Cover page

a. Award #: 2022-68013-36439

b. Project Title: "Leveraging high-throughput genotyping and phenotyping technologies to accelerate wheat improvement and mitigate the impacts of climate change"

c. Project Director: Jorge Dubcovsky

d. Project website: https://www.triticeaecap.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: $3,000,000

WheatCAP Year 3 report

1. SUMMARY YEAR 3: During the third year of the project, WheatCAP breeders have released 28 commercial varieties (7 with PVP) and deposited five improved germplasms in GRIN-Global. Good productivity of the WheatCAP participants was demonstrated by 28 new peer-reviewed papers. The project also generated HiFi genomic data for 10 diverse accessions of wheat and expression data for 200 accessions across five tissues. We also used quantitative multiplexed in single molecule FISH and single-cell transcriptomics to identify distinct cell populations within the developing wheat spike and to generate an expression atlas of wheat spike development. Several genotyping platforms were explored, and markers from different platforms present in T3 were integrated using imputation with the Practical Haplotype Graph v1. The development of an improved PHG v2 has been initiated. Agronomic data was collected for 57,800 plots from 290 field trials, and additional field data were collected from 282 UAS flights over 119 acres including 35,376 plots. A total of 48,900 samples were genotyped by the breeding programs and the Genotyping labs generating >350 M datapoints. This information was used to implement genomic selection in the public wheat breeding programs and is also a valuable research resource. In 2024, 8 students successfully completed their PhD and another 50 participated in multiple educational activities organized by the project educational coordinator, including in-person workshops and online activities. Students' personal profiles and projects as well as links to project meetings and educational resources are available at the WheatCAP website (https://www.triticeaecap.org/). The complete lists of released varieties and publications are available in Appendices 1 and 2, respectively. Community resources are in Appendix 3 and students trained in Appendix 4. Appendix 5 presents the results of a survey conducted by the Genotyping laboratories.

2. Education

In 2024, 8 students graduated and another 50 (46% female) participated in the WheatCAP educational activities. Ten of the WheatCAP students are funded through external sources at their respective universities. Training was also provided for an additional 6 PhD students outside the WheatCAP at the Functional Genomics Workshop.

In 2024, the project held four workshops and will host a fifth in November 2024 (Genomics and bioinformatic resources for wheat in the UK). In January, the WheatCAP Student Workshop was led by students Kyle Parker and Maria Rottersman at the Plant and Animal Genome conference (PAG). Twenty-three students gave short presentations with updates on their research projects to the group. Students also attended the Annual WheatCAP Meeting at PAG, where they presented posters on their research projects. At the WheatCAP Student Workshop and the Annual Meeting, students had time to discuss their projects with fellow students and WheatCAP CoPIs.

In the Spring, twelve students in their 3rd or 4th year of their PhD program (Cohort 1) participated in one-on-one coaching and group workshops with BONSAI, a company specializing in leadership coaching and development of graduate students. WheatCAP students took the Gallup 34-strengths assessment and learned about their results during a one-on-one coaching session with a leadership coach. During these sessions, students learned about their unique strengths and how to utilize these talent themes to be more productive at work and better lead and collaborate. Students also participated in two workshops designed to build off their strengths. The first workshop focused on being more effective as individuals, appreciating their strengths, while

learning about those that others possess. Understanding how one's strengths impact the ability to communicate and influence others was a key component of this session. A second workshop focused on utilizing strengths in teams. This helped students understand how to interact strategically with others, such as their PI or lab mates. Students learned how to predict and navigate team dynamics. A process for effective delegation was taught and practiced.

In April, twenty-four WheatCAP students attended a two-day online course on hybrid breeding strategies offered by the UC-Davis Seed Biotechnology Center. Students were provided copies of all lectures for future reference. This course supplements the students' training on breeding self-pollinated crops, provided by their respective breeding programs.

The Functional Genomics workshop was held in Manhattan, KS at Kansas State University August 5th-9th and attended by 26 WheatCAP students and 6 externally funded graduate students. The first day students learned about cytogenetics of wild wheat introgression, completing a genomic in situ hybridization laboratory to identify wild wheat introgressions via fluorescence microscopy. Next, the students created RNA-seq libraries in the lab and conducted RNA-seq data analyses. Students also learned about sequencing technologies, genotyping, practical haplotype imputation, and marker design, applying what they learned in hands-on computer exercises using R. Students toured the Kansas Wheat Innovation Center, the Wheat Genetics Resource Center, and the KSU Integrated Genomics Facility.

In Spring 2024, Zachary King, a corn breeder at Syngenta, led a meeting talking about his experiences finding, applying, and interviewing for industry breeding positions and advice for a successful career in plant breeding. Sarah Davidson Evanega, Vice President of External Relations at Okanagan Specialty Fruits and former Founding Executive Director at Alliance for Science, led a meeting discussing the current state of biotechnology use in crops and public perception of genetically modified organisms and gene editing. Recordings of meetings were shared with students via e-mail.

In 2024, the WheatCAP student mentorship program was created. The Education Coordinator identified and matched 19 professionals in industry (13), non-profit (1), and academic (5) careers in plant breeding and data science fields with interested students. Sixteen of the mentors work in the United States, two work in Canada, and one works in Germany. Mentors and mentees were matched based on their responses to an application with questions evaluating their expectations for mentorship and what they hope to gain from and contribute to the experience.

This fall, students resumed monthly meetings with invited speakers. Jennifer Yates, the wheat breeding lead at Bayer, and Masha Trenhaile, Head of University Strategy and Outreach, led an information session about the Bayer co-op and internship programs for graduate students. Jennifer also shared her career journey. Cohort 1 will participate in mock interview panels where they will both be interviewed and interview others and receive feedback on their performance. Sara Tirado Tolosa, Global Precision Phenotyping Lead at Corteva Agriscience, will discuss the applications of machine learning and AI in trait discovery and plant breeding. In November, the students will attend the final workshop of 2024, "Genomics and bioinformatic resources for wheat in the UK" hosted by Designing Future Wheat. In December, students will have a monthly meeting focusing on effective networking, including having a positive presence on LinkedIn, prior to attending PAG in January 2025.

The WheatCAP students are planning a plant science symposium for graduate students in January 2025. They formed a symposium planning team, with symposium Co-Chairs, a secretary

and program, finance committee, communications, and registration committees. They have applied for and received funding from Corteva as part of their Symposium Series. The students are seeking additional sponsorships for the symposium. They selected the theme "From AI to Ag: Using Data-Driven Decisions to Inform the Future of Crop Improvement".

New students have joined the "WheatCAP students and alumni" LinkedIn group, which has 50 current members. Primarily, the Education Coordinator shares jobs (permanent, post-doctoral, and internships/co-cops), scholarships, awards, and opportunities with group members, but members are encouraged to share as well.

Interested students continue to participate in writing groups, offering feedback on manuscripts, proposals, and theses. Currently, there are 18 students participating in 4 small writing groups.

3. T3 database

3.1. Personnel: David Waring continues to support uploading and curating all incoming data. He also writes code to add and improve features on T3. Clay Birkett (full time USDA-ARS employee) works on integrating the PHG and uploads all genotypic data. A postdoc, Dr. Tesfahun Alemu Setotaw, started in 2023. He runs genomic predictions for breeding programs and is developing a robust pipeline for this work.

3.2. New wheat datasets made Findable, Accessible, Interoperable, and Reusable

Data Category	2022 Report	2023 Report	2024 Report	Increment from '23 to '24
Accessions with phenotypes	34,059	48,250	61,975	13,725
Accessions with genotypes	16,656	18,533	45,851	27,318
Total Accessions	57,407	75,717	107,785	32,068
Phenotyping Trials	3,283	3,612	4,120	508
Plots with Observations	286,000	427,672	517,712	90,040
Phenotype Observations	1,138,372	2,070,545	4,711,855	2,641,310
Genotyping Projects	43	77	112	35

In this third year of WheatCAP, uploads of phenotypic data continue to be strong. T3 received high numbers of observations coming from drone-based phenotyping. Close to 2,260,000 phenotype observations in the increment from the 2023 report to the 2024 report came from high-throughput phenotyping. The remaining ~380,000 observations were of traditional traits. ***In the past year the number of phenotypic observations in T3 has more than doubled***, reflecting the fact that each has an average of ~30 phenotypes. Unlike last year, the number of accessions with genotype data is outpacing the number of accessions with phenotype data. Genotypic data should continue apace now that the AgriSeq and Illumina 3K platforms are close to being finalized. As in the last report, there might be some discrepancies between the sum of numbers of plots and accessions reported by breeders and the T3 content status report. These discrepancies originate because breeders may report historical data from years prior to the current year. These data are not included in the above T3 tally. Also, Breeders may have reported trials that are still in the process of uploading to T3 and are therefore not yet included in the T3 tally.

WheatCAP is meeting the challenge of assembling large phenotypic datasets and the next challenge will be to convert those datasets into a greater ability to understand and model wheat growth and performance in different environments.

3.3. Improvements to T3 usability for breeders and data analysts

1. Genotype data download. An intended benefit of T3 is to be able to concatenate genotype data from multiple projects without breeders having to engage in any data wrangling. Unfortunately, T3 is still working through performance constraints such that when the total number of data points to be downloaded exceeds ~1M, web timeouts may occur. As a temporary stopgap, for large download requests, T3 issues a warning and makes it possible for users to download the original VCF files, which is fast.

2. Practical Haplotype Graph (PHG) imputed genotype data. A large majority of genotyping protocols have now been imputed using PHG v1. The resulting imputed data are available through T3's search wizard. The genotype download section of the Wizard provides links. All imputed datasets are also shown on this page: https://wheatcap.triticeaetoolbox.org/imputation
T3 is are working with the Small Grains Genotyping Labs (SGGLs) and with the Akhunov Lab to develop PHG v2. That PHG should come online before the next annual report.

3. Simplified genotype data submission. This form prompts the user to fill in all the related metadata such as the protocol and a description of the sample population. Once the user fills out the form, they upload their VCF file to a cloud service (up to 50 GB in size) for Clay to access. Once T3 has the file and the metadata, they can start the process of adding the data to the database. This submission process will streamline uploads from the genotyping labs.

4. Improved phenotyping trial upload template. The template is now more flexible in terms of its column headers. T3 can now store entry numbers in the databases.

5. T3 created a new annual submission summary page hat shows for each program what T3 has uploaded each year https://wheatcap.triticeaetoolbox.org/guides/annual-summary. The page also links to detail and download pages for all trials and genotyping projects so that programs can easily recover well formatted data.

6. Genotyping project uploads now go through the T3 synonym finder to ensure that genotyped accession names are correctly linked to phenotyped accession names.

3.4. Outreach: Database curator David Waring engages in frequent one-on-one interactions with WheatCAP graduate students to facilitate data uploads.

3.5. Quantitative genetic research relying on T3/WheatCAP data: This research is being driven by the T3 postdoc, Dr. Setotaw and includes three different projects.

Project1: Spatial analysis: Spatial variation is a major source of error in agricultural field experiments. Statistical modeling of the spatial effect will improve the prediction of the genotype performance. To assess the impact of statistical control of spatial effects, the T3 group evaluated the impact on prediction accuracy of statistical models that included these effects: 1. Block, 2. Block + Spatial, 3. Block + Marker and 4. Block + Marker + Spatial. The efficiency of the methods was compared by estimating heritability and prediction accuracy parameters and the model fit parameter AIC. Models with statistical control of spatial effects gave higher prediction

accuracy than those without that control. Dr. Setotaw and David Waring developed a shiny app based on heritability and AIC values to simplify spatial adjustments from T3. This app will be integrated into the T3/WheatCAP database and will be available to breeders.

Project 2: GxE interaction analysis with a factor analytic model: Genotype x environment interaction is a major problem for any breeding program. Breeders need better ways to identify experimental lines that will perform well and stably early in the breeding pipeline, before enough seed has been accumulated to test across many environments. One approach is to use "sparse testing" in which individual lines are tested in few environments, but their performance is predicted for other environments based on relatives that are tested elsewhere. The T3 group compared four sparse testing approaches, and all worked equally well. Not surprisingly, they found that increasing the number of observations per trial increased the accuracy of genomic prediction.

Project 3: Generating environmental covariates to model environment similarity: All selection is based on predictions of experimental line performance in future environments. A recent approach to this challenge uses environmental covariates to estimate pairwise similarity between environments. These similarities can be assembled into an "environment relationship matrix" that can be used in much the same way as the genomic relationship matrices used in genomic prediction. Then, predictions for unknown environments in which observations have never been made (i.e., future environments or those outside of the breeder's testing network) are made based on known similar environments. T3 is learning to use a parsimonious wheat crop growth model to enable reaction norm modeling of wheat trials on T3/WheatCAP.

4. Genomics resources

4.1. Wheat PanGenome and Practical Haplotype Graph: For discovering SNPs, small-scale indels and large-scale structural variants, the KSU group generated HiFi data for 10 diverse accessions of wheat. The genome assembly statistics and annotation results have been provided in the previous report. The variant analyses were performed in whole genome alignments generated by AnchorWave against the Chinese Spring reference genome v. 2.1. On average, they detected ~100 M. SNPs, 2.9 M. insertions and 2.6 M deletions differentiating each of the 10 wheat genomes from the reference genome. On average, the largest insertion and deletion detected in the genomes were ~7.5 Mbp and 6.3 Mbp, respectively.

Further, for studying the functional impact of variation discovered by whole genome sequencing, the KSU team characterized structural variation (SV) in the promoter regions of the wheat genome. Prior studies indicate that SVs in the genome have a strong impact on gene regulation. Promoter regions were defined as 5 kb upstream of high-confidence genes in the Chinese Spring version 2.1 genome, resulting in a set of 106,925 regions. On average, each line had 2.0 M. SNPs, ~166,000 insertions and ~161,000 deletions in the promoter regions compared to the reference genome. These variants are currently being tested for association with variation in gene expression levels across tissues in a diverse panel of lines.

The sequenced 10 genomes from WheatCAP project were combined with 13 genomes available from the public databases to construct a diversity dataset for building the Wheat PHG2 based on the whole genome alignments. The reference ranges in PHG were defined using the gene model boundaries. The KSU group tested the imputation accuracy using whole genome sequence data generated at 10x and 0.1x coverages (2 x 150 bp) for two wheat lines, GF336 and GF263. The

accuracy was assessed based on the match of the non-reference alternative alleles in the imputed and a test dataset that included nearly 2 million variable sites. The accuracy of imputation at 10x coverage was 92.4% and 92.7% for GF336 and GF263, respectively. No significant changes in imputation accuracy were detected at 0.1x coverage, which were 92.0% and 92.0% for GF336 and GF263, respectively. These results indicate that the new version of PHG provides a powerful tool for imputation in wheat. Further testing of PHG imputation accuracy across diverse germplasm is underway.

4.2. Spatial and single cell expression analyses of wheat spike development

The UCD group used quantitative multiplexed in situ hybridization and single-cell transcriptomics to identify distinct cell populations within the developing wheat spike. They conducted spatial expression analysis using single-molecule fluorescence in situ hybridization (smFISH) to examine the expression of 99 genes across three developmental stages: early reproductive induction (W1.5), late double ridge stage (W2.5), and lemma primordia stages (W3.25). This was followed by single-cell RNA sequencing (scRNA-seq) profiling of 13,660 cells at the W2.5 stage and 13,734 cells at the W3.25 stage, where cell differentiation increases. Through clustering analysis, they identified distinct cell types, including meristem, ground tissue, lateral organs, and vasculature cells. They annotated these clusters using known developmental markers and conducted co-expression analysis to uncover novel marker genes associated with key regulators. Those included *ULTRAPETALA1, TCP24*, and *FRIZZY PANICLE* (*FZP*), which play critical roles in spikelet and floral meristem regulation. This integrative study provides a comprehensive cell-type atlas of the wheat inflorescence spike and reveals key regulatory networks involved in spike development. This work offers new insights for developing more productive wheat spikes.
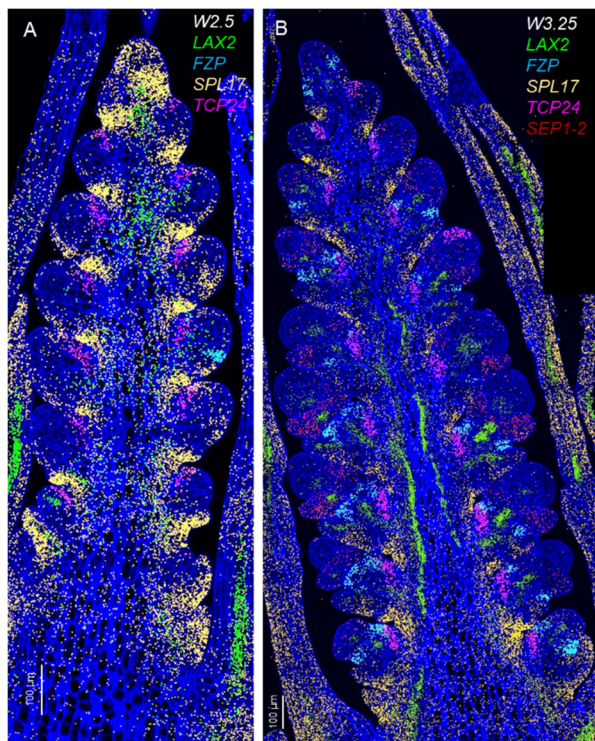

Fig. 1. Expression profiles of diagnostic genes for different cell clusters at the base of the spikelet and glumes/lemmas. (**A**) Late double ridge stage (W2.5). (**B**) Lemma primordia stage (W3.25). *SPL17* marks the lower ridge region (LRR), *LAX2* marks the spike central region and the central part of the base of the spikelet meristem, *FZP* marks the glume axilla and its border with the lemma, *TCP24* marks the spikelet meristem adaxial boundary region, and *SEP1-2* marks the glumes and lemmas, but is expressed in very few cells at W2.5.

4.3. Characterization of regulatory diversity in the wheat genome: To generate genomics resources for functional analysis of genes and investigating the role of regulatory variation in gene expression, the KSU group assembled a diverse panel of 200 lines including wheat, wild relative and synthetic lines. This panel is being characterized by sequencing transcripts from multiple tissues. Currently, tissue sampling and RNA extraction have been finished for 5 tissues (985 samples): 1-week seedling, 4-week crown, root meristem at 15 days, flag leaf sheath +

lamina at complete emergence, and spikelet at complete gynoecium development. For 709 samples, RNA-seq libraries were constructed and sequenced. For two tissues (404 samples), the RNA-seq datasets were filtered for quality. The read count and TPM estimates were obtained using *kallisto* program and pan-transcriptomic reference build using the gene models from multiple wheat genomes. Previously, they have shown that pan-transcriptomic reference is necessary for correcting the reference bias in polyploid RNA-seq datasets. The data will be deposited to the T3 database before the end of 2024.

5. Genotyping Laboratories

Regional genotyping labs supported breeding programs doing marker-assisted selection through genotyping samples using trait associated markers as requested. The USDA-ARS Genotyping Labs conducted a survey to obtain input on mid-density platforms from all U.S. public wheat breeding programs and the results are summarized in Appendix 5.

A total of 14,366 samples were submitted to the genotyping labs by WheatCAP breeding programs that were evaluated with 1 to 102 KASP, STS or SSR assays. Large numbers of samples were genotyped using genome-wide sequence-based approaches as part of ongoing genomic selection and gene mapping efforts. This includes 12,892 samples processed in the regional genotyping labs for genotyping by sequencing (Table 5.1). The Central lab also genotyped 670 samples using the MRASeq platform. Research into the development of the AgriSeq and Illumina3K mid-density genotyping platforms continues.

During 2024, a larger number of samples were genotyped using mid-density platforms than with non-targeted sequencing approaches. The Northern lab genotyped 11,808 samples with the Illumina 3K platform. A total of 6,484 samples from four ARS genotyping labs were genotyped using the AgriSeq platform. In addition, the Eastern lab genotyped 768 samples with the Allegro targeted genotyping platform and 702 samples were genotyped with the Axiom 35K array. Scripts were prepared that combine the output from the AgriSeq and KASP genotyping platforms and apply consistent calling criteria for reporting alleles of major loci that are compliant with the USDA-ARS T3 database. More than 40 M datapoints were deposited in T3.

The number of markers included in the initial AgriSeq design that passed quality filters ranged from 3,284 marker for the Northern region to 3,636 for the West. However, analysis of linkage disequilibrium (LD) determined that several hundred markers were highly redundant ($r^2 > 0.95$). For the next round of primer synthesis, a strategy was implemented to improve information content with a target of 4,000 primers pairs total. When one or more markers with $r^2 > 0.95$ were identified, the marker with the least amount of missing data was retained, resulting in 2500 selected markers. The U.S. wheat exome capture SNP data set was used to select approximately 1200 additional markers to fill in intervals greater than 1 Mbp with SNP having $r^2 < 0.25$ with their nearest neighbor. When possible, a SNP in common with the Illumina 3K marker platform was selected to improve overlap between the platforms. The new AgriSeq Wheat version 2 design includes primers for 3,696 genome-wide markers. The number of primers targeting loci of known effect was increased to 306 and includes new target loci nominated by breeding programs in the survey. Synthesis of primers sufficient for 18,000 samples was purchased by the genotyping labs. Members of the U.S. wheat community have access to genotyping services with

the AgriSeq Wheat platform for $10 per sample through ThermoFisher and North American Genetics.

Table 5.1. Overview of WheatCAP samples processed by the genotyping labs for 2024

Genot. Lab	Program	No. of samples	Platform	Genot. Lab	Program	No. of samples	Platform
Central	Regional	576	KASP, SSR, STS	Eastern	ARS-NC	706	Axiom 35K
	Regional	576	AgriSeq		UMD	576	GBS
	Regional	670	MRASeq		Regional	776	GBS
	KS/ARS	2500	KASP		Regional	1278	28 KASP
	OK	384	KASP, SSR, STS		Regional	1278	AgriSeq
	NE	384	KASP, SSR, STS		KS	768	Allegro
	KS/ARS	1248	KASP, SSR, STS		NCSU	288	GBS
	KS/ARS	864	GBS		ARS-NC	2,118	KASP
	TX	672	GBS	Western	OR	22	KASP
	ND	192	KASP, SSR, STS		CA	192	SNP panel
	CO	192	KASP, SSR, STS		OR	96	KASP SSR
	MT	192	KASP, SSR, STS		WA	240	GBS
	MT	2208	GBS		WA	384	SNP panel
	KS	288	AgriSeq		WA	192	SNP panel
	SD	288	KASP, SSR, STS		OR	192	SNP panel
Northern	Regional/Validation	576	Illumina 3K		ID	192	SNP panel
	ERSGGL	960	Illumina 3K		WA	231	SNP panel
	ERSGGL/UMaryland	288	Illumina 3K		WA	231	SNP panel
	UW	960	Illumina 3K		OR	154	GBS
	USDA-Fargo	720	Illumina 3K		WA	2,304	GBS
	Montana	432	Illumina 3K		WA	2,304	SNP panel
	SDSU	480	Illumina 3K		ID	96	SSR KASP
	NDSU	3888	Illumina 3K		WA	1,728	SSR
	Ohio State	144	Illumina 3K		WA	192	SNP panel
	U of Nebraska	288	Illumina 3K		WA	768	GBS
	UC Davis	576	Illumina 3K		WA	2,304	KASP
	UMN	2,496	KASP		ID	96	KASP SSR
Eastern	Cornell	384	GBS		WA	232	SNP panel
	U IL	1920	GBS		WA	682	GBS
	VA Tech	1056	GBS				

The non-targeted MRASeq platform developed at the Central lab was further optimized. A set of seven primers was designed and validated to provide the highest number of quality SNPs compared to other primer combinations tested. Increased sequence depth significantly increased the number of SNPs of MRASeq. When 50% missing data were used to filter SNPs, MRASeq generated the same or more SNPs compared to those from GBS. The cost of MRASeq is lower from $5 to $7 depending on sequence depth. The preliminary data from Hays' breeding program indicated that SNPs imputed from 50% missing MRAseq data provided a similar prediction accuracy as GBS-SNPs in a genomic selection experiment.

6. UAS-hub (Texas A&M University and Purdue University)

6.1. UASHUB-Data receiving, processing, feature extraction: The UAS-hub continued processing the 2023 data and completed 142 UAV flights, covering an area of approximately 30

acres (15 environments). In 2024, the focus has been on continuing phenotypic feature extraction, with UAV data collected from 130 UAV flights covering 30 acres (Table 6.1). The initial step of Level 1 data processing—which includes generating orthomosaics and digital surface models—has been completed for all locations. Raw UAV images have been received, orthomosaics have been uploaded to UAS-Hub, and phenotypic features have been extracted from 85 flights covering 22 acres. Data are being transferred to T3. The TX group is waiting for plot boundary files from 6 wheat breeding programs to complete the data extraction and upload process for 2024. Nine other breeding programs opted to process their data in-house (131 flights), showcasing an increase in their data-handling autonomy.

Table 6.1. Data received by the UAS-hub in 2024

No.	Location	Flights	UAS-Hub Project created	Processing & Data Delivery	Approx. Size (ac)
1	TX-Amarillo Irrigated	24	2024 Amarillo Irrigated	Extract features	3.5
2	TX-Amarillo Dryland	18	2024 Amarillo Dryland	Extract features	4.5
3	TX-Amarillo Chillicothe	4	2024 Amarillo Chillicothe	Extract features	6.2
4	TX-McGregor	5	2024 McGregor	CC, CH, CV, ExG	0.5
5	UW-Madison (WI)	8	2024 Wisconsin-Madison	Extract features	0.9
6	NDSU (ND)	4	2024 NDSU	Upload MOSAICS	0.4
7	Cornell (NY)	9	2024 Cornell	CC, CH, CV, ExG, NDVI, NDRE	0.5
8	UIUC (IL)	5	IL 2024 UIUC – St. Peter	Pend. boundaries	2.1
9	NCSU	3	2024 N Carolina State	Pend. Boundaries	0.4
10	Kansas (KS)	10	2024 Manhattan	Pend. Boundaries	1.0
11	Kansas (KS)	9	2024 Russell	Pend. Boundaries	1.0
12	Kansas (KS)	6	2024 Tribune	Pend. Boundaries	1.0
13	MSU (MT)	5	2024 Montana	CC, CH, CV, ExG, NDVI, NDRE	2.4
14	Idaho (ID)	6	2024 Idaho	Extract features	2.5
15	Kansas-Colby (KS)	8	2024 Colby	Missing GCPs	1.0
16	U. Minnesota (MN)	6	2024 UMN	Extract features	1.0

The increased independence of the breeding programs to process their data aligns well with the initial goal set by the UAS-Hub and WheatCAP leadership to empower the breeding programs to perform their own analyses of UAV-based high-throughput phenotyping. See section 9.4 to see the new tools that are being planned to increase the independence of the breeding programs.

6.2. UAS data management tools and training programs development

The UAS-hub group has developed a pipeline to upload plot coordinates extracted from orthomosaics for all the breeding programs into T3 automatically. Also, they have developed another pipeline to upload orthomosaic and flight acquisition date metadata onto T3 to provide the right basemap layer to visualize the GeoJSON plot maps. With this file on T3, breeders can see the correct spatial maps of their plots on T3.

To provide more support for the breeding programs, the UAS-hub developed a training video titled "How to Convert from Julian dates to Days After Planting (DAP)". In this video, they show how to convert Julian dates to DAP which is important when relating genomic and phenomic information. This video has been uploaded into the T3 BreedBase training archives and shared with all the programs.

The UAS-hub has also created a training video that guides breeders on how to delineate plot boundaries and attach plot identifiers. This resource aims to reinforce breeding program knowledge in digital agriculture. By following the video, breeders can accurately define plot areas and associate them with the correct identifiers, facilitating better data integration and management within digital platforms.

7. Spring and winter wheat IWYP hubs

7.1. NIFA IWYP Winter Wheat Breeding Innovation (WWBI) Hub at KSU.
WheatCAP collaborates with the NIFA IWYP WWBI Hub at KSU on introgression of gene alleles of controlling yield component traits. The donor germplasm for eight yield component genes were provided by the WheatCAP to the WWBI Hub for introgression into winter wheat cultivars. For the reporting period, the BC_4F_1 populations were developed for introgression of *Elf3* and *Tagw2* gene alleles. The QTL affecting heading (*Vrn-B1* and *Ppd-A1a.1*) and yield (1AL: 472.2-531.6 Mb) from AGS 2000 were crossed with winter wheat cultivars to develop 1) BC_3F_1 for Bob Dole, KS090387K-20, and OK11D25056; 2) BC_3F_2 for OK13625, Hilliard, TAM114, and NE10589, and 3) BC_3F_3 for Tekoa. The heterogeneous inbred families (HIF) of the above-mentioned lines selected from BC_3F_3 will be planted for evaluation in the field trials in 2024-2025. In fall of 2024, the winter-hub will finalize the crosses of *Ae. tauschii*-derived lines carrying low canopy temperature and *Ppd1* alleles with three winter wheat cultivars to produce BC_1F_1.

7.2. Spring Wheat hub at CIMMYT

7.2.1. CSU experiments: $BC_1F_{2:4}$ populations were developed for the introgression of *WAPO-A1b* and *Qsn.csu-6BLb* alleles conferring greater spikelet number into two CYMMIT high-biomass lines. The donor parent was 'Platte' and the recurrent parents were 'Nadi' and GID:4314513 developed by CYMMIT. BC_1F_2 individuals were genotyped for both alleles. Lines homozygous at both loci were advanced to $BC_1F_{2:3}$. Twenty $BC_1F_{2:3}$ lines were selected for each recurrent parent with five lines representing each allelic combination, *WAPO-A1a/Qsn.csu-6BLa, WAPO-A1a/Qsn.csu-6BLb, WAPO-A1b/Qsn.csu-6BLa,* and *WAPO-A1b/Qsn.csu-6BLb.* These lines were used for subsequent field evaluation at CYMMIT (RCBD with three replications, 3 environments). The 'Nadi'*2/'Platte' population expressed a significant increase in spikelet number conferred by the *WAPO-A1b* allele, with an effect size of 1.15 spikelets. Significant interactions between *WAPO-A1* and *Qsn.csu-6BL* were observed for grain yield, grain number, and infertile spikelet number.

The GID:4314513*2/'Platte' population showed no significant effect on spikelet number from *WAPO-A1*, however, a significant interaction between *WAPO-A1* and *Qsn.csu-6BL* was observed for spikelet number in the population. Significant interactions were also observed for infertile

spikelet number and heading date. Significant effects on grain yield were not observed in this population. Notably, the *WAPO-A1* locus had significant effects on grain number, plant height, and grain length in this population.

7.2.2 WSU experiments: The WSU team is focused on a strong QTL for kernel weight (LOD= 6.5) and kernel number (LOD= 6.6) identified in the cross between elite spring cultivars Kelse and Scarlett and introgressed into five high-biomass CIMMYT lines. Lines were 'late' planted at CIMMYT in January 2022 and 2023, along with 'early' plantings in November 2022 and 2023. Preliminary analysis of isolines with and without the QTL indicates that presence of the QTL significantly increases thousand grain weight by an average of 2.6 grams, increases grain filling period by an average of two days (through earlier heading and anthesis dates), and significantly increases seed length (0.12 mm) and seed width (0.06 mm). The effects of the QTL were variable with overall trends for lower grain yield and grain number per plant. These appear to be dependent on background and environment, and additional analysis needs to be conducted to identify the full effect.

7.2.3. MT experiments: The collaborative project with CIMMYT included NILs created to test the effects of *QTn.mst-6B*, associated to productive tiller numbers, and *WAPO-A1,* associated to spikelet number per spike, on yield component traits in two high biomass spring wheat varieties from CIMMYT, BAJ and Kingbird. For this analysis, the MT group used data from the NILs grown at CIMMYT in 2022-2023 and 2023-2024. The spikelet number per spike (SNS) per meter square was not significantly different between NILs carrying the low and high tillering *QTn.mst-6B* alleles in both BAJ and Kingbird backgrounds. In the BAJ background, NILs with the high tillering *QTn.mst-6B* alleles had significantly lower 1000-Grain weight (g). Although not significant, grain yield was lower in the high tillering allele NILs. In the Kingbird background, NILs with the high spikelet number *WAPO-A1* allele significantly increased spikelet number and grain number per spike, but yield was not significantly different. However, grain yield was significantly decreased in the NILs containing the high tillering *QTn.mst-6B* allele, suggesting that it is not a useful allele for this environment.

8. Progress by individual breeding programs

8.1. CA. University of California, Davis. Jorge Dubcovsky & Xiaofei Zhang

Education: PhD student Maria Rottersman completed her QE. She organized the WheatCAP student workshop at PAG 2024 in San Diego and submitted her first manuscript to bioRxiv as first author (Rottersman et al. 2024). She attended all workshops organized by the WheatCAP education team, except the Expression workshop at KSU that was attended by new UCD PhD student Connor Tumelty. He will be working on genes affecting grain number per spike. A third PhD student, Elmer Flores, began his PhD in September 2024 and will be working closely with the wheat breeding program. He will participate in future WheatCAP educational activities.

Germplasm: The UCD team deposited in GRIN Global three bread wheat germplasm lines (PI 704906, PI 704907, and PI 704908) with radiation induced deletions that eliminated all the alpha gliadins from chromosome arms 6AS (*Gli-A2*), 6BS (*Gli-B2*) and 6DS (*GliD-2*), respectively (Apdx. 2). Two years of yield evaluations demonstrated that these deletions have no negative

effects on grain yield or breadmaking quality, and that the *GliD-2* deletion significantly improved gluten strength and breadmaking quality. These deletions, released by the University of California, Davis, eliminate alpha gliadin epitopes that are immunogenic for people with Celiac Disease.

Publications: The UCD team published five papers in Development, The Plant Journal, Plant Biotechnology Journal, Theoretical and Applied Genetics and bioRxiv (Apdx. 1).

UAS: The UCD wheat breeding program integrated drone-based phenotyping technology to enhance data collection across breeding trials. They captured RBG and multi-spectral images throughout the growing season. A total of 32 trials, comprising 4,922 plots, were surveyed, covering 20 acres. Data from seven flights were uploaded to the T3-WheatCAP database. The CA breeding program submitted 7 flights covering 6.3 acres to the UAS-hub.

Genotyping: The UCD group evaluated the USDA-SoyWheOatBar-3K array developed at the USDA Genotyping lab in Fargo. In 2024, they submitted 190 samples across three small grains crops – bread wheat, barley, and oat – for genotyping. Among 3,421 total markers analyzed, 1,950 high-quality markers were identified for bread wheat, 2,811 markers for barley, and 2,229 for oat (missing < 0.2, MAF > 0.05). These markers are now being used to implement genomic selection strategies across the Small Grains breeding programs. They are currently assessing the performance of the genotyping platform in cross-crop applications, combining samples from two crops to reduce genotyping expenses.

T3 Trials and genomic selection: The UCD group submitted 32 field trials, comprising 4,922 plots, to T3. Key agronomic traits such as grain yield, heading date, plant height, stripe rust severity, and grain protein content were uploaded for each trial. Once the genotyping platform is fully validated, this data will be used as a training set for genomic selection, allowing for the integration of phenotypic and genotypic data to enhance selection accuracy. The CA group received GEBVs from T3 in 2023 and used this information to decide which of the 2024 planted lines to advance. The genotyping from this year and the resulting GEBVs will be used in selections for this coming planting.

8.2. CO. Colorado State University. Esten Mason

Education: Three graduate students and 18 undergraduates worked in the wheat breeding program in FY24. Meseret Wondifraw graduated in Spring 2024. Currently, she is a Postdoctoral Associate at Breeding Insight, Cornell University. PhD student Mik Hammers participated in all WheatCAP educational activities. PhD student Andrew Katz is on continued enrollment as he is employed full-time and plans to graduate in Spring 2025. Emily Billow, MS, Female, started in Summer 2024.

Germplasm & publications: Obtained PVP 202400244 for 'AP24-AX' – PVP 202400244 and released 3 new varieties that will be submitted to PVP in Augus 2025 varieties (Apdx. 2). Three publications, two in The Plant Genome and 1 in Crop Sciences (Apdx. 1).

UAS: CO processes all flight images/data in-house. In 2024, there were 4 usable flights totaling 20 acres (~3000 plots). Data collected included multispectral wavelengths used to calculate indexes NDRE, NDVI, and plant height. Data will be uploaded in T3 before the end of the year.

Genotyping: a total of 170 lines were submitted to the USDA-ARS Genotyping Lab in KS for genotyping. Data was received for single marker assays. Will deposit breeding line information into T3. Data was generated on ~110 SNPs using KASP assays. In-house whole genome genotyping: CSU sequenced 4,992 unique lines in 2024 in collaboration with DNA Services, University of Illinois.

In-house KASP genotyping: CSU generated 10,730 single marker KASP datapoints including 2,040 for loci resistant to Aggressor herbicide, 1,620 datapoints for resistance to Beyond herbicide, 6,520 datapoints for the *Sst1* stem solidness locus and 550 markers for other traits.

T3 agronomic traits: For 2024, CSU will upload 10 trials to T3 containing 4,446 plots of yield and agronomic data including grain yield, test weight, heading day, plant height, and lodging.

Genomic Selection: CSU generates and utilizes genomic estimated breeding values (GEBVs) for selection starting at the preliminary yield nursery (PYN) stage for conventional crosses and the head-row stage for doubled haploids on a yearly basis through elite level testing. In 2024, GEBVs were generated for 4,992 unique lines. Predicted traits included grain yield, test weight, heading, height, lodging, loaf volume, flour yield, bake mix time, protein, stripe rust severity, sawfly cutting and stem solidness, and wheat streak mosaic virus severity. Prediction accuracy using a univariate G-BLUP model ranged from r = 0.10 to 0.77. In addition, whole genome genotype data was used to predict haplotypes using the HaploCatcher package developed by CSU (https://cran.r-project.org/web/packages/HaploCatcher/index.html). HaploCatcher was used to postulate the presence of the *Sst1* locus for stem solidness, a wheat curl might resistance locus (*WCM6D*), the wheat streak mosaic virus resistance locus *WSM2*, and the soil-borne mosaic virus resistance locus *Sbm1*, amongst other loci.

8.3 ID. University of Idaho. Jianli Chen

Education: Yaotian Gao (PhD, *FT-D1* CRISPR and genomic prediction) and Pabitra Joshi (PhD, drone phenotyping and genomic prediction) participated in various educational and professional development activities organized by WheatCAP including the monthly meetings, the PAG student workshop in 2024, the mentorship program and the soft skills and leadership training. Ms. Pabitra Joshi was awarded the 2024 Borlaug Scholarship from the National Association of Plant Breeders and will be graduating in November 2024.

Germplasm & publications: variety UI Warrior was submitted for PVP 202400308 in March 2024. UI Warrior is a soft white spring wheat with high grain yield, excellent end-use quality and test weight (Apdx. 2). One publication in "Agriculture".

UAS: For year 2024, the ID team submitted 10 flights to the UAS-HUB along with the plot boundary files covering 2.01 acres and 1,296 plots.

Genotyping: A total of 192 samples were submitted to Deven See at WA Genotyping Lab for the Agriseq 5K SNP datasets. Genotypic data was received for 187 samples, and this data will be

deposited in T3 before the end of 2024. On average, each line contained 4000 SNPs. Regarding data quality, 29.4% of the lines had less than 10% missing data, 94.1% had less than 30% missing data, and 99.4% had less than 50% missing data. They also genotyped in-house two populations of 442 lines using 32 KASP breeding markers, including *Vrn1*, *Ppd1*, *FT1*, *Rht1*, and *Glu1*.

Breedbase: They are planning to submit two trials from spring wheat diversity panel Gp502 and Gp503 with phenotypic data for grain yield, plant height, heading date, test weight, protein content, thousand kernel weight, and spikelet number per spike. The Gp502 diversity panel includes 250 plots with two replications, and the Gp503 diversity panel contains 192 plots with two replications. This data will be deposited in T3 before the end of 2024.

Genomic selection: ID is still in the early stages of the implementation of Genomic Selection in the breeding program. They are working on prediction model optimization that can better suit for specific yield or quality traits in their program. They have optimized some prediction models for wheat quality trait that can give high accuracy as reported in their reported publication. They have observed that the RKHS model and the RF model outperform traditional genomic selection models in terms of prediction accuracy across nearly all yield traits. Between the two, the RF model has a shorter computation time than the RKHS model, making it a promising model for genomic selection.

8.4. IL. University of Illinois. Jessica Rutkoski

Education: Graduate students Lucas Berger Munaro (Male) and Raysa Gevartosky (Female) and undergraduate students Zachary Bradley (Male), Maria Vasquez (Female), and Noam Kramer (Female) collaborated with the WheatCAP project. Lucas Berger Munaro and Raysa Gevartosky both attended the HTP workshop in Amarillo and the GS workshop in Raleigh. Lucas Berger Munaro attended the genotyping workshop in Kansas. Both graduate students were awarded the 2024 Borlaug Scholarship from the National Association of Plant Breeders.

Germplasm: three new varieties were licensed to companies: IL2020-5376, 5, IL16LCSDH-04-T-2659-3 6, and IL16LCSDH-04-T-2659-216. Companies provide labelling and IP protection so no PVP plans.

UAS: The 2023 data including 10 flights at 3 locations were submitted to T3. In 2024, the IL group collected HTP data from 7 acres and 3,178 plots, these were spread across 3 locations. Data will be deposited in T3 by February 2025.

Genotyping: In 2024, IL group submitted 1,337 samples for GBS and obtained ~8,000 SNP per line. This data was submitted to T3.

Breedbase: The IL program routinely uses Breedbase for trial data management and seed lot management. For the 2024 season, 43 trials were uploaded to the University of Illinois private instance of T3. Among those 43 trials, the 20 breeding trials containing 7,439 research plots will be submitted to T3 by February 2025. The IL program provided valuable insights on the integration of public breeding programs into T3.

Genomic selection: The IL group routinely uses GS for parent selection and for advancement decisions. Thanks to GS, the IL group was able for the first time this year to select some parents that had not yet been phenotypically evaluated.

8.5. KS. USDA-ARS Manhattan & KSU. M. Guttieri, E. Akhunov, G. Bai, A. Fritz, G. Zhang,

Education: Under the supervision of G. Bai, PhD student Xiaoting Xu graduated and continues to work in the genotyping lab as a post-doc fellow, and Ms. Jinan Park initiated a M.S. and will participate in the WheatCAP educational activities. They both participated in the Functional Genomics workshop at KSU. Under the supervision of M. Guttieri, PhD student Adriano Abreu Moreira participated in the Functional Genomics Workshop, the Spring Writing Group and the Mentorship Program. Undergraduate student Luke LeBar is expected to graduate 12/2024 and is applying to PhD programs in plant breeding and 3 other undergrad students are participating in the program. Under the supervision of E. Akhunov, three PhD student (Nicholas Stelling, Buket Sahin, and Gabriela Romero Campos) and two MS students (Dwight Davidson and Jaime Knight) participated in the Functional Genomics workshop at KSU and in other activities organized by the WheatCAP educational program.

Germplasm Two new varieties were released in 2024, the HRWW KS20H124 (a two-gene Clearfield wheat with competitive yield, good drought tolerance, good disease resistance, and excellent baking quality, and the HWWW KS20HDW185, with very competitive yield, good drought tolerance, good disease resistance, and very strong gluten strength via the *Glu-B1* Bx7oe allele. Both varieties were evaluated in the regional nursery testing by the HWW Regional Genotyping laboratory (App1)

Publications: The KS group generated 10 publications in scientific journals including Phytopathology (2), TAG, Front Plant Sci (2), J Integrative Agriculture, bioRxiv, Genome Biology, BMC Biology and Crop Journal (App2)

UAS: the USDA-ARS group has deposited 7 flights including 1220 plots into the T3 database. The Kansas State University deposited 23 flights covering 2240 plots into T3. USDA-ARS is reporting additional 19 flights over three locations that will be processed in-house and deposited in T3.

Genotyping: The KS genotyping lab provided genotyping services for 12,767 lines including 864 lines with AgriSeq, 5,568 lines with GBS and the rest with various KASP, SSR, and STS markers. Of this, 576 lines genotyped with AgriSeq markers (2,640,230 datapoints) and 471 lines genotyped with MRASeq (14,169,710 datapoints) have been deposited n T3 by the Genotyping lab. In addition, Allegro data was received from the Eastern Genotyping lab for 158 lines (~3900 SNP/line).

Breedbase: The KSU group entered 13 trials and 4,017 plots with agronomic data into T3.

Genomic selection: The KSU-ARS team is fitting the first genomic selection models. Design of possible cross-validation schemes that would be useful to the program is at early developments stage. The KSU group has identified several introgression lines that outperform the recurrent parents in grain yield and is investigating the causal loci.

The KSU team analyzed the root architecture of one of the most drought tolerant *Aegilops tauschii* introgression lines, WGR004327. Compared to the parental cultivar, this line showed 14-36% increase in canopy temperature depression in irrigated and water-limiting conditions. The analysis of root architecture showed that lower canopy temperature was associated with ~50% increase in the total root length compared to the parental line. In addition, WGR004327 showed higher stomata opening, which suggests that reduction in canopy temperature is associated with more effective evapotranspiration. The QTL mapping of loci controlling these traits is underway.

8.6. MI. Michigan State University. Eric Olson.

Education: Jonathan Concepcion is the PhD student supported by WheatCAP at MSU and is expected to graduate in the spring of 2025. Jonathan and another MSU PhD student, Libby Ross, participated in the WheatCAP Functional Genomics Workshop at KSU. Libby is conducting gene expression experiments on pre-harvest sprouting, and she greatly benefited from the training.

Germplasm & publications: soft red winter wheat 'MI20R0210' has been proposed for release in 2025, pending commercialization negotiations. (Apdx. 1). MSU published one paper in Sci. Rep, with the WheatCAP PhD student as the first author (Apdx. 2).

Genotyping: A total of 2,280 breeding lines, parents and GS training lines were genotyped in-house using GBS in 2024 and 15,085 SNPs with a 70% call rate and 5% minor allele frequency were generated. These data will be deposited in T3 by the end of 2024. A total of 70 MSU entries in the Big6 nursery were evaluated with KASP markers for large-effect genes.

UAS: Four flights were done in 2024, and the data is being processed for uploading into T3.

Breedbase: 34 trials for 6,240 plots were submitted to T3. Agronomic data will be submitted to T3 before the end of the year.

Genomic selection: Genotyping was completed in August of 2024 and 700 selections were identified to have predicted high grain yield across 10-year x location combinations, low FHB severity, low DON and low levels of preharvest sprouting. Selected genotypes are currently being planted for observation and selection in 2025. Genotypic data has not been submitted to T3 yet.

8.7. MN. University of Minnesota. Jim Anderson.

Education: New PhD student Marcos Winicius Goncalves de Souza started in June 2024, so he has not participated in any WheatCAP educational activities yet.

Germplasm & publications: No germplasm on variety released. The MN group published one paper in the Journal of Plant Registrations reporting the previously released variety MN-Torgy'.

Genotyping: 2,392 samples (2,101 unique lines) were submitted to the Fargo Genotyping Center for genotyping using 14 KASP markers targeting 12 genes (33,488 datapoints). The MN group performed GBS on the same samples and obtained on average 4,239 SNPs for each of the 2,101 unique lines (9 M datapoints). The 2023 and 2024 genotyping data from MN have not been deposited into T3 yet.

UAS: 8 flights from the St. Paul location (0.7 acres each, 5.6 acres total). The 2023 and 2024 UAS data from MN have not been submitted yet to T3.

Breedbase: collected agronomic data included 4 traits: Grain yield, and protein from 7 locations (1 location lost due to hail); heading date, 3 loc.; and height, 2 loc. URSN trial data included disease-related traits (up to 8 traits per environment). The data has not been uploaded yet to T3.

Genomic selection: In 2023, GS was used to predict FHB response and seed size in 2,032 F6 lines. This data was used to reduce the FHB nursery by 405 entries. Additional data on FHB parameters, pre-harvest sprouting, and gluten strength were incorporated to improve prediction models in February 2024. Based partly on the prediction models, 625 out of the 1,627 F6 plots were selected for harvest from the winter nursery. In 2024, GS is currently in progress to predict FHB response, seed size, glutopeak quality traits, and pre-harvest sprouting in 2,101 F6 lines. Historical data from the previous breeding cohort entering PY trials in 2024 is being incorporated in the model training. Post-harvest FHB and quality trait data will be added to refine prediction models for the February 2025 winter nursery harvest selections.

8.8. MT. Montana State University. Jason Cook

Education: The two PhD students funded by the WheatCAP project, Jared Lile and Mei Ling Wong participated in the WheatCAP annual meeting at PAG2024 and the Functional Genomics Workshop hosted in Kansas. They participated in the monthly online meetings and in the Mentor Match opportunity. Jared participated in the BONSAI training workshops. Both students passed their comprehensive exams this summer and made good progress in their projects.

Germplasm & publications: Montana State University breeding program released the new spring wheat variety MT Ubet (MT 2030) and applied for PVP for MT Dutton (PVP 202400204) and MT Carlson (PVP 202400205), both on December 28, 2023.

UAS: 6 UAV flights were flown over 2024 Bozeman yield trial that contained 468 plots. The images have been submitted and processed by the UAS-hub and the data has been submitted to T3 database.

Genotyping: The data for the 433 lines genotyped in 2023 have been deposited in T3. These data are being used to develop the MT genomic selection models. In 2024, genotyping was delayed due to seed supply problems, but the MT group is sending ~414 breeding lines to the Fargo, ND USDA-Genotyping Lab for genotyping with the USDA-SoyWheOatBar-3K array.

Breedbase: For 2024, MT submitted their field books and maps for the yield trial. Data submission will be completed after harvest and before the end of 2024.

Genomic selection: MT tested 5-fold cross validation models for GS on 12 end-use quality traits using historic multi-environmental yield trial data. Accuracies were similar between RR-BLUP and Bayesian Alphabet models. Validation using leave-one-year-out (LOYO) showed high variation in prediction accuracy depending on the year that was removed for all quality traits. The use of GWAS-informed fixed effect SNPs in the models gave substantial gains in prediction accuracy in the LOYO validations in most cases. The MT group is also testing a new modeling

approach that directly predicts phenotypic values instead of estimated breeding values. This method yielded good prediction accuracies.

8.9. NC. USDA-ARS Raleigh. Gina Brown-Guedira

Education: PhD student Nico Lara advanced to candidacy in March 2024. Nico participated in most WheatCAP-organized activities and is currently undergoing a 6-month data science co-op at Syngenta, working with their Discovery Breeding and Advanced Analytics and Data Science teams. Matthew Willman started his PhD program during Spring 2024 and attended the Functional Genomics workshop at KSU. Daniela Miller completed her PhD during Spring 2024.

Germplasm & publications: Two mapping populations were deposited in the USDA-NSGC: LA95135 x SS-MPV57 (UX1443; 358 lines) and AGS2000 x LA95135 (UX1444; 291 lines). These populations were evaluated for spike and seed morphology traits, heading date and plant height, glaucousness and disease resistance in multiple environments, contributing to the characterization of multiple genes (App1). One paper was published in TAG (App2).

UAS: In 2024, there were 7 UAV flights flown at Raleigh, NC and 7 flights flown at Kinston, NC. The two locations each had 560 small plots consisting of two 1-m rows. Ground truthing of biomass measurement was done through destructive sampling. There were ~0.4 acres/flight, total of ~5.6 acres. All 14 flights are in the process of being uploaded for processing.

Genotyping: A total of 2118 samples were submitted to the Eastern genotyping lab at Raleigh for evaluation with six KASP assays for four genes (*Rht24*, *Rht25*, *Vrn1* and *Ppd1*). A total of 706 samples from three of the SunRIL mapping populations were submitted for evaluation with the Axiom 35K array. Data were received for 35,143 SNP per line (24.8 M datapoints). The data will be uploaded in T3 before the end of this year.

Breedbase: During 2024, agronomic data including powdery mildew incidence, winter dormancy release, flowering time, and height for two trials having 560 total plots each submitted to T3.

Genomic selection: GEBVs were generated for multiple traits, including grain yield and quality measures. Results were used in decisions about line advancement and will contribute to expansion of the training population for genomic selection.

8.10 ND. USDA-ARS and ND State University, Fargo. Justin Faris and Andrew Green

Education: Ms. Pooja Kumari joined as a WheatCAP student in August 2022, and she is working on her PhD at NDSU in the Genomics, Phenomics, and Bioinformatics program. She has participated in all the monthly meetings and the workshops, and she is acting as one of the coordinators of the student symposium to be held in January 2025.

Germplasm & publications: The hard red spring wheat variety 'ND Stampede' (PVP 202400409, PVP applied for July 2024) was released. ND Stampede is widely adapted throughout the Northern Plains region. Marker-assisted selection was performed throughout line development. (Apdx. 1). No publications.

UAS: Canopy Cover (CC) and ExG data have been provided by TX group for 2023 UAV flights data. In 2024, 12 flights have been submitted to TX this year. Each flight covered an area of ~0.42 acres.

Genotyping: 4500 samples were submitted to the USDA-ARS Genotyping Lab at Fargo, ND and approximately 2,300 SNPs were received per line using 3k Illumina (~10.3 M datapoints). Data was not submitted yet to T3.

Breedbase: The 2024 field designs for UAV flights have been submitted to T3.

Genomic selection: Training population data are being collected in 2024. Implementation of GS is progressing slowly. At this point, major gene MAS is being used more extensively. The Fargo group has discovered new QTLs with major effects for resistance to bacterial leaf streak and has developed KASP markers for MAS.

8.11. NE. University of Nebraska. Katherine Frels

Education: PhD student Sydney Graham participated in the Functional Genomics Workshop and in the Strength Coaching Individual & Group Sessions. Undergraduate student Cole Hammett graduated in spring 2024 and is a current M.S. student at NCSU.

Germplasm & publications: E Prism CLP (NHH17612) is a hard red winter wheat with two-gene Clearfield herbicide resistance targeted towards the Nebraska Panhandle and western South Dakota region. It will be submitted for PVP soon (Apdx. 1). No new publications.

UAS: 16 UAV flights were conducted at four Nebraska locations that spanned 2.25, 2.20, 0.75, and 0.70 acres in size. Flights were conducted at 4 timepoints during the summer. NDVI and NDRE (for 1,824 plots) will be uploaded to T3 pending completion of data extraction. 2023 UAS data is still not uploaded to T3.

Genotyping: 404 lines were genotyped at the Fargo Genotyping Lab for USDA-SoyWheOatBar-3K Array and ~2300 SNP per sample were received 4 weeks after submission (~900,000 SNPs). Data was deposited in T3.

Breedbase: UNL submitted 9 trials containing 1,824 plots with agronomic data to T3

Genomic selection: Genomic selection is regularly used for agronomic traits and a GS model training for wheat stem sawfly is in progress.

8.12. NY. Cornell University. Mark Sorrells

Education: Timothy Mulderrig is the PhD student working on the WheatCAP at Cornell. He attended the virtual monthly meetings, submitted a recorded presentation for the student meeting at PAG, participated in the industry-mentor program, and attended the Functional Genomics Workshop at KSU. Tim is working on fine mapping a grain size QTL on chromosome 2DS.

Germplasm & publications: No new wheat germplasm or publications.

UAS: For 2023-2024 the NY group performed multispectral imaging over the Winter Wheat Master (PYT) trial. The trial consisted of 210 plots covering ~0.5 acres. The trial was

phenotyped at 9 timepoints across the growing season and raw flight data for all flights were uploaded to the UAS hub. The UAS hub generated CC, CH, CV, ExG, NDVI, NDRE data and uploaded it into T3.

Genotyping: 2423 samples were submitted from the 2024 Winter Wheat Master Trial to the Eastern Regional Small Grains Genotyping Lab for 72 markers. In addition, 258 lines were genotyped by GBS (8,920 markers average). Both datasets have been uploaded into T3.

Breedbase: In 2024, NY uploaded the Winter Wheat Master (PYT) to T3 (210 plots). All trial metadata, agronomic data, and phenotype scores have been uploaded to T3. Genomic selection has not been completed using the 2024 data from this trial yet, but the NY group will continue implementing GS in their wheat breeding program.

8.13. OK. Oklahoma State University. Liuling Yan, Brett Carver & Phil Alderman

Education: PhD student Sanju Shrestha attended the WheatCAP Annual Meeting in San Diego 2024 and with PhD student Juan Luzuriaga attended the Functional Genomics Workshop in Kansas in August 2024. PhD student Wenxuan Zhai graduated in December 2023 and is working as a postdoctoral fellow at the Carnegie Institution for Science, California.

Germplasm & publications: The HRW wheat cultivar 'Scab Stryker' (OK16103083) was released to commercial seed producers by OSU Ag Research in April 2024 and will be submitted to PVP in December 2024. It provides a reliable source of Fusarium head blight (FHB) resistance in eastern and north central Oklahoma. Scab Stryker resulted from marker-assisted introgression of *Fhb1* with the assistance of the USDA-ARS Hard Winter Wheat Genotyping Laboratory (Apdx. 1). The OK group has 3 papers under review that will be included in the 4th year report.

UAS: Nineteen UAS flights covering 3.3 acres of the breeding trial field were processed using OpenDroneMap (1743 plots). High-level phenotypic traits data such as canopy cover, ExG, NDVI, canopy height, NDRE, and GNDVI, have been submitted to T3. All data processing was in-house. The field map is being uploaded to T3.

Genotyping: 1,504 lines were genotyped using GBS approach at the Manhattan Genotyping Laboratory, which resulted in an average of 32,636 GBS markers (6.2M datapoints). In addition, 1,743 lines were genotyped with MRASeq (2,887 markers) and data was submitted to T3.

Breedbase: Eight trials with a total of 1,742 plots were submitted to T3 including yield data.

Genomic selection: No data reported.

8.14. OR. Oregon State University. Margaret Krause (moved from UT to OR in 2024)

Education: Dalton Jones graduated from Utah State University with an MS in Plant Science in May 2024. He is now pursuing a PhD at Oregon State University with Margaret Krause. Dalton attended the project meeting in San Diego 2024 and the functional genomics workshop at Kansas State University. He participated in the monthly student meetings and is currently in one of the writing groups.

Germplasm & publications: OR released two SWWW cultivars in 2024: 'Gale' (PVP 202500001, 10/03/2024), and 'Mallory CL+' (PVP soon).

UAS: due to the move between UT and OR it was not possible to collect WheatCAP UAS data in 2024. Flights will be resumed in 2025.

Genotyping: 175 lines were submitted to the genotyping lab in WA. Data was received in March 2024 and showed an average of 3,921 SNPs per line. Data was uploaded to T3 under genotyping project "OSU_2024_AgriSeq".

Breedbase: 7 trials (846 plots) from the 2023 last season at UT and the corresponding agronomic data were submitted to T3. In 2024, the OR group generated data for 103 trials and 10,050 plots and 93 trials and 8,882 plots have been uploaded to T3.

Genomic selection: The AgriSeq 5K were the first genome-wide marker data generated on Oregon State University breeding material and will be used to generate the first training sets. Additional lines will be sent for genotyping to USDA Genotyping lab before the end of the year.

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

Education: Two PhD students, Swas Kaushal and Mandeep Singh, participated in most of the WheatCAP educational activities including the monthly meetings, the writing groups, the in-person 2024 PAG meeting, and the Functional Genomics Workshop in KSU Manhattan. Swas is participating in the WheatCAP Planning Committee, assisting in organizing events and contributing to planning efforts. He also did oral presentations at the U. of Illinois and the Nebraska Plant Science Symposia and in the AI in Agriculture. He presented posters at the North American Plant Phenotyping Network Annual Conference and at the National Association for Plant Breeding, St. Louis, MO. Two undergraduate students (Mackenzie Henning, Female and Chunki Lau, Male) participated also in the WheatCAP project.

Germplasm & publications: 'SD PHEASANT' was released in 2023 and PVP was obtained in 2024, (PVP No. 202400437 submitted 8/16/24) (Apdx. 1). The SD group published 5 peer-reviewed articles in 2024 (summarized in Apdx. 2).

UAS: In-house processing of the UAV data was performed and high-level data from 11 flights from 2 locations for 2024 Elite and Advanced yield trials (720 plots) has been uploaded to T3 (3 acres). Data from 5 additional flights are being processed and will be uploaded when completed.

Genotyping: 157 samples for which phenotypic data was collected over multiple locations in 2024 were submitted for GBS to HWW Genotyping Lab, Manhattan, KS. On average 14,200 SNP per line were received and were uploaded into T3.

Breedbase: Six trials constituting 1,080 plots with their corresponding agronomic data were submitted to T3 for 2024.

Genomic selection: The SD group has completed forward validation of GS for several traits including AI-assisted FDK and DON and is currently working on GS for grain size. The SD group found a novel and major stable QTL for flag leaf angle QTL (qFLANG.1A) on chromosome 1A that accounts for 9–13% variation. The allele for narrow leaf angle showed a

high frequency likely associated with positive selection. A KASP assay was developed for allelic discrimination of qFLANG.1A and was used for its independent validation.

8.16. TX. Texas A&M. Shuyu Liu, Juan Landivar, Jackie Rudd

Education: PhD student Kyle Parker was awarded the 2024 Borlaug Scholarship from the National Association of Plant Breeders, graduated in August, and is currently working for Bayer Crop Science. PhD student Shannon Baker presented a poster at the PAG meeting and made an oral presentation at the 3rd International Wheat Congress. PhD students Luke Whiteley and Abdullah participated in the WheatCAP educational activities. Kyla Diaz and Himabindhu Badavath started their PhD programs in 2024.

Germplasm & publications: No new varieties or publications in 2024.

UAS: the TX breeding programs completed 51 flights across five locations and 14.7 acres and images were sent to the hub (4328 plots). Two locations have been processed and data will be uploaded to T3.

Genotyping: A total of 327 breeding lines and 326 doubled-haploid lines from 4 parents, 8 parents and nested association mapping populations were analyzed by GBS; gene-linked KASP markers were screened on 8 Texas lines in 2024 SRPN, and 90 lines in RGON were screened by genotyping center at Manhattan, KS. Data will be deposited in T3 before the end of the year.

Breedbase: Four filed trials with 120 plots each (480 plots) and their respective agronomic data have been uploaded into T3.

Genomic selection: Approximately 100k SNPs from GBS were used for GEBV. GEBVs were evaluated and compared with phenotypic evaluation and selection. On average, ~ 5-10% were selected by both GEBV and phenotypic selection, ~55% were discarded by both methods and ~15-20% were selected by GEBV or phenotypic selection. Due to high rust pressure, phenotypic selection based on rust data was much higher than usual.

8.17. VA. Virginia Tech. Nick Santantonio

Education: PhD student Sunilda Frias participated in the student workshop at PAG and the Functional Genomics Workshop at KSU.

Germplasm & publications: The VA group released four new SRWW varieties that are being privately licensed or are up for license (Apdx. 1). No new publications.

UAS: In 2024, 230,052 plot images from 2022 and 2023 were processed and submitted to T3, representing 16 trials, 5,602 plots, 2,491 lines and 5 to 9 flights per trial and 5 bands per flight. New 2024 flights included 5 flights over 3282 plots in Warsaw and 7 flights over 2651 plots in Blacksburg (total 34,697 images).

Genotyping: 1,060 lines were submitted to Wheat Genotyping Lab at NCSU. These lines are part of a set of 6,597 VT lines genotyped *in house* by GBS in 2024 resulting in an average of 15,124 markers per line. Genotyping data was deposited in T3.

Breedbase: In 2024 the VA group generated 64 trials (36 yield trials, 13 scab trials, 14 disease trials) including 13,204 plots (9,342 yield plots, 1,856 scab plots, 2,006 disease plots). Agronomic data for the 9,342 plots in the 36 yield trials will be submitted to T3.

Genomic selection: The VA program is currently using genomic selection at all stages of their breeding program. Genome-wide markers are used to estimate marker effects, and to predict all possible pairs of selected parents. A selection index is used to prioritize crosses from lines that are available to cross any given day. F1s are selected for DH based on the selection index and prior knowledge of parents. All F4:6 lines advanced to yield trials are genotyped with GBS. Genomic prediction of all lines across all environments is used to select lines and target them to mega environments after the initial year-1 yield trials. For 2024 crossing, a genomic prediction model was trained using data from the VT breeding program across five years (2019-2023).

8.18. WA. Washington State University. Arron Carter and Mike Pumphrey

Education: PhD student Andrew Herr graduated in 2024 and is working as a breeder at Corteva. PhD students P. Schmucker, M. Zubrod, and A. Jamalzei participated in 2024 WheatCAP meetings at PAG. Two undergraduate researchers from underrepresented minority groups were interns in the UAV program in 2024.

Germplasm & publications: the WA group released 3 new varieties (Perrine, Rydrych MZ and Bush) in 2024 (Apdx. 1) and published three new papers (Apdx. 2.).

UAS: WA analyses their UAS data in-house. In 2024, they completed 43 unique winter wheat trials at eight different locations which totaled 16 acres. The 2024 winter wheat UAV data include 12 trials, and 1,764 plots with data collected on them (22,932 datapoints). Data will be deposited in T3 by the end of 2024. The UAS data is used in different prediction models that helped the WSU programs advance breeding lines with more stable agronomic performance across diverse environments.

Genotyping: In 2024, the WA group submitted 2,304 winter wheat samples for GBS in collaboration with the Eastern Regional Genotyping Laboratory. These same samples were submitted to the ThermoFisher 5K genotyping platform in conjunction with the Western Genotyping Lab to compare platforms. The samples were also screened with markers for resistance genes against stripe rust, eyespot foot rot, and SBWMV. Data will be submitted to T3 by the end of the year.

Breedbase: In 2024, the WA group will be submitting trials from six locations on preliminary and advanced breeding trials. This will include 1,764 plots with 22,932 phenotypic datapoints. Agronomic and spectral data will be submitted to T3 by the end of the year.

Genomic selection: The WA team runs genomic selection in the winter wheat program on all 1st year observation plots, primarily to estimate end-use quality performance, disease resistance, and grain yield.

8.19. WI. University of Wisconsin. Lucia Gutierrez

Education: PhD student Rishap Dhakal finished his course work and completed his written preliminary exam. He has participated in the annual student WheatCAP meeting in 2024 in San Diego and presented a poster in PAG 31. He also participated in 3 monthly meetings, 1 writing workshop, 1 training workshop and 2 lecture and discussion series from WheatCAP. Rishap is the Treasurer for 2025 WheatCAP-Plant Sciences Graduate Student Symposium in San Diego. PhD Pablo Sandro also participates in the WheatCAP as a research technician and will be graduating in Fall 2024. Gopika Gopinathan is a third PhD student who participated in 2 lectures and discussion series from the WheatCAP and is part of one of the writing workshops.

Germplasm & publications: The new HRWW variety 'Bickford' (WI13WW260.06) has been released and PVP is in preparation. One wheat publication in preparation.

UAS: For the 2023-2024 season, a total of 8 flights - including the first flight used for topography were submitted to T3. Each flight covers a field of 1.4 acres, for a total of 12.6 acres (1510 plots). The shape file and field map were sent to the Texas A&M team, but data have not been received back yet.

Genotyping: A total of 960 winter wheat genotypes were sent for genotyping at the Fargo genotyping lab with the 3k Illumina Infinium II array technology that includes 3000 SNPs on a multi-species platform. The data will be uploaded in T3/Wheat CAP by the genotyping lab.

Breedbase: A total of 3 trials consisting of 2 yield trials (Madison and Spooner) and 1 headrow nursery (Madison) with a total of 1511 plots have been uploaded to T3/WheatCAP database. The trials include agronomic traits such as grain yield, grain test weight, plant height, heading date, stripe rust, Fusarium head blight, barley yellow dwarf virus (total 6284 data points).

The breeding program has been using the Field Book app for collecting data on several traits. However, due to the need for quality checks, the WI group has not been able to implement a direct upload into T3. The collected data is saved locally, and it will be uploaded into T3 after a quality check.

Genomic selection: In 2024, the WI team genotyped their nurseries (where they cannot have yield data) and selected families with the best performance. The WI program has now a functional GS pipeline that is helping them to accelerate generation advancement by combining speed breeding with estimated GEBV.

9. Priorities for year 4

9.1 Education priorities for Y4: Education priorities for 2025 include several workshops, expanding the mentorship program for new students, and continuing writing groups and monthly meetings. One workshop will be in person, the other three will be online.

The first workshop will be held online and hosted by CIMMYT and will include topics such as the role of CGIAR and CIMMYT, the CIMMYT traditional breeding program, physiological breeding strategies and approaches, the integration of molecular technologies in breeding programs, and available genomic and germplasm resources.

In the Spring, the second set of students in their 3rd or 4th year (Cohort 2) will participate in one-on-one leadership coaching with BONSAI, followed by two group workshops, as described in 2. Education. In the fall, Cohort 2 will participate in mock interviews. An in-person workshop focused on science communication will be led by the company "Something Else".

9.2. Genomic Resources priorities Y4: In 2025, the KSU team will conduct analysis of tissue-specific eQTL data generated for a diverse panel of 200 wheat lines and wheat wild relatives. The relationship between marker-trait associations detected in the WheatCAP mapping experiments and eQTL will be investigated and used for building improved genomic prediction models. The deposition of RNA-seq and eQTL data to the T3 database will be completed in 2025.

The new PHG based on whole-genome sequence alignments will be further expanded to include additional lines sequenced at intermediate levels of sequence coverage (5-6x) using PacBio technology. These lines will be selected from the set of 200 diverse wheat lines collected from the US wheat breeding programs and used for eQTL study. This dataset will be incorporated into the Wheat PHG to take full advantage of a new PHG tool that has higher imputation accuracy. In addition, KSU team will integrate into new PHG genomic data generated using short-read technologies at 5-10x coverage for a subset of 200 diverse WheatCAP lines. This will permit more accurate imputation of genome-wide data generated for 200 WheatCAP lines and conduct more accurate eQTL mapping. The updated PHG will be used to impute all deposited genotyping data in the T3 database.

The UCD team will explore the expansion of spatial transcriptomics resources by using the 10X Xenium platform. A new experiment with 240 genes is in preparation for early 2025 to characterize developing spikes of wildtype and mutant wheat lines and to expand the spike development expression atlas. Efforts are being coordinated internationally to test different spatial transcriptomics platforms and to generate a uniform nomenclature for genes involved in wheat spike development.

9.3. T3/Breedbase priorities Y4

1. Practical Haplotype Graph version 2. The new version of the PHG requires whole genome assemblies. There are currently a limited number of assemblies available publicly. The T3 group is organizing an effort among the genotyping labs and the Akhunov Lab to bring together assemblies that are not yet publicly available to populate a PHGv2 that is effective for North American germplasm. This project is delicate because each lab has its own research and publication plans for its assemblies. Nevertheless, the T3 group expects that for next year's annual report they will have results from a PHG v2 that incorporates over 30 assemblies.

2. In addition to anonymous genome-wide markers, the genotyping labs score many Known Informative Markers (KIMs), which are typically KASP markers. Downstream, the calls at those markers are used to infer what alleles are present at major loci affecting height, vernalization, photoperiod, disease resistance, etc. The process is complicated and is currently managed independently by each genotyping lab. The T3 group is working with the

labs to coordinate efforts so that common KIMs and inference algorithms are used to call Major Locus Alleles. Controlled vocabularies for the trait categories, the genes, and the known alleles at the genes are being defined. A new genotyping protocol will be defined on T3, the Major Locus Alleles genotyping protocol, for which the allele scores will not be directly uploaded but will be determined based on genotypes at the AgriSeq+KASP or Illumina 3K+KASP (from the Fargo Lab). Definition of that protocol will enable the following new functionality:

    a. Links to available literature on all genes through GrainGenes. GrainGenes already curates many literature citations to genes. These will be linked to the Major Locus detail pages on T3.

    b. Accession search based on major locus genotypes. Breeders will be able to seek among all genotyped accessions for those carrying specific alleles at those loci. Such accessions could then be used as parents to introgress the alleles into the breeder's program.

    c. Display accession's major locus profiles. The breeder will enter an accession's name in the interface and its genotype at relevant major loci will be displayed.

3. UAS data from programs analyzing their own drone data. As software for analyzing UAS images has improved, more individual programs are analyzing their own data. In year 4, T3 will develop a system to capture data from programs that are not going through the UAS-Hub. The D2S platform described below can be that option.

9.4. UAS-hub priorities Y4

The UAS-hub plans to switch the UAV data management platform to Data to Science (D2S) to further enhance data accessibility and analysis tools. The D2S will provide a complete UAS data processing pipeline to breeding program so that they can perform all the UAS-based HTP feature extraction and submit the extracted features back to the T3 database without relying on other programs. They plan to train a smaller set of breeding programs in 2024 to test the new D2S platform and switch to the new platform starting in 2025. The switch will be announced in the PAG 2025 meeting with a live demonstration. A full training workshop (online training) will be provided in Q1 of 2025 to complete the transition.

As the project enters its fourth additional efforts will be made to make the breeding programs more independent in the analysis of their own data, so they can continue their UAS programs beyond the WheatCAP project. The D2S platform will help with this transition.

Challenges: So far, the TX group has successfully downloaded plot map information from T3 and uploaded spatially referenced plot maps and phenotypic information onto T3, but more datasets need to be uploaded. However, there are still communication challenges between the breeding programs and the UAS-hub that result in late uploading of raw images, and delays in the submission of plot boundaries and upload of the plot map information to T3. These delays then limit the ability of the TX group to complete other tasks on time. The transition to D2S platform is expected to reduce these delays.

9.5. Genotyping labs priorities Y4

During year 4 of the grant, the genotyping labs will evaluate new versions of the AgriSeq and Illumina 3K genotyping platforms for genome-wide information content and reliability of trait related markers. The genotyping labs will share information across labs for automated generation of marker reports and add a process to calculate concordance of new samples against those previously genotyped in the labs. The mid-density platforms will be broadly available for genotyping breeders' samples as requested.

The genotyping labs will work with breeders to incorporate new genotyping platforms into their existing genomic selection pipelines. Their analyses indicate that using subsets of samples with overlapping marker datasets (i.e., GBS and AgriSeq markers) is an option for "bridging the gap" between genotyping platforms. Work will be done to utilize a Practical Haplotype Graph for US wheat in this effort. Imputation could allow breeders to use existing training populations for genomic prediction without the need for re-genotyping large numbers of lines. The labs will work to evaluate imputation accuracy and genomic prediction accuracy in breeding materials.

Ongoing meetings involving the PI and staff for the four regional genotyping labs and the T3 database will continue in Year 4. The goal is to improve communications across regions, share new results and develop a standardized nomenclature for known informative markers for loci of major effect. This effort is particularly focused on loci evaluated with the targeted mid-density platforms. Standardization of protocols and data handling at the genotyping labs is aimed at uniform reporting for marker data that is conducive to the inclusion of more information in T3.

10. Budget and personnel changes

There have been no changes in the original objectives of the grant and no changes in the total. The transfer of WheatCAP subaward from Utah State University to Oregon State University (Margaret Krause) mentioned in the previous report was completed smoothly.

11. Concluding remarks

The third year of the WheatCAP has been very productive with the project outputs disseminated through 28 new peer-reviewed publications including some in high-profile scientific journals (e. g. Development, The Plant Journal, Plant Biotechnology Journal, BMC Biology, among others). New genomic resources included HiFi genomic data for 10 diverse accessions of wheat, expression data for 200 accessions across five tissues, and the generation of a spatial and single cell transcriptomics atlas for wheat spike development. Markers from different platforms present in T3 were integrated using imputation with the Practical Haplotype Graph v1.

Agronomic data was collected from 57,800 plots from 290 field trials and was supplemented with 282 UAS flights over 119 acres (35,376 plots). Genotypic data was collected from 48,900 samples generating >350M datapoints. WheatCAP breeders released 28 new varieties and 5 improved germplasms that benefitted from high-throughput genotyping and phenotyping platforms. Presentations of these varieties in field days across the country showcased the value of these new technologies and of the USDA-NIFA support to the growers and to the wheat industry. The increased productivity, disease resistance and quality of these new varieties provides high-

quality products for consumers while reducing the use of fungicides and pesticides and boosting the competitiveness of USA wheat growers and industry.

The genomic tools and databases generated by the WheatCAP continue to accelerate the pace of discoveries in wheat improvement which is essential to anticipate the changes caused by climate change. One of the most important contributions of the WheatCAP is the development of a framework for coordinating activities across all the major wheat breeding and research programs in the country, which eliminates unnecessary duplications and generates positive and synergistic collaborations. Finally, the WheatCAP PIs have trained 50 PhD students in modern plant breeding techniques, who will guarantee the continuity of the US agricultural enterprise into the future. None of these would have been possible without this integrated Collaborative Agricultural Project. The wheat research and breeding communities and the wheat industry are grateful for this support.

12. Deliverables & Other Outputs:

- **Appendix 1.** Germplasm releases
- **Appendix 2**. Publications
- **Appendix 3**. Community resources
- **Appendix 4.** Graduate students. https://www.triticeaecap.org/educational-activities/
- **Appendix 5.** Results of a survey conducted by the genotyping laboratories

APPENDIX 1. Varieties and Germplasm releases 2024 (28 varieties and 5 germplasm).

2024 PVP that were Pending in previous reports

1. 'AP24-AX' (CO14A136-135 = [(AF10/2*Byrd)//(AF26/Byrd)]) is a new CoAXium wheat variety released by Colorado State University in 2023. PVP 202400244, submitted 02/01/2024.

2. 'UI Warrior' was submitted for PVP 202400308 in 03/13/2024. UI Warrior is a soft white spring wheat with high grain yield, excellent end-use quality and test weight (App. 2).

3. 'MT Dutton' is a spring wheat variety that was released from Montana State University breeding program in 2023. PVP 202400204, submitted 12/28/2023.

4. 'MT Carlson' is a spring wheat variety that was released from Montana State University breeding program in 2023. PVP 202400205, submitted 12/28/2023.

5. 'ND Stampede' is a hard red spring wheat variety released by North Dakota State university that is widely adapted throughout the Northern Plains region. PVP 202400409, submitted 07/05/2024.

6. 'Gale' (OR2180377) is a SWWW variety adapted to the Willamette Valley in western Oregon. It was developed using molecular markers for hybrid necrosis, photoperiod insensitivity, stripe rust resistance, straw breaker foot rot resistance, FHB resistance, pre-harvest sprouting, and polyphenol oxidase activity. PVP 202500001, submitted 10/03/2024.

7. 'SD PHEASANT' is a HRWW developed by the South Dakota Agricultural Experiment Station that was released in 2023 and was PVP in 2024. PVP 202400437 submitted 8/16/24.

2024 Variety releases with pending PVP or licensed without PVP

1. CO19D087R (CO12D1777/Langin) is a high yielding hard red winter wheat, developed utilizing the wheat by maize doubled haploid system. Released by Colorado State University in 2024. PVP will be submitted in August 2025.

2. CO18042RA (CoAxium Byrd/Langin) is a new three gene CoAXium wheat variety. Released by Colorado State University in 2024. PVP will be submitted in August 2025.

3. CO200037R (Canvas//X170868/Canvas) is a new hard red winter wheat variety containing *Wsm3* which provides broad resistance to curl mite vectored viruses. Released by Colorado State University in 2024. PVP will be submitted in August 2025.

4. IL2020-5376. This is an ultra-early maturing variety with moderate scab resistance, good test weight, and high yield for its maturity class. Molecular markers were used in this line's evaluation process. Licensing agreement with seed company (no PVP).

5. IL16LCSDH-04-T-2659-3. This is a very early maturing variety with moderate scab resistance, good test weight, and excellent yield for its maturity class. Licensing agreement with seed company (no PVP).

6. IL16LCSDH-04-T-2659-216. This is a very early maturing variety with good scab resistance, good test weight, excellent standability, and high yield for its maturity class. It also has good

resistance to multiple diseases including septoria, leaf rust, and stripe rust. Licensing agreement with seed company (no PVP).

7. KS20H124, released as KS Homesteader CL+ for western Kansas, is a hard red, two-gene Clearfield winter wheat with competitive yield, good drought tolerance, good disease resistance, and excellent baking quality. It was evaluated in regional nursery testing by the Hard Winter Wheat Regional Genotyping laboratory.

8. KS20HDW185, released as KS Snow Fox in 2024 for western Kansas, is a HWWW with very competitive yield, good drought tolerance, good disease resistance, and very strong gluten strength via the $Glu\text{-}B1_{Bx7oe}$ allele. It was evaluated in regional nursery testing by the Hard Winter Wheat Regional Genotyping laboratory.

9. MI20R0210 is a soft red winter wheat being proposed for release in 2025 by the Michigan Wheat Program, pending commercialization discussions.

10. MT Ubet (MT 2030) is a spring wheat variety released from Montana State University breeding program in 2024. The PVP application will be submitted this fall.

11. NE Prism CLP (NHH17612) is a hard red winter wheat with two-gene Clearfield herbicide resistance targeted towards the Nebraska Panhandle and western South Dakota region. It will be submitted for PVP soon.

12. 'Scab Stryker' (OK16103083) is a HRW wheat cultivar released to commercial seed producers by OSU Ag Research in April 2024. It provides a reliable source of FHB resistance. Scab Stryker resulted from marker-assisted introgression of $Fhb1$ with assistance of the USDA-ARS Hard Winter Wheat Genotyping Laboratory

13. 'Mallory CL+' (ORI2190025 CL+) is a SWWW variety adapted to the low to intermediate rainfall production areas of Oregon. It contains two genes for resistance to the Beyond herbicide and markers for photoperiod insensitivity, stripe rust resistance, straw breaker foot rot resistance, FHB resistance, and reduced pre-harvest sprouting. PVP in preparation.

14. 16VDH-SRW03-023 is a Virginia Tech high yielding, early, semi-dwarf, awnletted SRWW broadly adapted to the eastern US with high levels of resistance to leaf and stripe rusts, Hessian fly and barley yellow dwarf virus, as well as moderate resistance to powdery mildew and fusarium head blight. Privately licensed.

15. 15VTK-1-101 is a Virginia Tech high yielding, full season, short stature semi dwarf, awned SRWW adapted to the southern coastal states (VA, NC, SC, GA) with high levels of resistance to leaf rust, powdery mildew, Hessian fly and barley yellow dwarf virus, moderate levels of resistance to fusarium head blight and stripe rust. Up for license.

16. 17VTK4-29 is a Virginia Tech high yielding, early, semi-dwarf, awnletted SRWW adapted to the Mid-Atlantic with high levels of resistance to leaf rust and powdery mildew, moderate resistance to FHB ($Fhb1$) and Hessian fly, but susceptible to stripe rust. Up for license.

17. VA19FHB-36 is a Virginia Tech high yielding, high TW, early, semi-dwarf awned soft red winter wheat broadly adapted to the eastern US, with high resistance to powdery mildew and leaf rust, moderately resistant to FHB, but susceptible to Hessian fly. Privately licensed.

18. 'Perrine' is a WSU SWWW adapted to the irrigated regions of South Idaho. It was developed using markers to incorporate eyespot and nematode resistance genes. PVP pending.

19. 'Rydrych MZ' is a WSU SWWW tolerant to the herbicide metribuzin. This line will allow growers to rotate herbicide options with other herbicides. PVP pending.

20. 'Bush' is a WSU SWSW with high and stable grain yield, stripe rust resistance, aluminum tolerance, and Hessian fly tolerance and excellent end-use quality. PVP pending.

21. 'Bickford' (WI13WW260.06) is a new HRWW variety release from the University of Wisconsin-Madison. PVP is in preparation.

2024 Germplasm

1. PI 704906. Bread wheat RIL143 with a radiation induced deletion (4.2 Mb) on chromosome 6AS that eliminates all the *Gli-A2* α-gliadins. No negative effect on yield or quality. Released by the University of California, Davis (Rottersman, M. et al. 2024).

2. PI 704907. Bread wheat RIL143 with a radiation induced deletion (2.1 Mb) on chromosome 6BS that eliminates all the *Gli-B2* α-gliadins. No negative effect on yield or quality. Released by the University of California, Davis (Rottersman, M. et al. 2024).

3. PI 704908. Bread wheat RIL143 with a radiation induced deletion (14.7 Mb) on chromosome arm 6DS that eliminates all the *Gli-D2* α-gliadins. No negative effect on yield and significant improvements on gluten strength and breadmaking quality. Reduced allergenicity for Celiac Disease. Released by the University of California, Davis (Rottersman, M. et al. 2024).

4. Mapping population LA95135 x SS-MPV57 (UX1443; 358 lines) was deposited in the USDA- NSGC. This population was evaluated for spike and seed morphology traits, heading date and plant height at multiple environments, contributing to characterization of genes underlying the *B1* awn suppressor, *Rht25*, *WAPO-A1* and *FT-A2* loci.

5. Mapping population AGS2000 x LA95135 (UX1444; 291 lines) was deposited in the USDA- NSGC. This population was evaluated for heading date, plant height, glaucousness and disease resistance at multiple field environments and contributed to identification of candidate genes underlying *Pm54* and *Sr2Ak* conferring resistance to powdery mildew and to stem rust race TTKSK, respectively.

APPENDIX 2. Peer reviewed publications WheatCAP 2024

Google Scholar Impact of previous WheatCAP publications (measured 10/8/2024)

TriticeaeCAP (2011-2016): 28,502 cross-references (h index = 79).

WheatCAP (2017-2021): 8,504 cross-references (h index = 52).

WheatCAP (2022-2026): 1,149 cross-references (h index = 20).

Reported in 2024. 28 new publications

1. Zhang, J., G.F. Burguener, F. Paraiso, J. Dubcovsky. 2024. Natural alleles of LEAFY and WAPO1 interact to regulate spikelet number per spike in wheat. bioRxiv https://doi.org/10.1101/2024.08.17.608376 accepted in Theoretical and Applied Genetics.

2. Zhang, C., J. Hegarty, M. Padilla, D.M. Tricoli, J. Dubcovsky, J.M. Debernardi. 2024. Manipulation of the microRNA172 - *AP2L2* interaction provides precise control of wheat and triticale plant height. bioRxiv https://doi.org/10.1101/2024.08.05.606718. Accepted in Plant Biotechnology Journal.

3. Rottersman, M.G., W. Zhang, J. Zhang, G. Grigorian, G.F. Burguener, Carter C., T. Vang, J. Hegarty, X. Zhang, and J. Dubcovsky. 2024. Deletion of wheat alpha-gliadins from chromosome 6D improves gluten strength and reduces immunodominant celiac disease epitopes. bioRxiv. https://doi.org/10.1101/2024.07.19.604379

4. Paraiso F., H. Lin, C. Li, D.P. Woods, T. Lan, C. Tumelty, J.M. Debernardi, A. Joe, J. Dubcovsky. 2024. LEAFY and WAPO1 jointly regulate spikelet number per spike and floret development in wheat. Development 151:dev202803. https://doi.org/10.1242/dev.202803 (in bioRxiv previous report).

5. Li, C., H. Lin, J.M. Debernardi, C. Zhang, and J. Dubcovsky. 2024. GIGANTEA accelerates wheat heading time through gene interactions converging on FLOWERING LOCUS T1. The Plant Journal. 118, 519–533. https://doi.org/10.1111/tpj.16622

6. Winn, Z.J., E. Hudson-Arns, M. Hammers, N. DeWitt, J. Lyerly, G. Bai, P. St. Amand, P. Nachappa, S. Haley, R.E. Mason. 2024. HaploCatcher: An R package for prediction of haplotypes. The Plant Genome 17:e20412. https://doi.org/10.1002/tpg2.20412

7. Wondifraw, M., Z.J. Winn, S.D. Haley, J.A. Stromberger, E.E. Hudson-Arns, R.E. Mason. 2024. Advancing water absorption capacity in hard winter wheat using a multivariate genomic prediction approach. Crop Science In press: https://doi.org/10.1002/csc2.21321

8. Wondifraw, M.A., Z.J. Winn, S.D. Haley, J.A. Stromberger, E.E., R.E. Mason. 2024. Elucidation of the genetic architecture of water absorption capacity in hard winter wheat through genome wide association study. The Plant Genome 17:e20500. https://doi.org/10.1002/tpg2.20500

9. Joshi P, G.S. Dhillon, Y. Gao, A. Kaur, J. Wheeler, and J. Chen. 2024. An optimal model to improve genomic prediction for protein content and test weight in a diverse spring wheat panel. Agriculture, 14:347. https://doi.org/10.3390/agriculture14030347

10. Zhao, L., A. Bernardo, F. Kong, W. Zhao, Y. Dong, H. Lee, H.N. Trick, J. Rupp Noller, G Bai. 2024. A glutathione S-transferase from *Thinopyrum ponticum* confers *Fhb7* resistance to Fusarium head blight in wheat. Phytopathology 114 (7): 1458-1461 https://doi.org/10.1094/PHYTO-03-24-0106-SC

11. Kumar, P., H.S. Gill, M. Singh, K. Kaur, D. Koupal, S. Talukder, A. Bernardo, P. St Amand, G. Bai, S.K. Sehgal. 2024. Characterization of flag leaf morphology identifies a major genomic region controlling flag leaf angle in the US winter wheat (*Triticum aestivum* L.). Theor Appl Genet 137 (205): 1-17 https://doi.org/10.1007/s00122-024-04701-1

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APPENDIX 3. Community resources generated in Y3

2024 Public databases

1. T3 database https://triticeaetoolbox.org/wheat/ large increase in data deposits

2. Sequenced mutant populations https://dubcovskylab.ucdavis.edu/wheat_blast. Data has been deposited in GrainGenes and ENSEMBL.

3. Additional ATAC-seq data incorporated into the GrainGenes genome browser.

5. New protocols for Marker Assisted Selection https://maswheat.ucdavis.edu/

   1. Updated information for spikelet number per spike gene *bZIPC1* (https://maswheat.ucdavis.edu/protocols/bZIPC1).

   2. CAPS Marker for the A10 allele of the spikelet number per spike gene *FT-A2* (https://maswheat.ucdavis.edu/protocols/FTA2)

   3. Updated KASP markers for stripe rust resistance gene Yr78 (https://maswheat.ucdavis.edu/protocols/Yr78)

Appendix 4. Graduate Students (8 completed in 2024 + 50 current = 57, 46% female)

Institution	PI	1st name	Last name	Deg.	Gen.	Start Date[1]	*
U. of California-Davis	J. Dubcovsky	Maria	Rottersman	PhD	F	9/1/2022	
U. of California-Davis	J. Dubcovsky	Connor	Tumelty	PhD	M	9/1/2023	
U. of California-Davis	J. Dubcovsky	Elmer	Flores	PhD	M	9/1/2024	
Colorado State U.	Eston Mason	Emily	Billow	MS	F	5/1/2024	
Colorado State U.	Eston Mason	Mereset	Wondifraw	PhD	F	1/1/2019	*
Colorado State U.	Eston Mason	Mikayla	Hammers	PhD	F	9/1/2022	
U. of Florida	Ali Babar	Jordan	McBreen	PhD	M	1/1/2021	
U. of Idaho	Jianli Chen	Pabitra	Joshi	PhD	F	1/1/2021	
U. of Idaho	Jianli Chen	Yaotian	Gao	PhD	M	7/1/2021	
U. of Illinois	Jessica Rutkoski	Lucas	Munaro	PhD	M	1/1/2021	
U. of Illinois	Jessica Rutkoski	Raysa	Gevartosky	PhD	F	8/1/2021	
Purdue University	Jinha Jung	Ismail	Olaniyi	PhD	M	8/1/2021	
Kansas State U.	Eduard Akhunov	Buket	Sahin	PhD	F	8/1/2023	
Kansas State U.	Eduard Akhunov	Dwight	Davidson	MS	M	8/1/2021	
Kansas State U.	Eduard Akhunov	Gabriela	Campos	PhD	F	5/1/2024	
Kansas State U.	Eduard Akhunov	Jaime	Knight	MS	F	8/1/2023	
Kansas State U.	Eduard Akhunov	Nicholas	Stelling	PhD	M	1/1/2023	
USDA-Kansas State U.	Katherine Jordan	Lawrence	Tidakbi	PhD	M	1/1/2022	
USDA-Kansas State U.	Mary Guttieri	Adriano	Moreira	PhD	M	1/1/2023	
USDA-Kansas State U.	Guihua Bai	Jinan	Park	MD	F	5/1/2024	
USDA-Kansas State U.	Guihua Bai	Xiaoting	Xu	PhD	F	1/1/2020	*
Louisiana St. U	Noah DeWitt	Jolean	McClane	MS	F	1/1/2024	
Louisiana St. U	Noah DeWitt	Julio	Sellani	MS	M	1/1/2023	
U. of Maryland	Vijay Tiwari	Adam	Schoen	PhD	M	8/1/2020	*
U. of Maryland	Vijay Tiwari	Anmol	Kajila	PhD	F	8/1/2021	
U. of Maryland	Vijay Tiwari	Arielís	Morales-Salvá	PhD	F	1/1/2024	
U. of Maryland	Vijay Tiwari	Ian	Moot	PhD	M	8/1/2023	
Michigan State U.	Eric Olson	Jonathan	Concepcion	PhD	M	8/1/2021	
U. of Minnesota	James Anderson	Marcos	De Souza	PhD	M	6/1/2024	
Montana State U.	Jason Cook	Mei Ling	Wong	PhD	F	8/1/2022	
Montana State U.	Jason Cook	Jared	Lile	PhD	M	6/1/2021	
USDA-NC State U.	G. Brown-Guedira	Nico	Lara	PhD	M	8/1/2020	
USDA-NC State U.	G. Brown-Guedira	Daniela	Miller	PhD	F	1/1/2019	*
USDA-NC State U.	G. Brown-Guedira	Mathew	Willman	PhD	M	1/1/2024	
USDA-ND State. U.	Justin Faris	Pooja	Kumari	PhD	F	8/1/2022	
U. of Nebraska-Lincoln	Katherine Frels	Sydney	Graham	PhD	F	4/1/2022	
Cornell U.	Mark Sorrells	Timothy	Mulderrig	PhD	M	8/1/2022	
Oklahoma State U.	Liuling Yan	Wenxuam	Zhai	PhD	F	NA	*
Oklahoma State U.	Liuling Yan	Juan	Luzuriaga	PhD	M	1/1/2022	
Oklahoma State U.	Phillip Alderman	Sanju	Shrestha	PhD	F	8/1/2023	
Oregon State U.	Margaret Krause	Dalton	Jones	PhD	M	5/1/2024	[2]
South Dakota State U.	Sunish Sehgal	Swas	Kaushal	PhD	M	5/1/2022	
South Dakota State U.	Sunish Sehgal	Mandep	Singh	PhD	M	5/1/2024	
Texas A&M	Shuyu Liu	Kyle	Parker	PhD	M	9/1/2020	*
Texas A&M	Shuyu Liu	Luke	Whiteley	PhD	M	1/1/2024	
Texas A&M	Shuyu Liu	FNU	Abdullah	PhD	M	8/1/2021	
Texas A&M	Shuyu Liu	Himabindhu	Badavath	PhD	F	8/1/2024	
Texas A&M	Jackie Rudd	Kyla	Diaz	PhD	F	8/1/2024	
Texas A&M	Jackie Rudd	Shannon	Baker	PhD	F	9/1/2023	
Virginia Tech	N. Santantonio	Sunilda	Frias	PhD	F	8/1/2021	
Washington State U.	M. Pumphrey	Peter	Schmuker	PhD	M	8/1/2020	
Washington State U.	Arron Carter	Andrew	Herr	PhD	M	8/1/2019	*
Washington State U.	Arron Carter	Adele	Jamalzei	PhD	F	9/1/2023	
Washington State U.	Arron Carter	Melinda	Zubrod	PhD	F	9/1/2023	
U. of Wisconsin-Madison	Lucia Gutierrez	Rishap	Dahakal	PhD	M	6/1/2022	
U. of Wisconsin-Madison	Lucia Gutierrez	Pablo	Sandro	PhD	M	8/1/2020	
U. of Wisconsin-Madison	Lucia Gutierrez	Gopika	Gopinathan	PhD	F	9/1/2022	

[1] Date started degree program. * = graduated

[2] Dalton Jones graduated with his MS from Utah State University, advised by Dr. Margaret Krause in Spring 2024 and is currently in a PhD program at Oregon State University.

APPENDIX 5. Survey performed by the Genotyping laboratories

The ARS Genotyping Labs conducted a survey to obtain input on mid-density platforms from all U.S. public wheat breeding programs. There were 30 projects that responded to the survey, including most WheatCAP breeding program participants.

The preferred lab for submitting samples reflected the distribution of U.S. breeding programs across regions, with fourteen, seven, six and five indicating they would send samples to the labs in NC, KS, ND and WA, respectively. Just under half of respondents preferred to utilize the AgriSeq (7) and Illumina 3K mid-density genotyping platforms (7) developed with support of the WheatCAP project, while the remaining programs either preferred to use GBS (8) or indicated no platform preference (8). When asked about the minimum number of polymorphic markers needed for mid-density genotyping, 87% of respondents indicated that 2500 to 3000 markers were required, while 13% indicated a need for 5000 markers.

Breeders were asked how many samples their programs expect to evaluate if the genotyping costs was $7 versus $10 per sample, and approximately half of the individuals responding to this question indicated that they would genotype the same number of samples at both price points, with 8 programs genotyping >1,536 samples and five genotyping either 384-768 or 192-384 samples. For eleven programs, the number of samples evaluated would be halved at the $10 per sample price compared to $7 per sample and for three projects the number of samples would be less than half.

Breeding programs were also asked to rank the relative importance of including markers associated with 145 genes affecting disease and insect pest resistance, flowering time, plant height, kernel characteristics and end-use quality in the platform. While the relative importance of loci varied by regions and breeding programs, mots loci (87%) were ranked as "must have" by at least one program and all loci were given a "high priority" ranking by at least one program. The most critical resistance loci were the *Sbm1*, *Fhb1* and *Fhb7* resistance genes, along with multiple rust resistance loci *Lr37/Yr17/Sr38* and *Lr34/Yr18*. Other highly ranked markers included those for the hardness loci, reduced height genes, glutenin alleles, vernalization and photoperiod genes. The figure below includes traits that were highly ranked by at least 2/3 of participants. Survey results have informed the development of an updated version of the AgriSeq genotyping platform for wheat.

Number of breeding programs ranking trait markers as "high priority" or "must have"