## **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 2 report

1. SUMMARY YEAR 2: During the second year of the project, WheatCAP breeders have released 32 commercial varieties and deposited five improved germplasms in GRIN-Global. The WheatCAP team published 51 new peer-reviewed papers. In the second year, the WheatCAP team made good progress in automation, data sharing, and communication between the UAS-hub and the T3/Wheat database. Field data were collected from 248 UAS flights over 37 acres. A total of 17.815 samples were sent to the Genotyping labs for genotyping, and several genotyping platforms were explored. Approximately 75,000 plots with phenotypic data from 249 field trials were deposited by breeders into T3. This information was used to implement genomic selection in public wheat breeding programs and represent an invaluable resource for investigating the effects of different alleles and their epistatic interactions across environments and germplasm. This year, 10 students completed their PhD and another 41 participated in multiple educational activities organized by the project including three in-person workshops and additional online activities. Students' personal profiles and projects as well as links to project meetings and educational resources are available at the WheatCAP web site (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.

#### 2. Education

In 2023, the WheatCAP team trained 51 students, 10 of which graduated this year. Of those that graduated, 5 started in the previous WheatCAP. The project currently trains 41 students, 44% of which are female. Six of the current WheatCAP students are funded by other sources, but were able to participate in the educational activities by leveraging funds available at their respective universities. In addition to the WheatCAP students, the project provided training to an additional 6 PhD students and 2 Post-docs at the Genomic Selection Workshop.

The project held four workshops in 2023. The WheatCAP Student Workshop, was held in January at the Plant and Animal Genome conference (PAG). Twirty-two students met each other for the first time in-person and gave short presentations to introduce their research projects to the group. Students also attended the Annual WheatCAP Meeting at PAG and presented posters on their research projects after the meeting and discussed them with the WheatCAP CoPIs.

The T3/Breedbase Online Workshop was held online over Zoom across four mornings in February. Between 12 and 17 students attended each day of the workshop, which covered information pertaining to the use of T3 and Breedbase, with the goal of increasing the number of trials uploaded to T3. Topics covered included databases, locations, accessions, field layouts, trait observations, lists, data aggregation, data sub-setting and downloading, accessing the PHG/Imputed data, use of the Android App "Field Book," seedlots, barcodes, and submission of genotyping data to T3. Each day of the workshop was recorded and both recordings and written instructions are available on the Plant Breeding Training Network.

The UAS Workshop, was held in Amarillo, TX May 23rd-25th, 2023 and was attended by 25 WheatCAP students (1 as instructor). Students were provided with a manual to guide them in UAS data collection and analysis. The workshop covered how to plan a mission and prepare for a UAS flight followed by demonstration at the Conservation and Production Laboratory (Bushland, TX). The students learned how to complete all steps of data analysis from initial upload of UAS images to calculating vegetation indices. They attended the Small Grains Field Plot Tour in the morning at the Bushland field site and learned about analysis and interpretation of generated attributes, focusing on the practical application in a breeding program.

The fourth workshop, the Genomic Selection Workshop, was held in Raleigh, NC July 21st – 23rd. A total of 23 graduate students and 2 Postdocs participated in the workshop. Topics covered included introductions to genotyping and data processing for genomic selection, mixed models, genomic prediction, breeding program design for genomic selection in wheat, and future directions in genomic selection. Students were led through many hands-on exercises using example datasets and R code, conducting imputation, cleaning and preparing phenotypic data, calculating BLUEs, and conducting genomic prediction. Four students gave presentations at the workshop on incorporating HTP and UAV data into predictions of genetic merit. Recordings from the workshop, datasets, and code are available on the Plant Breeding Training Network.

In addition to the workshops, monthly meetings with invited speakers were held during the spring and fall semesters. Monthly meeting topics included wheat quality genes, data-driven participatory research to expand the Ethiopian wheat breeding toolbox, physiological traits to breed for drought adaptation and climate resilience, and a panel with four wheat breeders. Recordings from monthly meetings were shared with students via email, or in the case of the breeders panel, on the Plant Breeding Training Network.

Students connected with each other through Slack. Two student-writing groups met in both the spring and fall semester, each with 3-5 students. In the fall semester, one group was dedicated to students writing their thesis or dissertation. Students share written pieces and give each other feedback. Students have also taken on leadership roles, organizing a meeting, symposium, or workshop and a mentorship program is being set up (see future plans section 9.1).

## 3. T3 database

*3.1. Personnel*: David Waring continues to support uploading and curation of 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 new postdoc (Dr. Tesfahun Alemu Setotaw) started in 2023. He is available to run genomic predictions for breeding programs and is developing a robust pipeline for this work. Spatial analysis of field trials and improved estimation in the presence of GxE using Factor Analytic models are being included in that pipeline.

3.2. New wheat a	datasets made l	Findable. Ad	ccessible. Ir	nteroperable.	and Reusable
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Data Category	2021 Report	2022 Report	2023 Report	Increment from '22 to '23
Accessions with phenotypes	26,612	34,059	48,250	14,191
Accessions with genotypes	16,375	16,656	18,533	1,877
Total Accessions	37,261	57,407	75,717	18,310
Phenotyping Trials	3,087	3,283	3,612	329
Plots with Observations	NA	286,000	427,672	141,672
Phenotype Observations	898,949	1,138,372	2,070,545	932,173
Genotyping Projects	40	43	77	34

Table 1. Summary T3/Wheat data

In this second year, uploads of phenotypic data have strongly picked up. The uptick is particularly visible in the number of phenotyping trials uploaded, the number of plots with observations, and the total number of phenotype observations. Among others, a high number of observations is now coming to T3 from drone-based high throughput phenotyping, which account for 407,225 new phenotypic observations from 50 trials. Breeders are now also depositing to T3 data on accessions that are at earlier stages of the breeding pipeline and that is translating to a much higher number of accessions being documented. In addition, T3 is now tracking pedigrees more carefully. That effort leads to the addition of accessions to T3 that themselves do not have phenotypes but that are parents of phenotyped accessions. The number of accessions with genotype data is currently not keeping track with the number of accessions with phenotype data. The number of genotyped accessions is expected to pick up once WheatCAP has settled on its mid-density platforms. Finally, T3 has started to impute all GBS, 90K and 9K genotyping projects to exome-capture level marker densities. Each imputed genotyping project is considered as a new project and that has led to a strong increase in the number of genotyping projects counted on T3. In addition, separate genotyping projects are maintained when aligned against RefSeqV1 versus RefSeqV2.

#### 3.3. Improvements to T3 usability for breeders and data analysts

An important feature of T3 is that it is compliant with the Breeders Application Program Interface (BrAPI, www.brapi.org). BrAPI has been critical to the integration of the Texas / Purdue UAS-hub that has enabled rapid deposit of image analysis derived traits to T3. That integration is the most prominent example of an external computational service both pulling data and pushing data to T3 without human intervention. Extensive effort from both the UAS-hub and T3 teams enabled this success.

Another smaller example of the use of BrAPI is a new synonym tool developed by T3 (https://synonyms.triticeaetoolbox.org/). This tool enables a user to upload a list of accession names. It then checks those names against all accessions in a BrAPI compliant database for accessions with the same or similar names. It checks the primary name field as well as synonym fields. It knows a number of ways in which the same breeding line may have different names, for example, inclusion or not of a prefix, punctuation differences, case sensitivity, as well as allowing for an edit distance. Because this tool is BrAPI compliant, it is now used during the upload of accession metadata to ensure that the same accession is not uploaded twice with slightly different names, and to check the names of the accession's pedigree. The incorporation of this tool is maximizing the validity of the data stored on T3.

T3 has added sundry data and tool features:

• Barcode improvements. For breeders, it is now easier to customize barcode labels and to order the labels in different ways to facilitate application of the labels to field plots or samples.

• Associated with this ordering feature, it is now possible to order labels or downloads of plots from more than one trial together. This feature is useful when more than one trial is planted in the same field and harvest or other data collection occurs across trials.

• It is now possible to upload metadata for multiple trials in a single step rather than one trial at a time.

• T3 has long used lists to aggregate information. For example, lists of accessions, of traits, or of trials allow users to download or analyze data from all listed objects. T3 has extended this

functionality now to the concept of a dataset. A dataset corresponds to multiple lists and enables the data to be used in an analysis to be specified completely. For example, a genome wide analysis requires listing accessions, genotyping projects, and traits. Datasets make analyses performed on data pulled from T3 more reproducible.

• The T3 team is working to improve the analysis of cooperative nurseries. Data from such nurseries are typically reported in analyses that give overall means across all nursery locations or means at single nursery locations. Breeders, however, frequently want customized sets of locations, for example, those locations that are closest to their target environments. T3 now has a tool to summarize trials that generates customized reports on the basis of a specified list of trials. The tool provides an output much like a cooperative nursery report.

*3.4. Practical Haplotype Graph integration*: T3 has begun to impute genotyping projects done using Illumina 90K and 9K protocols as well as GBS protocols. The resulting imputed data are available through T3's search wizard. When a genotyping project is selected that has been imputed, the download section of the Wizard shows that an imputed dataset is available. All imputed datasets are also shown on this page: <u>https://wheatcap.triticeaetoolbox.org/imputation</u>

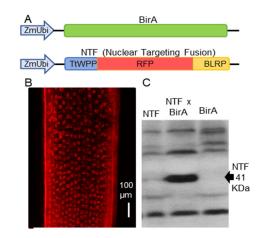
The current WheatCAP PHG was processed using RefSeqV2. Thus, all imputed datasets are relative to that reference.

*3.5. Outreach*: T3 held a data upload workshop with WheatCAP graduate students from Feb. 6th to 9th. Almost all WheatCAP students were trained in data submission practices for T3.

#### 4. Genomics resources

4.1. Sequenced mutant populations: The value of the initial Kronos and Cadenza EMS sequenced populations (Krasileva et al. PNAS 2017) was limited by the fragmented and poorly annotated genome available at that time, so the UCD group generated an improved version by remapping the >50 billion reads to the first wheat reference genome RefSeq v1.0. By using an improved error-threshold method and better annotation, the UCD group identified 560,000 more mutations, reaching a total of 4.7 million mutations in the coding regions, and annotated the effects of 2.7 million more mutations than in the initial version. In addition, 4.3 million new sequenced induced mutations were incorporated in the promoter regions of all wheat genes. The results are published in ref. 4, App. 2 and the data is publicly available through the GrainGenes genome browser and ENSEMBL.

4.2. New regulatory capture for wheat: In 2023, the UCD group published a new capture for wheat regulatory regions (ref. 14, App. 2). Based on the experience with the first promoter capture, a second-generation wheat regulatory capture was developed including 23.5 Mbp of non-overlapping open-chromatin regions identified by ATAC-seq data from roots (ref. 9, App. 2). To generate high-quality root ATAC-seq data, the UCD group implemented the INTACT technology in wheat (Isolation of Nuclei Tagged in specific-Cell-Types, Fig. 1). Filtering of repetitive regions was improved achieving a 60% increase in read-pairs on target and a 3-fold increase in coverage depth relative to the first capture. The new capture is commercially available and the ATAC-seq data was integrated into the public GrainGenes genome viewer.



**Fig. 1. A)** Constructs for INTACT transgenic plants expressing *E. coli* biotin ligase (BirA) gene and nuclear targeting fusion protein (NTF). The chimeric NTF protein includes the wheat WPP domain for nuclear envelope targeting, Red fluorescence protein (RFP) for visualization and a biotin ligase recognition peptide (BLRP), for biotinylation by BirA **B**) Nuclei showing RFP signal in roots of Kronos transformed with NTF. **C**) Western blot of proteins extracted from NTF and BirA lines and from their  $F_1$  hybrid showing the biotinylated NTF protein

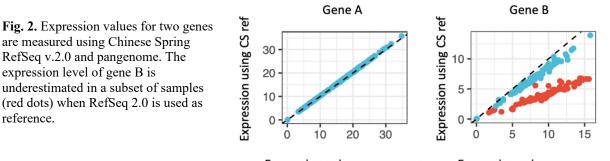
4.3. Expanding the Wheat PanGenome to improve the PHG tool: The PHG tool improvement efforts were directed towards sequencing and incorporating data from more diverse sets of wheat lines to capture the broader range of haplotypic diversity present in wheat. Per recommendation of the SAB, the WheatCAP project has incorporated exome capture data for additional 25 lines from the CIMMYT's HiBAP panel. The new and expanded PHG includes 497 lines and is designated PHG500. The PHG500 will be used in the next round of T3 genotype data imputation.

The KS group sequenced and assembled genomes of additional four accessions of wheat bringing the total number of genomes assembled using long-read sequencing technologies to ten. The average N50 of assembled genomes was 15.9 Mb and the total average size of the assemblies was 14.5 Gb. The data has been combined with the dataset generated by the Wheat Pangenome consortium and comparative genomic analyses aimed at detecting structural variation are underway for creating the updated wheat pangenome. The new pangenome will be used as a reference for variant calling in the exome capture and whole-genome re-sequencing datasets generated for the Wheat CAP germplasm.

4.4. Characterization of regulatory diversity in the wheat genome. To generate genomic resources for functional analysis of genes and investigating the role of regulatory variation in gene expression, the KSU team has assembled a diverse panel of 200 lines including wheat, wild relative and synthetic lines. This panel is being characterized by RNA sequencing conducted on 10 tissues collected from different plant parts at different developmental stages. During the reporting period, RNA sampling and library construction has been accomplished for two tissues: 1) seedling root and vegetative at one week, and 2) crown with axillary tillers and roots at four weeks. The RNA-seq libraries have been submitted for sequencing. The sampling of tissues from other stages of wheat development will be completed by the summer of 2024.

For detailed characterization of regulatory diversity and mapping eQTL, the same panel of 200 lines is being sequenced using a tiered sequencing approach. For a set of 10 lines from this panel, the HiFi data was generated, and genomes assembled using reference-guided strategy (see above). The additional set of 10 lines has been subjected to deep sequencing at 10x coverage. The entire set of lines has been subjected to low-pass sequencing at 1x coverage. The sequence data is aligned to the set of assembled genomes from WheatCAP and the international wheat pangenome project. The genotype calling using pan-genome reference is underway.

The accuracy of eQTL mapping depends on how accurate the estimates of gene expression are. The KSU team has discovered that the sequence divergence between the analyzed wheat lines and the Chinese Spring reference genome caused by introgression of diverged haplotypes could result in biased gene expression estimates. To correct for reference bias in RNA-seq analysis, the KSU team has developed a strategy that uses a pan-transcriptomic reference prepared using predicted gene models from multiple genomes of tetraploid and hexaploid wheat and their wild relatives. Comparison of expression values based on using RefSeq 2.0 and pangenome, showed that the latter provides more accurate estimates of expression (Fig. 2). The manuscript describing this approach is available in bioRxiv (ref. 52, App. 2).



Expression using pangenome Expression using pangenome

#### 5. Genotyping Laboratories

Large numbers of samples were genotyped using non-targeted sequence-based approaches (GBS, MRASeq) as part of ongoing genomic selection and gene mapping efforts. This includes 11,351 samples processed in the regional genotyping labs, in addition to 12,274 samples processed inhouse by the breeding programs at University of Minnesota, Michigan State University and Colorado State University (Table 2). Mid-density genotyping platforms (Allegro, AgriSeq, Illumina 3K and GBMAS) were also implemented to genotype 10,151 samples in total.

Regional genotyping labs supported breeding programs doing marker assisted selection by genotyping samples using known informative markers (KIMs) using KASP and STS assays. A total of 6,990 samples were submitted to the genotyping labs by WheatCAP breeding programs that were evaluated with 1 to 102 KASP or STS assays. The WA lab genotyped 20,976 breeding samples with requested SSR markers. An additional 1,104 wheat lines were evaluated with a PlexSeq primer pool developed in collaboration with Agriplex Genomics. Data for 81 agronomically important alleles were determined to be reliable and reported to breeders. This small amplicon sequencing primer pool continues to be available from Agriplex as a targeted genotyping service.

Research into development of mid-density genotyping platforms continues and this year progressed into the implementation phase. The evaluated genotyping platforms provide uniform genome coverage with high information content markers and include KIMs previously used in marker assisted selection. The GBMAS approach developed by the Pullman lab targeting approximately 2,000 markers was used for 4,487 samples.

Genotyping Lab	Program	No. of samples	Platform	Genotyping Lab	Program	No. of samples	Platform
Central	KS/ARS	96	AgriSeq	Eastern	MSU	2280	GBS
	CA/ARS	480	GBS		U IL	960	GBS
	KS/ARS	288	GBS		VA Tech	1056	GBS
	KSU	288	GBS		ARS-NC	599	GBS
	MSU	960	GBS		UMD	1440	GBS
	NDSU	384	GBS		Regional	576	GBS
	OSU	672	GBS		Regional	1104	28 KASP
	SDSU	864	GBS		Regional	1872	Allegro AgriSeq,
	TX A&M	672	GBS		Regional test	96	Illumina 3K, Axiom
	CSU	5260	GBS		Regional	1104	Agriplex
					WSU ID	1,248 585	GBS GMS
	KS/ARS	96	KASP, STS	Western	CA	402	GMS
	MSU	96	KASP, STS		PNW breeding	4,487	GBMAS
	NDSU	96	KASP, STS		PNW breeding	510	KASP
	OSU	480	KASP, STS		PNW breeding	20,976	SSR
	Regional	576	KASP, STS		PNW breeding	576	AgriSeq
	SDSU	288	KASP, STS	Northern	Regional/Validation	1152	Illumina 3K
	TX A&M	480	KASP, STS		ERSGGL	384	Illumina 3K
	UNL	384	KASP, STS		UW	192	Illumina 3K
	ND/ARS	96	MRASeq		USDA-Fargo	384	Illumina 90K
	OSU	192	MRASeq		NDSU	1	Illumina 90K
	Regional	576	MRASeq		USDA-Albany	480	Illumina 90K
Eastern	KSU/KS-ARS	1200	Allegro		UCD HRSW Breeding MSU	177 2880 433	Illumina 3K KASP Illumina 3K
	MSU	2280	GBS		UMN	5184	GBS

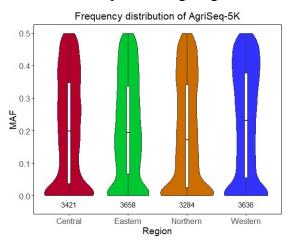
Table 2. Overview of Wheat CAP Genotyping samples for 2023

The genotyping lab at Fargo has developed a multi-species Illumina Infinium II array (USDA-SoyWheOatBar-3K) that targets 3,000 SNPs each in wheat, oat, barley and soybean. At an array cost of \$14 per sample, this multi-species format enables multiple-sample hybridization to decrease the per-sample cost (\$3.50 if four species are included). Extensive validation indicated good technical performance and high concordance results with wheat data from other platforms. The platform is robust, requires only 200 ng of DNA and has a turnaround time of three days for delivering usable data. In particular, the concordance of 178 KIMs associated with traits was high when compared with data using KASP or STS markers. The Fargo Laboratory has built automatic processes that convert Illumina array data into standardized genotyping file formats (Variant Call Format), generate a MAS report that provides predicted alleles of the wellperforming KIMs, and checks the concordance of biological replicates. When individual HRSW breeding programs were evaluated, at least 2,000 markers were informative within a given program that were sufficient for the development of genome prediction models with accuracies similar to the GBS based models. A total of 2,593 samples from all growing regions were processed during this grant period and the number of markers with minor allele frequency greater than 0.05 was greater than 2,000 for all projects, indicating that the USDA-SoyWheOatBar-3K

can be used to genotype wheat germplasm from all US market classes. Uploading of these data to T3 databases is underway. Future efforts with this array include adding content to better overlap with the AgriSeq platform, improve KIM information, and add a process to calculate concordance of new samples against those previously genotyped in the lab.

The Eastern genotyping lab processed 3,072 breeding samples with the Allegro platform that utilizes the single primer extension technology. Samples were sequenced using two lanes of Illumina NovaSeq S1, each having 1,536 pooled samples. Genomic estimated breeding values obtained using the Allegro marker data were highly correlated with those obtained with GBS data, indicating that individuals selected were mostly the same using both genotyping platforms. Although the probe pool targeted 3,680 genome-wide loci, the higher pooling level in 2023 resulted in data for 1,621 variants after filtering for MAF>0.05 and less than 20% missing data. This is compared with 2,158 variants after filtering genotypes in 2022 when libraries were sequenced in pools of 768, suggesting that the higher sequence coverage may be necessary to obtain useful data for larger numbers of markers. Close examination of the 48 sample pools from the 64 Allegro library preparations revealed variation in the average number of sequencing-reads per sample across pools (4 x  $10^5$  to  $1.2 \times 10^6$ ) also contributed to the observed variability in useful marker numbers. This lack of consistency may be due to the small reagent volumes necessary for the protocol to be cost effective for breeding applications.

A platform targeting 5,000 loci was developed collaboratively by the four regional genotyping labs with ThermoFisher using their AmpliSeq targeted amplicon sequencing technology. Sequences flanking 1,500 exome capture SNPs with high information content in each wheat market class and even distribution throughout the genome were selected for submission to ThermoFisher for an initial design. SNP were selected that were also present in the Allegro, GBMAS, or USDA-SoyWheOatBar-3K marker sets to aid in data translation across platforms. A total of 194 sequences targeting KIMs were also included. The genotyping labs supported design



and synthesis of the primer pools sufficient for 6,000 samples by ThermoFisher. Samples provided for validating the pool included controls for KIMs, nullitetra lines and diverse US breeding germplasm.

In total, data were recovered for 5,100 variants with less than 10% missing data. The number of markers that passed quality filters in each region ranged from 3,284 in the North to 3,636 in the West (Figure 3).

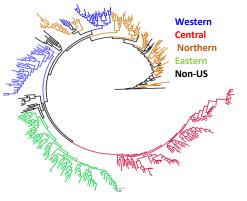
**Figure 3**. Number of markers at different minor allele frequencies (MAF) that passed quality filters in each of the four US region

In the HRS wheat germplasm, 1,294 and 1,209 AgriSeq markers matched the 1,500 common calls generated with the USDA-SoyWheOatBar-3K assay and Allegro genotyping platform, respectively. These results indicate a high level of common data between the AgriSeq with other platforms. Analysis in eastern germplasm identified 62 KIMs requiring adjustment of pipeline parameters to improve genotyping accuracy (ie. non-concordant dominant markers) or requiring primer redesign. Future directions include trouble-shooting problematic KIMs and adding new trait related markers based on new research and existing literature. Genotyping labs will work

with ThermoFisher to synthesize a new primer pool(s) and to maintain cost-effective pricing. Members of the US wheat community have access to genotyping service with the existing AgriSeq platform for \$10 per sample through ThermoFisher and North American Genetics.

The labs coordinated submission of 384 US wheat samples from all market classes for genotyping with the AXIOM Wheat Breeders Array having 42,000 SNP. A publication describing the array by collaborators at University of Bristol is in process. Although target loci

for this array were selected based on SNP discovery in European wheat germplasm, the level of monomorphic markers was low in the US lines evaluated (8.6%). Relationships of lines were consistent with sample origin, with soft winter wheat submissions from the Eastern region being quite distinct from hard winter wheat from the North Central region (Figure 4). Deposition of these data into T3 is in process. North American Genetics provides genotyping with the Axiom array as a service. These results suggest the Axiom array can be used to genotype mapping populations given that the Illumina 90K SNP chip for wheat is no longer manufactured.



**Figure 4.** Relatedness of US Wheat based on Axiom Array SNP data

On-going research to gather information about important variation in US wheat germplasm continued. Exome capture data for US wheat were reanalyzed with alignment to the Chinese Spring RefSeq version 2.1. Promoter capture sequencing of 139 samples of PNW wheat germplasm was completed and 96 eastern SRW wheat samples are in progress. Additionally, a WheatCAP graduate student developed long read assemblies of two SRW wheat cultivars, AGS2000 and Hillard, that have been deposited at NCBI. These new genomes will enrich resources for winter wheat and serve as a basis of genomic analyses in this market class. The AGS2000 assembly is the first PacBio long read assembly of a wheat cultivar having the widely deployed 1RS.1BL chromosome.

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

6.1. Communication with the UAS-hub: The UAS-hub developed a 'data pull' feature, enabling users to retrieve plot map information from T3 with a single click. This feature has undergone multiple updates to accommodate the diverse formats from the breeding programs. Additionally, The UAS-hub developed a comprehensive pipeline for pushing phenotypic information back to T3. This pipeline is fully operational and has been successfully used to upload data from the phenotypic information generated in the 2021 – 2022 growing season to the T3/Breedbase database. The UAS-hub is currently working on pushing phenotypic information generated in the 2022 - 2023 growing season to the T3/BreedBase databases (Table 3).

The UAS-hub is developing a quality assurance and quality check pipeline to verify the uploaded phenotypic information to the T3 database. Following this, the next step is to create a QGIS plugin to even further streamline phenotypic feature extraction and push the extracted phenotypic features back to the T3 database. This plugin will assist individual breeding programs in matching T3 plot IDs with those from UAS data. In the long term, the UAS-hub aims to develop

an API for accessing the UAS-hub's phenotypic database. This API will adhere to OpenAPI standards and be designed for easy integration with T3, similar to BrAPI. The development of the UAS-hub API will be crucial in later project stages, enabling the development of applications that leverage both genomic and phenotypic data for advanced analysis.

No.	Location	Flights	UAS-hub Project created	Processing & Data Delivery	Acres
1	TX-Amarillo Irrigated	21	2023 Amarillo Irrigated	CC, CH, CV, ExG	3.5
2	TX-Amarillo Dryland	17	2023 Amarillo Dryland	Mosaics	4.5
3	TX-College Station	10	2023 College Station Wheat	Mosaics	1.2
4	TX-McGregor	5	2023 McGregor Wheat	CC, ExG	1
5	UC Davis (CA)	7	2023 DA Wheat	CC, ExG, CH, CV, NDVI, NDRE	6.3
6	UW-Madison (WI)	10	2023 WI-Madison Wheat	CC, ExG, NDVI, NDRE	1.2
7	Kansas-Colby (KS)	8	2023 Colby Wheat	CC, ExG, CH, CV, NDVI, NDRE	1.6
8	NDSU (ND)	10	2023 NDSU Wheat	Mosaics	0.4
10	SDSU (SD)	6	2023 SDSU Wheat	CC, ExG, NDVI, NDRE	0.95
11	Cornell (NY)	2	2023 Cornell Wheat	CC, ExG, NDVI, NDRE	0.5
12	UIUC	13	2023 UIUC Wheat - St. Peter	Mosaics	2.9
13	NCSU	11	2023 NC State Wheat	CC, ExG, CH, CV	0.5
14	Idaho (ID)	9	2023 Idaho Wheat	CC, ExG, CH, CV, NDVI, NDRE	3
15	Kansas-Hays (KS)	9	2023 Manhattan Wheat	CC, ExG, CH, CV, NDVI, NDRE	1
16	MSU (MT)	9	2023 Montana Wheat	Mosaics	0.9
17	UMN (MN)	-	2023 UMN Wheat	-	
18	USU (UT)	-	2023 Utah Wheat	-	
19	Virginia Tech (VA)	-	-	Will process their own data.	
20	UNL Lincoln	-	-	Will process their own data.	
21	WSU (WA)	-	-	Will process their own data.	
22	CSU (CO)	-	-	Will process their own data.	
23	OSU (OK)	-	-	Will process their own data.	

Table 3. Summary of UAS-data received and processed by the hub

6.2. UAV data collection and processing: The Texas A&M/Purdue team supported programs new to UAS technology to help them identify platforms for their needs, develop data transfer pipeline to integrate UAS hub with T3 database, and to develop training modules in UAS-HTP.

## 6.3. Centralized online platform developed by TAMU/Purdue UAS-HTP group

(<u>https://wheatcap.uashubs.com/</u>) for data receiving, processing, and importation: In Year 2, the UAS-hub received raw UAS images for processing from 142 flights coming from 15 fall planted wheat programs as shown in Table 3 below. The team processed the raw images and generated orthomosaics and Digital Surface Models (DSMs) for feature extraction for all flights. The UAS team has completed data analyses for 88 flights and submitted the data back to the breeders, and completed mosaics for another 54 flights. After extraction of the phenotypic features, data will be transfer to the T3 database. In year 2, the UAS-hub team focused on enhancing UAS data

processing capabilities of breeding program. WheatCAP students received UAS data processing and feature extraction training in May of 2023, and are now capable to delineate plot boundaries and integrate with field layout/plot information. In addition to the in-person training program, the UAS-hub team has developed a video module to perform this task.

6.4. List of spectral vegetation indices and canopy features developed by the UAS-hub team: Canopy cover (CC), Canopy Height (CH), Canopy Volume (CV), Average Excess Green Index (ExG), Average Normalized Difference Veg. Index (NDVI), and Average Normalized Difference Red Edge Index (NDRE) were generated and sent back to breeders.

6.5. Bottlenecks for efficient data processing & delivery: the UAS-hub team has identified several areas that can be improved for more efficient data processing and delivery: a) some of the breeding programs had to ground their DJI platforms which impacted routine data collection in year 2, b) the ban on DJI manufactured platforms and sensors will force breeding programs to look at alternatives which may require time to learn and validate results. c) programs are still learning and sometimes do not follow data collection and transfer protocols, document the field layout poorly, or fail to collect GCP survey results, d) to improve the efficiency of data transfer, processing, and final delivery to breeding programs can help the UAS team to generate plot boundary delineation, e) timely upload of raw imagery data is critical for planning data processing. All these issues will be discussed at the PAG24 annual meeting.

6.6. *Program Design and Integration*: The UAS-hub accessed the plot-level data, uploaded to the T3 Database by each breeding program, using several breeding application programming interfaces (BrAPI) calls within a Python programming environment. Utilizing the unique IDs assigned to each plot, the UAS-hub processed this data and extracted the phenotypic information, which was then uploaded to T3.

6.7. *Workflow*: The current setup involves a process that connects two database systems: the UAS-hub and the T3 Breedbase databases. The first step in this process is to pull plot map information from the T3 database, which is a key parameter for the UAS data processing phase. In the second step, the processed data is prepared and uploaded back to T3 for further analysis. This upload is facilitated using the trait database IDs (db ids) provided by the T3 database team. These IDs are extracted for each piece of phenotypic information based on the UAS flight dates. The data upload pipeline is then employed to transfer the processed data back to T3.

6.8. *Limitations*: The major limitation was encountered during the data pull process. While most programs adhered to the established T3 formats, some breeding programs do not follow the guidelines provided by the T3 team. Additional problems included overlapping plots and problems with private lines. The UAS-hub is encouraging users to review their data and to discuss any identified challenges with the T3 Breedbase team to mitigate these issues.

Delimitation of plot boundaries is a time-consuming task. The division of responsibilities for this task between the UAS-hub and the individual programs for the remining years of the grant will be discussed at the PAG24 annual project meeting.

6.9. *Training video*: The training video created and uploaded under the support tab has contributed to the educational activities, particularly in ensuring that individual breeding programs adhere to established plot boundary map creation standards. In addition, the UAS-hub organized a workshop last summer led by the Texas A&M AgriLife Research Center in

Amarillo, TX. At this workshop, 25 students from 19 universities were trained in the basics of UAS high-throughput phenotyping.

## 7. Spring and winter wheat IWYP hubs

## 7.1. Winter Wheat Breeding Innovation (WWBI) hub at KSU

Wheat CAP collaborates with the NIFA IWYP WWBI hub at KSU on introgression of gene alleles 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<sub>3</sub>F<sub>2</sub> populations were developed for introgression of *Elf3* and *Tagw2* gene alleles. The 1AL grain yield QTL allele was crossed to generate BC<sub>3</sub>F<sub>2</sub> or BC<sub>4</sub>F<sub>1</sub> generation lines with recurrent parents KS090387K-20, Hilliard, TAM114, and Tekoa. The CRISPR-Cas9-edited alleles of *TaGW7*, *TaGW2*, *TaCKX2-1*, *TaCKX2-2* and *TaARF4* developed in collaboration with WheatCAP were used to generate BC<sub>2</sub>F<sub>2</sub> populations with the WWBI hub parents OK16D101089, Bob Dole, OK13625, KS090387K-20, SF Fortify, Tekoa and IDO676. The lines are ready for distribution to the breeding programs upon request.

For the next round of WWBI hub introgression crosses, WheatCAP provided germplasm and marker data for introgression of *FT-2A* and *Rht13* gene alleles. In addition, the low canopy temperature (chromosomes 1D, 2D and 7D) and photoperiod alleles (chromosome 2D) identified in the *Ae. tauschii* introgression population (see above) characterized as part of the WheatCAP project are currently being used for introgression into the adapted germplasm in the NIFA IWYP Winter Wheat Breeding Innovation hub.

## 7.2. Spring Wheat hub at CIMMYT

Four WheatCAP experiments were performed at CIMMYT, three at the common wheat Springhub, and one in collaboration with the CIMMYT durum breeding program. The experiments are summarized in Table 4. Mapped QTLs and cloned genes identified in the US programs were introgressed into high-yield and high-biomass breeding lines that are being evaluated in the spring wheat hub in Obregon, CIMMYT.

7.2.1. CA: Materials sent to CIMMYT for 2022-2023 field season: The UCD team sent four groups of germplasm (101 lines total, 11/2022) with genes or traits of interest for the CIMMYT global durum breeding program. The durum breeder (Karim Ammar), sowed the trials in Obregon, Mexico in December 2022 and harvested in May 2023. The germplasm include:

- Desert Gold+: These 27 lines are the result of marker assisted back crossing of the stripe rust resistance genes *Yr5*, *Yr15*, and *Yr36*. This line also includes the functional allele of *GPC-B1* for high grain protein (linked to *Yr36*), a gene for reduced grain cadmium content, the Bx7OE allele of *Glu-B1* (*GluB1al*) for improved gluten strength and two QLT for improved yellow pigment.
- Durum-*WAPO-A1-F47*. These are 3 pairs of near isogenic lines with and without the beneficial allele of *WAPO-A1-47F*. This allele is associated with increased spikelet number per spike (SNS) and is not frequent in durum. The recurrent parents include UC Davis breeding lines UC1850 and UC1771 and the released variety UC-Miwok.

- Durum-*FT-A2-10A*. These are 3 pairs of near isogenic lines with the *FT-A2-10A* or *FT-A2-10D* alleles. The 10A allele is associated with an increase in spikelet number and is rare in tetraploid wheat.
- Polonicum backcross into durum wheat. These 62 lines are the result of a backcross experiment done by Adam Lukaszewski at UC Riverside. Phenotypically selected traits of interest coming from Polonicum were introgressed into the cultivar UC-Desert King. These BC<sub>2</sub> to BC<sub>4</sub> lines differ in seed length, glume length, and awns presence.

**Table 4.** List of the WheatCAP experiments performed in the CIMMYT IWYP- hub during Obregon cycle Y22-23.

Trial	lines	blocks	plots	Scientists	Sowing details
Wheat Cap	14	8	112	Jason Cook	-hill plots Y20-21 (10 lines, 5 reps)
MT Y21					-small plots Y21-22 (10 lines, 8 reps; 4 lines only increase seed in hill plots)
					-small plots Y22-23 (14 lines, 8 reps)
Wheat Cap	40	3	120	Andrew Katz	-hill plots Y20-21 (50 lines, 3reps)
CSU Y21				Esten Mason	-small plots Y21-22 (40 lines, 3reps (discard Bonsu))
				Stephen Pearce	-small plots Y21-22 (40 lines, 3reps (discard Bonsu))
Wheat Cap	24	4	96	Arron Carter	-small plots late sowing in Y21-22
WSU Y22					-small plots late sowing in Y22-23; small plots normal sowing in Y22-23
WheatCAP	101	-	101	J. Dubcovsky	-seed increase of new lines for the CIMMYT durum program (see description CA below)
	Wheat Cap MT Y21 Wheat Cap CSU Y21 Wheat Cap WSU Y22	Wheat Cap14MT Y2114Wheat Cap40CSU Y2140Wheat Cap24Wheat Cap24	Wheat Cap148MT Y21403Wheat Cap403CSU Y21404Wheat Cap244WSU Y224	Wheat Cap148112Wheat Cap403120CSU Y2140496Wsu Y22496	Wheat Cap MT Y21148112Jason CookWheat Cap CSU Y21403120Andrew Katz Esten Mason Stephen PearceWheat Cap Wheat Cap WSU Y2224496Arron Carter

7.2.2. CO: CSU introgressed the high-value *Qsn.csu-6Bb* and *WAPO-A1b* alleles for greater spikelet number into three CIMMYT high biomass parents; 'Bonsu', 'Nadi', and 'GID:4314513'. Field trials were conducted at CIMMYT in 2022-2023 using 40 lines and 3 blocks. The *Qsn.csu-6B* associated with thousand kernel-weight was mapped within a 108.0 Mbp region between markers KS0617\_761651 (Chr6B: 545.3Mbp) and KS0617\_768340 (653.3Mbp).

7.2.3. *MT*: The 6B QTL for number of fertile tillers from Vida was introgressed into the CIMMYT lines BAJ and KINGBIRD to generate isogenic lines. The 2022-2023 experiment was performed as an RCBD with 14 lines and 8 blocks. A significant increase in total number of grains per m<sup>2</sup> was detected. The increase in grain number per m<sup>2</sup> reflects an increase in the number of pikes per m<sup>2</sup>, which is the expected result for the high tillering alleles of the 6BQTL. The MT group is repeating the experiment one last year at CIMMYT and Bozeman, MT (2023-2024) to generate the information required for publication.

7.2.4. WA: 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. Near-isogenic BC<sub>3</sub> hard red spring wheat lines were developed with the QTLs 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. The last field

season of data is expected to be returned summer 2024 to complete two field seasons. For all trials, CIMMYT collaborators collected data on phenological, morphological, and agronomic traits. The same lines were also planted at two locations in Washington in both the 2022 and 2023 seasons with similar data collection. Initial data analysis indicates significant differences between isogenic lines for thousand kernel weight, grain number, grain filling period, and days to heading. The WA team intends to have data fully analyzed in 2024 after the final harvest season, looking at interactions between locations, planting dates, and isogenic lines to understand the interaction of these QTL with high biomass parental lines from CIMMYT.

#### 8. Progress by individual breeding programs

#### 8.1. CA. University of California, Davis. Jorge Dubcovsky

*Education*: PhD student Priscilla Glenn graduated in 2022 and PhD student Maria Rottersman started in 2022. She presented her first results at PAG 2023 in San Diego and is currently writing her first manuscript. She attended all workshops organized by the WheatCAP education team and has taken a leadership position in the organization of the 2024 student workshop. *Germplasm & publications*: The UCD team released the HWS variety UC-Central White,

deposited 5 germplasm accessions (App. 1), and published 14 peer-reviewed papers (App. 2).

*UAS*: The CA breeding program submitted 7 flights covering 6.3 acres to the UAS-hub. *Genotyping*: The UCD group evaluated multiple marker platforms in 2023.

- 1. Genotyping-by-multiplex-sequencing (GMS) developed by the Genotyping lab in Pullman WA. The UCD group submitted 402 lines and received 1,774 markers. However, only 186 markers showed consistent genotyping scores among check replicates and a MAF>0.05.
- USDA-SoyWheOatBar-3K array developed at the Fargo Genotyping lab. The UCD group submitted 177 lines and received 3421 markers. Of those 1,693 were good markers (missing < 0.2 and MAF > 0.05).
- 3. Thermo-Fisher AgriSEQ SNP platform. The UCD group submitted 179 lines and received 5,171 markers. Of those, 2,558 were good markers (missing < 0.2 and MAF > 0.05).
- 4. AXIOM Wheat Breeder Array. The UCD group submitted 11 lines and received 43,373 markers. Of these 9,370 (21.6%) were polymorphic for the UC lines. For the whole population, 15,121 out of 43,373 markers have MAF > 0.05 (34.9%).
- 5. Finally, The UCD group did in-house GBS for UCD lines from the year 2022: Out of 51,740 total SNPs; 30,344 were good SNPs (missing < 0.2 and MAF > 0.05);

In summary, the USDA-SoyWheOatBar-3K and the AgriSEQ platforms were the most promising platforms for breeding applications based on price and number of polymorphic markers. The Axiom platform produced the largest number of markers and is suitable for mapping populations, but expensive for breeding applications.

KASP markers were developed for the *bZIPC1* allele associated with increased SNS and the protocol was deposited in MASwheat <u>https://maswheat.ucdavis.edu/protocols/bZIPC1</u>.

*T3 Trials and genomic selection*: The UCD group submitted to T3 23 trials including 3,000 plots and uploaded data for grain yield, heading date, plant height and grain protein content for these trials. In collaboration with the T3 team, the UCD group performed their first Genomic Selection

test for 6 traits using the 2022 data. All traits showed good prediction accuracy (> 0.5). Pedigree data will be sent next year to improve predictions and incorporate GEBVs into the program.

## 8.2. CO. Colorado State University. Esten Mason

*Education*: Andrew Katz (PhD) completed all course and prelim exam requirements for graduation in Spring 2024. Mik Hammers started a PhD in fall 2022 and completed her research proposal 1<sup>st</sup> committee meeting. Selena Lopez (PhD) started in Spring 2022 completed her third semester but is taking a leave of absence. Zachary Winn started a postdoc at CSU in September 2022 and has published three first author papers in 2022-2023. Both Selena and Mik have been participating in regular project update meetings and small writing groups.

Germplasm & publications: 5 varieties (App. 2) and 5 publications (App. 1).

*UAS*: CO processes all flight images/data in-house. In 2023 there were 8 flights totaling 20 acres. Data collected included multispectral wavelengths used to calculate indexes NDRE, NDVI, and plant height.

*Genotyping*: 35 lines submitted to the Manhattan, KS genotyping center as part of the SRPN and RGON cooperative nurseries. CSU generated 20,519 single marker KASP datapoints in house.

*Breedbase*: CSU has uploaded 16 trials to T3 containing 5,460 plots, and 11 trials with 5,197 plots with agronomic data.

*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 which continues on a yearly basis through elite level testing. In 2023, GEBVs were generated for 5,260 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 0.80. 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 the monthly meetings, writing groups. Joshi attended the UAS workshop in Amarillo and took on the role of Communication Coordinator for the Plant Sciences Graduate Student Symposium, scheduled for 2025. Pabitra Joshi passed preliminary exam, submitted a manuscript, and presented two oral and one poster presentations at two international meetings this year.

*Germplasm & publications*: variety UI Gold was submitted for PVP (202300355, submitted 08/10/2023, App. 1) and was published in the Journal of Plant Registrations (App. 2).

*UAS*: 10 flights submitted for 1340 plots in 2.75 acres in 2023. They also received canopy coverage, greenness index, NDVI, and NDRE for >1400 plots grown in 2022. 250 lines grown in 2022 genotyped with 90K were used to develop genomic prediction models. Additional 420 lines grown in 2023 with 3K GMS were used to predict yield components and other agronomic data, using the UAS indexes.

*Genotyping*: 585 spring wheat lines were genotyped using 3K GMS panel at Western Genotyping LAB and were deposited in T3. In-house genotyping was conducted on more than 2500 lines using KASP technology for multiple traits,

*Breedbase*: Agronomic data from 770 lines from a spring wheat diversity panel were evaluated in two replications (1340 plots submitted to T3).

*Genomic selection*: Preliminary analysis for test weight and protein content in 250 diverse lines have shown prediction accuracies of 65.03%, and 74.35%, respectively. The project will use 580 spring wheat lines grown in 2023 and indexes to predict yield and yield components using 90K SNP and 3K GMS system.

## 8.4. IL. University of Illinois. Jessica Rutkoski

*Education*: Lucas Berger Munaro and Raysa Gevartosky both attended the HTP workshop in Amarillo and the GS workshop in Raleigh.

*Germplasm*: IL19-5632 was released in 2023 for brand labeling. IL19-5632 is a very early maturing soft red winter wheat variety that yields well in the northern half of the eastern US and is moderately resistant to scab, caused by *Fusarium graminearum* (App. 1).

UAS: 11 flights over 2 acres were submitted to the UAS hub. 2022 data was received and 2023 is pending. Data is being processed in-house and compared with the hub, but they are still not using HTP data for breeding decisions.

*Genotyping*: Genotypic data from 2022 and 2023 have been generated, but are not yet in Breedbase (~8000 SNP per line).

*Breedbase*: The IL program routinely uses Breedbase for trial data management and seed lot management. 40 trials with 15,086 plots were submitted in 2023 to T3. Agronomic data has been deposited in T3 for 14,905 of these plots. The IL program provided valuable insights on the integration of public breeding programs into T3.

*Genomic selection*: The IL group routinely implements Genomic Selection for parent selection and for advancement decisions based on Genomic Estimated Breeding Values (GEBVs).

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

*Education*: Yuzhou (Bruce) Xu (Ph.D) graduated in 2023 and is working as post-doc in the USDA Genotyping Lab at Manhattan KS. KSU PhD students Xiaoting Xu (GB), Dwight Davidson (EA), Nicholas Stelling (EA), Adriano Abreu Moreira and Lawrence Tidakbi (MG and K Jordan) attended student meetings, participated in the UAS, T3, and GS workshop, and actively participated in the education meetings and writing groups.

*Germplasm & publications*: Four varieties reported in 2022 without PVP were submitted for PVP in 2023 and two new varieties were released pending PVP (App. 1). 11 new papers were published in 2023 by the different KS coPIs (App.2).

UAS: 17 flights over 2.6 acres were sent to the UAS hub in 2023.

*Genotyping*: 1060 lines were genotyped and 500 were deposited in T3. New markers were developed for plant height gene *Rht8*, Hessian fly resistance gene *H13*, a FHB resistance QTL on chromosome arm 2DS, and canopy temperature QTL on chromosomes 1D, 2D, and 7D.

*Phenotypic data deposited in Breedbase*: The KSU group entered agronomic data for three trials harvested in 2023 in T3 including 1,018 accessions and 1,648 plots. The USDA-ARS group deposited an additional 2,000 plots from 4 trials harvested in 2023. In addition, data were entered

in 2023 for three trials, each including 1722 accessions and 2930 plots phenotypes collected in 2022 (6-9 flights each), and including agronomic and UAS data.

*Genomic selection*: The KSU team used UAS data collected for wild-relative derived germplasm for phenomic predictions of yield in multi-environmental trials. The machine learning approaches showed high prediction accuracies achieving 70% within environments, and 57-69% across environments. The collected data is used for prioritizing wild-relative derived germplasm with improving environmental adaptation for pre-breeding.

## 8.6. MI. Michigan State University. Eric Olson.

*Education*: Jonathan Concepcion (PhD) attended the PAG student workshop, the UAS Workshop in TX and the Genomic Selection workshop in NC. He participates in the planning of the WheatCAP Breeding Symposium.

*Germplasm & publications*: soft red winter wheat 'MI20R0012' selected based on genomic prediction of grain yield and disease resistance (App. 1). No publications.

*UAS*: A total of 8 flights from 2022 have been submitted in 2023 to the TX hub. All plot level data has been extracted and a total of 30 vegetation indices have been developed. Processing is underway for 2023 images.

*Genotyping*: 3115 accessions were genotyped in-house by GBS and were entered in T3. In addition, the MI group evaluated the entries in the Big6 nursery with KASP markers for large-effect genes influencing grain quality, disease resistance and phenology.

Breedbase: 19 trials for 1520 plots with agronomic data were submitted to T3.

*Genomic selection*: Phenomic predictions were made for grain yield using four UAS flights from 2022 capturing multi-spectral data. The fourth flight had the highest cross validation accuracy of 0.60. The MSU wheat breeding program at has been using genomic selection since 2019. In 2023, predictions from a set of 10 location x year combinations were used to make selections among 2,280 selection candidates. An 80/20 cross validation strategy using the entire data set was used to assess prediction accuracy. Cross validation accuracy for grain yield ranged from very low at 0.23 for at Huron county, MI 2023 to very high at 0.62 at Monroe, MI. Moderate cross validation accuracies were achieved for DON mycotoxin, preharvest sprouting and response to leaf rust.

## 8.7. MN. University of Minnesota. Jim Anderson.

*Education*: Previous WheatCAP PhD student Max Fraser graduated in 2023 and is now a barley breeder at Anheuser-Busch. MS student Nanthicha Krueger was funded by WheatCAP as an MS candidate from June 2022 – August 2023 and she participated in the WheatCAP project meeting in San Diego and UAV workshop in Texas. This position is currently vacant and MN is seeking an MS or PhD candidate to continue her research.

*Germplasm & publications*: HRS variety MN-Rothsay was submitted for PVP in 2023 (PVP 202300138, submitted 02/03/2023, App. 1). No publications 2023.

*Genotyping*: in 2023 MN submitted 2389 lines with 15 KASP markers to T3. This year, they also deposited in T3 960 lines evaluated with the 3K chip at ND Genotyping lab and 3698 evaluated with 14 KASP markers generated in 2022.

UAS: 10 flights covering 1 acre were submitted to the UAS-hub

*Breedbase*: In 2023, a total of 1920 plots were entered in T3 representing 192 experimental lines evaluated in 8 locations (1 replication each + 4 checks replicated 8-12 times in 10 locations).

*Genomic selection*: In 2023, the MN teams used GS FHB reactions and seed size of 2,032  $F_6$  lines using a training population consisting of a subset of 210  $F_6$  lines and 49 parents. Further training data on FHB parameters, pre-harvest sprouting and gluten strength will be used to develop and refine prediction models prior to final 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 UAS workshop in Texas, and the Genomic Selection workshop in NC. Mei Ling is the secretary for the 2025 WheatCAP Plant Science Symposium, and Jared is participating in a writing group.

*Germplasm & publications*: Spring wheat varieties MT Carlson and MT Dutton were released from Montana State University breeding program in 2023 (App. 1). The MT group published two papers in the Journal of Plant Registrations and Crop Sciences in 2023 (App. 2).

*UAS*: 10 UAS flights over Bozeman trials including 468 plots were submitted to the UAS- hub. *Genotyping*: 433 breeding lines were sent to the ND Genotyping Lab in 2023. The lines were genotyped with the USDA-SoyWheOatBar-3K array and the data was deposited in T3. In addition, 418 lines genotyped in 2022 were deposited in T3 in 2023.

Breedbase: 1 trial with 468 plots with the associated agronomic data has been entered in T3.

*Genomic selection*: MT genotyped all their breeding lines since 2010, and they are using the genotypic data and historical yield trial data to develop genomic selection models. They have made good progress developing genomic selection models for end-use quality.

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

*Education*: PhD student Nico Lara completed coursework and should finish preliminary exams to advance to candidacy in the Spring. Nico participated in the Wheat CAP UAV and Genomic Selection Workshops and in writing group sessions, group meetings. PhD student Daniela Miller who continued from previous WheatCAP participated in the Genomic Selection Workshop and is scheduled to defend her dissertation in January 2024. The NC-ARS group hosted the 2023 Wheat CAP Genomic Selection Workshop on the campus of North Carolina State University. The workshop had approximately 40 participants, including graduate students and post-docs working in programs of Wheat CAP PIs and other US public wheat breeding programs.

*Germplasm & publications*: 3 publications in The Plant Genome, Crop Science and Journal of Experimental Botany were published in 2023 (App. 2).

*UAS*: In 2023, there were 11 flights at Kinston, NC that have been processed and 9 flights at Raleigh, NC that are in progress. The UAV aspect of the project is aimed at QTL mapping of growth rate in two RIL populations grown in small plots. These two locations had 560 and 600 plots consisting of two 1-m rows. Ground truthing of biomass measurement was done through destructive sampling.

*Genotyping*: The NC-ARS program submitted 599 lines grown in a single-rep observation nursery to the genotyping lab in Raleigh that were evaluated using the GBS protocol and with 11 KASP assays for disease resistance and quality traits. A new KASP marker for Rht24 and a new variant of TaELF-A1 were evaluated on multiple RIL populations. Data are being evaluated to determine marker reliability prior to depositing into T3.

*Breedbase*: WheatCAP student Nico Lara evaluated a mapping population consisting of 1980 RILs, plus 13 parent lines (30 reps) in three environments in 1-m row plots and submitted data to T3. Data was uploaded for two additional UAV experiments including 560 plots at one location and 600 at the other.

*Genomic selection*: Genotypic data described above were used in conjunction with training data obtained during 2022 to generate GEBVs 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 North Dakota State University, Fargo. Justin Faris and Andrew Green

*Education*: The education coordinator Katherine Running provided excellent support to all WheatCAP students. She coordinated the 2023 PAG student workshop, two in-person workshops, one on-line workshop, plus monthly meetings and coordination of writing groups. PhD student Pooja Kumari participated in all the monthly meetings and the workshops that have occurred since her arrival.

*Germplasm & publications*: The HRSW variety 'ND Thresher' (PVP 202400025, submitted 10/20/2023) released by the ND breeding program is adapted to eastern and central ND (App. 1) Marker-assisted selection was performed throughout line development. One paper was published in The Plant Genome in 2023.

UAS: ND submitted 5 UAV flights over 0.4 acres

*Genotyping*: 2352 lines were sent to the Fargo genotyping lab where they were genotyped with KASP markers, 90k Illumina, and 3k Illumina. Genotyping data for 492 lines were deposited in T3. The ND group has discovered new QTLs with major effects for resistance to bacterial leaf streak and is in the process of developing markers for the QTLs.

Breedbase: The 2023 field designs for UAV flights have been submitted to T3 (820 plots).

*Genomic selection*: the NDSU HRSW breeding program is refining genomic prediction models for basic agronomic traits and FHB. The ND group is genotyping 5000 F<sub>5:6</sub> lines from the winter nursery to use genomic selection and major gene selection for key traits in 2024.

## 8.11. NE. University of Nebraska. Katherine Frels

*Education*: PhD student Sydney Graham participated in the 2023 WheatCAP Meeting at PAG; UAV workshop Amarillo; online T3 workshops, monthly webinars and the writing group.

*Germplasm & publications*: HRWW NHH17612, released in 2023 by the University of Nebraska is a two-gene Clearfield herbicide resistance variety (App. 1).

*UAS*: UNL completed 4 UAV flights at 4 Nebraska locations in 2023 for a total of 16 data collection timepoints over multiple research trials. UNL is switching to in-house image processing due to the acre limitation for TAMU processing. NDVI data for each trial is ready for T3 upload.

*Genotyping*: 1,000 UNL breeding lines in preparation for submission to USDA-Fargo (not counted in this report). 384 KASP markers were generated at the Manhattan Genotyping lab.

*Breedbase*: UNL submitted 10 trials to T3 representing 2,100 plots and uploaded agronomic data for all 10 trials in 2023.

*Genomic selection*: Genomic selection is regularly used for agronomic traits. The NE group is working to add genomic selection for the wheat stem sawfly resistance program.

## 8.12. NY. Cornell University. Mark Sorrells

*Education*: PhD student Timothy Mulderrig attended the Wheat CAP student workshop at PAG, the UAS training at TX and about 10 of the 2023 virtual professional development and education meetings.

Germplasm & publications: 1 new publication (App. 2).

UAS: two flights were conducted over the Cornell Winter Wheat Master (PYT) Trial (0.5 acres) and data were submitted to the Wheat CAP UAS hub. Data was received back 12/2023.

*Genotyping*: 285 samples were submitted from the 2024 Winter Wheat Master Trial and the Male Sterility population to the Eastern Regional Small Grains Genotyping Lab.

*Breedbase*: In 2023, 1 trial consisting of 140 plots with its agronomic data was submitted to the T3 database.

Genomic selection: the NY group has not yet completed the genomic selection on 2023 data.

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

*Education*: 4 graduate students are currently working in the OK program. Jennifer Tapia completed her MS in 2023 and is currently a Ph.D. student at NC State U. She attended the WheatCAP meeting in San Diego and the student monthly meetings. Sunja Shrestha, Ph.D. joined the WheatCAP project in May 2023 and works on UAS under the supervision of Dr. Phil Alderman. She attended the UAS training workshop in TX. Ph.D. student Wenxuan Zhaiwill graduate in 12/ 2023. She attended the WheatCAP meeting in San Diego and the T3/Breedbase workshop. Juan Carlos Luzuriaga-Yañez completed his MS in Dr. Yan's lab and will initiate a PhD in the same lab in 2024.

*Germplasm & publications*: 4 HRWW varieties were released in early 2023 by Oklahoma State University, three of them included the *Glu-B1*7BxOE allele for strong gluten (App. 1). The OK group published three papers in 2023 (App. 2).

*UAS*: 6 flights collected from 3.3 acres are being processed in-house using OpenDroneMap (ODM). High-level data will be deposited in T3 before the PAG meeting.

*Genotyping*: The USDA-ARS, Manhattan lab provided 192 lines with MRASeq data select parents for crosses, 480 samples with KASP markers, and GBS data for 672 lines for GWAS for disease resistances. The OK genotyping lab provided genotyping data for 62 lines and 500 individual plants for the 2-gene BASF trait, 354 lines for the *sbeII* mutants for increased dietary fiber, and 3,000 hexaploid wheat and 100 tetraploid lines for the *TaCOL-B5* alleles

*Breedbase*: 9 trials with 1,863 plots and the associated agronomic data were submitted to T3. *Genomic selection*: No data reported.

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

*Education*: Previous WheatCAP PhD student Harsimardeep Gill graduated in 2023 and is a Postdoc in MN. PhD student Swas Kaushal attended the WheatCAP Genomic Selection Workshop in Raleigh, the UAS Workshop in Texas and the education group monthly meetings.

*Germplasm & publications*: HRWW variety 'SD Pheasant' is a HRWW developed by the SD Ag. Exp. Station and is recommended for release in fall 2023 (App. 1). The SD group published 5 peer-reviewed articles in 2023 (summarized in App. 2).

UAS: The SDSU breeding program completed 18 flights from three locations (3 acres) and after in-house analysis, the vegetation indices from 1,080 plots were submitted to T3. In addition, SD has submitted 6 flights to the UAS hub.

*Genotyping*: 155 lines were genotyped by GBS at the UDSA HWW Genotyping Lab, Manhattan in 2023, and the data were entered into T3 (~10,000 SNP per line). In addition, 157 lines that were genotyped in 2022, were entered into T3 in 2023.

Breedbase: 6 trials with 1,080 plots and their agronomic data were uploaded into T3 for 2023.

*Genomic selection*: GS models are being evaluated for grain yield, grain protein, test weight, end-use quality, and FHB (Visual and NIR/Hyperspectral Imaging) resistance (Disease index, FDK, and DON) in the program (3 articles were published in this area).

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

*Education*: PhD Zhen Wang graduated and is now a postdoc in Texas A&M. Kylie Scott also graduated and is a crop advisor for a company. PhD Kyle Parker participated in all online meetings, and attended the PAG-2023 student workshop and the UAS workshop in Amarillo. He is a student co-organizer of the PAG student meeting 2024. Shannon Baker started her Ph D in 2023. She has attended monthly meetings and presented the Texas wheat breeding UAV program at the WheatCAP meeting at PAG-2023. She will be attending PAG and the Hybrid Breeding Course. She also co-organized the Amarillo UAV workshop. The TX group organized an excellent in-person UAS workshop at Amarillo TX that was attended by 25 WheatCAP students from 19 universities.

Germplasm & publications: No new varieties and four new publications (App. 2).

*UAS*: the TX breeding programs completed 53 flights across three locations and 10.2 acres and images were sent to the hub. Maps and plots for 2023 Texas elite lines in Bushland irrigated and dryland plots were uploaded in 2023. UAS data products generated by Amarillo and College Station were uploaded onto T3. Data was processed and final data was delivered to the Texas breeders for 33 flights (88 flights when all WheatCAP breeders are considered). The TX group developed a tool that can be used to download plot layout maps from T3 and another tool that can be used to upload phenotypic information to the T3 database (see details in section 6).

*Genotyping*: 588 lines were screened by GBS and 23 primary synthetic lines were analyzed by exome capture by the UDSA-ARS lab. The 23 synthetics and 425 Synthetic Derived lines were evaluated by skim sequencing by the lab of Eduard Akhunov. Genotypic data for 73 lines was submitted to T3.

*Breedbase*: 9 trials with 1080 plots were submitted to T3 and agronomic data were entered for 480 plots.

*Genomic selection*: Late-stage breeding material from 26 (year x location) environments were evaluated for genomic prediction accuracy. 1497 unique Texas A&M AgriLife Research breeding lines from preliminary, advanced, and elite yield trials were evaluated for grain yield, including 6 field locations across five years. All unique lines were genotyped through ddRAD seq. Single environment predictions using a 5-fold cross-validation ranged from 0.27-0.72. Multi-environment models, including weather covariates, increased prediction accuracy for most

environments, but eight environments decreased. The 2023 yield prediction was confirmed as valuable by breeders after they compared it with their phenotypic selection.

## 8.16. UT. Utah State University. Margaret Krause (moving to OR in 2024)

*Education*: MS student Dalton Jones attended all but one monthly student meeting, the UAV workshop in Amarillo, the GS workshop in Raleigh, and the project meeting in San Diego. He is also in one of the student writing groups.

Germplasm & publications: None to report

*UAS*: 4 flights covering 6 acres were completed and the data have been submitted to the UAS-hub. Additional flights were analyzed in-house and the high-level data will be submitted directly to T3.

*Genotyping*: 465 lines were submitted to the genotyping lab in WA. Data was received for 393 lines that showed an average of 900 SNP and was submitted to T3.

Breedbase: Agronomic data was entered in 2023 for the 2,424 plots from 2022.

*Genomic selection*: The UT program used genomic BLUP to make selections and select parents for crossing in 2023.

## 8.17. VA. Virginia Tech. Nick Santantonio

*Education*: WheatCAP supported PhD student Sunilda Frias has taken part of the online meetings and attended the student workshop at PAG, and both the UAS and Genomic Selection Workshops. New MS student Francis Reith will join the WheatCAP educational activities in 2024.

*Germplasm & publications*: One SRWW is publicly released as 'VT Pitman' and one HRWW is licensed to Mennel Milling Co. as 'Phoenix 96' (App. 1).

*UAS*: 15 flights were conducted across two locations (7 in Warsaw VA, 5.6 acres, 8 in Blacksburg VA, 5.2 acres). Data is being processed in house and high-level data will be submitted directly to T3.

*Genotyping*: 1,032 lines genotyped with GBS at the Eastern Genotyping Lab (9286 imputed GBS markers). Pending submission to T3.

*Breedbase*: 57 trials including 11,537 yield plots were entered into T3/Wheat. They represent data for 2304 lines and include 64,355 phenotypic records.

*Genomic selection*: The VA program is currently using genomic selection at the following stages:1) Parent selection, 2) mate pair selections (which parents to mate) 3) F<sub>2</sub>-F<sub>4</sub> population advancement, 4) year 1 yield trials, prediction in unobserved environments (sparse testing in 4 locations, 5) advancement of lines in later stage trials to target specific environments.

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

*Education*: PhD student Morgan Hasler graduated in 2023 and is working as an agronomist at Nutrien Ag Solutions. Current PhD student Peter Schmuker participated in the 2023 WheatCAP student workshop. PhD student Andrew Herr participated in UAS hub Orientation at TX and presented at the Genomic Selection forum hosted by NC State University. Melinda Zubrod and

Adele Jamalzei just started their PhD programs. Two undergraduate researchers from underrepresented minority groups were interns in the program in 2023.

*Germplasm & publications*: the WA group released 5 new varieties (Rollie, Windust, Gemini, Nova AX, & Butch CL+) in 2023. Nine new papers in peer-reviewed journals were published by the WA group.

*UAS*: WA analyses their UAS data in house. In 2023 they flew 19 winter wheat trials at four different locations (12 acres). The UAV data was submitted to T3 for both the 2022 (1354 plots) and 2023 years (2976 plots). The UAS data have been helpful to select varieties at different locations and have been incorporated in WA selection models.

*Genotyping*: The WA group submitted 960 winter wheat samples for GBS in collaboration with the Eastern Regional Genotyping Laboratory (average 42,000 SNP per lines received). They also submitted 384 winter wheat samples to the Small Grains Genotyping Laboratory in Fargo, ND that have been deposited in T3 (average 3,000 SNP per line). Additional 186 were submitted to T3 through the WA Genotyping lab.

*Breedbase*: The WA team submitted the 2023 winter wheat data, which entails 2,976 plots. In addition, the phenotypic data for 1,354 plots collected in 2022 were deposited in T3 in 2023. Both spectral and agronomic data were submitted. The WA team uses the internal Genovix database, where they have all their historical data.

*Genomic selection*: The WA team runs genomic selection in the winter wheat program on all 1<sup>st</sup> year observation plots, primarily to estimate end-use quality performance, disease resistance, and grain yield. They have an internal robust procedure for running genomic selection, and plan on continuing to apply this tool annually for selection.

## 8.19. WI. University of Wisconsin. Lucia Gutierrez

*Education*: PhD Rishap Dhakal participated in the WheatCAP education monthly meeting and presented his research at the PAG annual meeting in 2023. PhD student Pablo Sandro participated in the monthly meetings, lectures, and discussion series and has a paper accepted for publication. Finally, PhD student Gopika Gopinathan has participated in a few of the monthly meetings, lectures, and discussion series.

*Germplasm & publications*: Foundation seeds for advanced inbred lines 47.04 and 260.04 was produced in 2022-2023 field season, and a larger increase has been planted. PVP paperwork has been initiated. The WI group published 2 papers in peer reviewed journals.

UAS: 10 flights were submitted to the UAS hub in 2023 (1.1 acres each)

*Genotyping*: A total of 182 genotypes were sent for genotyping at the Fargo genotyping lab and were deposited in T3 in 2023. Genotyping through 3k Illumina Infinium II array technology provided 3000 SNP per sample.

*Breedbase*: A total of 14 trials, consisting of 2127 plots from the years 2017 to 2023 have been submitted to T3/WheatCAP database. The trials were grown in three locations and include grain yield, grain test weight, grain moisture content, plant height, barley yellow dwarf virus incidence, and lodging incidence (total of 12,151 data points). WI is in the process of migrating their system to BreedBase and starting to use the field-book app for data collection.

*Genomic selection*: WI team focuses on developing new strategies and data analysis tools for incorporating various yield related source and sink components and environmental covariates accounting for GEI into the genomic prediction models.

#### 9. Priorities for Year 3

**9.1.** Education priorities Y3: Education priorities for 2024 include four workshops, a hybridbreeding course, and continuing writing groups, the mentorship program, and monthly meetings. Monthly meetings have primarily been scientific presentations by invited speakers. While this will continue, students will also help select topics for monthly meetings related to soft-skills and/or career prep.

The first workshop will be the 2024 WheatCAP Student Workshop at PAG-2024. The second workshop will focus on functional genomics and the genomics/genetics/cytogenetics of wild relative introgression breeding, hosted in-person by Dr. Eduard Akhunov at Kansas State University. The third workshop will focus on genomic and bioinformatic resources for wheat in the UK, hosted on-line by Dr. Cristobal Uauy and Designing Future Wheat. The fourth workshop will focus on strengths-based leadership for students in their 3<sup>rd</sup> or 4<sup>th</sup> year.

Prior to this workshop, students will complete Clifton-Strengths 2.0, the strengths-based leadership assessment and conduct one-on-one debriefing with Loriana and Patrick Sekarski, who are Gallup Certified Strengths Coaches and soft skills experts. In the one-on-one debriefing, students will identify their strengths, how they currently use them in work/life, how to apply their strengths to achieve their professional goals, and how to manage their weaknesses. In the strengths-based leadership workshop, students will practice applying their strengths in teambuilding activities, designed to develop their interpersonal communication and teamwork skills. Twenty-five students indicated they were interested in the Hybrid Breeding Strategies course hosted by UC-Davis Seed Biotechnology Center. WheatCAP education funds will be used to cover the registration costs for interested students. The course will supplement the students' training with breeding for self-pollinated species within the wheat breeding programs.

During the third year, students will take more leadership roles: Two students are planning the WheatCAP Student Workshop (January 2024), one student is planning a monthly meeting (Spring 2024), and 14 students are planning a graduate student plant science symposium for January 2025. The students have set up Program, Finance, Communication, and Registration Committees and identified Symposium Chairs and a Secretary.

A WheatCAP mentorship program is being set up. Twenty students filled out a mentee application designed to identify what they were hoping to gain from the program to best match them with potential mentors. Students are looking for someone to ask questions about their experiences in graduate school and/or work, network with, give advice on soft-skill development, and/or talk with about their own future career plans. Based on student responses, potential mentors were identified and contacted in industry, academic, government, and nonprofit/NGO careers in plant science. Responses to a mentor application are being collected, which will be used to identify beneficial mentee-mentor matches.

**9.2.** Genomic Resources priorities Y3: In 2024, the KSU team will finalize annotation of 10 wheat reference genomes assembled using long-read sequencing technologies. This genomic resource will be used to characterize structural variation in wheat genome and developing pangenome reference for integration into the wheat PHG to improve accuracy and completeness of genotype imputation. The updated PHG will be used to impute all deposited genotyping data in the T3 database.

The sequencing and analysis of RNA-seq data for a diverse panel of 200 lines and eQTL mapping in multiple wheat tissues will be finalized in 2024. The genome sequence variation data generated for this panel will be deposited to T3. RNA-seq expression values for a diverse panel of 200 wheat lines and wheat wild relatives will be deposited to T3 database. A workshop on application of genomic and phenomic tools for wheat pre-breeding using wild relatives will be organized at KSU.

The UCD group will focus on transferring the 9M EMS sequenced mutations mapped to CS into the recently published Kronos genome, and in the development of spatial transcriptomics resources for wheat spike development.

## 9.3. T3/Breedbase priorities Y3

- Increase automation of genotype uploads. T3 is BrAPI compliant, which means that external servers and computer scripts can both download and upload data to T3. This feature has been key to the rapid incorporation of hundreds of thousands of phenotypic measurements from UAS hub. Getting to this level of automation required sustained effort and communication with the UAS hub, in the form of monthly meetings to discuss bottlenecks and the specification of tasks to overcome them. In 2024, the T3 team proposes to engage in the same effort with the USDA Small Grains Genotyping Labs so that results from new genotyping assays will more rapidly be incorporated into T3. Broadly the data types to fully specify are:
  - a. Identities of genotyped lines. To ensure that the genotyping labs receive line IDs that exist in T3, the synonym tool described above will be used, ensuring that once markers are scored, they will be properly tied to T3 accessions.
  - b. Genotyping protocols. Marker positions and reference / alternative nucleotides (or presence / absence) need to be specified in a genotyping protocol. Now that the genotyping labs are finally using their mid-density assays, it should be straightforward to define these protocols.
  - c. Genotyping experiments. If tissue samples were collected from specific trials that exist on T3, those will need to be tied to a genotyping project.
- 2. Improved handling and search features for Known Informative Markers (KIMs). The data storage structure that T3 uses for KIMs was initially developed for morphological markers (called "Genetic Characters" on T3) and needs improvement:
  - a. The specification of new KIMs, given that new KIMs arise more frequently and in higher numbers than the data structure was designed for
  - b. Upload of KIM data on lines in T3. This process will follow from point 1 above.
  - c. Search queries to identify lines in T3 with specified combinations of KIM alleles. Breeders may want to use such queries for decision support in choosing crosses to make.

## 9.4. UAS-hub priorities Y3

Currently, the UAS-hub is enhancing the website by adding functionality that will enable users to push phenotypic information to T3 with a single click. They are also developing a QGIS plugin to expedite the plot map creation process and quality assurances to ensure the integrity of data uploaded to T3. The ban of DJI platforms has impacted routine data collection in year 2.

The UAS-hub team will elaborate a list of suggested platforms to standardize UAV data collection and ameliorate this problem as programs update their equipment.

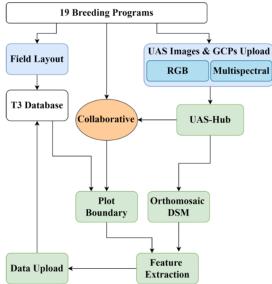
*QGIS plugin to delineate plot boundaries:* The UAS-Hub website is being enhanced by enabling users to push phenotypic information to T3 with a single click. Moreover, the Purdue group is developing a new QGIS plugin to expedite the plot map creation process. The new QGIS plugin will automate the plot boundary delineation process using a new zero-shot learning algorithm to lighten the manual plot boundary delineation process. The new QGIS plugin will also communicate with the T3 database so that *plot\_id* (a unique identifier used in the T3 database) will be automatically assigned to each plot. *Plot\_id* is important information when pushing processed phenotypic features back to the T3 database. Users have to type in this information in the previous workflow manually. The new QGIS plugin will help users streamline the UAS data processing pipeline. Furthermore, the Purdue group will work on developing software to check UAS phenotyping data integrity. This new software will ensure the integrity of data uploaded to T3 from UAS-Hub, focusing on preventing any missing and duplicate data.

*Streamlined UAS data processing, feature extraction, and data delivery:* In year 3, our primary focus will be on efficiently processing UAV raw data obtained from all Wheat CAP programs. We will continue to process the data from a field size of up-to 2 acres across all programs, utilizing both RGB and Multispectral datasets and derive comprehensive phenotypic features as appropriate to the sensors used. Currently, the establishment of precise plot boundaries for the transition from level 1 to level 2 data demands intensive manual effort and is done at the hub. To overcome this bottleneck, a discussion is underway to streamline the generation of plot boundaries and transfer that responsibility to the breeding programs, which are more familiar with their field.

*Enhancing communication and collaboration between UAS-hub team and breeding programs:* To improve communications between the UAS-hub and the breeding programs, we plan to develop a protocol that sends email notifications for updates or data collection, upload, and data retrieval. Additionally, a dedicated Team folder will be established on Microsoft Teams. Within this platform, individual programs will have exclusive access to folders, ensuring data security and compliance with program-specific restrictions. Notably, each program's folder will be accessible only to the specific program and the project owner, Jose Landivar, thus fostering a secure and organized environment. We will also continue the development of training materials on UAS-HTP to educate incoming graduate students.

*Workflow for efficient data transfer from breeding programs to UAS-Hub, and T3 database:* For the next year we propose to establish the workflow described below. This workflow delineates the specific steps for each program to follow, as well as the corresponding responsibilities for the Purdue/Texas A&M team. This structured approach aims to facilitate seamless coordination and efficient execution throughout the project's third year. The following steps are proposed: 1) All breeding programs upload field layout and information to T3 database as soon as the crop is planted. 2) Programs collect UAS data and use UAS-hub and upload the data within a week of data collection following the protocols described in the manual as well as in the Hub. When the programs send their first flight data, they include field layout and information and upload level 1 data products (orthomosaics) to the hub. 4) UAS-hub team and breeding programs co-develop plot boundaries and integrate with field information to seamlessly send the data back to breeding

programs and deposit in T3 data base. 5) UAS-Hub team will extract the phenotypic features and share the data to breeding programs and T3 database using the automated tool developed.



**Figure 5**. Overall workflow of communication between breeding programs, UAS hub, and T3 database. Activities for breeding programs and activities for TX-Purdue UAS-Hub team.

## 9.5. Genotyping labs priorities Y3

In 2024, the genotyping labs will further optimize mid-density genotyping platforms for the wheat community. We will collaborate to select SNP targets that enhance the level of overlap between the USDA-SoyWheOatBar-3K Illumina Infinium II and the Thermo Fisher AgriSeq platform. Discussion with Thermo Fisher will focus on synthesis of new, improved AgriSeq primer pools for use by the US wheat breeding programs. To reduce per sample costs, we will explore the development of primer pools targeted to growing regions. Research will be done with breeding programs to evaluate use of data from the new platforms to impute markers. Accuracy of genomic selection using existing training sets genotyped using other technologies (ie. GBS) will be assessed.

Efforts will be made to increase the Known Informative Marker (KIM) content in the middensity marker sets based on new research results. We will work to improve the performance of problematic KIMs. The labs will also continue development of KASP assays for traits of interest to breeding programs.

Pipelines will be developed to automatically process AgriSeq data for upload to T3, generate MAS reports that provide predicted alleles of the well-performing KIMs, and check the concordance of biological replicates. The labs will collaborate with T3 to improve handling of genotyping data, as noted in 9.3.

#### 10. Budget and personnel changes

There have been no changes in the original objectives of the grant and no major changes in the budget. A total of \$9000 transfer from KS to TX to cover some unexpected expenses for the Oracle Cloud. In Year 3, there is also a reduction of \$3,969 in the UCD supplies budget to accommodate the indirect rate adjustment at UCD. There are no changes in the total budget.

There are two changes in personnel planned for 2024.

10.1, Change of PI at Texas A&M. Dr. Amir M. H. Ibrahim has been promoted to Texas A&M AgriLife Research associate director, chief scientific officer. In his new role he will provide leadership to Texas A&M College of Agriculture and Life Sciences. He will be replaced by Dr. Juan A. Landivar, from Texas A&M University, who is already listed as key personnel in the original WheatCAP grant application. Juan Landivar is a UAS-HTP specialist and he was in charge of the UAS-HTP pipeline and image analyses from breeders, so he is very familiar with all the processes. Dr. Amir Ibrahim and Juan Landivar are overlapping during the months of November and December 2023 to facilitate the transition.

10.2. Transfer of WheatCAP subaward from Utah State University to Oregon State University: Margaret Krause, the wheat breeder at Utah State University and coPI of the WheatCAP grant was recently hired as the new wheat breeder for Oregon State University. UC Davis will transfer the current subaward to Dr. Margaret Krause from Utah State University to Oregon State University starting from the start of the third year of the WheatCAP project (1/1/2024). The residual funding at Utah State University will remain there to continue paying WheatCAP graduate student Dalton Jones, who is expected to complete his MS by May 2024 at Utah State University, and then transfer to a PhD program at Oregon State University under the direction of Dr. Margret Krause. Dr. Paul Johnson at Utah State University will help Margaret Kraus to administer the residual WheatCAP funds for the completion of Dalton Jones graduation. Any residual funding after the graduation of Dalton Jones will be transferred to Margaret Krause at Oregon State University.

## 11. Concluding remarks

The second year of the WheatCAP has been very productive with the project outputs disseminated through 51 new peer-reviewed publications including some in high-profile scientific journals (e. g. Nature Communications, Proceedings of the National Academy of Sciences of the USA, and PLOS Genetics, among others). Data was collected from 248 UAS flights over 37, 17,815 samples were sent for genotyping, and phenotypic data from 75,000 plots from 249 field tria1s were uploaded in T3. WheatCAP breeders released 32 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. Improved disease resistance of the new varieties will help reduce the applications of fungicides, reducing costs, benefiting the population, and the environment. The increased productivity of these new varieties will provide high-quality products for consumers while 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 accelerating 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 training of the next generation of plant

breeders guarantees 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.

# 11. Deliverables & Other Outputs:

- Appendix 1. Germplasm releases
- Appendix 2. Publications
- Appendix 3. Community resources
- Appendix 4. Graduate students. <u>https://www.triticeaecap.org/educational-activities/</u>

APPENDIX 1. Varieties and Germplasm releases 2023 (30 varieties and 5 germplasm).

#### 2023 Variety releases with PVP (3)

- 'UC-Central White' (PVP 202300187, submitted 4/17/2023) is a hard white spring wheat variety released by the University of California, Davis adapted to the Sacramento and San Joaquin valleys. It has excellent breadmaking quality and is the highest yielding variety in CA among the varieties in the "Recommended" CWC list base on quality. It was selected with molecular markers for stripe rust resistance gene *Yr15* and strong gluten allele *Glu-D1d*.
- 2. 'ND Thresher' (PVP 202400025, submitted 10/20/2023) is a HRSW variety released by the ND breeding program adapted to eastern and central ND. Marker-assisted selection was performed throughout line development.
- 3. 'Fortress' (PVP 202400022, submitted 10/15/2023) (Antero/Judee//Antero), a semi-solid stemmed, hard red winter wheat, released by Colorado State University in 2021 will be marketed by MonTech.

## 2023 PVP that were Pending in previous reports (7)

- 'KS Providence' (PVP 202300377, submitted 08/09/2023). KS Providence is a Kansas HRW with excellent yield potential and stability. It has demonstrated good drought tolerance and is expected to be well adapted to the Central Corridor of Kansas. It carries *Lr34*, *Lr46* and *Lr68*, giving it very good resistance to leaf rust but has intermediate resistance to stripe rust. It benefitted from genotyping lab analysis of regional nursery germplasm.
- 2. 'KS Big Bow' (PVP 202300090, submitted 12/13/2022). KS Big Bow is a Kansas Hard-White wheat with excellent yield potential. Viewed as a Joe replacement having better yield, sprout tolerance and coleoptile length than Joe. Carries the *Wsm2* gene for WSMV. It benefitted from genotyping lab analysis of elite/regional nursery germplasm.
- 3. KS Territory (PVP 202300091, submitted 12/13/2022). is a Kansas Hard-Red Winter wheat with good resistance to WSMV based on *Wsm2*. It benefitted from genotyping lab support of elite/regional nursery germplasm.

- 4. KS Ahearn (PVP 202300010, submitted 10/10/2022). KS Ahearn was released as a latematuring hard red winter wheat with excellent straw strength, good resistant to leaf, stripe and stem rusts and acceptable test weight. It has excellent yield potential and has performed well across widely disparate growing seasons. It does carry the 1B.1R translocation.
- 5. 'Windom SF' (PVP 202400023, submitted 10/15/2023) (CO18SF009W = Warhorse/Breck//CO12D1028), a hard white semi-solid stemmed winter wheat, released by Colorado State University.
- 6. 'UI GOLD' (PVP 202300355, submitted 08/10/2023) is a Hard-White Spring wheat cultivar released by the University of Idaho. It has very high grain yield and excellent bread-baking quality and good resistance to stripe rust.
- 7. 'MN-Rothsay' (PVP 202300138, submitted 02/03/2023) is a HRS released in 2022 by the University of Minnesota. It has high grain yield and strong straw. DNA markers generated by the USDA Genotyping lab at Fargo were used in the selection and development of MN-Rothsay

#### 2023 Variety releases with pending PVP (22)

- 1. 'AP18-AX' (CO14A136-135 = [(AF10/2\*Byrd)//(AF26/Byrd)]) is a new CoAXium wheat variety released by Colorado State University in 2023. It will be marketed by Syngenta. PVP will be submitted in August 2024.
- 2. 'CO18D007W' (CO12D906/CO07W722-F5) is a high yielding hard white winter wheat, developed utilizing the wheat by maize doubled haploid system. Released by Colorado State University in 2023. PVP will be submitted in August 2024.
- 3. 'CO18D297R' (CO12D906/CO11D1353//Monarch) is a high yielding hard red winter wheat, developed utilizing the wheat by maize doubled haploid system. Released by Colorado State University in 2023. PVP will be submitted in August 2024.
- 4. IL19-5632 is a very early maturing SRWW released by the University of Illinois that yields well in the northern half of the eastern US and is moderately resistant to scab, caused by *Fusarium graminearum*. Genomic Selection was used to advance IL19-5632 from stage 2 to stage 3 of testing. IL19-5632 was released in 2023 for brand labeling.
- 5. KS Mako is a medium maturity HRW with excellent yield, protein deviation and quality. It is moderately susceptible to leaf and stripe rusts, moderately resistant to stem rust and resistant to SBMV and WSMV (Wsm2). Benefitted from marker lab.
- 6. KS Bill Snyder was released in 2023. With its high yield potential, good drought tolerance, good disease resistance package, good quality, and good straw strength, KS Bill Snyder can be used for both dryland and irrigated production in western Kansas and surrounding regions. Its wheat steak mosaic virus resistance was confirmed with the Wsm2 marker testing during its development.
- 7. MT Carlson (MT 1939) is a spring wheat variety that was released from Montana State University breeding program in 2023.
- 8. MT Dutton (MT 1809) is a spring wheat variety that was released from Montana State University breeding program in 2023.

- 9. NHH17612, released in 2023 by the University of Nebraska is a HRWW with two-gene Clearfield herbicide resistance targeted towards the Nebraska Panhandle and western South Dakota region. NHH17612 was advanced in part based on genome estimated breeding values, and molecular markers for specific biotic and abiotic stress resistance genes.
- 10. 'SD PHEASANT' is a HRWW developed by the SD Ag. Exp. Station and is recommended for release in 2023. SD Pheasant is a high-yielding line with good test weight and grain protein content. SD Pheasant is resistant to leaf rust and moderately resistant to stem rust, hessian fly and moderately tolerant to stripe rust, FHB, and Bacterial Leaf Streak. Along with excellent grain yield potential, SD Pheasant has good milling characteristics and excellent baking characteristics.
- 11. 15VDH-FHB-MAS25-15 is a SRWW released by Virginia Tech that is publicly released as 'VT Pitman'.
- 12. VA18HRW-96 is a HRWW released by Virginia Tech and is licensed to Mennel Milling Co. as 'Phoenix 96'.
- 13. 'Rollie' is a SWWW released by Washington State University that was developed using marker-assisted selection to incorporate stripe rust resistance and eyespot resistance genes into a low-rainfall adapted cultivar; PVP pending.
- 14. 'Windust' is a SWWW released by Washington State University that was selected using marker-assisted selection as a standard height cultivar with excellent emergence, stripe rust resistance, eyespot resistance, and high end-use quality; PVP pending.
- 15 'Gemini' is a HRWW released by Washington State University that was selected using marker-assisted selection for end-use quality traits, targeted for production in intermediate rainfall zones, and has very good stripe rust resistance and tolerance to low pH soils; PVP pending.
- 16. 'Nova AX' is a SWWW released by Washington State University with tolerance to the Aggressor herbicide, confirmed to be homozygous for tolerance genes using markers, and approved for use in the CoAXium production system; PVP pending.
- 17. 'Butch CL+' is a SWSW released by Washington State University with tolerance to the Beyond herbicide, confirmed to be homozygous for tolerance genes using markers, and approved for use in the Clearfield production system. This line has very good stripe rust and Hessian fly tolerance (confirmed with markers) and very high grain yield across environments; PVP pending.
- 18. Paradox is a hard red winter wheat variety released in early 2023 by Oklahoma State University for contracted seed wheat and grain production in a controlled supply chain (farmer to baker). Its development benefited from the molecular markers for the Bx70e highmolecular-weight glutenin subunit.
- 19. Breadbox is a hard red winter wheat variety released in early 2023 by Oklahoma State University for contracted seed wheat and grain production in a controlled supply chain (farmer to baker). Its development benefited from the molecular markers for the Bx70e highmolecular-weight glutenin subunit.
- 20. Firebox is a hard red winter wheat variety released in early 2023 by Oklahoma State University. It was released for contracted seed wheat and grain production in a controlled

supply chain (farmer to baker). Its development benefited from the molecular markers for the Bx70e high-molecular-weight glutenin subunit.

- 21. High Cotton (OK18510) (PVP application in preparation) is a hard red winter wheat variety released in early 2023 by Oklahoma State University for adoption throughout Oklahoma and the southern Great Plains. Molecular markers were used to select desirable leaf rust resistance genes that corroborated phenotypic results and to confirm the presence of *Sbm1* in the absence of more reliable phenotypic data.
- 22. 'MI20R0012' is a SRWW released by Michigan State University with high grain yield and test weigh as well as resistance to leaf rust, stripe rust and FHB. The variety was advanced based on genomic prediction of grain yield and disease resistance. GBS marker sets developed at MSU were used for prediction of quantitative traits. KASP markers for quality, disease resistance and phenology were evaluated by the genotyping lab in Raleigh, NC. The variety has been licensed by the Michigan Wheat Program.

#### 2023 Germplasm

- 1. PI 701905. Released by UCD. Isogenic line of tetraploid wheat *T. turgidum* ssp. *durum* cultivar Kronos carrying the photoperiod sensitive allele *Ppd-A1b*.
- 2. PI 701906. Released by UCD. Isogenic line of tetraploid wheat *T. turgidum* ssp. *durum* cultivar Kronos carrying the combined knock-out mutant alleles *elf3 phyB*.
- 3. PI 701907. Released by UCD. Isogenic line of tetraploid wheat *T. turgidum* ssp. *durum* cultivar Kronos carrying the combined knock-out mutant alleles *elf3 ppd1*.
- 4. PI 702421. Released by UCD. BC<sub>4</sub>F<sub>3</sub> isogenic line of tetraploid wheat *T. turgidum* ssp. *durum* cultivar Kronos carrying combined mutations *platz-A1 platz-B1*.
- 5. PI 702944. Released by UCD. Isogenic line of tetraploid wheat *T. turgidum* ssp. *durum* cultivar Kronos carrying the adult plant stripe rust resistance gene *Yr78*.

## **APPENDIX 2.** Peer reviewed publications WheatCAP 2023

Google Scholar Impact of previous WheatCAP publications (measured 12/8/2023)

TriticeaeCAP (2011-2016): 26,598 cross-references (h index = 78).

WheatCAP (2017-2021): 7,343 cross-references (h index = 48).

WheatCAP (2022-2026): 569 cross-references (h index = 13).

#### Reported in 2023. 51 new publications

- Yu S, Li M, Dubcovsky J, Tian L (2022). Mutant combinations of *lycopene ε-cyclase* and β-*carotene hydroxylase 2* homoeologs increased beta-carotene accumulation in endosperm of tetraploid wheat (*Triticum turgidum* L.) grains. Plant Biotech. J. 20: 564– 576. <u>https://doi.org/10.1111/pbi.13738</u>
- Zhang J, Nirmala J, Chen S, Jost M, Steuernagel B, Karafiatova M, Hewitt T, Li H, Edae E, Sharma K, Hoxha S, Bhatt D, Antoniou-Kourounioti R, Dodds P, Wulff BBH, Dolezel J, Ayliffe M, Hiebert C, McIntosh R, Dubcovsky J, Zhang P, Rouse MN, Lagudah E (2023) Single amino acid change alters specificity of the multi-allelic wheat stem rust resistance locus *SR9*. Nature Communications 14:7354. <u>https://doi.org/10.1038/s41467-023-42747-9</u>
- 3. Glenn P, Woods DP, Zhang J, Gabay G, Odle N, Dubcovsky J (2023) Wheat bZIPC1 interacts with FT2 and contributes to the regulation of spikelet number per spike. Theoretical and Applied Genetics 136:237. <u>https://doi.org/10.1007/s00122-023-04484-x</u>
- Zhang J, Xiong H, Burguener GF, Vasquez-Gross H, Liu Q, Debernardi JM, Akhunova A, Garland-Campbell K, Kianian SF, Brown-Guedira G, Pozniak C, Faris JD, Akhunov E, Dubcovsky J (2023) Sequencing 4.3 million mutations in wheat promoters to understand and modify gene expression. Proceedings of the National Academy USA 120:e2306494120. <u>https://doi.org/10.1073/pnas.2306494120</u>
- 5. Li H, Hua L, Zhao S, Hao M, Song R, Pang S, Liu Y, Chen H, Zhang W, Shen T, Gou J-Y, Mao H, Wang G, Hao X, J. L, Song B, C. L, Li Z, Wang Deng X, Dubcovsky J, Wang X, Chen S (2023) Cloning of the wheat leaf rust resistance gene *Lr47* introgressed from *Aegilops speltoides*. Nature Communications 14:6072. <u>https://doi.org/10.1038/s41467-023-41833-2</u>
- 6. Bekkering CS, Yu S, Isaka NN, Sproul BW, Dubcovsky J, Tian L (2023) Genetic dissection of the roles of beta-hydroxylases in carotenoid metabolism, photosynthesis, and plant growth in tetraploid wheat (*Triticum turgidum* L.). Theor Appl Genet 136:8. https://doi.org/10.1007/s00122-023-04276-3
- Li H, Luo J, Zhang W, Hua L, Li K, Wang J, Xu B, Yang C, Wang G, Rouse MN, Dubcovsky J, Chen S (2023) High-resolution mapping of *SrTm4*, a recessive resistance gene to wheat stem rust. Theor. Appl. Genet. 136:120. <u>https://doi.org/10.1007/s00122-023-04276-3</u>
- Boden SA, McIntosh RA, Uauy C, Krattinger SG, Dubcovsky J, Rogers WJ, Xia XC, Badaeva ED, Bentley AR, Brown-Guedira G, Caccamo M, Cattivelli L, Chhuneja P, Cockram J, Contreras-Moreira B, Dreisigacker S, Edwards D, Gonzalez FG, Guzman C, Ikeda TM, Karsai I, Nasuda S, Pozniak C, Prins R, Sen TZ, Silva P, Simkova H, Zhang

Y, Wheat I (2023) Updated guidelines for gene nomenclature in wheat. Theor. Appl. Genet. 136:72 <u>https://doi.org/10.1007/s00122-023-04253-w</u>

- Debernardi JM, Burguener G, Bubb K, Liu Q, Queitsch C, Dubcovsky J (2023) Optimization of ATAC-seq in wheat seedling roots using INTACT-isolated nuclei. BMC Plant Biology 23:270. <u>https://doi.org/10.1186/s12870-023-04281-0</u>
- 10. Zhang J, Li C, Zhang W, Zhang X, Mo Y, Tranquilli GE, Vanzetti LS, Dubcovsky J (2023) Wheat plant height locus *RHT25* encodes a PLATZ transcription factor that interacts with DELLA (RHT1). Proc. Natl. Acad. Sci. USA. 120:e2300203120. https://doi.org/10.1073/pnas.2300203120
- Alvarez MA., Li C, Lin H, Joe A, Padilla M, Woods DP, Dubcovsky J. (2023). EARLY FLOWERING 3 interactions with PHYTOCHROME B and PHOTOPERIOD1 are critical for the photoperiodic regulation of wheat heading time. PLoS Genetics 19:e1010655. <u>https://doi.org/10.1371/journal.pgen.1010655</u>
- Gabay G, Wang HC, Zhang JL, Moriconi JI, Burguener GF, Gualano LD, Howell T, Lukaszewski A, Staskawicz B, Cho MJ, Tanaka J, Fahima T, Ke HY, Dehesh K, Zhang GL, Gou JY, Hamberg M, Santa-María GE, Dubcovsky J (2023) Dosage differences in *12-OXOPHYTODIENOATE REDUCTASE* genes modulate wheat primary root growth. Nature Communications. 14:539. <u>https://doi.org/10.1038/s41467-023-36248-y</u>.
- Chen Z, Debernardi JM, Dubcovsky J, Gallavotti A (2022). Recent advances in crop transformation technologies. Nature Plants. 8:1343–1351. <u>https://doi.org/10.1038/s41477-022-01295-8</u>
- Zhang J, Debernardi JM, Burguener GF, Choulet F, Paux E, O'Connor L, Enk J, Dubcovsky J (2023) A second generation capture panel for cost-effective sequencing of genome regulatory regions in wheat and relatives. The Plant Genome. 16:e20296 <u>https://doi.org/10.1002/tpg2.20296</u>
- Wong ML, Bruckner PL, Berg JE, Lamb PF, Hofland ML, Caron CG, Heo H-Y., Blake NK, Weaver DK, Cook JP (2023). Evaluation of wheat stem sawfly-resistant solid stem *Qss.msub-3BL* alleles in hard red winter wheat. *Crop Science*, 63, 556–567. <u>https://doi.org/10.1002/csc2.20866</u>
- Bian R, Liu N, Xu Y, Su Z, Chai L, Bernardo A, St. Amand P, Fritz A, Zhang G, Rupp J, Akhunov E, Jordan KW, Bai G (2023) Quantitative trait loci for rolled leaf in a wheat EMS mutant from Jagger. Theor Appl Genet 136(3):52. <u>https://doi.org/10.1007/s00122-023-04284-3</u>
- Chen J, Wheeler JJ, Marshall JM, Chen X, Windes S, Wilson C, Su M, Yimer B, Schroeder K, Jackson C (2023) Release of 'UI Gold' hard white spring wheat. Journal of Plant Registrations. <u>https://doi.org/10.1002/plr2.20309</u>
- Crutcher FK, Lamb PF, Nash D, Fiedler JD, Eberly J, Kephart KD, McVay K, Torrion J, Beiermann CW, Vetch JM, Chen C, Holen D, Blake NK, Heo H-Y, Cook JP (2023) Registration of 'MT Sidney' hard red spring wheat. Journal of Plant Registrations 17(2):368–375. <u>https://doi.org/10.1002/plr2.20268</u>
- 19. DeWitt N, Lyerly J, Guedira M, Holland JB, Murphy JP, Ward BP, Boyles RE, Mergoum M, Babar MA, Shakiba E, Sutton R, Ibrahim A, Tiwari V, Santantonio N, Van Sanford

DA, Howell K, Smith JH, Harrison SA, Brown-Guedira G (2023) Bearded or smooth? Awns improve yield when wheat experiences heat stress during grain fill in the southeastern United States. Journal of Experimental Botany. 74(21):6749-6759. https://doi.org/10.1093/jxb/erad318

- 20. Dixon L, Bellinger B, Carter AH (2023) A gravimetric method to monitor transpiration under water stress conditions in wheat. The Plant Phenome Journal 6(1):e20078. https://doi.org/10.1002/ppj2.20078
- 21. Dogan M, Wang Z, Cerit M, Valenzuela-Antelo JL, Dhakal S, Chu C, Xue Q, Ibrahim AMH, Rudd JC, Bernardo A, St. Amand P, Bai G, Zhang H, Liu S (2023) QTL Analysis of Yield and End-Use Quality Traits in Texas Hard Red Winter Wheat. Agronomy 13(3):689. <u>https://doi.org/10.3390/agronomy13030689</u>
- 22. Garst N, Belamkar V, Easterly A, Guttieri MJ, Stoll H, Ibrahim AMH, Baenziger PS (2023) Evaluation of pollination traits important for hybrid wheat development in Great Plains germplasm. Crop Science 63(3):1169–1182. <u>https://doi.org/10.1002/csc2.20926</u>
- 23. Gill HS, Brar N, Halder J, Hall C, Seabourn BW, Chen YR, St. Amand P, Bernardo A, Bai G, Glover K, Turnipseed B, Sehgal SK (2023) Multi-trait genomic selection improves the prediction accuracy of end-use quality traits in hard winter wheat. The Plant Genome:e20331. <u>https://doi.org/10.1002/tpg2.20331</u>
- 24. Guttieri MJ, Bowden RL, Zhang G, Haley S, Frels K, Hein GL, Jordan KW (2023) Agronomic and quality impact of a shortened translocation for wheat streak mosaic virus resistance. Crop Science 63(2):622–634. <u>https://doi.org/10.1002/csc2.20876</u>
- 25. Halder J, Gill HS, Zhang J, Altameemi R, Olson E, Turnipseed B, Sehgal SK (2023) Genome-wide association analysis of spike and kernel traits in the U.S. hard winter wheat. The Plant Genome 16(1):e20300. <u>https://doi.org/10.1002/tpg2.20300</u>
- 26. Hammers M, Winn ZJ, Ben-Hur A, Larkin D, Murry J, Mason RE (2023) Phenotyping and predicting wheat spike characteristics using image analysis and machine learning. The Plant Phenome Journal 6(1):e20087. <u>https://doi.org/10.1002/ppj2.20087</u>
- 27. Herr AW, Adak A, Carroll ME, Elango D, Kar S, Li C, Jones SE, Carter AH, Murray SC, Paterson A, Sankaran S, Singh A, Singh AK (2023) Unoccupied aerial systems imagery for phenotyping in cotton, maize, soybean, and wheat breeding. Crop Science 63(4):1722–1749. <u>https://doi.org/10.1002/csc2.21028</u>
- 28. Herr AW, Carter AH (2023) Remote sensing continuity:a comparison of HTP platforms and potential challenges with field applications. Frontiers in Plant Science 14:1233892. https://doi.org/10.3389/fpls.2023.1233892
- 29. Kaur S, Gill HS, Breiland M, Kolmer JA, Gupta R, Sehgal SK, Gill U (2023) Identification of leaf rust resistance loci in a geographically diverse panel of wheat using genome-wide association analysis. Frontiers in Plant Science 14:1090163. <u>https://doi.org/10.3389/fpls.2023.1090163</u>
- Liu Z, Li Y, Bernardo A, Amand PSt, Zhang P, Sehgal S, Bai G (2023) Development of diagnostic SNP markers for identification of rye 1RS translocations in wheat. Crop Science 63(1):255–265. <u>https://doi.org/10.1002/csc2.20841</u>

- 31. Merrick LF, Burke AB, Zhang Z, Carter AH (2022) Comparison of single-trait and multitrait genome-wide association models and inclusion of correlated traits in the dissection of the genetic architecture of a complex trait in a breeding program. Frontiers in Plant Science 12. <u>https://doi.org/10.3389/fpls.2021.772907</u>
- 32. Merrick LF, Herr AW, Sandhu KS, Lozada DN, Carter AH (2022) Optimizing plant breeding programs for genomic selection. Agronomy 12(3):714. https://doi.org/10.3390/agronomy12030714
- Merrick LF, Herr AW, Sandhu KS, Lozada DN, Carter AH (2022) Utilizing genomic selection for wheat population development and improvement. Agronomy 12(2):522. <u>https://doi.org/10.3390/agronomy12020522</u>
- 34. Merrick LF, Lozada DN, Chen X, Carter AH (2022) Classification and regression models for genomic selection of skewed phenotypes: A case for disease resistance in winter wheat (*Triticum aestivum* L.). Frontiers in Genetics 13:835781. https://doi.org/10.3389/fgene.2022.835781
- 35. Montesinos-López OA, Herr AW, Crossa J, Carter AH (2023) Genomics combined with UAS data enhances prediction of grain yield in winter wheat. Frontiers in Genetics 14. https://doi.org/10.3389/fgene.2023.1124218
- 36. Mustahsan W, Guttieri MJ, Bowden RL, Garland-Campbell K, Jordan K, Bai G, Zhang G (2023) Mapping the quantitative field resistance to stripe rust in a hard winter wheat population "Overley" × "Overland." Crop Science 63(4):2050–2066. https://doi.org/10.1002/csc2.20977
- 37. Peters Haugrud AR, Sharma JS, Zhang Q, Green AJ, Xu SS, Faris JD (2023) Identification of robust yield quantitative trait loci derived from cultivated emmer for durum wheat improvement. The Plant Genome:e20398. <u>https://doi.org/10.1002/tpg2.20398</u>
- 38. Wang H, Bernardo A, St. Amand P, Bai G, Bowden RL, Guttieri MJ, Jordan KW (2023) Skim exome capture genotyping in wheat. The Plant Genome:e20381. <u>https://doi.org/10.1002/tpg2.20381</u>
- 39. Winn ZJ, Amsberry AL, Haley SD, DeWitt ND, Mason RE (2023) Phenomic versus genomic prediction-A comparison of prediction accuracies for grain yield in hard winter wheat lines. The Plant Phenome Journal 6(1):e20084. <u>https://doi.org/10.1002/ppj2.20084</u>
- 40. Winn ZJ, Larkin DL, Lozada DN, DeWitt N, Brown-Guedira G, Mason RE (2023) Multivariate genomic selection models improve prediction accuracy of agronomic traits in soft red winter wheat. Crop Science 63(4):2115–2130. <u>https://doi.org/10.1002/csc2.20994</u>
- 41. Winn ZJ, Lyerly JH, Brown-Guedira G, Murphy JP, Mason RE (2023) Utilization of a publicly available diversity panel in genomic prediction of Fusarium head blight resistance traits in wheat. The Plant Genome 16(3):e20353. https://doi.org/10.1002/tpg2.20353
- 42. Wold-McGimsey F, Krosch C, Alarcón-Reverte R, Ravet K, Katz A, Stromberger J, Mason RE, Pearce S (2023) Multi-target genome editing reduces polyphenol oxidase

activity in wheat (Triticum aestivum L.) grains. Frontiers in Plant Science 14:1247680. https://doi.org/10.3389%2Ffpls.2023.1247680

- 43. Wu J, Jia H, Qiao L, Fu B, Brown-Guedira G, Nagarajan R, Yan L (2023) Genetic basis of resistance against powdery mildew in the wheat cultivar "Tabasco". Mol Breeding 43(7):56. <u>https://doi.org/10.1007/s11032-023-01402-3</u>
- 44. Xu Y, Li Y, Bian R, Zhang G, Fritz AK, Dong Y, Zhao L, Xu Y, Ghori N, Bernardo A, St. Amand P, Rupp JLS, Bruce M, Wang W, Akhunov E, Carver B, Bai G (2023) Genetic architecture of quantitative trait loci (QTL) for FHB resistance and agronomic traits in a hard winter wheat population. The Crop Journal. Online first. https://doi.org/10.1016/j.cj.2023.09.004
- 45. Rebollo I, Aguilar I, Pérez de Vida F, Molina F, Gutiérrez L, Rosas JE (2023) Genotype by environment interaction characterization and its modeling with random regression to climatic variables in two rice breeding populations. Crop Science 63(4):2220–2240. https://doi.org/10.1002/csc2.21029
- 46. Bhandari M, Chang A, Jung J, Ibrahim AM, Rudd JC, Baker S, Landivar J, Liu S, Landivar J (2023) Unmanned aerial system-based high-throughput phenotyping for plant breeding. The Plant Phenome Journal 6:e20058. <u>https://doi.org/10.1002/ppj2.20058</u>
- 47. Cerit M, Wang Z, Dogan M, Yu S, Valenzuela-Antelo JL, Chu C, Wang S, Xue Q, Ibrahim AMH, Rudd JC, Metz R, Johnson CD, Liu S (2023) Mapping QTL for Yield and Its Component Traits Using Wheat (*Triticum aestivum* L.) RIL Mapping Population from TAM 113 x Gallagher. Agronomy 13:2402. <u>https://doi.org/10.3390/agronomy13092402</u>
- 48. Wang Z, Dhakal S, Cerit M, Wang S, Rauf Y, Yu S, Maulana F, Huang W, Anderson JD, Ma X-F, Rudd JC, Ibrahim AMH, Xue Q, Hays DB, Bernardo A, St. Amand P, Bai G, Baker J, Baker S, Liu S (2022) QTL mapping of yield components and kernel traits in wheat cultivars TAM 112 and Duster. Frontiers in Plant Science 13:105701. https://doi.org/10.3389/fpls.2022.1057701
- 49. Rooney TE, Sweeney DW, Kunze KH, Sorrells ME, Walling JG (2023) Malting quality and preharvest sprouting traits are genetically correlated in spring malting barley. Theor Appl Genet 136(3):59. <u>https://doi.org/10.1007/s00122-023-04257-6</u>
- 50. Jordan KW, Fernandez-de Soto M, Korol A, Akhunova A, Akhunov E (2023). Allelic variation in the wheat homolog of topoisomerase II is associated with crossover rate. BioRxiv, <u>https://doi.org/10.1101/2023.07.06.548017</u>
- **51.** Coombes B, Lux T, Akhunov E, Hall A (2023). Introgressions lead to reference bias in wheat RNA-Seq analysis. BioRxiv, <u>https://doi.org/10.1101/2023.10.04.560829</u>.

#### **APPENDIX 3.** Community resources generated in Y2

#### 2023 Public databases

- 1. T3 database https://triticeaetoolbox.org/wheat/
- 2. Sequenced mutant populations <u>https://dubcovskylab.ucdavis.edu/wheat\_blast</u> with the addition of 4.3 million new mutations in regulatory regions. Reported in reference 4, Appendix 2. Data has been deposited in GrainGenes and ENSEMBL.
- 3. New ATAC-seq data incorporated into the GrainGenes genome browser (reference 9, Appendix 2).
- 4. New capture for wheat regulatory regions developed in collaboration with Arbor Biosciences and published in 2023 (reference 14, Appendix 2)
- 5. New protocols for Marker Assisted Selection https://maswheat.ucdavis.edu/

#### 2023 Tools for marker-assisted selection (MAS)

- Molecular markers for new alleles for increased number of spikelets per spike in genes bZIPC1 (reference 3, Appendix 2) and LFY (<u>https://doi.org/10.1101/2023.11.02.565263</u>). These two papers also report significant epistatic interactions between bZIPC1 and FT2, and between WAPO1 and LFY, and identify the best allele combinations to maximize the number of spikelets per spike and grains per spike.
- 2. Cloned genes providing perfect functional markers for wheat improvement:
  - a. Stem rust resistance gene Sr9 (ref. 2, App. 2).
  - b. Leaf rust resistance genes *Lr47* (ref. 5, App. 2).
  - c. Wheat plant height *Rht25* (ref. 10, App. 2).
  - d. OPRIII genes regulating root length and architecture (ref. 12, App. 2).
- 3. Improved markers for:
  - a. Stem rust resistance gene SrTm4 (ref. 7, App. 2).
  - b. Sawfly resistance QTL Qss.msub-3BL (ref. 15, App. 2).
  - c. Wheat Streak Mosaic Virus shorter translocations (ref. 25, App. 2)
  - d. Different 1RS.1BL translocations in wheat (ref. 31, App. 2)

Institution	PI	1 <sup>st</sup> name	Last name	Deg.	Gender	Start Date <sup>1</sup>	Gra
Colorado State U.	Eston Mason	Mikayla	Hammers	PhD	F	9/1/2022	
Colorado State U.	Eston Mason	Selena	Lopez	PhD	F	1/1/2022	
U. of California-Davis	Jorge Dubcovsky	Maria	Green Rottersman	PhD	F	9/1/2022	
U. of Florida	Ali Babar	Jordan	McBreen	PhD	М	1/1/2021	
U. of Idaho	Jianli Chen	Pabitra	Joshi	PhD	F	1/1/2021	
U. of Idaho	Jianli Chen	Yaotian	Gao	PhD	М	7/1/2021	
U. of Illinois	Jessica Rutkoski	Lucas	Munaro	PhD	М	1/1/2021	
U. of Illinois	Jessica Rutkoski	Raysa	Gevartosky	PhD	F	8/1/2021	
Purdue University	Jinha Jung	Ismaila	Olaniyi	PhD	М	8/1/2021	
Kansas State U.	Eduard Akhunov	Nicholas	Stelling	PhD	М	1/1/2023	
Kansas State U.	Eduard Akhunov	Dwight	Davidson	MS	М	8/1/2021	
USDA-Kansas State U.	Katherine Jordan	Lawrence	Tidakbi	PhD	М	1/1/2022	
USDA-Kansas State U.	Mary Guttieri	Adriano	Moreira	PhD	М	1/1/2023	
USDA-Kansas State U.	Guijua Bai	Xiaoting	Xu	PhD	F	1/1/2020	
USDA-Kansas State U.	Guijua Bai	Yuzhou	Xu	PhD	М	8/1/2017	*
Louisiana St. U	Noah DeWitt	Julio	Sellani	MS	М	1/1/2023	
U. of Maryland	Vijay Tiwari	Adam	Schoen	PhD	М	8/1/2020	
U. of Maryland	Vijay Tiwari	Anmol	Kajila	PhD	F	8/1/2021	
Michigan State U.	Eric Olson	Jonathan	Concepcion	PhD	М	8/1/2021	
U. of Minnesota	James Anderson	Max	Fraser	PhD	М	7/1/2017	*
Montana St. UBozeman	Jason Cook	Mei Ling	Wong	PhD	F	8/1/2022	Ī
Montana St. UBozeman	Jason Cook	Jared	Lile	PhD	М	6/1/2021	1
USDA-North Carolina St. U.	G. Brown-Guedira	Nico	Lara	PhD	М	8/1/2020	
USDA-North Carolina St. U.	G. Brown-Guedira	Daniela	Miller	PhD	F	1/1/2019	
USDA-North Dakota St. U.	Justin Faris	Pooja	Kumari	PhD	F	8/1/2022	
North Dakota St. U.	Andrew Green	Lucas	Batista	MS	М	1/1/2021	*
U. of Nebraska-Lincoln	Katherine Frels	Sydney	Graham	PhD	F	4/1/2022	
Cornell U.	Mark Sorrells	Timothy	Mulderrig	PhD	М	8/1/2022	
Oklahoma State U.	Liuling Yan	Jennifer	Tapia	MS	F	8/1/2021	*
Oklahoma State U.	Liuling Yan	Wenxuam	Zhai	PhD	F	NA	
Oklahoma State U.	Liuling Yan	Juan	Luzuriaga	MS	М	1/1/2022	
Oklahoma State U.	Brett Carver	Abby	Tucker	MS	F	8/1/2022	*
Oklahoma State U.	Phillip Alderman	Sanju	Shrestha	PhD	F	8/1/2023	
South Dakota State U.	Sunish Sehgal	Harsimardeep	Gill	PhD	М	1/1/2021	*
South Dakota State U.	Sunish Sehgal	Swas	Kaushal	PhD	М	5/1/2022	
Texas A&M	Shuyu Liu	Zhen	Wang	PhD	М	9/1/2019	*
Texas A&M	Shuyu Liu	Kyle	Parker	PhD	M	9/1/2020	t
Texas A&M	Jackie Rudd	Kylie	Scott	MS	F	9/1/2020	*
Texas A&M	Amir Ibrahim	Russ	Garretson	PhD	M	9/1/2021	t
Texas A&M	Jackie Rudd	Jose	Landivar Scott	MS	M	9/1/2021	*
Texas A&M	Jackie Rudd	Shannon	Baker	PhD	F	9/1/2023	$\mathbf{f}$
Utah State U.	Margaret Krause	Dalton	Jones	MS	M	5/1/2022	1
Virginia Tech	Nicholas Santantonio	Sunilda	Frias	PhD	F	8/1/2022	$\mathbf{f}$
Washington State U.	Mike Pumphrey	Peter	Schmuker	PhD	M	8/1/2020	$\mathbf{I}$
Washington State U.	Arron Carter	Andrew	Herr	PhD	M	8/1/2020	┢──
Washington State U.	Arron Carter	Adele	Jamalzei	PhD	F	9/1/2023	┢──
Washington State U.	Arron Carter	Melinda	Zubrod	PhD	F	9/1/2023	$\vdash$
Washington State U.					F		*
U. of Wisconsin-Madison	Mike Pumphrey Lucia Gutierrez	Morgan	Hasler Dahakal	MS PhD	г М	1/1/2022 6/1/2022	$\vdash$
U. of Wisconsin-Madison	Lucia Gutierrez	Rishap Pablo	Sandro	PhD	M		┢
U. of Wisconsin-Madison	Lucia Gutierrez	Gopika	Gopinathan	PhD PhD	F	8/1/2020 9/1/2022	<u> </u>

# **APPENDIX 4. Graduate students** (10 completed + 41 current = total 51, 44% female)

Individual student projects and profiles at https://www.triticeaecap.org/meet-our-students/

<sup>1</sup>Date started degree program