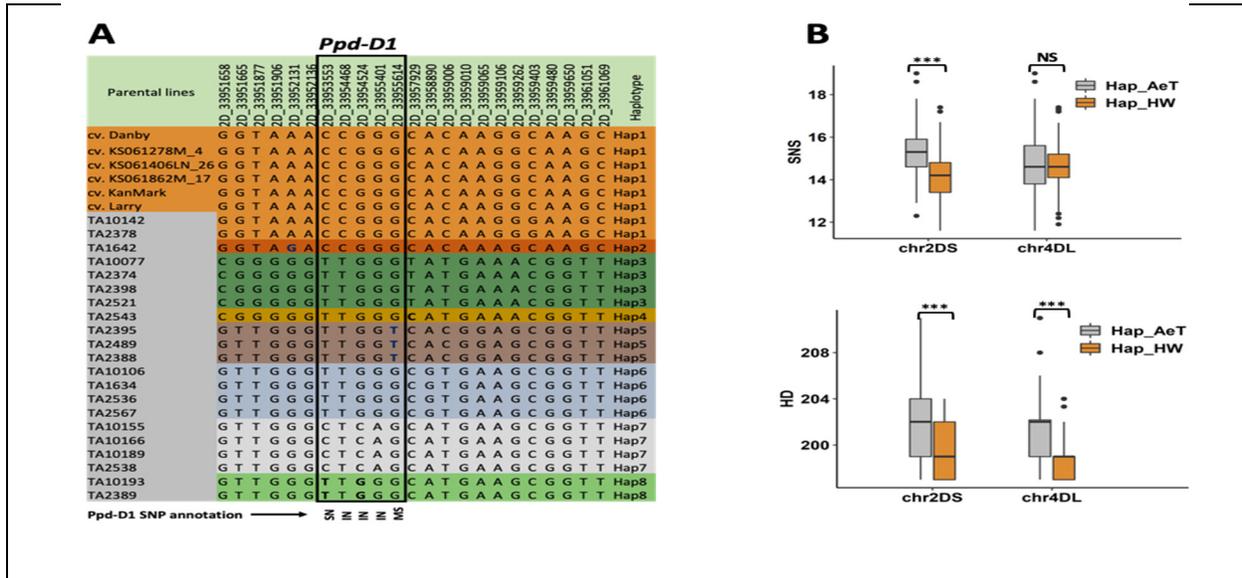


Fig. 3. A) *Ppd-D1* haplotypes identified in winter wheat (top six) and 21 *Ae. tauschii* accessions. The black rectangle shows SNPs within the coding region of *Ppd-D1*. SN - synonymous SNP, IN - intronic SNP, MS - missense SNP. **B)** Boxplot shows the impact of introgression from *Ae. tauschii* (Hap_AeT) into 2DS and 4DL on SNS and HD traits.

Low-cost genotyping assay for breeding programs: The KSU group performed in-house evaluation of the NimaGen’s molecular inversion probe (MIP) assay for low-cost genotyping in wheat. The assay includes 363 SNPs based on functional markers and SNPs from the wheat 90K iSelect assay. A total of 32 wheat cultivars have been genotyped. The NGS of pooled MIP amplicons produced data for 287 SNP sites (79%), of which 210 were captured in all wheat lines with the depth of read coverage > 10 reads. Comparison with genotyping data generated using the 90K SNP assay showed nearly 85% concordance rate. To simplify genotype calling in MIPs data, they developed an alignment-free genotype calling pipeline. The pipeline uses a database of predefined k-mers representing the alleles of SNP sites to call genotypes using the raw fastq reads generated by sequencing MIP amplicons. Combined together with the MIP-based genotyping assay, this pipeline provides an effective low-cost genotyping solution for breeding applications.

Sequenced tilling populations: The UCD group remapped the TILLING Kronos exome capture to the Chinese Spring RefSeq v1.0 and called mutation effects on all annotated genes using a new error threshold methodology adjusted by individual captures. The final dataset available in the UC Davis web browser and GrainGenes includes 4,774,529 sequenced mutations with an estimated error rate of 0.33% (99.67% accuracy) and a mutation density of 40 mutations per kb for the complete population. In addition, the UCD group completed the sequencing of the promoter regions (2 kb) of all annotated high-confidence genes in 1,513 lines of the EMS mutagenized Kronos population using a promoter capture developed by Anthony Hall in the UK.

This large sequencing effort resulted in 4,287,361 sequenced mutations with an estimated error rate of 0.21% (99.79% accuracy), and a mutation density of 36 mutations per kb. The promoter mutations, together with the re-mapping of the exome capture data, is publicly available through ENSEMBL, GrainGenes genome browser and the Dubcovsky's lab website. Direct links to seed distribution are provided to the users.

A new transformation technology improves regeneration efficiency and editing in wheat. This new technology, developed by the Dubcovsky lab in collaboration with the transformation facility at UCD, has been published in *Nature Biotechnology* 2020 38: 1274-1279. This technology uses a chimeric protein including the wheat GROWTH-REGULATING FACTOR 4 (GRF4) and its cofactor GRF-INTERACTING FACTOR 1 (GIF1) that increases regeneration efficiency > 7-fold, reduces the transformation protocol from 91 to 56 days, and expands the number of transformable wheat genotypes. The combination of *GRF4-GIF1* and CRISPR-Cas9 facilitates the generation of large numbers of edited wheat plants. All vectors have been deposited in ADGEN and more than 130 requests were reported in 2021 documenting the impact of this WheatCAP resource.

A. 5. Genotyping Laboratories

The role of the genotyping labs in the current WheatCAP project is to provide KASP and other marker genotyping services for QTL analysis and high-resolution mapping of candidate genes for yield-related QTL, and to support GS and MAS in the public wheat breeding programs. In total, the Regional Genotyping labs provided well over 1 billion data points on genome-wide markers to breeding programs during the past year alone. The Fargo lab continues to support breeding and genetics with Illumina 90K SNP genotyping, processing 4,944 wheat samples during 2020-2021. The Kansas lab evaluated more than 7,000 wheat samples from 15 genetics and breeding programs with various types of genome-wide marker platforms, (GBS, GBMAS, MRaseq) which generated about 350 million data points. The NC lab continued collaboration with eastern wheat breeding programs performing genomic selection and genotyping by sequencing (GBS) done for more than 15,000 lines submitted by all 13 public wheat breeding programs, resulting in approximately 0.5 billion data points. The use of genotyping-by-multiplexed sequencing (GMS) has increased in both wheat and barley. In wheat new oligos have been tested and added to the panel. The bioinformatics have been optimized for use on USDA SciNET system which has decreased the long analysis time bottleneck.

The GMS protocol was used to evaluate genetic diversity in 320 Pacific Northwest (PNW) wheat cultivars and to examine population structure and changes in genomic-level and agroecosystem-level genetic diversity of PNW wheat over the past 120 yr. At the genome level, no long-term shifts in gene diversity were detected in either spring or winter wheat, but fluctuations were significant within market classes and within the most widely grown spring and winter varieties. At the agroecosystem level, diversity has been on a rising trend since the 1990s as the dominance of acreages by a few varieties has become less common. Cultivation of multiple market classes and periodic incorporation of new germplasm by breeding programs have been able to maintain the quantitative level of genetic diversity in PNW wheat over time.

To investigate a mid-density genotyping platform for replacing the two-enzyme GBS protocol, the NC lab piloted the single primer extension technology as a targeted sequencing approach. A pool of primers for the Allegro targeted genotyping platform was developed for SNPs selected from exome capture data of 400 North American wheat cultivars and breeding lines. Weighted LD thinning was used to select targets having MAF > 0.10 across all growing regions (599) or within Eastern U.S. winter wheat germplasm (741). Selection criteria (MAF and read depth) were relaxed to select an additional 1138 SNPs to fill gaps in genome-coverage. Recent genotyping of 95 eastern wheats determined that 99% of the 2,478 targets were covered when Illumina reads aligned to the reference genome. The targeted variants were identified for 96% and 84% of SNPs with high MAF in the overall set and in eastern germplasm, respectively. In contrast, variants were identified for 47% of SNPs selected to fill gaps. While this preliminary analysis is very encouraging, it highlighted the importance of selecting high-quality SNPs for inclusion in future primer pool designs. Preliminary analyses also identified more than 1000 off-target SNPs, many of which were located in regions flanking the target, particularly for reads originating from alien introgressions commonly used in wheat breeding. Multiple analyses are ongoing, including for causal variants (that are often indels) included in the primer pools. Cross-validation in the eastern winter wheat training population for resistance to Fusarium head blight is underway to assess utility of markers in the Allegro mid-density panel for genomic prediction in this germplasm. Liquid handling instrumentation for miniaturization of genotyping reactions and NGS library preparation has been purchased and protocols will be deployed that reduce genotyping costs.

Sequences of genes identified as part of the WheatCAP project, including *BI*, *WAO-A1*, *TaGW-A2*, *Vrn-A3*, *FT-A2* and others were targeted in an amplicon sequencing primer pool developed in collaboration with Agriplex Genomics. Overall, the primer pool included 170 assays targeting loci affecting plant growth and development, pest resistance and end-use quality. A total of 2,524 public and privately developed wheat lines from all US wheat growing regions were evaluated. Regional differences in allele frequency were notable for many of the target loci, reflective of selection for end-use qualities specific to market class, as well as local adaptation and pest pressure. Individual project data were provided to breeding programs and are being used for analysis of gene/QTL effects across diverse environments. Genotypes for the 622 entries in eastern winter wheat collaborative nurseries will be posted on the USDA-ARS Plant Science Research website <https://www.ars.usda.gov/southeast-area/raleigh-nc/plant-science-research/docs/small-grains-genotyping-laboratory/regional-nursery-marker-reports/> and provided to the T3 database. The Kansas lab genotyped 2,200 wheat lines, including parental lines from regional breeding programs, two HWW regional wheat performance nurseries (RPN) and germplasm observation nursery (RGON) with 100 sequence-specific markers linked to important traits, including those identified in this grant, resulting in 230,000 sequence-specific (KASP, SSR, STS) data points. These data are available at <https://www.ars.usda.gov/plains-area/lincoln-ne/wheat-sorghum-and-forage-research/docs/hard-winter-wheat-regional-nursery-program/research/>. The Fargo Lab genotyped 109,000 wheat lines with trait-linked allele-specific PCR and SSR markers for regional breeding and genetics programs; providing 1.8 M data points. This includes genotyping entries to the Uniform Regional Hard Red Spring Wheat Nursery and

Uniform Regional Scab Nursery with 23 markers for disease, quality, and flowering time. This data are available at <https://wheat.pw.usda.gov/GG3/germplasm>

The Regional genotyping labs developed and validated new KASP assays for wheat breeding. The Kansas lab developed two highly predictive KASP markers for *Fhb7* for resistance to head scab, two diagnostic markers for *Cmc4* for curl mite resistance, and two diagnostic markers for *H34* for HF resistance. The new markers have been validated in more than 500 germplasm lines and are reliable in different genetic backgrounds. The Kansas lab collaborated with Mike Smith in KSU to identify a candidate gene of *Cmc4* for curl mite resistance on chromosome 6D using map-based cloning. Currently the candidate gene has been sequenced from both resistant and susceptible parents and transformed into Bobwhite for gene overexpression. M0 plants were obtained and the M₁ will be phenotyped for curl mite resistance this fall.

The Fargo lab also validated the markers for *Fhb7* in spring wheats, as well as new co-dominant markers for *Tsn1* and *Snn1* loci conferring susceptibility to the tan spot and Septoria nodorum pathogens, respectively. They are supporting a WheatCAP student project to clone a tetraploid wheat kernels-per-spike QTL on 2BL and are assisting in validating the markers. They also collaborated with the breeding program at NDSU to validate and deploy markers for *Wsm2* conferring Wheat Streak Mosaic Virus resistance and the *SMI* locus for Orange wheat blossom midge resistance. Collaboration with the University of Saskatchewan was established to validate the *Usw275* KASP assay for hollow stem/sawfly resistance in durum wheat. The NCSU lab developed and validated a KASP assay linked to the stem rust resistance locus that is either the same or allelic to *SrTnp* resistance.

The NC lab collaborated with NCSU graduate students fine-mapping glaucousness loci. Fifteen assays targeting a 30 Mbp region in on chromosome 2AL and a 10 Mbp region on 3AL were evaluated on 768 samples from heterozygous RILs for development of NILs. The NC lab also collaborated with the NCSU breeding program to fine-map the *Pm54* locus conferring resistance to powdery mildew. This gene was mapped to a region on chromosome arm 6BL where there are three candidate genes. EMS mutants of resistant cultivar AGS2000 have been evaluated and sequencing of susceptible M3 lines is underway.

A. 6. CIMMYT and Winter HUBs

A.6.1. CIMMYT hub Matthew Reynolds, Sivakumar Sukumaran & Karim Ammar

A.6.1.2 Lines from MT with the *QTn.mst-6B* for number of reproductive tillers: The MT team completed the introgression of the *QTn.mst-6B* for increased number of reproductive tillers into CIMMYT high yield line BAJ #1 and sent them to CIMMYT for testing. The experiment, performed as an RCBD with 5 blocks, showed a significant 10.2% increase in total number of grains per m² ($P = 0.0108$). No significant differences were detected for the number of spikelets per spike, suggesting that the increase in grain number per m² reflects an increase in the number of spikes per m², which is the expected result. The high tillering allele of *QTn.mst-6B* showed a positive effect on grain yield of ~4.4%, but the differences were not significant, likely due to the negative correlation between grain number and grain weight in this experiment. The lines with

the high tillering allele for *QTn.mst-6B* showed a 5% decrease in TGW that offset some of the gains obtained by the increased grain number per m². This result, suggests that the plants were not able to fill the extra grains, possibly due to some limitations in the environment, a trend observed during this season in other experiments testing lines with increased GNS. These results will be validated by a similar experiment in 2021-2022 using larger plots.

A.6.1.2 Lines from CO with a 6B QTL for SNS

The CO team validated their target QTL for spikelet number on chromosome 6BL (position 492-502 Mb, RefSeq v1.0, *Q6BL-SNS* henceforth) across three field seasons in Fort Collins, CO in a Platte/CO940610 RIL population (F5:8, n=222), with the Platte 6BL allele conferring an average increase of 0.83 spikelets per spike (% R² = 7.6, *P* < 0.0001). Platte was also the donor of the *WAPO-A1* allele from high SNS (haplotype 2). The CO team introgressed the *Q6BL-SNS* and *WAPO-A1* favorable alleles into the CIMMYT High Biomass line 2 (henceforth, HB2 = GID4314513) and the high-yielding variety NADI by two crosses followed by self-pollination (BC₁F_{2:4}). Preliminary experiments at CIMMYT were done in Hill Plots (0.5 m long x 1.2 m width and 4 rows) under drip irrigation, which also served as seed increases for the 2021-2022 season. Experiments were organized in a CRD with VARIETY as block (random factor) and 10 replications.

The results from these experiments revealed an interaction between *Q6BL-SNS* and *WAPO-A1*. *WAPO-A1* showed significant differences in SNS, grain number per m² and estimated grain yield (10% increase *P* = 0.04) only in the presence of the favorable Platte allele for *Q6BL-SNS*. In addition, *Q6BL-SNS* showed opposite effects in the different *WAPO-A1* backgrounds for SNS and grain number per m², and was significant for estimated yield only in the presence of the favorable *WAPO-A1* allele (10% increase). The Platte alleles for the two genes showed contrasting effect on grain weight. The *WAPO-A1* Platte allele associated with high SNS was significantly associated with a significant decrease in grain weight, whereas the Platte allele in *Q6BL-SNS* was associated with a positive and significant effect on grain weight. These interesting results will be validated by a similar experiment in 2021-2022 using larger plots.

A.6.1.3 Lines from CA with the *WAPO-A1*, *TmElf3* and *gw-A2* alleles

The effect of the introgression of the *WAPO-A1-H2* into the high-biomass line HB2 (GID4314513) was evaluated in homozygous BC₅F₃ sister lines generated at UCD in the CIMMYT spring hub using a RCBD with 8 blocks. The *WAPO-A1-H2* allele was associated with an 8.8% increase in SNS and a 5.6% increase in grains per m². The increase in grain number was partially offset by a 1.3% decrease in grain weight, which resulted in a 4.1% increase in grain yield. These results, together with the experiments with lines from CO described below demonstrated that the *WAPO-A1-H2* allele is a useful tool to increase grain number and grain yield in wheat.

In 2021, the CIMMYT hub also planted a second year of an experiment to evaluate the effect of an introgressed allele of *ELF3* from *T. monococcum* (*TmElf3*) associated with increased SNS and a loss-of-function mutation in the *gw-A2* gene associated with increases in grain weight and protein content. These two genes were introgressed into the high yielding CIMMYT line

Kingbird and three high-biomass lines and tested in a RCBD with 10 replications. In both the 2020 and 2021 experiments *TmElf3* was associated to small but significant increases in SNS and 1-2 d delayed heading, whereas the *gw-A2* mutant allele was associated with increases in grain weight. However, none of the genes showed significant improvements in total grain yield over the two years, suggesting a limited effect of these genes in this environment.

These two genes were also evaluated in three different tetraploid wheat varieties (Cirno, GID6420253 and Kronos) in 2020 and 2021 under both full and limited irrigation. The combined ANOVA using years as blocks showed no significant increases in grain yield for either of the two genes. However, *gw-A2* showed a positive effect on grain size and weight and on grain protein content. Similar effects for this gene were reported in previous experiments at UCD in different locations. Based on these results the *TmElf3* allele will not be incorporated into the CYMMIT or UCD breeding programs but the *gw-A2* will be used based on its beneficial effect on grain protein content.

For the 2021-2022 season experiments will be repeated with larger plots for the MT *QTn.mst-6B* for increased number of reproductive tillers, the CO *Q6BL-SNS* x *WAPO-A1* interaction, and the CA *WAPO-A1* lines in tetraploid wheat. New seed increases will be initiated for the *FT-A2* A10 and D10 isogenic lines in Kronos and for new lines from WSU for the 4AL Scarlet QTL for kernel weight.

A.6.2 NIFA IWYP Winter Wheat Breeding Innovation (WWBI) Hub at KSU

In 2020, KSU established the WWBI Hub funded by USDA NIFA which works in close collaboration with the WheatCAP project and IWYP (International Wheat Yield Partnership). The Hub is a public-private partnership aimed at evaluating the research outputs of the WheatCAP and IWYP in elite winter wheat germplasm relevant for U.S. breeding programs. The WWBI Hub's Steering Committee composed of representatives from industry and public breeding programs selected four genes and QTL identified in the WheatCAP program with potential positive effects on grain yield (*IAL:472.2-531.6* Mb, *WAPO-A1*, *Elf3* and *TaGW2*) for introgression into eleven recipient lines, which include eight hard red winter, one soft red winter and two soft white winter wheat lines from Kansas, Nebraska, Oklahoma, Colorado, Texas, Idaho and Washington.

A. 7. QTL cloning and deployment projects

During the WheatCAP 16 valuable genes have been identified, validated and/or published:

- Five stem rust resistance genes (*Sr13*, *Sr21*, *Sr60*, *Sr22b* and *Sr9e*).
- Five genes affecting grain size (*GW2*, *GW7*, *GS3*, *CKX2-1*, *CKX2-2*)
- Five genes affecting number of spikelets/spike (*WAPO-A1*, *LFY*, *SVPI*, *FUL2*, *VRT2*).
- The awn suppressor B1

2021 progress of individual research projects:

AR. University of Arkansas. Esten Mason

Education: Dylan Larkin was a Ph.D. student from Fall 2017 and graduated Spring 2021. Dylan is now working as potato line breeder at Aardevo North America. Zachary Winn started on August 21, 2017 as an M.S. student and completed his M.S. in Aug 2019. Zachary is attending North Carolina State University for his doctoral research and will graduate in Spring 2022. Mikayla Hammers, began her MS degree in Jan 2019 at the University of Arkansas and is continuing her degree at Colorado State University.

Research project: The University of Arkansas validated a QTL on chromosome 1AL in the field over two growing seasons (2018-2020). On average, the additive effect of the allele substitution increased as the position moved distally, with the AGS 2000 allele favorable for kernels spike⁻¹, area spike⁻¹, kernel weight spike⁻¹, and 1000 kernel weight. In addition, a moderate throughput method was developed for quantifying spike characteristics including area spike⁻¹ as a surrogate for spike length using image analysis and for anther extrusion, an important trait for hybrid wheat production (Winn et al. 2021). With the exception of 1000 kernel weight, the additive effect for all yield components was consistent across years and in the combined analysis. Based on current marker density, the QTL was finally mapped between 521.3 and 531.6 MB. This QTL was introgressed into CIMMYT spring wheat and BC₃F₁ lines were developed. On Aug 17, 2020, Dr. Mason started as an Associate Professor and Wheat Breeder at Colorado State University and since staff did not remain at the University of Arkansas the work was concluded with the high-density mapping of the QTL. This work cannot be continued at CSU because the Southern soft red winter wheat is not well adapted to Colorado. Remaining resources were shifted toward the CSU cloning project and are being used to complete Mikayla Hammers MS research project using machine learning to determine wheat spike characteristics.

CA. University of California, Davis. Jorge Dubcovsky

Education: Saarah Kuzay completed her PhD with one paper published in TAG and one currently in preprint and submitted to PLoS Genetics. Her papers demonstrate that *WAPO-A1* is the causal gene of the 7AL QTL for spikelet number per spike (SNS), completing a very successful research project. Priscilla Glenn published her first paper on the high-density mapping of a 3AS QTL for SNS. Her study provides compelling evidence that the gene underlying this QTL is *FT-A2* and that an amino acid change D10A is the most likely causal mutation. She is making progress on her second paper and she is on track to complete her PhD in the Summer of 2022. During 2021, she completed a productive summer internship in industry (BASF).

Research project: The UCD group identified a chromosomal rearrangement that resulted in the triplication of a 4Mb chromosome segment on the short arm of chromosomes 1BS-1RS, in the introgression line Hahn-1RS^{RW}. In a paper published in The Plant Genome (2021:e20079), and using radiation mutants and exome capture, the UCD team demonstrated that the change in gene dosage was responsible for the difference in root length and drought tolerance between Hahn-1RS^{RW} and Hahn-1RS isogenic lines. More recently the team identified the causal gene and validated it using CRISPR mutants and transgenic complementation. The manuscript reporting the first cloning of a drought resistance gene is in preparation.

The UCD team completed the cloning and validation of a QTL for spikelet number per spike (SNS) on the long arm of chromosome 7A using CRISPR mutants and transgenic

complementation. The study demonstrated that there are three alleles of *WAPO-A1*, with H2 being the one associated with the highest SNS, H3 with intermediate results and H1 with the lowest number of SNS. The results have been published as a preprint in bioRxiv and they are under revision in PLoS genetics (<https://doi.org/10.1101/2021.07.29.454276>).

The UCD team also completed the high-density mapping and validation of *FT-A2* as the underlying gene of the 3AS QTL for SNS. The results have been published as a preprint in Research Gate and are in press in Theoretical and Applied Genetics. This study showed that the increase in SNS was associated with a D10A polymorphisms in the encoded protein, and that the favorable allele has been under positive selection in hexaploid wheat but is almost absent in tetraploid wheat. A similar low frequency in tetraploid wheat was observed for the favorable allele of *WAPO-A1* suggesting that these two genes can be used to improve yield in durum wheat. Tetraploid wheat isogenic lines with the *WAPO-A1* and *FT-A2* favorable allele have been submitted to the CIMMYT hub for evaluation in the 2021-2022 season

Finally, the UCD team completed an extensive study on the role of the SVP MADS-box genes on spike development that was published as a preprint in bioRxiv and is in press in The Plant Cell. This study showed that the *VRT-A2* and *SVPI* genes interact with the SQUAMOSA MADS-box genes in the regulation of heading time, SNS, plant height and spikelet development. The study also showed that mutations on the *VRT-A2* and *SVPI* genes are associated with significant increases in SNS, which may have direct practical application for the improvement of wheat grain yield.

Deployment: In 2021, the UCD lines combining the *Elf-A^m3* allele for increased spikelet number from *T. monococcum* and the *gw-A2* mutants into tetraploid varieties Kronos, ‘Cirno C 2008’ and ‘GID₆₄₂₀₂₅₃’; and into hexaploid wheat Kingbird HB1 (GID4878569), HB2 (GID4314513) and HB3 (GID4577963) were evaluated in small plots in CIMMYT. Results for the experiments performed with the UCD seeds are described in the CIMMYT section (A.6.1.3). UCD group also introgressed the *WAPO-A1* allele into a CIMMYT high-biomass bread wheat line and into the tetraploid variety Kronos (BC₄F₂) and the *FT-A2-A10* allele into Kronos. Seeds were sent to CIMMYT in September 2021 for evaluation in the 2021-2022 growing season.

CO. Colorado State University. Stephen Pearce, Pat Byrne and Scott Haley

Education: PhD student Andrew Katz has completed all course credit requirements for his PhD degree (GPA 4.00). He submitted his first thesis chapter that has been published as a preprint in Research Square <https://orcid.org/0000-0002-1794-7618> and has been submitted to Molecular Breeding. Andrew Katz is expected to graduate in Spring 2022. Andrew has supervised four undergraduate students (Forrest Wold-McGimsey, Mallory Wilemon, Alejandro Benitez and Jack Mentzer) during the project, and presented his research at departmental and university-wide seminars at CSU and at the Texas A&M Plant Breeding Symposium.

Research project: The CO team validated a QTL for spikelet number on chromosome 6BL (position 493-503 Mb, RefSeq v1.0) across three field seasons in Fort Collins, CO in a Platte/CO940610 RIL population (F_{5:8}, n=215), with the Platte 6BL allele conferring an average increase of 0.74 spikelets per spike (% R² = 13.8, P < 0.0001). They further validated the QTL

using eight HIFs ($F_{5:6}$, $n=18$ sown in three replications as headrows). Each HIF was segregating for the 6BL peak marker but fixed for different combinations of the *VRN-D3a/b*, *PPD-B1a/b*, and *WAPO-A1a/b* allelic variants, allowing us to account for epistatic effects between these loci. The 6BL QTL was significantly associated with spikelet number ($P < 0.05$) in seven of the eight families, with an effect size ranging from 0.25 to 0.50 spikelets per spike. The study has been published as a preprint in Research Square <https://orcid.org/0000-0002-1794-7618> and a manuscript describing these results has been submitted to *Molecular Breeding*.

They screened 2,592 $F_{5:6}$ individuals from these HIFs using 17 KASP markers spanning the peak QTL region and identified 10 critical recombinants. Progeny tests of three independent critical recombinant families with 100 individual $F_{7:8}$ derived lines each further delimited the 6BL QTL to a region between markers 500-494 Mb, RefSeq v1.0. The 6BL QTL was significantly associated with spikelet number ($P < 0.05$) in two of the three families with a mean effect size of 0.46 spikelets per spike. They developed two new critical recombinant families of $F_{7:8}$ derived lines, which will be planted as headrows in the fall of 2020 to further delimit the 6BL QTL region. They performed RNA-seq analysis to compare the transcriptomes of segregating HIF individuals at two stages of early spike development and identified candidate genes that are highly and differentially expressed. For one candidate gene, they are developing CRISPR/Cas9 knockout lines for functional characterization.

Deployment: They have introgressed the high-value 6BL and *WAPO-A1b* alleles for increased spikelet number into three CIMMYT high biomass parents; ‘Bonsu’, ‘Nadi’, and ‘GID:4314513’. They developed BC_3F_2 seed segregating for these alleles in ‘Nadi’, and the lines were tested in the CIMMYT hub. See results in the CIMMYT section for $BC_1F_{2:3}$ segregating families in ‘Bonsu’, ‘Nadi’, and ‘GID:4314513’. $BC_1F_{2:3}$ materials segregating for the 6BL QTL have also been developed in the winter wheat variety ‘Monarch’ and advanced breeding line ‘CO15D098R’, which will be further backcrossed and characterized in field experiments.

ID. University of Idaho. Jianli Chen

Education: Kyle Isham earned his M.S. degree in December 2019 and is now operating a farm in Idaho. Katrina Johnson earned her M.S. Degree in December 2019 and is now a lab technician at Washington State University. Meng Su plans to complete her Ph.D. in Spring 2022. She will complete the fine-mapping of the SNS QTL on chromosome arm 5AL. Meng Su attended the WheatCAP organized training workshop (Summer Institute in Plant Breeding) in June and made an oral presentation for the 2021 CSSA meeting in 2021. Postdoc Dr. Wang Rui (50% support from the WheatCAP) started a new job in a private company in the US.

Research project: The ID team work was concentrated in two different mapping populations:

a. Fine-mapping of *QtSNS.ui-5A*. Using the UI Platinum (UIP) x SY Capstone (SYC) DH mapping population, the ID team mapped the QTL for SNS on chromosome arm 5AL (*QtSNS.ui-5A*) 639 to 641 Mb. To achieve this, Ph.D. student Meng Su screened 3,615 F_7 plants derived from three HIFs and identified 185 recombinants, which were evaluated for SNS in one dryland and one irrigated location in 2020. To validate the candidate gene region, the 185 pairs of NILs plus parental lines were assessed for SNS, HD, and FD in two dryland environments in Moscow

and in one irrigated environment in Aberdeen in 2021. 115 pairs were also assessed for SNS in two greenhouse experiments. Five candidate genes were selected in the candidate gene interval. Using the Kronos sequenced mutant populations, early stop codon or splicing site mutants were obtained to validate these candidate genes. The ID team obtained CRISPR CAS9 loss-of-function mutants for the *AMY3* gene in the candidate region and is phenotyping them to test if this is the causal gene.

b. Fine-mapping and candidate gene analysis for QTL on 7DS and 7BL. In the UI Platinum x LCS Star population, the ID team mapped a QTL on chromosome arm 7DS associated with grain yield, thousand kernel weight (TKW), SNS, and heading date and published the results in Theoretical and Applied Genetics. *FT-D1* is proposed as a candidate gene with pleiotropic effects on the multiple traits detected in this region. In the same mapping population, the ID team identified a novel QTL and a candidate gene on chromosome arm 7BL associated with grain cadmium content (a manuscript submitted for publication). The ID team performed a whole genome sequencing for the two parents to accelerate the fine-mapping and candidate gene analysis. The ID team also identified a DHL (CddH-266) that has more spikelets per spike than any of the parental lines (25 spikelets). The allele increasing SNS has been introgressed into elite lines in the ID wheat breeding program

Deployment: The ID team introgressed the high-SNS QTL on 4AL and 7AL from UI Platinum into CIMMYT high biomass lines GID431453 and GID3613474. The GID3613474 background looks better than GID431453 and both lines will be submitted to the CIMMYT hub.

KS. Kansas State University. E. Akhunov.

Education: PhD student Elina Adhikari successfully completed her PhD program and is currently a postdoctoral researcher at the Department of Horticulture, University of Wisconsin-Madison, Wisconsin, USA. Bliss Betzen completed his MS program and is currently a Plant Pathologist-Domestic Identifier at USDA APHIS, Manhattan, KS. A new MS student, Dwight Davidson, joined the program in Aug. 2021. Postdoctoral researcher Katherine Jordan involved in WheatCAP joined USDA ARS as Wheat Research Geneticist and continues her collaboration with the WheatCAP on the development of the Wheat Practical Haplotype Graph Database. Postdoctoral researcher Fei He, who was also involved in the WheatCAP, was hired for a faculty position at the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences (Beijing, China), one of the top international research institutes in China.

Research: The research performed at Dr. Akhunov's lab is described in the section describing the genomic resources developed by the WheatCAP (see A.4).

Deployment: Gene editing events for the *TaGW7*, *TaGW2*, *CKX2-1*, and *CKX2-2* genes are being introgressed into three CIMMYT high-biomass lines, but phenotypic evaluation of these introgression lines was delayed due to COVID-19. These mutations are being crossed to generate different combinations. Plants carrying mutations in two different genes have been identified by next-generation sequencing and will be used to investigate the effects of multigene editing on yield component traits in the 2021-2022 season.

KS. USDA-ARS Manhattan. Mary Guttieri

Education: Wardah Mustahsan is a PhD student and has completed her written comprehensive exam. Her target graduation date is August 2022. She made an online poster presentation at the National Association of Plant Breeders entitled “Mapping Wheat Stripe Rust Resistance in Overlay × Overland Population”. She also attended a special training session on SciNet Bioinformatics Training (4/28-4/29/21). The course provided an introduction for newer scinet/ceres users to help them get oriented with HPC, but also covered useful programs and basics of UNIX, HPC, SLURM, Galaxy. Wardah completed an 8-week summer intern experience working in the breeding program of Dr. Guorong Zhang at Hays, KS.

Research: Progeny (n=433) of 10 previously identified recombinant HIF individuals were evaluated, and from these progeny, fourteen pairs of homozygous recombinant/non-recombinant families were identified, and seed was increased. Field trials (10 reps, 2.55 m² plots) were established at both Hays and Ashland Bottoms, KS. The Hays trial was lost to a post-anthesis hail storm. Analysis of the Ashland Bottoms trial demonstrated that a line with a recombination event between 323.6 Mb and 326.2 Mb, at the proximal end of the target region, resulted in a 13.3% yield increase in which the Overland allele was favored.

Six pairs of near-isogenic lines for the target region were also evaluated in yield trials (16 reps) in 2021 at the same trial sites. As in the 2020 trial, HIF family x genotype was highly significant. In those families for which genotype was significant, the Overland allele was favorable by 12.7 to 13.8%. The effect was independent of the allele at *Ppd-D1*. On average, the Overland allele was favorable in the NIL trials by 5%. These results were counter to expectation from previous GWAS studies. However, the potential value of the region has been demonstrated. Additional genomic characterization of the recombinants and NILs is underway to better define the candidate region.

The WheatCAP support also enabled the genetic analysis of adult plant stripe rust resistance in the Overlay x Overland population. Four QTL regions were identified, one of which aligned with the 2N^VS translocation that confers *Yr17* in Overlay. KASP markers were developed for the three remaining QTLs using exome capture resequencing data for Overlay and Overland. Together, the KASP assays explained 55% of the phenotypic variation in field resistance to stripe rust.

Deployment: Introgression of the Overlay allele of the QTL region from KS11WGGRC53-O into CIMMYT lines was completed, with NILs created in the GID:4314513 and PRL/2*Pastor backgrounds. NILs of the 5M^S introgression conferring *Yr40-Lr57* also have been created in the Baj, Borlaug 100, Chipak, Mucuy, and PRL/2*Pastor backgrounds. Seed is under increase for distribution.

MI. Michigan State University. Eric Olson.

Education: MS student, Jonathan Turkus, took a leave of absence from fall 2019 to fall 2020. Jonathan is currently employed as a research assistant at the University of Nebraska. A dissertation has been completed but a Jon has yet to formally defend his thesis. A second MS student, Tommy Reck, began work on the project in January, 2019. Tommy made crosses to introgress the 2DS QTL into CIMMYT backgrounds and participated in all elements of the breeding program. His strong technical background has helped him to acquire a research

assistant position with Corteva in Garden City, KS. Tommy will defend his Thesis in September, 2021. M.S. student Melissa Winchester contributed to the WheatCAP project by analyzing and compiling results for the yield testing done in 2021. Mel defended her thesis on August 16, 2021 and is employed as an assistant grower by Fluresh, a supplier in Grand Rapids, MI.

Research project: The MI project is focused on a QTL for grain yield located on chromosome arm 2DS derived from KS05HW14. A total of 13 recombinants have been identified in the region from 23.3 to 29.4Mb. Recombination events can be divided into four bins across the region. A yield trial was conducted in 2021 to determine the chromosome interval carrying the gene conferring higher grain yield. Lines sampling 13 recombination events were planted in three replicate yield plots at the Saginaw Valley Research and Extension Center along with the recurrent parent KS05HW14 and the local check, Whitetail. Two two-foot sections were cut from each plot. Multiple yield components were evaluated including sample dry weight, grain weight, spikelets per spike and thousand kernel weight and harvest index. Significant variation was identified for all traits except harvest index. All traits were significantly correlated with dry weight and grain weight being the most highly correlated. Grain weight was used as a proxy for grain yield and the gene conferring grain yield in 2DS was assigned to the recombination bin from 24.9Mb to 29.4Mb. A set of 12 F7:8 HIFs were identified to be segregating for this region and will be tested in 2021-22 to refine the interval and identify candidate genes.

In addition to the high-density map, the MI group developed a forward breeding strategy to deploy the D genome introgressions from *Ae. tauschii*. A set of 407 BC₂F₄ lines derived from direct hybridizations between hexaploid wheat and seven *Ae. tauschii* accessions were developed and yield tested across winter wheat growing regions. The highest yielding lines derived from different *Ae. tauschii* donors were intercrossed to develop segregating populations to develop transgressive segregant plants carrying genes from multiple D genome sources. Yield data were used to train genomic selection models and grain yield was predicted among segregating F₂ plants. The F₂ individuals with the highest breeding values were intercrossed to generate a subsequent selection cycle. Segregating populations were advanced in bulk in the greenhouse to the F₄ generation where single F₄ heads were planted in rows in the field for visual selection. Single F₅ plants were selected and grown a second year in single rows. Single rows were harvested and planted in single plots for observation and seed increase for yield testing. A two-rep yield trial was conducted in 2021 at the Saginaw Valley Research and Extension Center. Entries included 19 introgression parental lines, the recurrent parent KS05HW14, three local checks and 91 lines derived from D genome intercrosses. Among the lines tested, 17 yielded significantly higher than the recurrent parent (LSD (0.05) = 6.8 bushels per acre). This result suggests that a combination of direct hybridization, genomic selection and conventional phenotypic selection can be used to pyramid D genome alleles from multiple sources that confer higher grain yield.

MN. University of Minnesota. Jim Anderson.

Education: PhD student Max Fraser successfully completed his written and oral preliminary examinations during the 2020-2021 academic year. In addition to giving an oral presentation at

the 2021 Hard Red Spring Wheat Rust Symposium, he presented a poster at the Minnesota Supercomputing Institute Research Exhibition. Undergraduate student Catherine Li completed her bachelor's degree and is beginning her graduate education at the University of Illinois in Urbana-Champaign.

Research Progress: The MN team continued to work on dissecting two QTL influencing spikelet number per spike, *Qsns.mna.4A.1* and *Qsns.mna.4A.2*. In 2020, 402 F₅ individuals were genotyped for several loci using the mapping population 18X023 (MN10201-4-116/Shelly). This population segregates for *Rht24* and for *bdv2* (resistance to Barley Yellow Dwarf Virus) in addition to *Qsns.mna.4A.1* and *Qsns.mna.4A.2*. Recombinant individuals were not found for the QTL on chromosome 4A. However, a single F₅ individual was discovered with a recombination event within the *Rht24* region. A heterogeneous inbred family derived from this individual is in development with the long-term goal of fine mapping *Rht24*. Further screening of progeny from heterozygous F₅ individuals will continue in the fall of 2021. 258 F_{5:6} RILs from 18X023 were phenotyped for height and maturity in a field nursery in St. Paul in 2021. Additionally, the population will be evaluated for kernel traits using the MARVIN seed analyzer in the fall of 2021. Height data will be used to validate the markers for *Rht24*. The population has been also genotyped for *bdv2* and a subset of 97 RILs were phenotyped by the University of Illinois in summer 2021 to gain a better understanding of the effects of *bdv2* on BYDV in an inoculated field environment.

In addition, PhD student Max Fraser has been leading efforts with Dr. Matthew Rouse from the USDA Cereal Disease Lab to map and characterize a novel stem rust resistance QTL from a wild relative of wheat. Using population (15xR012) developed from a cross between a UMN hard red spring wheat line (MN07098-6) and a *Triticum turanicum* accession (CItr 11390; 'Sun Ray'), The MN group identified one QTL conferring adult plant resistance to the Ug99 rust family and another impacting all-stage resistance to the Digalu race. 15XR012 was also found to segregate for seed length. Mapping for this trait, as well as other yield components is also underway.

Deployment: Crossing between University of Minnesota and CIMMYT high-biomass lines exhibited hybrid necrosis, so the recurrent parent was changed to 'Kingbird'. Successful backcrosses of *Rht24*-containing germplasm into Kingbird were made during the fall 2020 and spring 2021 greenhouse seasons. Using speed breeding techniques, NILs should be ready to send to Mexico for the 2022 season. The MN group published three manuscripts in 2021.

MT. Montana State University. Jason Cook

Education: PhD student Brittney Brewer-Jones graduated with her PhD in plant breeding in November 2020. Brittney now works for Bayer Crops Science as a soybean breeder. Mei Ling Wong, MS student, temporarily took over the phenotyping portion of Brittney's gene cloning project during the spring semester 2021. A new PhD student, Jared Lile, started his graduate program during the 2021 summer semester and is now working on the gene-cloning project. Jared has also participated in online plant breeding short courses hosted by the UC-Davis Plant Breeding Academy and the University of Minnesota.

Research project: The MT researchers are fine mapping a QTL, designated *QTn.mst-6B*, for early tiller number (at Zadoks stage 31) located on chromosome 6B. Brittney identified heterozygous inbred families (HIFs) from heterozygous F₅ individuals derived from biparental crosses between Reeder x Choteau and Vida x McNeal. Reeder and Vida possess the high *QTn.mst-6B* tillering allele. Population sizes of the HIF families are 540 and 556 F₂ lines from the Reeder/Choteau and Vida/McNeal crosses, respectively. Genotyping has been completed using SNPs and markers developed from the 2018 WheatCAP Exome Capture Assay. Phenotyping of the HIFs has been done in the greenhouse. Brittney was able to fine-map the QTL down to a 226,868,798 - 247,209,591 bp interval on the short arm of chromosome 6B (IWGSC RefSeq v1.0). This region contains approximately 120 high confidence gene models. Before graduating, Brittney published three manuscripts and will be a co-author on a future fine-mapping manuscript.

For the remainder of the grant, additional recombinant lines will be phenotyped to reduce the size of the QTL interval. Jared is also analyzing RNA-seq data obtained from tiller bud and stem axis tissue collected from NILs with the different alleles. The goal is to identify differentially expressed genes in the candidate region that correspond to tiller number variation.

Deployment: The MT team completed successfully the introgression of the *QTn.mst-6B* into Montana adapted varieties McNeal and Duclair as well as CIMMYT high yield lines BAJ #1, and Kingbird. Grains for the last two were sent to CIMMYT for testing in 2020 and encouraging results were received in 2021 (see CIMMYT report).

NC. USDA-ARS Raleigh. Gina Brown-Guedira

Education: Graduate student Noah DeWitt has completed seven semesters of classes and was selected to participate in the USDA-ARS pathways program that prepares students for a career with the agency. Noah presented his research work to examine pleiotropic effects of QTL for yield component traits and differential effects of QTL across environments using the LA95135 x SS-MPV57 (LM) mapping population at the ASA-CSSA annual meetings. He published a manuscript describing an oligogenic trait architecture of plant height and heading date in the LM biparental population in BMC Genomics. Noah is submitting an additional manuscript using structural equation modeling (SEM) to dissect effects of genes underlying component traits on spike yield across environments. This work demonstrated that variations in QTL effects across environments resulted from variable direct effects on phenotypes and environment-by-environment differences in the observed pleiotropic effects on component traits, contributing to a conceptual model for yield genotype-by-environment interactions in wheat.

Graduate student Daniela Miller completed five semesters of classes toward her plant breeding degree while also pursuing a minor through the statistics department. Daniela presented her research work to examine epicuticular wax in wheat using the LA95135 x AGS200 mapping population to peers in plant breeding at NCSU.

Research: The NC team completed the cloning and validation of their initial WheatCAP target for the gene controlling awn suppression. The NC PhD student Noah DeWitt identified the underlying gene *AWNS1*, characterized its effect on grain yield and published the results in 2020

in *New Phytologist*, 225:326-339. He recently published a second manuscript in *BMC Genomics* 2021, 22:1-8 describing the oligogenic trait architecture of plant height and heading date in the LM biparental population. The manuscript included a longitudinal data analysis of QTL effects on plant growth over time that will be applied to future work involving high-throughput phenotyping of plant height and biomass over time. In identifying a handful of genes that explain most of the additive genetic variation in this population, and are sufficient for prediction, the manuscript supports the WheatCAP grant approach of identification and marker development for genes underlying traits.

Noah is collaborating with breeders at Virginia Tech, Clemson, and UGA to study the efficacy of breeding program designs that leverage genomic selection in the early-generation stage. Random genotypes selected out of five SunRIL biparental populations were planted in a partially replicated design in four locations, similar to that of an observation yield test. Genomic data was used to perform spatial corrections despite absence of checks or blocking, and to predict performance of lines in the biparental populations, including unobserved lines. Using a leave-one-environment-out approach where lines unique to a location were predicted using data from full-sibs at all other locations, correlations (r) between observed and predicted grain yield of 0.72, 0.48, and 0.40 and 0.18 were observed for Kinston, NC, Plains, GA, Warsaw, VA, and Florence, SC, respectively. Correlations tracked with the observed heritability of grain yield at each location; in particular, heritability was close to zero at the Florence, SC location that experienced severe drought prior to and during grain fill. Unobserved full-sib and half-sib lines selected using the biparental population models will be planted alongside lines selected with genomic selection models based on historical data to assess the relative efficacy of the two GS paradigms in a “real-world” situation.

Graduate student Daniela Miller is conducting studies on glaucousness of wheat spikes and flag leaves. The AGS2000 x LA95125 mapping population, comprised of 293 recombinant inbred lines, was planted and scored visually over two growing seasons in Raleigh, NC. A linkage map with 26 linkage groups was generated having 3,408 markers using the R ASMap and QTL packages. For spike glaucousness, two major QTL were detected; one associated with the 1RS:1BL chromosome translocation and another with a peak near 735 Mbp on chromosome 2A. For flag leaves, one major QTL was detected near 604 Mbp on chromosome 3A. A second mapping population is being used to support these results, the Hilliard x GA06493-13LE6 population with 189 recombinant inbred lines (linkage map in construction). A GWAS analysis of 286 diverse elite lines confirmed the significant locus for flag leaf glaucousness on chromosome 3A. RNAseq analyses of different tissue types are in progress to help target the causal genes underlying these significant genomic regions.

A novel plant height gene on chromosome 3D, *Qncb.PH.3D*, was validated in field trials using NILs derived from heterozygous LA95135 x SS-MPV57 (LM) F5-derived lines. NILs planted in the field at two NC locations were able to confirm the plant height QTL and data are being analyzed to evaluate kernel length. In the bi-parental population, the LA95135 allele at this QTL has large, stable positive effects on kernel length, and variable negative pleiotropic effects on SPS and kernels per spikelet. A high-throughput KASP design pipeline has allowed for the development of over forty KASP markers on chromosome 3D used to screen this population.

Analysis of the QTL for kernel weight and plant height on chromosome 6A continued. GBS data were obtained for 5,664 RIL derived from a series of crosses involving eastern SRWW cultivars and breeding lines (referred to as SunRIL populations). A subset of 699 individuals from four families were grown at Raleigh, NC during 2020 and a QTL identified for plant height on chromosome 6A. Interestingly, when these results were compared with results from the original populations derived from crosses between Massey x SS-MPV57 and LA95135 x SS-MPV57, the NC group concluded that LA95135, AGS2000 and others SunRIL parents possess a gene for reduced height on 6A that differs from the gene/allele present in SS-MPV57. This is supported by different marker haplotypes in the region. A total of 3238 SunRIL entries derived from 22 crosses were planted in head rows at locations in NC, VA, GA, and SC during the 2020-2021 growing season and data obtained for plant height and heading date are being analyzed to determine the relative effect of these genes in different backgrounds and environments.

ND. USDA-ARS Fargo. Justin Faris

Education: PhD student Amanda Peters Haugrud completed her fourth year as a WheatCAP-funded student, and she has set a tentative graduation date of 12/31/2021. She has completed all her coursework as well as her written and oral preliminary exams, and she is currently writing her dissertation and several manuscripts for publication. Before the pandemic, Amanda attended the WheatCAP workshop and the Plant and Animal Genome Conference in San Diego, CA where she presented a poster on her research.

Katherine Running is an additional WheatCAP PhD student working on the evaluation of the genetics of seed size in durum wheat. Katherine's major at NDSU is Genomics and Bioinformatics and she has completed most of her courses with a tentative graduation date set for 05/14/2022. Before the pandemic, Katherine attended the WheatCAP workshop and the Plant and Animal Genome Conference in San Diego, CA where she presented a poster on her research. Both Katherine and Amanda have been actively involved all WheatCAP student activities, meetings, and workshops.

Research Projects: The group at Fargo is focused on a QTL for kernels per spike (KPS) on chromosome 2BL in durum wheat. The 2BL QTL was first mapped in the RIL population BP025, which was derived from crossing Ben (*Triticum durum*) with the cultivated emmer accession PI 41025 (*T. dicoccon*), grown under greenhouse conditions. A field experiment conducted in the summer of 2017 showed the QTL was expressed under field conditions as well. This QTL spans a 38 Mb region at 445 Mb to 483 Mb on the Svevo reference sequence. Two RILs containing PI 41025 alleles in the QTL region were backcrossed to Ben so that the QTL can be studied and validated in isolation. Previously, the ND group screened BC₃F₂ plants with two flanking markers about 7 cM on either side of the peak QTL marker region, identifying 108 recombinants. They selected homozygous recombinant BC₃F₃ individuals from these plants, which were genotyped with 37 newly developed markers between our flanking markers. BC₃F₄ plants were phenotyped in a replicated trial and used to reduce the QTL region. They evaluated more BC₃F₂ plants with the flanking markers Chr2B446082351 and Chr2B538239714 to identify 76 recombinants from 1556 gametes. They selected homozygous recombinant BC₃F₃ individuals from these recombinants and are currently genotyping them with internal KASP markers.

Additionally, the Fargo group has been introgressing this 2BL QTL into the CIMMYT line GID4878569 and are currently at the third backcross.

Additional projects of the Fargo group related to the WheatCAP objectives include mapping yield component and seed morphology traits in three RIL populations derived from crossing different durum varieties with cultivated emmer accessions, and also evaluating seed size and morphology in the global durum panel (GDP). For the durum \times emmer RIL populations, the Ben \times PI 41025 population was evaluated under field conditions the summers of 2017, 2018, and 2019. The Rusty \times PI 193993 population was evaluated under field conditions the summers of 2018, 2019, and 2020. These two populations were previously studied under greenhouse conditions as well. The third population, Divide \times PI 272527, was evaluated under field conditions the summers of 2017, 2019, and 2020. Additionally, they evaluated this population under greenhouse conditions during the winters of 2019 and 2020. For all three populations under field and greenhouse conditions, they measured and performed QTL analysis for the following traits: days to heading, plant height, spikelets per spike, kernels per spike, grain weight per spike, thousand kernel weight, along with the grain dimension parameters including length, width, area, circularity, and length/width ratio. They are currently preparing two manuscripts for publication to describe the results of this research.

For the evaluation of the GDP, the panel was grown in three replications in the field near Casselton, ND in the summer of 2020 and evaluated for heading date and plant height. GWAS analysis of plant height identified one significant association on chromosome 5A. Three significant associations were identified for heading date on chromosomes 5A, 6A, and 7A. Other traits including spike length, spikelets per spike, thousand kernel weight, and grain weight, number, length, width, and area will be collected after harvest. The GDP trial will be repeated in the field for two more seasons.

NY. Cornell University. Mark Sorrells

Education: Nicholas Santantonio graduated in May 2019 and after a one-year post doc with Kelly Robbins, was hired by Virginia Tech as assistant professor to replace Carl Griffey on the small grains breeding program. Daniel Sweeney graduated in May 2021 and was hired by the Weaver Popcorn Hybrid Seed Company. He received several awards and gave invited talks at the John Innes Center, Norwich, UK and at the International Barley Improvement Conference. Shitaye Megerssa graduated in May 2021 and was hired by the Ethiopian Institute for Agricultural Research as a durum wheat breeder. Ellie Taagen is entering the 5th year of her PhD and is on track to graduate May 2022. She is currently a full-time intern as a data scientist with Bayer and will return to her dissertation research in December 2021. She will present an IWGSC webinar on her structural variation research in November 2021. She received a 2021 Women in Triticum early career award from the BGRI to attend the 2022 International Wheat Congress.

Research: The Cornell Small Grains research program culminated investigation of a causal variant on chromosome 5A in the W7984 \times Opata M85 HIFs and identified nine additional candidate genes for grain weight and morphology on the group 5 chromosome short arms that could be pursued collaboratively in a future renewal of the WheatCAP. Graduate student Ellie

Taagen has led these research efforts, and her findings present important lessons for the future of positional cloning in polyploid crops.

Genomic structural variations are common among polyploids, but SNP-based approaches to characterize genetic diversity are frequently insensitive to these variants. Since our last report, Ellie determined that a large deletion on wheat chromosome arm 5AS was misidentified as QTL harboring a causal gene for increased grain weight. Leveraging heterogeneous inbred families (>5,000 plants, 129 informative recombinants), genomic data (parent exome and regulatory capture, and 31 KASP markers spanning the QTL), phenotypic associations (heading date, grain fill duration, plant height, spikelets per spike, thousand grain weight, grain length, and grain width), early grain development transcriptome profiles (4 and 8 days post anthesis), and predicted gene function, the Cornell group determined the QTL was a result of strong linkage disequilibrium with chromosome arm 5AS presence or absence ($r^2 = 0.91$). These findings highlight that chromosome structural variation linkages can overpower the considerable resources required for positional cloning, and they developed the following recommendations for more successful approaches to identifying causal variants (see Taagen et al. 2021 for further details): i) Move from a SNP to haplotype-based approach to identify genetic diversity. ii) Invest in sequencing to detect structural variants. iii) Use the transcriptome to identify candidate genes. iv) Traits with broad overlapping QTL may not be pleiotropic. They also identified nine candidate genes based on chromosome arm 5AS presence or absence that may impact yield components, laying the foundation for identifying hidden variation of homoeolog dosage-dependent and functionally redundant genes on the group five chromosome short arms (Taagen et al. 2021). All analysis conducted in Taagen et al. 2021 is reproducible and publicly available as a learning resource on GitHub: https://github.com/etaagen/Taagen_2021_TPG.git

Due to COVID-19 regulations and personnel availability, the cloning of a QTL for grain length and grain weight on chromosome 2D has been placed on hold. Approximately 700 F6:3 HIF lines and 15 KASP markers within the 20 Mbp interval are available for fine mapping and cloning the causal variant upon renewal of the WheatCAP.

OK. Oklahoma State University. Liuling Yan and Brett Carver

Education: Forrest C.C. Kan completed his Ph.D. on gene mapping on chromosome 1BS in the Duster x Billing population and graduated in December, 2019. He published his results as first author in Molecular Breeding. He was hired as a postdoc in Texas A&M University. Xiaoyu Zhang, the Ph.D. student working on gene mapping on chromosome 7BL, graduated in December, 2019 and returned to China. Two new MS students have been incorporated to the WheatCAP project. Abby Tucker has part of her thesis research dedicated to field-validating the direct and indirect effects of the major gene on chromosome 7BL for spikelet development across multiple genetic backgrounds with Billings as the common ancestor. M.S. student Jennifer Tapia was assigned to work on the identification of the major gene on chromosome 1BS for grain yield in winter wheat cultivar ‘Duster’ and to collaborate in the 7BL QTL project.

Research projects: The OK team is pursuing several projects in parallel. The first project is focused on the identification of a major gene for grain yield on chromosome 1BS in winter wheat cultivar ‘Duster’. While they wait for the development of new populations in the *ph1b*

background to overcome the extremely low recombination rate observed in the candidate gene region, the OK team has made significant progress in the utilization of this QTL in wheat breeding by using three KASP markers to introduce the entire *QYld.osu-1BS* region into breeding lines and cultivar ‘Duster’ as the donor parent. They published a manuscript in the Journal of Plant Registrations.

The second project is focused on the identification of a major gene on chromosome 7BL controlling SNS and spikelet development. The OK team cloned a gene on the QTL region on 7BL from C1tr17600 that promotes spike development and increases grain yield. They have conducted field experiments on its effects on spike development and grain yield for two years. A revised manuscript was resubmitted in August 2021. The favorable allele for higher yield was found in the winter wheat cultivar ‘Billings’ and OK1059018. They used molecular markers to accelerate deployment of this gene in numerous breeding lines/populations.

Deployment: The favorable allele for *QSns.osu-7B* from C1tr17600 was crossed with two CIMMYT high-biomass lines (GID: 4577963 and GID: 4314513). The experiments have advanced to BC₂F₃ generation. The favorable allele from *QYld.osu-1B* was backcrossed into six high-yielding lines (NADI, CHIPAK, BONSU, BORLAUG100, KENYA SUNBIRD, KACHU#1) and one high-biomass line (GID:4314513) (PI683503). The introgressions have been delayed by the loss of multiple crossing lines by viruses during a greenhouse increase.

SD. South Dakota State University. Sunish K. Sehgal.

Education: Two graduate students (Ph.D.) were trained under WheatCAP. Jyotirmoy Halder is working on the fine mapping, cloning, and deployment of the yield QTL and GWAS for kernel and spike traits. He has finished all the necessary coursework, comprehensive written and oral examinations. He is writing his dissertation and is on track to graduate in Fall 2021.

Harsimardeep Gill is partly supported by WheatCAP and is working on developing genomic selection models for various economic traits in the SDSU winter wheat breeding program. In addition, two undergraduate students were trained in wheat breeding during this period and were closely involved in the project focused on mapping and prediction of grain yield-related traits.

Research: The SD team targeted grain yield QTL mapped in the distal 6-16 Mb on the short arm of chromosome 7DS. The team screened more than 2,500 progenies with 15 codominant markers and identified 36 recombinant plants. Thirty-six recombinants (8 haplotypes) were evaluated as single 1m rows at two locations in South Dakota in 2020. A single location yield trial was conducted in 2021 where yield and yield components were recorded. Variation for grain yield among the haplotype groups was not significant based on four replications (1 m rows) at the Brookings location. However, yield contributing trait, SNS data showed significant variations among the haplotypes. Using this trait, the candidate region was reduced to 6 Mb (6 to 11 Mb). Multi-location yield trials will be planted in 2021-22 to generate more data to further fine map the QTL region.

The SD team also conducted genome-wide association and genomic prediction for spike and kernel traits in hard winter wheat. In this study, the hard winter wheat association mapping panel (HWWAMP) was evaluated in replicated trials at three locations in South Dakota. GWAS was

conducted using 15,590 SNPs and BLUE values of various spike and kernel traits data with FarmCPU algorithm revealed a total of 53 significant ($P < 0.001$) marker-trait associations (MTAs). Other than GWAS, the predictive abilities of two different genomic selection (GS) models were compared in this study. The result showed that the bayesian multi-trait multi-environment (MTME) outperformed the single-trait model (GBLUP) for all the traits studied in all three environments. A separate multi-trait multi-environment genomic prediction of agronomic traits using 314 advanced and elite breeding lines of hard winter wheat evaluated in 10 site-year environments was also evaluated. The multi-trait (MT-CV2) model outperformed all the other models for predicting grain yield with significant improvement in PA over the single-trait (ST-CV1) model. The MTME model performed better for all traits, with average improvement over the ST-CV1 reaching up to 19, 71, 17, 48, and 51% for grain yield, grain protein content, test weight, plant height, and days to heading, respectively. Overall, the empirical analyses elucidate the potential of the MT-CV2 and MTME models when advanced breeding lines are used as a training population to predict related preliminary breeding lines.

Deployment: The DNAM donor line was crossed with CIMMYT lines (GID:3613474 and Kingbird) and flanking markers were used to select for the homozygous *Ae. tauschii* 7D region. BC₂F₄ plants are growing and a limited number of seeds will be available in fall 2021.

TX. Texas A&M. Shuyu Liu, Amir Ibrahim, Jackie Rudd

Education: *Smit Dhakal* finished his Ph.D. in Dec of 2018, and he is currently a wheat breeder in a private company. He published one paper as first author and one as co-author. *Jorge Valenzuela Antelo* completed his PhD in the fall of 2020 and took a job as a breeder at Bayer CropScience in Mexico. He presented at the meetings of ASA-CSSA-SSSA in 2019, Texas Plant Protection Association annual meeting in 12/2019 (got 2nd poster award), the WheatCAP annual meeting and soft skill training at San Diego 1/ 2020, and Corteva sponsored TAMU Plant Breeding Symposium in 2/2020 as a student speaker. *Zhen Wang* joined TAMU in August of 2019. He participated in the WheatCAP annual meeting and soft skill training at San Diego in January of 2020. Zhen presented in the NAPB meeting in 8/2020 for genomic prediction and presented at ASA-CSSA-SSSA meeting in November of 2020 for yield component mapping. He published two papers as a co-author. M.S. student *Mustafa Cerit* works on a TAM 113/Gallagher population for yield components and summarizes the kernel traits for additional mapping populations, TAM 204/IBA and TAM 111/TX05A001822. Ph.D. student *Kyle Parker* joined TAMU in the fall of 2020 and is working on the genomic prediction/selection for wheat breeding and joined training from WheatCAP. Previous WheatCAP postdoc, *Chenggen Chu*, moved to a USDA-ARS geneticist position in Fargo, ND in February of 2020.

Research: For the positional cloning of the 2BS QTL for grain weight, 23 KASP markers from 90K array and exome capture were employed to genotype HIFs in the QTL region (54.44 - 97.1Mbp of RefV2, cross CO960293-2/TAM 111) and identified 39 heterozygous plants for developing HIFs. From 13 of these HIFs, the TX group selected 116 plants with homozygous genotypes in the QTL region for comparing kernel weight and used 45 heterozygous plants to produce recombinants in the next generation to narrow down the QTL region. Total yield and biomass for yield components were evaluated following the 2021 harvest and significant

differences in TKW were observed. TKW showed a high correlation with kernel width ($R = 0.94$). Crucial recombination events at the interval between IWB4154 (60 Mbp, RefV2) and K2BS653 (72.9 Mb, RefV2) showed significant TKW and kernel width differences, which narrowed down the target QTL region to a 12.9 Mbp interval. The two flanking markers will be used to screen more F9 progenies to find new recombinants within the target region. In the fall of 2021, we will plant in plots for four NILs from CT200-15 and eight NILs from CT200-50 NILs to further confirm and validate the 2BS TKW QTL interval. New NILs will be planted as headrows in the GH and/or field and new HIFLs will be screened with markers to narrow down the target QTL region.

In addition to the positional cloning project, Shuyu Liu, Jackie Rudd, and Amir Ibrahim evaluated a mapping population of 124 recombinant inbred lines (RILs) from the cross TAM 112/TAM 111. Yield and agronomic traits from 28 environments, yield components from 11 environments, and end-use quality from 7 environments were collected and analyzed. Genetic maps were constructed from 5840 SNPs and three manuscripts were published. They tested a nested association panel of 298 synthetic-derived BC1F7 lines from a set of 20 synthetics backcrossed with TAM 111 or TAM 112. Data were collected on yield and agronomic traits from 17 environments, yield components from 9 environments, and end-use quality from 7 environments. Significant SNP-trait associations have been identified for all the traits using 100K SNPs from whole genome genotyping-by-sequencing. Two manuscripts are in preparation. Finally, they tested a bi-parental mapping population from TAM 111/TX05A001822 including 179 RILs. Yield and agronomic traits were collected in 6 environments, yield components and end-use quality were analyzed in 3 environments. A set of 3000 SNPs was used for genetic maps and QTL analyzes. One manuscript is under preparation.

Deployment: BC₃F₁ plants having the TAM 111 kernel weight increasing loci confirmed with the linked SNPs were developed for doubled haploid (DH). There were sets of 65, 33 and 47 plants derived from 3 CIMMYT lines, GID3855011, GID4314513, and GID4577963. Plants from the DH pipeline are growing in the growth chamber and greenhouse now. Seeds from fertile plants were harvested and will be validated using linked SNPs to confirm the QTL introgression.

WA. Washington State University. Mike Pumphrey & Arron Carter

Education: *Samuel Prather* will be completing his Ph.D. in Fall 2021, and has already interviewed for a private-sector plant breeder position. He has participated in numerous conferences including a quantitative genetics symposium, Borlaug Global Rust Initiative conference, University of Minnesota workshop courses, and Wheat-CAP opportunities. *Peter Schmuker* started his Ph.D. program in Fall 2020, and has progressed well despite COVID-19-associated limitations. He has also participated in numerous virtual regional, national, and international conferences, specialized phenomics and genomics training sessions, and farmer field days. Post-doc *Tyson Keopke* has focused his work on fine-mapping and genomic analysis. He has participated in most of the same training and conferences as the Ph.D. students.

Research Project: The WSU team is focused on a strong QTL on chromosome arm 4AL for kernel weight identified in the cross between elite spring cultivars Kelse and Scarlett. Kelse contributes the positive allele for kernel number and Scarlett the positive allele for kernel weight. The WSU team screened 2,000-progeny from two selected HIFs and 119 recombination events were identified. Families segregating for the Kelse and Scarlet alleles across the region of interest were identified from two heterozygous source plants (52 and 235). 28 of these families representing the various haplotypes across the QTL region were grown in the greenhouse in fall of 2019 where heading date, head length, seeds/head, and seed weight were analyzed. Seed from these greenhouse plants were grown under field conditions as paired rows in 2020. Analysis of heading date from both the greenhouse (fall 2019) and field (2020) demonstrated roughly 3 days difference in heading across families from each heterozygous source (52 and 235). Analysis of the genetic component of heading date showed the region between markers IWB4308 and IWB57074 to be more impactful on heading date. This reduces the region to ~15Mb (9cM). Similar analyses are being conducted on head length, seeds/head, and seed weight.

To dissect the 15 Mb subregion, 37 families from one heterozygous source (52) were planted in the greenhouse. Seed from these families were planted in a replicated, paired row field trial in 2021. The heading date data revealed 5 days difference and data from the other traits will be collected and analyzed. The WSU team continue to analyze the genomes from the 10+ wheat genomes project and other genomic data to fine map and identify candidate genes in the region. An inversion has been confirmed relative to the Chinese Spring reference genome, and they have shifted to using other reference genomes with the same orientation as Kelse and Scarlet.

Deployment. CIMMYT germplasm was used as the recurrent parent to generate backcrossed-derived near-isolines with both Kelse and Scarlet alleles. These lines were completed in 2020, and increased in the greenhouse. They also produced sufficient seed to conduct a field-based split-plot yield trial of these backcross NIL families in 2021 in WA, which have been harvested and data analysis is pending. Seeds of these lines were sent to CIMMYT in October 2021.

A. 8. Summary of WheatCAP varieties and germplasm releases

The WheatCAP group released 19 new commercial varieties and six improved wheat germplasms (Appendix 1). Twelve varieties reported in previous years were assigned PVP in 2021 (Appendix 1). During the five years of its duration the WheatCAP project released 98 wheat varieties, 24 germplasm and 23 mapping populations. The complete list of released varieties, germplasm and populations is presented in Appendix 1 at the end of this report. By releasing improved wheat varieties in all market classes grown in the US, the WheatCAP group continues to provide a direct contribution to the US economy. Based on a 2017 survey, the wheat varieties developed by the US wheat breeders and researchers covered 57% of the US wheat acreage and contributed \$ 4.3 billion in production value. This value is amplified several times through the milling and baking industries, which contribute additional jobs and value to the economy.

A. 9. Summary of WheatCAP publications and identified genes

A.9.1. Publications. In 2021, WheatCAP participants published 45 peer-reviewed publications acknowledging the USDA-NIFA support for the WheatCAP. During the five years of its duration the WheatCAP researchers published 237 peer reviewed papers. The complete list of peer-reviewed publications is presented in Appendix 2 at the end of this report. The WheatCAP publications have been cross-referenced 3,857 times in Google Scholar (10/10/2021) in spite of the short term since their publication. A similar analysis of the publications generated by the previous T-CAP project (2011-2016) indicates 20,517 cross-references in Google scholar until 2021, documenting the high-impact of the publications generated by this group.

<https://www.triticeaecap.org/publications-and-germplasm/>

A.9.2. Cloned genes. The list of genes identified during the 5 years of the WheatCAP project includes five stem rust resistance genes (*Sr13*, *Sr21*, *Sr60*, *Sr22b* and *Sr9e*), the awns suppressor (*AWNS1*), five genes affecting grain size (*GW2*, *GW7*, *GS3*, *CKX2-1*, *CKX2-2*) and five genes affecting the number of spikelets per spike (*WAP0-A1*, *LFY*, *SVPI*, *VRT2*, *FUL2*).

B. Budget

In 2021, UC Davis completed the subcontracts for the 19 institutions on time and the funding was available to all collaborators in early 2021. UC Davis is now closing all the subcontracts.

C. Deliverables & Other Outputs:

- **Appendix 1.** Germplasm releases
- **Appendix 2.** Publications
- **Appendix 3.** Community resources
- **Appendix 4.** Graduate students.
- **Appendix 5.** Education Survey.

D. Concluding remarks

In 2021, WheatCAP researchers had a very productive year that resulted in new knowledge (45 peer-reviewed publications) and improved wheat varieties (19) with increased yield, resistance to pathogens and/or improved quality. The improved disease resistance of the new varieties will reduce the applications of fungicides, directly benefiting the population and the environment. The increased productivity of these varieties will maintain the USA wheat growers' competitive advantage in international markets, whereas their improved quality will benefit consumers directly and contribute to the competitiveness of the USA wheat industry. Wheat varieties developed by this group of public breeders cover 57% of the US wheat acreage and contribute \$4.3 billion in direct production value (2017 survey).

The genomic tools and databases generated by the WheatCAP team continue to accelerate breeding cycles and the pace of new discoveries. Sixteen new genes have been cloned and validated during the project.

One of the most important contributions of WheatCAP is the coordination among the major wheat breeding and research programs in the country, which eliminates unnecessary duplication and generates positive and synergistic collaborations.

Finally, the training of a new generation of plant breeders guarantees the continuity of the US agricultural enterprise into the future.

None of this would have been possible without this integrated Collaborative Agricultural Project, and the wheat community is grateful for this support.

APPENDIX 1. Varieties and Germplasm releases 2021.

<https://www.triticeacap.org/publications-and-germplasm/>

2021 New Commercial Wheat Varieties

1. **‘Uncharted’** (OK16D101089, PVP 202100241, submitted 3/17/2021) is the result of a long-term, marker-driven effort since 2014 to maximize the frequency of *Bdv1+Bdv2* genotypes. No other currently available hard red winter (HRW) cultivar features this dual genotype or immunity to barley yellow dwarf in the southern Plains. Additionally, Uncharted confers resistance to stripe rust with *Yr17* as the foundation, and to leaf rust and powdery mildew.
2. **‘Strad CL Plus’** (OK12912C-138407-2, PVP 202100242, submitted 3/17/2021). Sharing more than 75% of its parentage with Doublestop CL Plus, Strad CL Plus features a similar agronomic and end-use quality package, but provides earlier maturity, improved resistance to stripe rust and leaf rust, and slightly higher yield potential in a HRW background.
3. **‘Big Country’** (OCW04S717T-6W, PVP 202100243, submitted 3/17/2021). This HW cultivar is positioned for an area of the southern and central Plains where most HW cultivars are not well adapted – central and eastern Oklahoma and northward into Kansas. This adaptation pattern is supported by excellent resistance to leaf rust, stripe rust, stem rust, powdery mildew, tan spot, Septoria leaf blotch, and wheat soilborne mosaic/wheat spindle streak mosaic complex. Big Country is susceptible to barley yellow dwarf.
4. **‘Butler’s Gold’** (OK14124-2, PVP 202100244, submitted 3/17/2021) has a reduced vernalization requirement and early maturity per se, this HRW cultivar is specifically targeted toward late-planted systems, either by design to circumvent fall weed infestations or as consequence of season-disrupting weather conditions. Butler’s Gold is expected to appeal to a non-commodity wheat marketing system with its unusually high protein content, high water absorption, and exceptional baking quality.
5. **‘Breakthrough’** (OK168512, PVP 202100245, submitted 3/17/2021) was part of a long-term, marker-driven effort since 2014 to distribute *Wsm1* throughout the OSU wheat improvement program. Breakthrough confers a highly effective level of resistance to wheat streak mosaic and to Triticum mosaic, with best adaptation to the High Plains. As its name suggests, Breakthrough presents a unique yielding ability and end-use quality in the presence of *Wsm1* in a HRW background.
6. **‘Balance’** (PVP 02100490, submitted 8/31/21) is a HRW wheat released by WSU for its excellent tolerance to low pH soils, short plant stature, and high grain protein content. It is resistant to stripe rust, of which the resistance is uncharacterized. Balance carries the *Rht-B1b* allele for dwarfing and the *Dx5+Dy10* alleles at Glu-D1.
7. **‘Piranha CL+’** (PVP 202100485, submitted 8/26/21) is a soft white winter wheat cultivar released by WSU with two-gene resistance to Imazamox. It also carries the *Pch1*, *Yr17*, and *Lr68* genes for disease resistance. Piranha CL+ also has tolerance to snow mold and *Cephalosporium* stripe. Piranha CL+ has the *Rht-B1b* dwarfing allele and carries the *Ax2** allele at Glu-A1 and the *Dx2+Dy12* alleles at Glu-D1. It is broadly adapted across the Pacific Northwest and has very good end-use quality.

8. **‘Sockeye CL+’** (PVP 202100491 submitted 8/31/21) is a soft white winter wheat cultivar released by WSU with two-gene resistance to Imazamox. It also carries the *Yr17* and *Lr68* gene for disease resistance. Sockeye CL+ also has tolerance to snow mold and *Cephalosporium* stripe. Sockeye CL+ has the *Rht-B1b* dwarfing allele and carries the *Ax1* allele at Glu-A1 and the *Dx2+Dy12* alleles at Glu-D1. It is broadly adapted across the Pacific Northwest and has excellent end-use quality.
9. **‘AR09137UC-17-2’** (PVP pending submission) is a common soft red winter wheat variety released by the Board of Trustees of the University of Arkansas in 2021.
10. **‘Steamboat’** (CO15D098R = TAM 114/Antero//Byrd) (PVP pending submission) is a HRW wheat, released by Colorado State University in 2020.
11. **‘Amplify SF’** (Bearpaw/Antero//Antero), a HRW wheat, released by Colorado State University in 2021. Has the semi-solid-stemmed trait for partial resistance to wheat stem sawfly. PVP not yet submitted.
12. **‘CO16SF070’** (Antero/Judee//Antero), a HRW winter wheat, released by Colorado State University in 2021. Has the semi-solid-stemmed trait for partial resistance to wheat stem sawfly. PVP not yet submitted.
13. **‘IDO1804’** (PVP pending) HWS wheat developed by University of Idaho Wheat Breeding and Genetics program. IDO1804 has high grain yield, excellent bread-baking quality, and good resistance to stripe rust. IDO1804 has desirable productive tillers and grain numbers contributing to higher grain yield.
14. **‘IDO2002S’** (PVP pending) HWS wheat developed by University of Idaho Wheat Breeding and Genetics program. IDO2002S has high grain yield, excellent bread-baking quality, and good resistance to stripe rust. IDO2002S was selected from the CAP mapping population and has desirable glutenin subunits contributing to good bread-baking quality.
15. **‘MI16R0906’** (licensed without PVP) is a new SRW wheat variety developed by Michigan State University Wheat Breeding and Genetics. This variety is ideal for production in Michigan and the Midwest with high yield potential and excellent milling and baking quality. ‘MI16R0906’ has the fourth highest two-year grain yield average of soft red winter wheat lines tested in Michigan from 2020 to 2021. ‘MI16R0906’ has milling and baking quality that meet the needs of the soft wheat industry.
16. **‘MI17R0357’** (licensed without PVP) is a new SRW wheat variety developed by Michigan State University Wheat Breeding and Genetics. This variety is ideal for production in Michigan with high yield potential and excellent milling and baking quality. MI17R0357 has the highest two-year grain yield average of SRW wheat lines tested in commercial yield trials in Michigan. MI17R0357 Flowering date is earlier than average and is well adapted to Michigan environments. MI17R0357 has milling and baking quality that meet the needs of the soft wheat industry.
17. **‘SD Midland’** (PVP application to be submitted in 2022) is a HRW wheat cultivar developed by the South Dakota Agricultural Experimental Station. It is a late maturity variety with medium height, good straw strength, and winter hardiness and is adapted to the western half of South Dakota. ‘SD Midland’ had good yielding potential with good end-use quality. It is moderate resistant-resistant to stripe rust and average for FHB tolerance.

18. **‘Hale’** (PVP to be submitted) hard red spring wheat was released by WSU in 2020 with an excellent combination of disease resistance, Hessian fly resistance, yield, test weight, protein, end-use quality, and broad adaptation to all dryland production regions in the Pacific Northwest. Hale has a combination of seedling stripe rust resistance (*Yr15*, +) and adult plant resistance genes (*Yr18*, ++). It has strong dough strength based on the presence of *Ax1*, *Bx7OE*, *Dx5+Dy10*, and *wbml*, with excellent bread-baking quality.
19. **‘Roger’** (PVP to be submitted) spring soft white club wheat was pre-released by WSU in 2020 as the first Hessian fly-resistant, spring club wheat to be available in the Pacific Northwest. Roger has good adult plant stripe rust resistance, excellent yield, test weight, and end-use quality.
20. **‘KS Ahearn’** (KS12DH0156-88) was released as a late-maturing hard red winter wheat with excellent straw strength, good resistant to leaf, stripe and stem rusts and acceptable test weight. It has had excellent yield potential and has performed well across widely disparate growing seasons. Its quality is acceptable. It does carry the 1B.1R wheat-rye translocation that came from Gallagher.

2021 Added PVP numbers to previously reported varieties

1. **‘MN-Torgy’** (PVP 202100218, submitted 2/19/2021 pending) is a HRS wheat variety developed by the University of Minnesota wheat breeding program with a good balance of high grain yield and grain protein content. MN-Torgy is moderately resistant to important diseases including Fusarium head blight and bacterial leaf streak.
2. **‘Yecora Rojo 515’** (PVP 202100356, submitted 3/10/2021) is a HRS variety released by the UC Davis wheat-breeding program. It is derived from the popular variety Yecora Rojo by introgression of the stripe rust resistance genes *Yr5* and *Yr15* using marker assisted selection.
3. **‘KS Hamilton’** (PVP 202100368, submitted 6/24/2021) is a HRW wheat cultivar developed by Kansas State University for western Kansas. It has very competitive yield potential. It has good resistances to wheat streak-mosaic virus, stem rust, soilborne mosaic virus, and Hessian fly.
4. **‘AR06146E-1-4’** (PVP 202100427, submitted 8/4/2021 and marketed as Delta Grow 1800) is a common soft red winter wheat variety released by the Board of Trustees of the University of Arkansas from the University of Arkansas in 2020.
5. **‘Guardian’** (PVP 202100003, application 10/08/2020), a hard-red winter wheat, released by Colorado State University in 2019. Has combined resistance for wheat curl mite (CMC-T112-6D) and wheat streak mosaic virus (*Wsm2*). PVP 202100003
6. **‘Fortify SF’** (PVP 202100004, application 10/08/2020), a HRW wheat, released by Colorado State University in 2019. Has the semi-solid-stemmed trait for partial resistance to wheat stem sawfly.
7. **‘Whitetail’** (PVP 201900422, application 09/18/2019). Soft wheat variety released by Michigan State University.

8. ‘SD Andes’ (PVP 202100498, submitted 8/24/21) is HRW wheat cultivar developed by the South Dakota Agricultural Experimental Station adapted to the eastern half of South Dakota. It has excellent straw strength and winter hardiness with medium height and late maturity. ‘SD Andes’ had good yielding potential with average protein and test weight and acceptable end-use quality. It is moderate resistant-resistant (MR-R) to stripe rust.

2021 Germplasm

1. Kronos mutants *vrn1 vrn2*: PI 698812. This isogenic line has more spikelets per spike than Kronos.
2. Kronos mutants *ful2 vrn2*: PI 698814. This isogenic line has more spikelets per spike than Kronos.
3. Kronos mutants *ful3 vrn2*: PI 698815.
4. Kronos mutant *vrt2*: PI 698811. This isogenic line has more spikelets per spike than Kronos.
5. Kronos mutant *svp1*: PI 698813. This isogenic line has more spikelets per spike than Kronos.
6. Kronos introgression of *Wapo-A1b* allele (haplotype H2) from hexaploid wheat: PI 698810. This isogenic line has more spikelets per spike than Kronos.

2021 Populations

Texas A&M University developed 3 mapping populations: a) A set of 222 F6 RILs from the cross of TAM 204/Iba: 10K GBS SNPS were used to construct genetic maps, and QTL analyses for yield components from five environments in Texas are underway. b) A set of 182 F6 RILs from a TAM 112/Duster cross: 5081 GBS SNPs for genetic maps and QTL analyses for yield and yield components in six environments in Texas and Oklahoma. c) A set of 179 F6 RILs from a TAM 111/TX05A001822 cross: 3000 GBS SNPs were used for genetic maps. QTL for bread-making quality were analyzed in three environments. Kernel traits including kernel length, width, perimeter, and area are being analyzed.

Total WheatCAP releases in five years

	Varieties	Germplasm	Populations
Year 1 2017	19	3	5
Year 2 2018	14	8	5
Year 3 2019	24	3	4
Year 4 2020	22	4	6
Year 5 2021	19	6	3
Total	98	24	23

APPENDIX 2. Peer reviewed publications WheatCAP 2021. 37 publications.

- <https://www.triticeaecap.org/publications-and-germplasm/>
 - 2017-2020 WheatCAP publications cross-references in Google Scholar=3,965 (11/2021).
 - 2011-2016 TCAP publications cross-references in Google Scholar = 20,779 (11/2021)
1. ALTameemi, R., H.S. Gill, S. Ali, G. Ayana, J. Halder, J.S. Sidhu, U.S. Gill, B. Turnipseed, J.L. Gonzalez Hernandez, S.K. Sehgal. 2021. Genome-wide association analysis permits characterization of *Stagonospora nodorum* blotch (SNB) resistance in hard winter wheat. *Scientific Reports*. 11:12570. <https://doi.org/10.1038/s41598-021-91515-6>
 2. Anderson, J.A., J.J. Wiersma, S.K. Reynolds, E.J. Conley, R. Caspers, G.L. Linkert, J.A. Kolmer, Y. Jin, M.N. Rouse, R. Dill-Macky, M.J. Smith, L. Dykes, J.-B. Ohm. 2021. Registration of 'Lang-MN' hard red spring wheat. *J. Plant Registrations*, <https://doi.org/10.1002/plr2.20099>.
 3. Anderson, J.A., J.J. Wiersma, S.K. Reynolds, E.J. Conley, R. Caspers, G.L. Linkert, J.A. Kolmer, Y. Jin, M.N. Rouse, R. Dill-Macky, M.J. Smith, L. Dykes, J.-B. Ohm. 2021. Registration of 'MN-Washburn' Hard Red Spring Wheat Containing Barley Yellow Dwarf Virus Resistance Gene *bdv2*. *J. Plant Registrations*, <https://doi.org/10.1002/plr2.20130>
 4. Ayalew, H., M.E. Sorrells, B.F. Carver, P.S. Baenziger, X-F. Ma. 2020. Selection signatures across seven decades of hard winter wheat breeding in the Great Plains of the United States. *The Plant Genome* 13: DOI: 10.1002/tpg2.20032.
 5. Chen, S., J. Hegarty, T. Shen, L. Hua, H. Li, J. Luo, H. Li, S. Bai, C. Zhang, J. Dubcovsky. 2021. Stripe rust resistance gene *Yr34* (synonym *Yr48*) is located within a distal translocation of *Triticum monococcum* chromosome 5A^mL into common wheat. *Theor. Appl. Genet.* 134: 2197–2211.
 6. Chu, C., S. Wang, J.C. Rudd, Q. Xue, A.M.H. Ibrahim, R. Metz, C.D. Johnson, and S.-Y. Liu. 2021. RNA-seq analysis reveals different drought tolerance mechanisms in broadly adapted wheat cultivars 'TAM 111' and 'TAM 112'. *Nature Scientific Reports*. 11:4301.
 7. Dhakal, S., X. Liu, A. Girard, C. Chu, Y. Yang, S. Wang, Q. Xue, J.C. Rudd, A.M.H. Ibrahim, J.M. Awika, K.E. Jessup, J.A. Baker, L. Garza, R.N. Devkota, S. Baker, C.D. Johnson, R.P. Metz, S.-Y. Liu. 2021. Genetic dissection of end-use quality traits in two widely-adapted wheat cultivars 'TAM 111' and 'TAM 112'. *Crop Sci.* 61:1944-1959.
 8. DeWitt, N., M. Guedira, E. Lauer, J.P. Murphy, D. Marshall, M. Mergoum, J. Johnson, J.B. Holland, G. Brown-Guedira. 2021. Characterizing the oligogenic architecture of plant growth phenotypes informs genomic selection approaches in a common wheat population. *BMC Genomics*. 22:1-8.
 9. Fan, M., F. Miao, H. Jia, G. Li, C. Powers, R. Nagarajan, P.D. Alderman, Z. Ma, B.F. Carver, L. Yan. 2021. O-linked N-acetylglucosamine transferase is involved in fine regulation of flowering time in winter wheat. *Nature Communications*. 12: 2303.
 10. Gabay, G., J. Zhang, G. F. Burguener, T. Howell, H. Wang, T. Fahima, A. Lukaszewski, J. I. Moriconi, G. E. Santa Maria, J. Dubcovsky. 2021. Structural rearrangements in

- wheat (1BS)–rye (1RS) recombinant chromosomes affect gene dosage and root length. *The Plant Genome*, 2021:e20079.
11. Gill, H.S., J. Halder, J. Zhang, N. Brar, T.S. Rai, C. Hall, A. Bernardo, P. St. Amand, G. Bai, E. Olson, S. Ali, B. Turnipseed, S.K. Sehgal. 2021. Multi-trait multi-environment genomic prediction of agronomic traits in advanced breeding lines of winter wheat. *Frontiers in Plant Science*. 12:709545. <https://doi.org/10.3389/fpls.2021.709545>
 12. Glenn, P., Zhang, J., Brown-Guedira, G., DeWitt, N., Cook, J.P., Li, K., Akhunov, E., and Dubcovsky, J. (2021). Identification and characterization of a natural polymorphism in *FT-A2* associated with increased number of grains per spike in wheat. *Theor. Appl. Genet.* 10.1007/s00122-021-03992-y.
 13. Hassan MA, Yang M, Rasheed A, Tian X, Reynolds M, Xia X, Xiao Y, and He Z. 2021. Quantifying senescence in bread wheat using multispectral imaging from an unmanned aerial vehicle and QTL mapping. *Plant Physiology*. 10.1093/plphys/kiab431
 14. He F., W. Wang, W.B. Rutter, K.W. Jordan, J. Ren, E. Taagen, N. DeWitt, D. Sehgal, S. Sukumaran, S. Dreisigacker, M. Reynolds, S. Liu, J. Chen, A. Fritz, J. Cook, G. Brown-Guedira, M. Pumphrey, A. Carter, M. Sorrells, J. Dubcovsky, M.J. Hayden, A. Akhunova, P.L. Morrell, L. Szabo, M. Rouse, E. Akhunov. 2021. The landscape of genetic effects on gene expression levels in allopolyploid wheat reveals the impact of homoeologous gene dysregulation on agronomic traits. *Nat. Communications*, (under 2nd revision)
 15. Isham, K., R. Wang, W. Zhao, J. Wheeler, N. Klassen, E. Akhunov, and J. Chen. 2021. QTL mapping for grain yield and three yield components in a population derived from two high-yielding spring wheat cultivars. *Theor Appl Genet*, 134:2079-2095.
 16. Jia, H., S. Xue, L. Lei, M. Fan, S. Peng, R. Nagarajan, B. Carver, Z. Ma, J. Deng, L. Yan. 2021. A semi-dominant NLR allele causes whole-seedling necrosis in wheat. *Plant Physiology*. 186:183-496. <https://doi.org/10.1093/plphys/kiab058>
 17. Jones, B.H., N.K. Blake, H.-Y. Heo, J.M. Martin, J.A. Torrion and L.E. Talbert. 2021. Allelic response of yield component traits to resource availability in spring wheat. *Theor Appl Genet* 134: 603-620.
 18. Jordan J., P. Bradbury, Z.R. Miller, M. Nyine, F. He, M. Fraser, J. Anderson, E. Mason, A. Katz, S. Pearce, A.H. Carter, S. Prather, M. Pumphrey, J. Chen, J. Cook, S. Liu, J. Rudd, Z. Wang, C. Chu, A.M.H. Ibrahim, J. Turkus, E. Olson, R. Nagarajan, B. Carver, L. Yan, E. Taagen, M.E. Sorrells, B. Ward, J. Ren, A. Akhunova, G. Bai, R. Bowden, J. Fiedler, J. Faris, J. Dubcovsky, M. Guttieri, G. Brown-Guedira, E.S. Buckler, J. Jannink, E. Akhunov. Development of the Wheat Practical Haplotype Graph Database as a Resource for Genotyping Data Storage and Genotype Imputation. G3, 2021. In press bioRxiv <https://doi.org/10.1101/2021.06.10.447944>.
 19. Katz A., P. Byrne, S. Reid, S. Bratschun, S. Haley, S. Pearce. 2021. Identification and validation of a QTL for spikelet number on chromosome arm 6BL of common wheat (*Triticum aestivum* L.). preprint in Research Square <https://orcid.org/0000-0002-1794-7618> submitted to *Molecular Breeding*.

20. Kolmer J, M.K .Turner, M.N. Rouse, J.A. Anderson. 2021. Adult plant leaf rust resistance in AC Taber wheat maps to chromosomes 2BS and 3BS. *Phytopathol.* 111:380-385. doi: 10.1094/PHYTO-03-20-0074-R.
21. Kuzay, S., Lin, H., Li, C., Chen, S., Woods, D., Zhang, J., and Dubcovsky, J. (2021). WAPO-A1 is the causal gene of the 7AL QTL for spikelet number per spike in wheat. bioRxiv: 2021.2007.2029.454276. In review in *PLoS Genetics*
22. Li, H., L. Hua, M.N. Rouse, T. Li, S. Pang, S. Bai, T. Shen, J. Luo, H. Li, W. Zhang, X. Wang, J. Dubcovsky, S. Chen. 2021. Mapping and characterization of a wheat stem rust resistance gene in durum wheat ‘Kronos’. *Frontiers in Plant Sciences* 12:751398. doi: 10.3389/fpls.2021.751398
23. Li K., J. M. Debernardi, C. Li, H. Lin, C. Zhang, J. Dubcovsky. 2020. Interactions between SQUAMOSA and SVP MADS-box proteins regulate meristem transitions during wheat spike development. bioRxiv:2020.2012.2001.405779. *The Plant Cell*. doi:10.1093/plcell/koab243
24. Liu S., D.F. Wang, M. Lin, S.K. Sehgal, L. Dong, Y. Wu, G. Bai. 2020. Artificial selection in breeding extensively enriched a functional allelic variation in TaPHS1 for pre-harvest sprouting resistance in wheat. *Theor Appl Genet.* 134:339-350 <https://doi.org/10.1007/s00122-020-03700-2>
25. Liu Y., H. Chen, C. Li, L. Zhang, M. Shao, Y. Pang, X. Xu, G. Bai.2021. Development of diagnostic markers for a wheat leaf rust resistance gene *Lr42* using RNA-sequencing *Crop J* doi.org/10.1016/j.cj.2021.02.012
26. Luo, J., M.N. Rouse, L. Hua, H. Li, B. Li, T. Li, W. Zhang, C. Gao, Y. Wang, J. Dubcovsky, S. Chen. 2021. Identification and characterization of *Sr22b*, a new allele of the wheat stem rust resistance gene *Sr22* effective against the Ug99 race group. *Plant Biotechnology Journal*. On line first. doi: 10.1111/pbi.13737.
27. Marburger, D.A., A. de Oliveira Silva, R.M. Hunger, J.T. Edwards, VL. an der Laan, A.M. Blakey, L. Yan, K.A. Garland-Campbell, R.L. Bowden, M-S. Chen, M. Tilley, Y.R. Chen, G. Bai, B.W. Seabourn, Y. Jin, J.A. Kolmer, G.D. Rassi, P. Rayas-Duarte, B.F. Carver. 2021. Gallagher and Iba hard red winter wheat: Half-sibs inseparable by yield gain, separable by producer preference. *Journal of Plant Registrations.* 15: 177-195.
28. Mason R.E., T.L. Roberts, R. Boyles, A. Acuna, M.N. Arguello, D. Ballesteros, N. Subramanian. 2021. Molecular breeding in wheat, maize and sorghum: strategies for improving abiotic stress tolerance and yield. In: M. A. Hossain, et al. (Eds.), *Molecular breeding in wheat, maize and sorghum: strategies for improving abiotic stress tolerance and yield.*, CABI. pp. 90-116. Book Chapter.
29. Megerssa, S. H., M.E. Sorrells, K. Ammar, M. Acevedo, G.C. Bergstrom, P. Olivera, G. Brown-Guedira, B. Ward, B., A.G. Degete, and B. Abeyo 2021. Genome-wide association mapping of seedling and adult plant response to stem rust in a durum wheat panel. *Plant Genome.* e20105. doi.org/10.1002/tpg2.20105.
30. Mohammed S., T. Huggins, E. Mason, F. Beecher, C. Chick, P. Sengodon, A. Paudel, A. Ibrahim, M. Tilley, D. Hays. 2021. Mapping the genetic loci regulating leaf epicuticular

- wax, canopy temperature, and drought susceptibility index in *Triticum aestivum*. *Crop Science* 61:2294-2305.
31. Nyine M., E. Adhikari, M. Clinesmith, R. Aiken, B. Betzen, W. Wang, D. Davidson, Z. Yu, Y. Guo, F. He, A. Akhunova, K.W. Jordan, A.K. Fritz, E. Akhunov. The haplotype-based analysis of *Aegilops tauschii* introgression into hard red winter wheat and its impact on productivity traits. 2021, bioRxiv: <https://doi.org/10.1101/2021.05.29.446303>. *Front. Plant Sci.* In press.
 32. Sandhu, K.S., S.S. Patil, M.O. Pumphrey, A.H. Carter. 2021. Multi-trait machine and deep learning models for genomic selection using spectral information in a wheat breeding program. *The Plant Genome* doi:10.1101/2021.04.12.439532
 33. Sandhu, K.S., D.N. Lozada, Z. Zhang, M.O. Pumphrey, A.H. Carter. 2021. Deep learning for predicting complex traits in spring wheat. *Frontiers in Plant Science* 11:613325 doi:10.3389/fpls.2020.613325.
 34. Sweeney, D.W., J. Rutkoski, G.C. Bergstrom, M.E. Sorrells, 2020. A connected half-sib family training population for genomic prediction in barley. *Crop Science* 60: 262– 281. <https://doi.org/10.1002/csc2.20104>.
 35. Taagen, E., Tanaka, J., Gul, A., Sorrells, M. E., Positional-based cloning ‘fail-safe’ approach is overpowered by wheat chromosome structural variation (2021) *The Plant Genome* <https://doi.org/10.1002/tpg2.20106>
 36. Ward, B.P., K. Merrill, P. Bulli, M. Pumphrey, R.E. Mason, M. Mergoum, J. Johnson, S. Sapkota, B. Lopez, G. Brown-Guedira. 2020. Analysis of the primary sources of quantitative adult plant resistance to stripe rust in U.S. soft red winter wheat germplasm. *The Plant Genome*. 2021;14:e20082.
 37. Winn Z.J., D.L. Larkin, J.T. Murry, D.E. Moon, R.E. Mason 2021. Phenotyping anther extrusion of wheat using image analysis. *Agronomy* 11(6), 1244.
 38. Yang, Y., S. Dhakal, C. Chu, S. Wang, Q. Xue, J.C. Rudd, A.M.H. Ibrahim, K. Jessup, J. Baker, M.P. Fuentealba, R. Devkota, S. Baker, C.D. Johnson, R. Metz, S.-Y. Liu. 2020. Genome wide identification of QTL associated with yield and yield components in two popular wheat cultivars TAM 111 and TAM 112, *PLOS ONE* 15(12) e0237293.
 39. Zemetra, R.S., S.N. Phipps, T. Kohler, A.B. Burke, A.H. Carter. 2021. Registration of the Coda/Brundage wheat recombinant inbred line mapping population. *Journal of Plant Registrations*. DOI: 10.1002/plr2.20147
 40. Zhang G, T.J. Martin, A.K. Fritz, R.L. Bowden, Y. Li, G. Bai, M-S Chen, Y. Jin, X. Chen, J.A. Kolmer, B.W. Seabourn, R.Y. Chen, D.S. Marshall. 2021 Registration of ‘KS Silverado’ hard white winter wheat. *J. Plant Regist.* 15:147-153. <https://doi.org/10.1002/plr2.20106>
 41. Zhang G, T.J. Martin, A.K. Fritz, Y. Li, G. Bai, R.L. Bowden, M-S Chen, Y. Jin, X. Chen, J.A. Kolmer, B.W. Seabourn, R.Y. Chen, and D.S. Marshall. 2020. Registration of ‘KS Dallas’ hard red winter wheat. *J. Plant Reg.* 15:154-160. <https://doi.org/10.1002/plr2.20108>.

42. Zhang G, T.J. Martin, A.K. Fritz, Y. Li, G. Bai, R.L. Bowden, M-S Chen, Y. Jin, X. Chen, J.A. Kolmer, B.W. Seabourn, R.Y. Chen, and D.S. Marshall. 2020. Registration of ‘KS Western Star’ hard red winter wheat J of Plant Reg. 15:140-146.
<https://doi.org/10.1002/plr2.20104>.
43. Zhang G, T.J. Martin, A.K. Fritz, R. Regan, G. Bai, M.S.Chen, R.L. Bowden. 2020. Registration of ‘KS Venada’ hard white winter wheat. J of Plant Reg. 14:153-158.
44. Zhang G., R.Y. Chen, M.Q. Shao, G.H. Bai, B.W. Seabourn. 2020. Genetic analysis of end-use quality traits in wheat. Crop Sci. 61: 1709-1723.
<https://doi.org/10.1002/csc2.20411>
45. Zhao L., S. Liu, N. R. Abdelsalam, B. F. Carver, G. Bai. 2020. Characterization of wheat curl mite resistance gene Cmc4 in OK05312. Theor Appl Genet. 134:993-1005.
DOI: 10.1007/s00122-020-03737-3

Total WheatCAP peer reviewed publications in five years

	Peer Reviewed Publications
Year 1 2017	60
Year 2 2018	46
Year 3 2019	51
Year 4 2020	35
Year 5 2021	45
Total	237
Cross-references Google Scholar	3,800

APPENDIX 3. Community resources generated

2021 Public databases

1. T3 database <https://triticeaetoolbox.org/wheat/>
2. Promoter and exome sequenced mutant populations https://dubcovskylab.ucdavis.edu/wheat_blast. Also available in Ensembl and GrainGenes
3. Expression database. WheatExp. <https://wheat.pw.usda.gov/WheatExp/>
4. Molecular Marker Protocols. <https://maswheat.ucdavis.edu/>

Mapping Populations developed by WheatCAP

1. Sequenced Kronos EMS mutant population. 1536 lines with exome and promoter capture data. More than 10,000 mutant seeds distributed worldwide.
2. Three EMS populations developed by University of Idaho from three broad grown wheat cultivars: UI Silver (HWW), UI Brundage (SWW), and UI Platinum (HWS).
3. 1000 exome capture population. SNP data (He et al. 2019) Nat. Genet. 51: 896-904. Data available in T3.
4. Panel 300 modern US cultivars characterized by Exome capture
5. Spring wheat NAM population. Published in J. Plant Regist. 2019. 13: 294-297.
6. Stem rust resistant wheat NAM population, consisting of 852 RILs from 10 stem rust resistant sources, all crossed with susceptible line LMPG-6, were deposited in the NSGC and published in the *Journal of Plant Registrations*. The 10 stem rust resistance sources, all with adult plant resistance, consist of 9 Kenyan cultivars and one Minnesota cultivar
7. Wheat D-Genome Nested Association Mapping (NAM) population registered in 2020 and published in the Journal of Plant Registrations by Eric Olson (Michigan State University).
8. Two DHL mapping populations (UI Platinum x SY Capstone and UI Platinum x LCS Star) generated under support of WheatCAP were deposited in NSGC in the spring of 2020.
9. One Doubled Haploid line from UCD: UC-Lassik-RS x UC-Patwin-RS.

APPENDIX 4. Graduate students (20 completed + 23 in progress)

Individual student WEB pages at <https://www.triticeacap.org/educational-activities/>

Institution	PI	1 st name	Last Name	Deg.	G	Start Date	Gr	Current Position
U. of Arkansas	Esten Mason	Dylan	Larkin	PhD	M	9/1/2017	*	Potato Breeder, Aardevo
U. of Arkansas	Esten Mason	Mikayla	Hammers	MS	F	8/1/2020		Continuing degree in CSU
U. of Arkansas	Esten Mason	Zachary	Winn	MS	M	8/21/2017	*	PhD student, NC State U.
U. of CA-Davis	Jorge Dubcovsky	Saarah	Kuzay	PhD	F	9/1/2016	*	Solanaceous Plant Scientist, Unfold
U. of CA-Davis	Jorge Dubcovsky	Priscilla	Glenn	PhD	F	9/1/2017		
U. of CA-Davis	Jorge Dubcovsky	Youngjun	Mo	PhD	M	3/1/2014	*	Assistant Professor, Jeonbuk Ntnl. U.
Colorado State U.	Stephen Pearce	Andrew	Katz	PhD	M	8/21/2017		
U. of Idaho	Daolin Fu	Katrina	Johnson	MS	F	8/1/2017	*	Lab technician at WSU
U. of Idaho	Daolin Fu	Meng	Su	PhD	F	8/1/2018		
U. of Idaho	Jianli Chen	Kyle	Isham	MS	M	1/1/2018	*	Farm operator in Idaho
USDA-Kansas St..	Mary Guttieri	Wardah	Mustahsan	PhD	F	6/1/2018		
USDA-Kansas St. U.	Guijua Bai	Yuzhou	Xu	PhD	M	8/1/2017		
USDA-Kansas St. U.	Guijua Bai	Yaoguang	Li	PhD	F		*	Post-doc, U. of Connecticut
USDA-Kansas St. U.	Guijua Bai	Abdul	Hashimi	PhD	M		*	Assist. Prof. King Saud U. (UAE)
Kansas State U.	Eduard Akhunov	Bliss	Betzen	MS	F	5/1/2018	*	Plant Pathologist-USDA APHIS, KS
Kansas State U.	Eduard Akhunov	Elina	Adhikari	PhD	F	9/1/2015	*	Postdoc U. of Wisconsin-Madison
Kansas State U.	Eduard Akhunov	Qianli	Pan	MS	F	9/1/2016	*	Assoc. Sci., Wave Life Science
Kansas State U.	Eduard Akhunov	Davidson	Dwight	MS	M	8/1/2021		Started the program recently
Michigan State U.	Eric Olson	Jonathan	Turkus	MS	M	5/22/2017	*	PhD student, U. of Nebraska - Lincoln
Michigan State U.	Eric Olson	Tommy	Reck	MS	M	1/1/2019		
U. of Minnesota	James Anderson	Max	Fraser	PhD	M	7/1/2017		
Montana St. U.	Talbert/Cook	Brittney	Brewer	PhD	F	9/1/2016	*	Bayer Crop Science, Soybean Breeder
Montana St. U.	Cook	Jared	Lile	PhD	M	5/1/2021		Started recently
Montana State U.	Cook	Mei Ling	Wong	MS	F	9/1/2019	*	Research Assistant, Montana State U.
USDA-NC St. U..	Gina Brown-Guedira	Eddie	Lauer	MS	M	4/1/2017	*	PhD student, North Carolina State U.
USDA-NC St. U.	Gina Brown-Guedira	Daniela	Miller	PhD	F	1/1/2019		
USDA-NC St. U.	Gina Brown-Guedira	Noah	DeWitt	PhD	M	1/8/2018		
North Dakota St. U.	Justin Faris	Amanda	Peters	PhD	F	6/1/2017		
North Dakota St. U.	Justin Faris	Katherine	Running	PhD	F	9/1/2019		
Cornell U.	Mark Sorrells	Ellie	Taagen	PhD	F	6/12/2017		
Cornell U.	Mark Sorrells	Nicholas	Santantonio	PhD	M	8/1/2013	*	Assistant Professor, breeder, Virginia Tech U.
Oklahoma State U.	Liuling Yan	Forrest	Kan	PhD	M	9/1/2016	*	Post-doc, Texas A&M
Oklahoma State U.	Liuling Yan	Xiaoyu	Zhang	PhD	M	1/1/2017	*	Returned to China
Oklahoma State U.	Liuling Yan	Jennifer	Tapia	MS	F	8/1/2021		
Oklahoma State U.	Brett Carver	Abby	Tucker	MS	F	8/1/2020		
South Dakota St. U.	Sunish Sehgal	Jyotirmoy	Halder	PhD	M	8/22/2017		
South Dakota St. U.	Sunish Sehgal	Harsimardeep	Gill	PhD	M	1/1/2021		
Texas A&M	Shuyu Liu	Smit	Dhokal	PhD	M	9/1/2014	*	Scientist, Power Pollen
Texas A&M	Shuyu Liu	Jorge	Valenzuela	PhD	M	8/1/2017	*	Bayer Sci., Breeder Lead - Mexico
Texas A&M	Shuyu Liu	Zhen	Wang	PhD	M	9/1/2019		
Texas A&M	Shuyu Liu	Kyle	Parker	PhD	M	9/1/2020		
Washington St. U.	A. Carter/M. Pumphrey	Samuel	Prather	PhD	M	8/1/2018		
Washington St. U.	A. Carter/M. Pumphrey	Peter	Schmuker	PhD	M	9/1/2020		

* = Student has graduated