

## Cover page

**a. Award #:** 2017-67007-25939

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

**c. Project Director:** Jorge Dubcovsky

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

- **Appendix 1.** Germplasm releases <https://www.triticeacap.org/variety-and-germplasm-releases/>
- **Appendix 2.** Publications <https://www.triticeacap.org/publications-2017/>
- **Appendix 3.** Community resources
  - T3 database <https://triticeaetoolbox.org/wheat/>
  - Sequenced mutant populations [https://dubcovskylab.ucdavis.edu/wheat\\_blast](https://dubcovskylab.ucdavis.edu/wheat_blast)
- **Appendix 4.** Graduate students. <https://www.triticeacap.org/educational-activities/>

**e. Institution name:** University of California, Davis

**f. Requested amount:** \$3,000,000

## A. Review of WheatCAP past accomplishments

**Wheat-CAP overall productivity:** Members of the WheatCAP team have published 51 peer-reviewed publications acknowledging the support from the WheatCAP or the previous T-CAP. None of these publications has been included in previous T-CAP reports. Breeders from the WheatCAP have released 19 commercial varieties (10 with PVP and 9 pending or public), 3 germplasms, and 5 mapping or TILLING populations. Complete lists of publications and released varieties are presented in Appendices 1 and 2, respectively. Those lists are also available through the WheatCAP web site (<http://www.triticeacap.org/>). Community resources are in Appendix 3 and students trained in Appendix 4. Students are linking their personal profiles to the project web site and a forum on gene positional cloning has been initiated. The web site includes links to each of the 15 positional cloning projects (<http://www.triticeacap.org/qtl-cloning-projects/>).

**Education:** We initiated the training of 20 graduate students in molecular plant breeding. By leveraging funds from participating universities, we have currently hired 15 PhD students and 5 MS students (45% female) with two additional PhD hires pending at Washington State University and North Carolina State University. All incoming Wheat-CAP students are being self-evaluated with an anonymous survey to assess their education level on various topics that are pertinent to the student's success in completing their Wheat-CAP research project and preparing for their future careers. The survey will be given annually to track Wheat-CAP student education progress, and the information will help the education team to meet the educational needs of the students. Annual survey results will be posted on the Wheat-CAP website.

An interactive online discussion forum has been created for Wheat-CAP participants to post questions and answers that are important to the success of the Wheat-CAP student projects. Questions and answers posted in the discussion forum that are determined to be of value to the general research community will be posted in a Frequently Asked Questions (FAQ) link on the Wheat-CAP website. Once the students start their fall 2017 semester at their respective institutions, video conferencing using the Zoom webinar software will be commenced to provide a live forum where Wheat-CAP students can discuss their projects.

Educational activities have also included updating our online education delivery platform infrastructure. Leah Sandall, who provides online education technical support for Wheat-CAP, worked with Dr. Deanna Namuth-Covert in the transfer of the electronic TCAP education materials to the eXtension Online Campus (<https://campus.extension.org>). Wheat-CAP has provided support for upgrading the Plant and Soil Sciences eLibrary (PASSeL), which houses PBTN, to make both sites mobile-friendly. These revisions will result in PBTN being easily accessible by all web-browsing devices. The target date for completion of the mobile-friendly upgrades is May 2018. Both Leah and DeAnna Crow have been removing out-of-date information and bad links in PBTN.

**T3 database:** The T3 team has developed a beta version of a whole database GWAS analysis pipeline. For any phenotyping trial including > 50 wheat lines genotyped at sufficient density,

standard GWAS is performed. For traits assayed in multiple trials, results are combined by meta-analysis. Genes are sorted by cumulative evidence of association and automated links are made to up to four external databases: the WheatExp (Pearce et al., 2015), expVIP (Borrill et al., 2016), the EMBL-EBI Expression Atlas (Petryszak et al., 2016), and the Plant Reactome (Tello-Ruiz et al., 2016).

The T3 team has massively expanded the number of phenotyping trials accessible through T3 by adding data from a number of US Cooperative Uniform Nurseries. The number of phenotypic data points in T3 increased by 44%.

A wheat pan-genome was published in February 2017 (Montenegro et al., 2017). The T3 team has added a JBrowse track to this pan-genome and blasted all DNA variants stored in T3 to it. T3 provides a report page linking currently selected markers and the Ensembl genome browser, which displays annotations and polymorphism-effect predictions.

Exome capture data from parents of biparental populations used for fine mapping in WheatCAP are available, including 976,558 polymorphisms on 36 lines. A beta version of a tool to select polymorphisms between two WheatCAP parents has been developed.

**Genomics resources:** The sequenced mutant populations of tetraploid and hexaploid wheat were published in PNAS in January 2017 (Krasileva et al., 2016). More than 10,000,000 mutations in the coding regions of the wheat genome have been sequenced and catalogued in a publically accessible database. These mutations are being used by IWYP participants to identify mutations affecting grain size and number and other yield components.

The exome capture assay targeting 162 Mb of the wheat genome (Krasileva et al., 2016) was used to re-sequence the coding sequences of 36 wheat lines used as the parents of the Wheat CAP mapping populations. On average, 57 million 150-bp paired-end reads were generated for each line and mapped to the latest version of the wheat genome reference (IWGSC, v. 1.1) providing a 50x coverage of the targeted genomic regions. The variant calling using the GATK-based pipeline developed in the Triticeae CAP (Jordan et al., 2015) was performed, resulting in 976,558 SNPs and small indels. The data has been deposited to the searchable T3 database in July 2017 and made available to the project participants.

The design of the regulatory sequence capture targeting 250 Mb of unique genomic regions including predicted miRNA binding sites and 2 kb upstream of each gene model in the wheat genome was completed and submitted for synthesis to the Nimblegen Inc. (collaboration with the UK IWYP project led by A. Hall).

The MNase and ATAC-seq chromatin accessibility assays were optimized for wheat. The MNase assay has been used to analyze functional regions of the wheat genome. For this purpose, we have prepared genomic libraries from the MNase treated nuclei of Chinese Spring. Two levels of MNase treatment (heavy and light) were used according to Rodgers-Melnick et al. (2016) to identify hypersensitive regions of the wheat genome with open chromatic structure. Genomic libraries were sequenced at 10x coverage and mapped to the wheat genome reference (IWGSC, v. 1.1). The MNase hypersensitivity score was calculated by subtracting the normalized depth of coverage obtained for heavy digest from that of the light digest according to

Rodgers-Melnick et al. (2016). Consistent with these results, the functionally active hypersensitive chromatin regions are associated with the regions upstream of the transcription initiation sites. The data are being prepared for deposition to the T3 database to provide wheat community with the tool for filtering SNPs located in the functional portion of the wheat genome. In maize, these regions were shown to harbor SNPs that explain up to 40% of heritable variation in agronomic phenotypes (Rodgers-Melnick et al. 2016).

The spring wheat NAM population was developed, genotyped using 90K iSelect, GBS and exome capture assays, and used to characterize the distribution of 102,000 recombination breakpoints across the wheat genome (Jordan et al. 2017). The data was used to identify genes controlling recombination rate variation across the wheat genome. This study demonstrated that the chromosomal distribution and frequency of crossovers can be manipulated using both genetics- and biotechnology-based approaches providing a valuable resource for developing the methods of recombination rate modification in polyploid crops. In the Wheat CAP, the crossover frequency distribution across the wheat genome will be used by the project participants to assess the number of lines in the progeny of mapping populations to obtain the adequate number of recombinants for QTL mapping. The data is prepared for deposition to the T3 database.

### **Genotyping Laboratories**

The Western Regional Genotyping lab designed and tested 23 KASP markers that were evaluated on 353 individuals in two separate populations provided by participants in Idaho and Washington.

The Eastern Regional Genotyping lab developed 25 KASP assays targeted to the 6A region for TWK, including two assays designed around polymorphisms in the *TaGW2-A1* locus. The assays were used to evaluate 786 F<sub>4:6</sub> individuals from the LA95135 x MVP57 mapping population. Evaluation with the marker for *TaGW2* was also done on the SWW panels from the T-CAP project to assay allele frequencies and marker effects on kernel weight.

The Central Regional Genotyping lab collaborated with the program at Kansas State and identified a QTL for kernel length and thousand-kernel weight on chromosome arm 7AL of soft wheat variety Clark, designated *TaTKW-7AL*, and identified two flanking markers, *IWB13913* and *IWA5913*, for *TaTKW-7AL* using 90K SNP chip. These markers were converted into KASPar markers and validated in a RIL population and a diversity panel, respectively. These markers are used in the Genotyping Lab for routine MAS. The genotyping lab also provided a RIL mapping population from cross between the cultivars Overland x Overley, and generated GBS data and constructed a GBS map for QTL mapping and characterization.

The Northern Regional Genotyping lab completed the evaluation of genetic diversity and host resistance to stem rust in a panel of USDA NSGC durum wheat accessions. The study was published in Plant Genome.

The genotyping labs have been working on development of amplicon sequencing approaches to genotyping. A protocol for next generation sequencing trait-linked markers published by researchers at the genotyping lab at Manhattan, KS (Bernardo et al. 2015) was modified for use with genome-wide markers. The group at Pullman, WA selected 768 markers from the iSelect

arrays based on allele frequency and genome distribution. A high degree of success was observed for primer pools using targeted amplicon sequencing. Research at the Eastern Genotyping lab has focused on polymorphisms in causal genes and trait-linked markers. Sequences of markers for 80 genes involved in plant growth and development (ie. *PPD1*, *VRN1*, *RHT1*), grain quality (ie. seed texture and color, *Glu-D1*, *Glu-B1*, *TaGW2-A1*, *TaSus-2B*), and multiple pest resistances gene were provided to AgriPlex Genomics for development of primer pools. A high level of agreement was observed for most markers in a comparative analysis of amplicon sequencing and KASP results involving 520 breeding lines. Analyses aimed at expanding the primer sets, improving genome specificity and improving variant calling parameters are ongoing. With both approaches, new markers from the WheatCAP cloning projects can be added to the primer pools as they are validated.

Exome capture data generated within the WheatCAP project are being leveraged for targeted mapping of other genes/QTL. For example, fine mapping in the AGS 2000 x Pioneer 26R61 population will also be done for genes/QTL conferring resistance to stripe rust (*YrR61*), powdery mildew (*Pm54*) and Hessian fly (*Qhf.uga-6AL*).

Of the 41 publications reported in Appendix 2, the leaders of the genotyping labs are co-authors in 29 (Shiaoman Chao in 11, Gina Brown-Guedira in 9, Guihua Bai in 8 and Deven See in 1), documenting the good integration of the genotyping laboratories with the research and breeding programs of the WheatCAP.

### CIMMYT HUB. Matthew Reynolds

CIMMYT did not have a budget assigned for the first year of the project because no lines were expected to be ready that early. However, CIMMYT was very involved in the selection of the recurrent parental lines for the backcrossing of the selected QTL and in the delivery of the corresponding seeds.

**Table 1.** “High biomass” lines provided by CIMMYT

WMAII subset II	Abbrev. ID	GID	YLD	TKW	GNO	Heading	Mat	Height	BM t/ha	HI
Ent. 1	69	4878569	7.32	52.38	14032	80.88	128.13	110.88	21.06	0.42
Ent. 2	13	4314513	7.10	46.98	15148	80.55	125.25	113.04	20.80	0.38
Ent. 3	63	4577963	6.69	48.25	13958	80.67	125.63	115.85	20.76	0.37
Ent. 4	74	3613474	8.11	43.43	18699	82.02	129.25	100.47	20.24	0.44
Ent. 5	11	3855011	7.71	46.45	16671	82.31	125.25	109.67	19.89	0.41

Two sets of potential recurrent parents were selected. The first set included five lines selected for high biomass and good harvest indexes from the WAMI panel in Obregon (Table 1).

International data is available for this material in a range of environments. The rationale for this selection was the dependence of yield gains on simultaneous increases in “source” and “sink”. Since many of the selected QTL affect the sink side of the equation (more and larger grains), we selected high biomass lines to maximize our chances of an adequate carbon and nutrient supply

from the “source”. The second set included 15 high yielding varieties selected by the wheat breeder at CIMMYT (Ravi Singh) and is detailed in Table 2

**Table 2.** Selected CIMMYT high yielding parents

Recurrent parent		Recurrent parent	
1	KINGBIRD #1	6	PBW65/2*PASTOR
2	MUTUS #1	7	FRANCOLIN #1
3	MISR 1	8	BAJ #1
4	KACHU #1	9	KUTZ
5	PRL/2*PASTOR	10	NADI
		11	CHIPAK
		12	MUCUY
		13	KENYA SUNBIRD/KACHU
		14	VENDA
		15	BONSU
		16	BORLAUG100 F2014

## QTL cloning projects

### *AR. University of Arkansas. Esten Mason*

**Education:** PhD student Dylan Larkin and MS student Zachary Winn started to work in August 2017. Before, PhD candidate Andrea Acuna worked in the development of heterogeneous inbred families. Dennis Lozada, Ph.D. candidate and Monsanto Beachell-Borlaug fellow, completed research at CIMMYT with Susanne Dreisigacker validating markers for winter wheat QTL in spring wheat. Paul Wolf is an undergraduate honors student who works as a research assistant on the project.

**Research project:** D. Larkin and Z. Winn are targeting yield QTL on chromosomes 1A (between markers IWA2922 and IWA6707) and 6B, respectively. These QTL were previously discovered in the Pioneer 26R61/AGS 2000 RIL population (Addison et al. 2016). This population was evaluated in 12 environments and the 1A QTL was confirmed in an association-mapping panel including 240 soft red winter wheat in eight environments. Lines carrying the AGS2000 allele showed on average 116.5 kg ha<sup>-1</sup> higher grain yield than those carrying the P26R61 allele.

To generate a fine map of these QTL, they screened 338 individuals representing 13 RILs with markers flanking the known QTL regions to identify heterozygous RILs to develop heterogeneous inbred families (HIF). Ten individuals were confirmed to be heterozygous. These lines are currently being grown and re-screened for markers flanking QTL and with five newly designed KASP markers providing a narrower interval around the QTL. Homozygous progeny for the two segregating alleles have been identified for ~50 recombinant lines that will be evaluated in replicated rows in 2017-2018. Heterozygous individuals have been self-pollinated to advance the HIFs and reduce the segregating alleles outside the target region.

The Arkansas group released the variety AGS 2055 (Appendix 1) and published three peer reviewed articles (Appendix 2). Crosses of lines carrying the two QTL selected by the AR team with the high-yielding CIMMYT lines have been completed and F<sub>1</sub> seed is available (Table 3).

**Table 3.** Traits, QTL, donor alleles, CIMMYT recurrent parents and status of crosses.

State	Trait	QTL/Gene	Donor allele	CIMMYT Background <sup>1</sup>	Status
AR	Yield	1A (IWA7173)	AGS 2000	All HY CIMMYT lines	F <sub>1</sub>
	Yield	6B (IWA755, IWA6428)	AGS 2000	All HY CIMMYT lines	F <sub>1</sub>
CA	Spikelet No.	7AL 670-680 Mb CSv1 <sup>3</sup>	Berkut	HB (3855011, 4314513, 4878563)	F <sub>1</sub>
	Spikelet No.	1A <sup>m</sup> L. <i>Elf3 = Eps-A<sup>m</sup>1</i>	<i>T. monococcum</i> DV92	Kingbird	BC <sub>2</sub>
	Grain Size	6AL <i>gw-A2</i>	EMS mutant	Kingbird	BC <sub>2</sub>
	Combined	<i>Eps-A<sup>m</sup>1 + gw-A2</i>	Same as above	Cirno & GID 6420253 (4x)	BC <sub>3</sub> <sup>2</sup>
CO	Kernel weight	6BL 145-149 Mb	CO940610	Kingbird.	F <sub>1</sub>
		7AL 670-680 Mb CSv1	Platte	(CO breeding lines)	F <sub>1</sub>
ID	Spikelet No.	5A	UI Platinum	HB (3855011, ...13, ...63, ...74)	BC <sub>1</sub>
	Productive tillers	6A	Capstone	HY/HB under selection	Screen
KS	Yield & diseases	2DL QTL, <i>Sr57/Yr40</i> , 2NS	KS11WGGRC53-O	18 HY/HB	F <sub>1</sub>
MI	Yield	2DL and 7DS QTL	TA1615 & TA1718	Kingbird & Heilo	F <sub>1</sub>
	Yield	2DS and 6DL QTL	KS05HW14	Kingbird & Heilo	F <sub>1</sub>
MN	Grain size	2AL <i>gwm339-IWB12880</i>	<b>MN98550</b>	HB (3855011, ...13, ...63, ...69)	F <sub>1</sub>
		2AL <i>gwm339-IWB12880</i>	<i>T. dicoccoides</i> 'Israel A'	HY/HB under selection	Screen
MT	Productive tillers	6B <i>QTn.mst-6B</i> 150Mb CSv1	Reeder	HB (GID 3613474)	BC <sub>1</sub>
NC	Grain weight	6A	Massey	HY/HB under selection	Screen
ND	Yield	3A <i>QGyld.unl-3A</i>	Wichita	HY/HB under selection	Screen
	Spikelet No.	1BS <i>QSps.fcu-1B</i>	<i>T. dicocum</i> PI41025	HY/HB under selection	Screen
	Grain No./ weight	2BS <i>QGws.fcu-2B</i>	Ben	HY/HB under selection	Screen
	Grain No./ weight	5BL <i>QGws.fcu-5B</i>	<i>T. dicocum</i> PI41025	HY/HB under selection	Screen
	Spikelet No.	7AL <i>QSpn.fcu-7A</i>	<i>T. dicocum</i> PI41025	HY/HB under selection	Screen
NY	Grain size	5BS (60 cM)	Synthetic	HB (all 5)	F <sub>1</sub>
OK	Yield	<i>QYld.osu-1B</i>	Duster	HY/HB under selection	Screen
	Spikelet No.	7BL 650-700 Mb CSv1	Citr17600	HY/HB under selection	Screen
SD	Yield	7DS 6-16 Mb CSv1	<i>Ae. tauschii</i> , TA1691 <i>Th. intermedium</i> ,	Kingbird & GID: 4314513	Vern.
	Grain size	1B	L-693, L658	Kingbird & GID: 4314513	Vern.
TX	Grain weight	2BS 65.5 Mb CSv1	TAM 111	HB (4314513, 4878563)	F <sub>1</sub>
	Grain No.	2AL 648 Mb CSv1	TAM 111	HB (3855011)	F <sub>1</sub>
WA	Grain No/weight	4AL	Kelse x Scarlett	HB under selection	Screen

<sup>1</sup> CIMMYT recurrent parents for high biomass (HB) are described in Table 1 and those for high yield (HY) in Table 2.<sup>2</sup> These BC tetraploid lines from California are from collaboration with earlier IWYP project.<sup>3</sup> Csv1= IWGS RefSeq v1.0

**CA. University of California, Davis. Jorge Dubcovsky**

**Education:** PhD student Saarah Kuzay started the characterization of a QTL for spikelet number on chromosome arm 7AL. She completed her first courses successfully and trained undergrad student Jonathan Hateley. Saarah obtained a Plant Sciences Graduate Student Researcher fellowship that allowed the UCD project to hire a second PhD student (Priscilla Glenn) who will start her PhD on September 2017. Dr. Junli Zhang, a previous T-CAP student, was hired as project manager.

**Research project:** The 7AL QTL for spikelet number was validated in two independent HIFs. Progeny tests of the first recombinant isogenic lines confirmed the location of the QTL in an eight Mb region between markers *IWB78945* and *IWB53096*. Five hundred heterozygous HIFs were screened with markers flanking the QTL and five additional recombination events were identified in the target region. The progeny of these lines were genotyped and seeds of the homozygous recombinant and non-recombinant sister lines will be planted in the greenhouse and in the field for phenotyping.

A potential candidate gene has been identified at the peak of the QTL. Mutants for this gene have been identified for both the A and B genome homoeologs in the Kronos sequenced TILLING population (Krasileva et al. 2016). The mutants were backcrossed to Kronos to reduce the mutation load.

Dr. J. Zhang and Dr. T. Howell refined the mapping of a yield QTL associated with the introgression of a small wheat segment in the distal part of the 1RS introgression (Howell et al. 2014) using radiation mutants. They also demonstrated that the target region was associated with differences in root length both in hydroponic experiments and in field trials.

For our collaboration with CIMMYT, we have developed BC<sub>3</sub>F<sub>2</sub> introgressions of the *Elf-A<sup>m</sup>3* allele for increased spikelet number from *T. monococcum* and BC<sub>3</sub> introgressions of the *gw-A2* mutants for increased grain size in two tetraploid CIMMYT varieties “Cirno C 2008” and breeding line “GID 6420253”. BC<sub>3</sub>F<sub>1</sub> combining both mutations have been produced. BC<sub>3</sub>F<sub>3</sub> seeds for the four allelic combinations of these two genes in tetraploid Kronos have been produced and seed has been increased. In hexaploid wheat, we have developed BC<sub>2</sub> crosses of *Elf-A<sup>m</sup>3* and *gw-2* in CIMMYT line Kingbird and UC Davis varieties Patwin 515 and Lassik. We have also produced F1 of the 7AL QTL for spikelet number with three high biomass lines from CIMMYT (GID 4314513, GID 4577963 and GID 3855011)

The California group completed the development of the germplasm Yecora Rojo 515 carrying stripe rust resistance genes *Yr5* and *Yr15* (Appendix 1). They published four peer-reviewed articles, one of them in the Proceedings of the National Academy of Sciences (Appendix 2).

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

**Education:** PhD student Andrew Katz started his studies at CSU in August 2017. Andrew has enrolled in classes in breeding for drought tolerance, sustainable agriculture and plant molecular genetics for the fall 2017. During the 2017 spring and summer field seasons, five undergraduate

students (Lilia Johnson, Jack Mentzer, Quincy Cobb, Marilee Stonis and Meghan Henderson) worked under the supervision of Drs. Pearce and Byrne on the QTL validation project and gained experience in field phenotyping and genotyping using molecular markers.

**Research project:** The CO team is characterizing a QTL that affects grain width ( $h^2=0.66$ ) and thousand kernel weight (TKW,  $h^2=0.59$ ) in hard winter wheat. The QTL was identified from a GWAS study of the Hard Winter Wheat Association Mapping Panel and was mapped on chromosome 6BL (position 145-149 Mb). Lines carrying the beneficial allele exhibit an average increase of 1.12 g in TKW. The QTL is being validated in the Platte/CO940610 F<sub>5:6</sub> RIL population (n=220, sowed in two replications). The population is currently being phenotyped for TKW, grain width and spikelets per spike. DNA from this population has been extracted and KASP assays are being optimized for the peak SNPs on chromosome 6B to validate their association with TKW and grain width.

The Colorado group completed the release of the common wheat variety ‘Sunshine’ (Appendix 1) and published one peer-reviewed article (Appendix 2). They have initiated crosses with CIMMYT line Kingbird to introgress the 6BL QTL from CO940610. This QTL, together with the 7AL QTL for spikelet number and the *gw2* mutation for grain size are being introgressed into red and white varieties and breeding lines from CO.

#### ***ID. University of Idaho. Jianli Chen and Daolin Fu***

**Education:** The ID team has hired postdoctoral fellow Rui Wang and MS students Katrina Johnson (Dr. Fu) and Kyle Isham (Dr. Chen). Katrina has been involved in the phenotyping of the QTL for spikelet number. Kyle Isham (currently a senior college student starting his MS in spring 2018) has been doing marker validation and marker assisted selection and collecting phenotypic data in a doubled haploid population (UI Platinum x LCS Star).

**Research project:** The ID team is targeting a QTL on chromosome 5AL associated with number of spikelets per spike (SNS) and a QTL on 6A associated with productive spike number per unit area (PSN). Both QTL were identified in the doubled haploid population UI Platinum x SY Capstone. To validate and dissect the two QTL, five KASP markers were developed for the SNS on 5AL and four for the QTL on 6A. 600 F<sub>4</sub>-derived lines were genotyped with these markers to select heterozygous inbred families (HIFs) for the two QTL. Seventeen HIFs were assessed for SNS and PSN in the greenhouse in spring, and 85 HIFs were assessed for SNS and PSN in field this summer. The ID team is also generating the four possible allelic combinations of the two QTL to study their epistatic interactions out of the 102 HIFs assessment. In addition, the ID team genotyped the UI Platinum x LCS Star with the 90K SNP platform and planted this population in three environments in southern ID, where it was evaluated for SPS and PTN in two environments. In preparation for the future validation of the 5AL QTL, the ID team generated an EMS population of UI Platinum (>4000 first-tiller spikes harvested). UI Platinum has been crossed and backcrossed to three high biomass CIMMYT lines and the BC<sub>1</sub>F<sub>2</sub> seed were harvested.

The ID team completed the PVP application for “UI Sparrow” (Appendix 1) and identified HRS line IDO1603S as a potential release for 2018 based on its high yield potential and excellent breadmaking quality. IDO1603S was developed using molecular marker assisted selection against stripe rust, Hessian fly, and high protein (*Gpc-B1*). The ID team published five peer-reviewed publications (Appendix 2).

***KS. USDA-ARS Manhattan. Mary Guttieri***

**Education:** Yuzhou Xu is an incoming graduate student starting in the fall of 2017. Hannah Gerardy is an undergraduate and will be a senior in the fall of 2017. She has been working with the Overley/Overland population in the greenhouse and from field increases to characterize grain weight, and she has worked in the laboratory, sampling all plants for DNA.

**Research project:** The KS group has targeted a yield QTL on the long arm of chromosome 2D contributed by the HWW variety Overley. SNP marker *IWA8562* was identified at the peak of a grain yield QTL on chromosome 2DL (111.02 Mb). Lines with the favorable allele out-yielded sister lines by 5 to 7%. F<sub>3</sub> RILs of Overland/Overley and F<sub>4</sub> RILs of Lyman/Overley were screened to identify families homozygous for *Ppd-B1a* and *Ppd-B1b* and heterozygous for the 2DL target region (*BS00043082-IWA8562-IWB987*). Progeny of these individuals are being grown to generate F<sub>2:4</sub> HIFs for each of the *Ppd-B1* alleles for high density mapping.

The focus of the first 6 months of the project was the preparation of the materials for the incoming graduate student. Seed of the Overley/Overland RIL population were increased in Arizona and Kansas to plant field trials in fall 2017. An early generation of the populations Overley/Overland and Overley/Lyman were grown in the greenhouse, isolating DNA, and harvesting seed. Primers sets are being identified for SNP assays in the 2DL QTL region from the 90K iSelect panel and the core Bristol BS markers on the arm. Exome capture data has been received and additional markers will be developed from those SNPs.

A donor parent was selected for backcrossing into IWYP-identified germplasm, KS11WGGRC53-O (Pedigree: WL711 [T5DL·5DS·5MgS(0.95)]/3\*Overley (-O)). The translocation in this germplasm carries *Sr57/Yr40*. This germplasm is also expected to be also a donor of the 2NS translocation, which has been shown before by WheatCAP researchers to provide resistance to rusts (genes *Lr37/Yr17/Sr38*), root knot nematodes (Williamson et al. 2013), and wheat blast (Cruz et al. 2016). This germplasm is also expected to be a donor of the 2DL yield QTL in Overley. Initial crosses to 18 of the IWYP donors were completed (Table 3).

The KSU team released three varieties (Appendix 1) and generated 12 peer-reviewed publications (Appendix 2). They have also initiated the backcrossing of the 2DL QTL from KS11WGGRC53-O into the 18 high yielding lines from CIMMYT (F<sub>1</sub> have been produced).

***MI. Michigan State University. Eric Olson***

**Education:** Jon Turkus is a first-year PhD student working to clone D-genome grain yield genes from wheat and *Aegilops tauschii*. He has developed KASP markers for high-resolution mapping and is currently validating families segregating for QTL regions. Jon has taken part in wheat field days highlighting new variety releases from the MSU wheat-breeding program.

**Research Progress:** The MI team is focused on a grain yield QTL identified in the distal region of chromosome arm 2DL from *Ae. tauschii* accession TA1615 (162.9 cM in reference D genome). This QTL explains 7% of the phenotypic variation in grain yield. A set of 14 RILs have been identified that are heterozygous across the 2DL QTL region and were self-pollinated to develop HIFs for high-resolution mapping. Additional QTL on chromosome arms 7DS (from TA1718, and on chromosome arms 2DS and 2DL (from KS05HW14) are being explored in parallel.

Twenty-nine KASP markers have been developed to characterize QTL intervals on chromosomes 2DS, 2DL, 6DL and 7DS. These markers are being evaluated on a set of 103 F<sub>5</sub> individuals expected to be segregating for the QTL interval. From segregating individuals, a set of 500 to 1000 lines will be derived for high resolution mapping of the grain yield QTL. SNPs derived from exome capture will be used to saturate QTL intervals. To date, 600 lines have been identified that are segregating for hexaploid wheat QTL on chromosome 2DS.

The MI team has released two lines derived from the D genome NAM population that are fixed for *Pm58*. They also advanced a D-genome nested association mapping population (DNAM), a BC<sub>2</sub>F<sub>4</sub> population derived from direct hybridization of seven *Ae. tauschii* accessions with a common hexaploid wheat line, KS05HW14. The population is comprised of 420 RILs with a family structure of lines derived from a common *Ae. tauschii* accession. Subpopulations are derived from individual BC<sub>1</sub>F<sub>1</sub> plants (Appendix 1).

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

**Education:** The MN PhD student Max Fraser will begin coursework in the fall of 2017. His approved courses for the upcoming semester include Agricultural Statistics, Plant Breeding Principles, Colloquium in Sustainable Research, and Research Methods in Crop Improvement and Production.

**Research project:** PhD student Max Fraser is working to categorize the seed weight and width QTL *QTKw.mna-2A* (LOD 4.60;  $R^2 = 9.55$ , 12.6 cM interval) residing on the long arm of chromosome 2A. RILs heterozygous for the target region flanked by *Xgwm339-IWB12880* were identified from a mapping population from the cross MN98550-5 x MN99394-1. Individual plants within these families were genotyped, and heterozygous plants were selected and self-pollinated. The fine mapping population for *QTKw.mna-2A* currently consists of 51 NILs derived from 17 recombination events. An additional 850 plants heterozygous for the target region are currently being screened to identify additional NILs.

Additionally, categorization of *QSpn.fcu-2A*, a spikelets per spike QTL in a similar region as *QTKw.mna-2A*, has begun using a homozygous recombinant population derived from the cross Langdon (LDN) durum x LDNIsA-2A (the LDN background with chromosome 2A substituted from *Triticum turgidum* ssp. *dicoccoides* accession IsraelA). Phenotyping and genotyping of the LDN x LDNIsA-2A HR population is currently underway.

The MN team released the HRS variety ‘Lang-MN’ (PVP application in progress) that has competitive grain yields, high grain protein, good resistance to Fusarium head blight, leaf rust, stripe rust, and bacterial leaf streak (Appendix 1). The MN team completed the publication of two manuscripts submitted in the previous T-CAP and published an additional one in TAG (Appendix 2).

**MT. Montana State University. Luther Talbert**

**Education:** Ms. Brittney Brewer began her Ph.D. studies funded by the WheatCAP grant. Brittney has completed two semesters of course work and begun the fine-mapping project for the QTL for number of productive tillers on chromosome 6B. She presented a departmental seminar on yield components in wheat and presented a poster at the NAPB meeting in Davis.

**Research Project:** The goal of this project is to fine map and clone a QTL for tiller number on chromosome 6B, designated *Q<sub>Tn.mst-6B</sub>*. SSR markers *gwm58* and *gwm88* were used to select heterozygous F<sub>5</sub> individuals from three populations. Seeds from these individuals were screened to identify homozygotes for the two alleles. Field experiments using these plants confirmed that the favorable allele from Reeder is associated with a high initial tiller formation, that mature into productive tillers only in high-moisture environments.

Near-isogenic lines have already been developed for this QTL, and the phenotypic distinction of alleles has been confirmed. Nine polymorphic KASP markers identified from SNPs from the consensus map and our own RIL population have been screened. A linkage map for the region is under development, currently with six KASP markers spanning a 40 Mb region. The relationship between physical and genetic distances in the region is approximately 1.5-2 Mb per cM based on current data. An HIF-F<sub>2</sub> population with 2,500 individuals was developed. DNA has been isolated from the population for fine map construction. Brittaney is currently selecting recombination events between the flanking markers.

A set of BC<sub>1</sub> individuals segregating at *Q<sub>Tn.mst-6B</sub>* and the 7AL spikelet number QTL discovered in CA will be further backcrossed to develop NILs with all four possible genotypes at these two QTL to test for epistasis.

The allele for high tiller number at *Q<sub>Tn.mst-6B</sub>* has been backcrossed once into CIMMYT high biomass line 3613474 and the local parents McNeal and Duclair. The high yield CIMMYT lines are being screened to find other CIMMYT parents with the alternative allele at the QTL.

The MT team completed the release and publication of the variety NS Presser CLP wheat, a Clearfield variety with two genes for resistance to imidazolinone herbicides (Appendix 1). The MT team published five peer-reviewed manuscripts in 2017 (Appendix 2).

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

**Education:** Graduate student Eddie Lauer has been working on the project to validate and fine map the kernel weight QTL on chromosome 6A. Eddie will complete an M.S. degree during fall 2017. The NC team is recruiting a new PhD student that is expected to begin in January 2018.

**Research project:** The NC team is focused on a QTL for TKW located on chromosome arm 6AL (Massey x MPV, LOD = 3.5,  $R^2=16.5\%$ ). Student Eddie Lauer identified five  $F_5$  lines are heterozygous in the QTL region. Homozygous NIL pairs derived from these RILs were planted in replicated rows in the field at Raleigh, NC to confirm QTL effects.

QTL analysis utilizing a high-density genetic map of 4,845 GBS and KASP markers indicated two loci on 6A together explaining 18.3% of the phenotypic variation, with the Massey alleles conferring 1.0 g and 1.8 g increases in thousand kernel weight (TKW). The same loci were associated with plant height, explaining 18.1% of the phenotypic variation for plant height (PH), with the SS-MPV57 alleles conferring 0.9 and 3.5 cm height reductions, respectively.

The 95% Bayesian confidence interval for one of the co-located PH/TKW QTLs on chromosome 6A span a physical region from 98 Mb to 450 Mb and 29 cM. This region overlapped the most significant PH marker trait association from the GWAS of 272 lines in the TCAP elite soft winter wheat panel. The LOD peak of the other QTL on 6A occurs at 23.4 Mb, closely coinciding with the previously reported *gw2-A1* mutation affecting grain size. Ten KASP assays were developed for segregating markers that span the two QTL regions.

Twenty inbred lines contrasting in the region were selected from three  $F_5$ -derived heterozygous inbred families (HIFs) and were evaluated for kernel weight in the field at Raleigh, NC during 2017. The effects of the chromosome 6A QTL region was confirmed, with the largest differences observed in RIL family 58, for which the Massey allele was associated with a 12% increase in TKW. A difference of 9 cm was observed for plant height in both years. This HIF is being targeted for fine mapping.

The NC team published nine peer-reviewed publications (Appendix 2).

#### ***ND. USDA-ARS Fargo. Justin Faris & Shiaoman Chao***

**Education:** Graduate student Ms. Amanda Peters, started to work on the WheatCAP objectives in 2016 as an MS student. She is now pursuing a PhD in the NDSU Genomics and Bioinformatics program and is dedicated full-time to the WheatCAP objectives. Amanda has already taken graduate courses in statistics, plant genetics, plant breeding, and plant pathology as part of her MS program and will take courses in regression analysis and molecular breeding as part of her PhD studies. Amanda attended and presented oral and/or poster presentations at the Durable Wheat Resistance meeting in Minneapolis, the PAG XXV Conference in San Diego, CA, and the Graduate Student Symposium held at the University of Saskatchewan, Canada. Ms. Samantha Steckler is an undergraduate student at NDSU that also works in the project as a greenhouse and field assistant.

**Research project:** In collaboration with Dr. Baenziger (University of Nebraska), the ARS group at Fargo is validating and fine mapping a QTL for increased grain yield on chromosome arm 3AL. This QTL was identified in a population of recombinant inbred chromosome lines (RICLs)

derived from a cross between the winter wheat variety Cheyenne (CNN) and CNN with a WI chromosome 3A substitution [CNN(WI 3A)]. This QTL is expressed mainly in high moisture environments. A RICL harboring WI alleles for the QTL and CNN alleles for the remainder of the chromosome was crossed to CNN to develop a large F<sub>2</sub> population for high-resolution mapping. Crosses were also made to introgress the QTL into North Dakota HRSW. A greenhouse trial including CNN, WI, CNN(WI 3A), and several RICLs with and without the 3A QTL region failed to show differences in yield components.

The GxE interactions affecting the expression of the 3A QTL may make it a rather difficult and risky target. Therefore, the group at Fargo is concomitantly exploring other possible yield related QTL as targets. A population of tetraploid wheat derived from the durum variety Ben crossed with an accession of cultivated emmer wheat was used to identify QTL for number of spikelets per spike on chromosome arm 1BS and for the number of grains per spike on chromosome arm 2BS under greenhouse conditions. This population is currently being evaluated under field conditions. These QTL will be backcrossed so that they can be studied and validated in isolation, and appropriate populations will be developed for high-resolution mapping and gene discovery.

A second tetraploid wheat population derived from a cross between a durum variety (Divide) and a cultivated emmer accession segregates for number of spikelets per spike, seed size, and possibly other yield related traits. This population was grown in multiple replicates in the greenhouse, and it is currently growing in the field for phenotypic analysis. The Illumina 90K SNP array was used to genotype the population and generate a linkage map. Once the phenotypic data is available, we will analyze it for QTL associated with yield traits.

In another effort to identify yield-related QTL, we conducted a greenhouse trial consisting of 10 replications of the Langdon (LDN) durum – *Triticum dicoccoides* (LDN-DIC) chromosome substitution lines. The phenotypic data is currently being collected and will be used to identify *T. dicoccoides* or LDN chromosomes harboring genes associated with yield traits.

The ND team was involved in 11 publications generated by the WheatCAP group. Dr. S. Chao is the first author in one of these publications and co-author in the other ten.

### ***NY. Cornell University. Mark Sorrells***

**Education:** Ella Taagen, a first year PhD student, is the primary graduate student involved with Cornell's collaboration on the Wheat-CAP. This fall she will be enrolled in two plant breeding courses and one statistics course. She is interested in research, as well as participating in extension activities related to agricultural policy and science communication.

**Research project:** The Cornell team is using the SynOpDH (200) and SynOpRIL (2000) mapping populations to clone three seed size QTL on 5AS, 5BS and 5BL. The primary target is a QTL on 5BS at position 60 cM that was mapped in four different populations in a total of 18 environments (in collaboration with Jim Anderson MN). The 5AS QTL was mapped in three different populations in 12 environments. It is located in a colinear region to the QTL on 5BS. The seed size QTL on 5BL (at position 130 cM) was mapped in three different populations

grown in 15 environments. Heterozygous plants were identified for all three seed size QTL in the SynOp RIL families to develop HIFs.

In addition, near isogenic lines (NILs) for the different QTL are being generated by backcrossing the 5BS QTL from the double haploids into Opata, Tom, and Glenn. Three backcrosses generations have been completed and the fourth will be obtained in summer 2017.

For the SynOpRIL population, phenotypes and genotypes will be obtained in the fall of 2017 using 100 seeds from each of the 30 heterozygous families from SynOpRIL and from the SynOpDH BC<sub>1</sub>F<sub>2</sub>. Tissue harvesting of 3,770 plants for DNA extraction is complete. Sixteen SSR markers were mapped on the three QTL regions in the DH population and 20 additional ones will be mapped soon. A single seed analyzer will be used to phenotype seeds, recording weight, length, height, width, and protein concentration. The genotype and phenotype data will be analyzed using R/QTL.

Crosses have been made between the five CIMMYT high biomass lines (Table 1) and SynOpDH lines carrying the seed size QTL. Backcrosses will be initiated this winter. The Cornell team published three peer-reviewed papers in 2017 not previously reported in TCAP (Appendix 2).

#### ***OK. Oklahoma State University. Liuling Yan and Brett Carver***

**Education:** PhD student Forrest C.C. Kan is cloning the *QYld.osu-1B* gene in the ‘Duster’ x ‘Billing’ population. Xiaoyu Zhang, a Ph.D. student, provides assistance in the cloning of the *QYld.osu-1B* gene and is cloning a major gene for spikelet number in common wheat.

**Research project:** Seeds of 19 lines that were identified from among approximately 1,000 plants to have crossovers in the *QYld.osu-1B* region have been increased to test grain yield in a field in fall 2017. Six PCR markers for the internal region of *QYld.osu-1B* have been developed to genotype the 19 recombinant lines.

The OK team developed 1,438 GBS markers to genotype 186 F<sub>2</sub> lines generated from a cross between CIt17600 and Yangmai18. A major QTL for spikelet number was found on the long arm of chromosome 7B, which has the LOD value of 13.75 and explained 35.4% of the total phenotypic variation. Duster, which has the *QYld.osu-1B* gene, and its offspring or grand-offspring were introgressed in the pedigrees of 25% of the elite germplasm resident to the OSU wheat improvement program.

The OK team has released four varieties (Appendix 1) and published four peer-reviewed papers not previously reported in TCAP. They are screening the high biomass and high yielding lines from CIMMYT to select those with contrasting QTL haplotypes for backcrossing of the 1B and 7B QTL.

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

**Education:** The Ph.D. graduate student Jyotiymoy Halder has joined the project on August 2017, and will be leading the cloning and deployment of the yield QTL. One undergraduate student

was trained in wheat breeding during this period and was closely involved in the project developing populations and germplasm focused on yield traits.

**Research project:** The SD team is focused on a grain yield QTL from *Aegilops tauschii* transferred to hexaploid wheat. This QTL is located on chromosome arm 7DS in the distal 6-16 Mb on the short arm of chromosome 7DS. The parents of the mapping population, KS05HW14 (HWWW) and TA1615 (*Aegilops tauschii*) were genotyped using exome capture and 800 SNPs were identified in the target region. Five heterozygotes spanning the QTL region were planted and 600, 301, 190, 415, and 820 progenies were harvested in summer 2017.

A second QTL for grain size on chromosome 1B is being targeted at SDSU. The beneficial allele was identified in *Thinopyrum elongatum* introgression lines L693, L658. The donor parent of these two QTL are being vernalized for crossing this fall with CIMMYT lines Kingbird and GID: 4314513. An F<sub>1</sub> was developed from the cross between these donor lines and the SDSU recurrent parents Redfield, Ideal and SY Wolf in summer 2017.

The SD team released the HRW variety ‘Thompson’ that has an excellent combination of high yield and disease resistance and developed an EMS mutagenized population of Overland (Appendix 1).

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

**Education:** PhD student Smit Dhakal has started the characterization of grain yield QTL. An undergraduate student, Jackie Avila from Amarillo College is working with Smit Dhakal on this project. Associate Research Scientist, Dr. Chenggen Chu will be involved in this project using his expertise to advise the graduate student. Smit Dhakal presented research progresses in Texas A&M’s field days and at the Annual Hard Winter Wheat Breeder’s field day in Amarillo. He is also going to present his research findings at the ASA-CSSA-SSSA tri-societies International Annual Meeting in Tampa, FL.

**Research Project:** The TX team is focused on a QTL on chromosome arm 2BS associated with yield, thousand kernel weight, and harvest index with the favorable allele from TAM 111 (*Qgy.tamu.2BS*). The QTL was identified in a population (217 F<sub>6:7</sub> RILs) derived from the cross of CO 960293-2/TAM 111 evaluated in eight environments across Texas, Kansas, Colorado, and Idaho. The genotyping was conducted using 90K array SNP. The QTL is located at approximately 65.5 Mb in the CS v1 reference and flanking KASP markers were designed (eight KASP in the region). The screening to identify heterogeneous F<sub>5</sub> inbred families to develop the larger segregating population is in progress.

A second QTL from TAM 111 located on chromosome arm 2AL (~648 Mb in IWGS RefSeq v1.0 linked to markers IWB64705 and IWB64706) is also being targeted. The peak marker IWB64705 is associated with an increase of 1.5 kernels per spike with the TAM 111 alleles explaining 12.8% of the phenotypic variation. Six KASP markers have been designed in the region.

The 2BS QTL is being crossed with CIMMYT High Biomass lines GID 4314513 and GID 4577963, whereas the 2AL QTL is being crossed with High Biomass lines GID 3855011. The TX team has published four peer-reviewed publications in 2017 (Appendix 2).

***WA. Washington State University. Mike Pumphrey***

**Education:** The WA team recently lost a top recruit that was going to take the Wheat-CAP position, and expects a graduate student to be in this position by spring 2018. Meanwhile, an already existing graduate student and post-doctoral research associate are contributing to advancing the development of the high-resolution mapping population.

**Research:** The WA team has developed 17 KASP markers spanning the QTL interval and screened 190 F<sub>4</sub>-derived progeny and identified/increased seed of RIL that are heterozygous in the QTL region. The targeted QTL affects spike length (LOD=7.87; R<sup>2</sup>=12%), kernels per spike (LOD=6.55; R<sup>2</sup>=13%), and thousand kernel weight (LOD=6.52; R<sup>2</sup>=11%). This QTL was detected on chromosome 4AL in a biparental population of 190 RILs developed from a cross between Washington elite spring wheat cultivars Kelse (PI 653842) and Scarlet (PI 601814), for which a manuscript was recently submitted to Theoretical and Applied Genetics.

The WSU team has released six common wheat varieties (Appendix 1) and published 16 peer-reviewed publications (Appendix 2).

**WheatCAP varieties and germplasm releases**

Twelve wheat varieties, three germplasm and three mapping populations not previously reported in the T-CAP project were released by the project participants. The improvement of these lines was initiated under the T-CAP project and completed under this project. None of these materials was reported before. The complete list of released varieties, germplasm and populations is presented in Appendix 1 at the end of this report.

**WheatCAP publications**

Fifty-one research articles were published or have been accepted and are in press by the time of this report. These wheat studies were initiated under the T-CAP project and were completed under this project, but none of them was reported before in the T-CAP project. These publications acknowledge USDA-NIFA support for either the T-CAP or the Wheat-CAP. The complete list of peer-reviewed publications is presented in Appendix 2 at the end of this report.

**Budget**

The subcontracts to the 19 institutions have been completed and the funding has become available to all collaborators by March 2017. Since this financial report is up to July 31, 2017, there has been very limited time to start invoicing the project. In these first months, only 28% of

the budget has been spent (including 10% in received invoices pending processing). From the second year, the budget of USDA-ARS Ithaca will be transferred to Cornell University to provide T3 more flexibility and to simplify administration. The new budgets, budget justifications and rationale of this change are being provided with this report as a separate set of documents. None of the original objectives or responsibilities described in the original WheatCAP proposal for Cornell and USDA-ARS Ithaca have been modified.

## **B. Prospectus – Plan of Work for Second Year**

**Education:** The first educational activity of 2018 will be the positional cloning workshop at UC Davis. The workshop will be held in conjunction with the USDA funded Wheat CRISPR project led by Dr. E. Akhunov. The workshop will be taught by Dr. J. Dubcovsky (PD), Dr. E. Akhunov (Co-director), Dr. Jean-Luc Jannink (T3), Dr. Junli Zhang (project coordinator), PhD student Hans Vasquez-Gross (Tilling), Dr. Tanner Sen (GrainGenes) and Dr. Sarah Davidson (scientific communication). Because many of the student's first semester will be the fall 2017 semester, we delayed the first Wheat-CAP online course to provide the students an opportunity to acclimate to their home institution course work. Our first online course will start January 2018 and will teach the students on how to use the tools available in the T3 and GrainGenes databases.

eXtension will provide the courses with an established course delivery infrastructure and user technical support. All current and future online courses on the Plant Breeding Training Network (PBTN) will be housed in eXtension and will be freely available to the public.

**T3:** Filters for the exome capture data by genomic region and by gene-annotation will be finalized in the next month. Annotations will come from the TGACv1 reference because the annotations from IWGSC RefSeq1 are not yet published. The annotations will be updated once IWGSC RefSeq1 is released.

The Akhunov Lab is processing polymorphisms through “Variant Effect Predictor” and SIFT pipelines. When these analyses are done, we will make results available to WheatCAP members for visualization and as filtering criteria. This sequence resource will be BLAST searchable leading to reports and JBrowse visualizations of all polymorphisms detected within a segment surrounding BLAST hits. This will facilitate the identification of natural loss-of-function variants among our accessions.

Whole genome and whole exome capture data are becoming common even in wheat. The original database structure for genotype data in T3 was designed for Illumina Golden Gate assay. To better handle massive genomic data, we will transition T3 genotype storage to the Genomic and Open-source Breeding Informatics Initiative (GOBII, [gobiiproject.org](http://gobiiproject.org)) platform. This will enable us to integrate legacy and ongoing sequence data into a unified framework

**Genomics Resources:** In the next year, the KSU group will focus on re-sequencing the regulatory regions of a diverse set of wheat lines using the newly designed capture assay for wheat promoter regions. Two hundred lines for which gene expression data is available will be used for sequence capture. The data will be used to identify regulatory SNPs contributing to the regulation of gene expression in wheat. The MNase and ATAC-seq approaches will be used to

characterize the chromatin accessibility in nuclei of different wheat tissues. The data will be analyzed to start creating the catalog of functionally active regions of the wheat genome. The KSU team will collaborate with the T3 team on developing various genomic tools for wheat geneticists and breeders based on the newly generated gene expression, SNP variation and chromatin accessibility data.

### **Cloning projects**

**AR:** The AR group will evaluate homozygous individuals with and without the targeted QTL in highly replicated field head-row trials using an augmented design with replicated check lines. ~50 recombinant lines will be phenotyped for all major yield components and agronomic traits. Data from exome capture will be used to target additional polymorphic markers within the delineated QTL regions. In addition, heterozygous individuals will be identified for continued self-pollination to improve the isogenic background and move toward stage 2 of cloning.

**CA:** In 2018, the CA group will evaluate the second round of recombinants for the 7AL QTL for spikelet number in both greenhouse and field experiments. Controlled environment chambers will be used to study the effect of different photoperiod and temperatures on the differences between HIFs in spikelet numbers. The truncation mutants of the A and B genome homoeologs of the candidate gene will be intercrossed and the number of spikelets per spike will be evaluated in greenhouse and field experiments. If the mutants confirm an effect on spikelet number, we will generate transgenic plants to complement the mutants and demonstrate that this candidate gene is both necessary and sufficient to determine the observed differences in spikelet number.

**CO:** The CO group will complete phenotyping (TKW and grain width) two replicates of the Platte/CO940610 population and improve markers for the 6B peak region. Upon validation of the QTL, they will select individual lines heterozygous for the peak region to develop heterogeneous inbred families. The progeny of these families will be used to develop high-resolution maps of the peak QTL. New markers within the region will be developed from the recently completed exome capture of the two parental lines. CO will backcross the identified 6B QTL for grain width and TKW into two advanced CO breeding lines (one HRW, one HWW), which have the non-favorable haplotypes for these QTL and into the CIMMYT variety Kingbird. The CO team will also introgress the 7A QTL for spikelet number and the *gw-A2* mutant allele for grain weight into the CSU Wheat breeding program, including backcrossing to the variety Byrd, which has poor TKW.

**ID:** During 2018, the ID team will characterize the 20 HIFs in F<sub>4:5</sub> (20 x 20 plants) to develop fine maps of the two QTL. SNS and PSN phenotypes will be assed for the 400 F<sub>4:5</sub> plants in greenhouse (winter-spring) and field (spring-summer) in both Aberdeen and Moscow. HIFs that showed 3:1 phenotypic segregation in both greenhouse and field trials and 1:2:1 marker segregation will be prioritized for high-density mapping. Once the QTL is delimited to a reasonable size, the recently released IWGSC RefSeq v1.0 will be used to select potential candidate genes. The SNPs already detected in the exon capture of the parental lines will be used to predict effects on gene function to prioritize candidate genes. If interesting mutants are identified, tilling EMS mutants will be selected from the TILLING database and seeds will be ordered. In 2018, 4000 M<sub>2</sub> headrows of the EMS treated UI Platinum will be planted for DNA

extraction. The secondary mapping population UI Platinum x LCS Star will be planted in three locations in S ID for phenotypic evaluation (SNS and PSN) by Kyle Isham.

**KS:** Field trials will be conducted with the Overlay/Overland RIL population to evaluate yield components under field conditions, and the population will be re-mapped. SNP markers on 2DL in the region of interest will be optimized and mapped, along with new markers derived from the exome capture information. Progeny of early-generation plants heterozygous in the region of interest will be grown and will be genotyped with QTL flanking markers to identify recombination events. F<sub>1</sub>s from the crosses to 18 IWYP donors will be validated with marker analyses in the fall of 2017 and backcrosses will be continued by the incoming student.

**MI:** The MI team will complete the characterization of HIFs segregating for QTL on 2DS, 2DL, 6DL and 7DS with KASP markers by fall 2017. QTL intervals will be further saturated with KASP markers developed from exome capture. Seed increases for all HIF populations will take place from fall 2107 through summer 2018. Yield testing of HIFs will commence in fall, 2018. PhD student Jon Turkus will be trained in and take part in all elements of the MSU wheat breeding program operation. In addition to all genotyping activities, Jon will oversee planting of HIF seed increases and yield testing in fall, 2018.

**MN:** Population development for the fine mapping of *QTKw.mna-2A* will be the focus for 2018. Further marker screening during the fall and winter 2017/18 greenhouse seasons is necessary to identify additional unique NILs. The full fine mapping population will be planted and phenotyped in two Minnesota locations as well as one location each in South Dakota and North Dakota with the collaboration of the SD and ND WheatCAP groups in summer 2018. Additional genotyping of the MN98550-5 x MN99394-1 mapping population and recombinant NILs will also be conducted using genotyping by sequencing.

**MT:** The primary objective of the MT team for 2018 is to identify recombinants in the HIF-F<sub>2</sub> individuals. This will entail screening the population with currently available KASP markers. In addition, we will develop KASP markers for genes in the region identified in the exome capture experiment. Seed from recombinant plants will be screened to identify homozygous types for all recombinants. These will be available for phenotyping in order to construct a fine map of the chromosome region containing *QTn.mst-6B*.

**NC:** The NC team will target two populations for fine mapping. HIF derived from RIL 58 (MVP57 x Massey) for which significant differences in kernel weight were observed in the field in 2017, will be used to map the 6A QTL for grain weight. Remnant seed of this family will be used as a segregating population to identify recombinants in the region. HIF derived from the second cross (MVP57 x LA95135), which also showed differences in kernel weight associated to the 6A QTL region will be also used for the fine mapping. The exome data already obtained for the MVP57 and LA95135 will be used to generate KASP assays in the QTL region for high-density mapping.

**ND:** The ND team will genotype several hundred F<sub>2</sub> plants with flanking markers to identify recombinants within the 3A grain yield QTL region. The recombinants will be self-pollinated to obtain F<sub>3</sub> seed, and F<sub>3</sub> families will be genotyped to identify the homozygous recombinants,

which will be used for phenotypic analysis under field conditions. Additional backcrosses to move the 3A QTL into different HRSW and durum wheat backgrounds will also be conducted. Phenotypic data regarding yield traits will be collected from the summer field experiments of the two tetraploid populations. That data, along with the greenhouse experiment data from the two tetraploid populations and the LDN-DIC substitution lines will be analyzed. If significant yield-component QTL are identified, crosses will be initiated to develop high-resolution maps for individual QTL and the QTL will be introgressed into the durum-breeding program. The homozygous recombinant populations available for each DIC chromosome substitution in LDN will accelerate the mapping and validation of the identified QTL.

**NY:** The first goal is to (re) genotype the SynOpRIL and SynOpDH BC<sub>1</sub>F<sub>2</sub> populations derived from the lines heterozygous for the QTL regions and phenotype seeds using a single seed analyzer. This data will be used to validate the QTL and to begin the fine mapping. Backcrossing of the seed size QTL into Tom, Glenn and Opatá will be advanced to BC<sub>4</sub> and BC<sub>5</sub>. The CIMMYT high biomass lines will also be backcrossed and used to validate the QTL. Specific markers closely linked to the QTLs will be developed for breeding application.

**OK:** Field-test 19 lines that have recombination events in the *QYld-osu-1B* region. Identify new crossovers that occur in the *QYld-osu-1B* region. Fine map *QYld-osu-1B* based on phenotypic data from the field. Identify candidate genes for *QYld-osu-1B*. Fine map the gene for spikelet number. Track molecular markers for *QYld-osu-1B* and grain yield in Oklahoma. The OK team will continue the introgression of the yield QTL from Duster and the spikelet number QTL from CItr17600 into Kingbird. They will also screen the high-biomass and high-yielding CIMMYT lines for the markers in the QTL region and select lines with contrasting haplotypes for backcrossing.

**SD:** KASP markers flanking the QTL region will be designed from the polymorphic SNPs identified and the lines will be screened for recombination in the fall of 2017. Seed will be increased from the recombinant lines in the greenhouse and will be planted in the fall of 2018. The crosses and backcrosses with recurrent CIMMYT and local parents will be continued. Single M<sub>2</sub> from each M<sub>1</sub> harvested from the Overland EMS mutagenesis population will be advanced to M<sub>3</sub> generation and the population will be phenotyped in the field for yield component traits.

**TX:** KASP markers in the QTL regions are being screened for the recombinants of CO960293-2/TAM 111 to confirm the 90K array genotypes and to identify if there are heterozygote F<sub>5</sub>:8 RILs. Effective KASP marker will be used to screen F<sub>5</sub> RILs. The F<sub>5</sub> HIF's will be used to develop more recombinants. Yield trials will be conducted for the contrast lines with and without the major QTL.

**WA:** The progeny of the HIF's identified in the first year will be used to generate the high-density map of the grain yield QTL from chromosome 4AL. Backcrosses with the CIMMYT lines will be advanced two generations.

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## C. Deliverables & Other Outputs:

- **Appendix 1.** Germplasm releases <https://www.triticeacap.org/variety-and-germplasm-releases/>
- **Appendix 2.** Publications <https://www.triticeacap.org/publications-2017/>
- **Appendix 3.** Community resources
- **Appendix 4.** Graduate students. <https://www.triticeacap.org/educational-activities/>

### APPENDIX 1. Varieties and Germplasm releases.

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

#### *2017 Variety releases. Ten PVP and nine public releases or pending PVP*

1. ‘UI Sparrow (IDO1108DH) soft white winter wheat (PVP 201700189, application submitted 2017-03-30). This cultivar was developed using double haploid system and has high grain yield in most areas in ID and WA. The high grain yield was contributed by more productive spikes and spikelet numbers. This cultivar also has a combination of resistance genes that provides protection to stripe rust, snow mold, and dwarf bunt. UI Sparrow has very good winter hardiness and low grain cadmium content.
2. “Melba”, Common wheat released by Washington State University. PVP 201700203. Application Submitted 2017-02-05.
3. “Ryan”, Common wheat released by Washington State University. PVP 201700204. Experimental synonym WA 8214. Application submitted 2017-02-05.
4. ‘Tekoa’, Common wheat released by Washington State University. PVP 201700124. Experimental synonym WA 8189. Application submitted 2017-03-09.
5. ‘NS Presser CLP’. Common wheat released by Montana State University. PVP 201700053, application submitted 2017-01-11. This is a Clearfield variety with two genes for resistance to imidazolinone herbicides. Initial selection of herbicide resistance was accomplished using molecular markers. The variety has been licensed to a private company for commercialization
6. ‘Stardust’ (PVP application 201700107, filed 21 Feb. 2017) is a hard white cultivar developed by the OAES with a sufficient level of pre-harvest sprouting tolerance and resistance to the wheat soilborne mosaic/wheat spindle streak mosaic complex appropriate to the central corridor of the southern Plains.
7. ‘AGS 2055’ is a common wheat variety released by the Board of Trustees of the University of Arkansas/ University of Georgia Research Foundation, Inc. from the University of Arkansas (PVP No. 201600401, Application submitted 2016-09-20).
8. ‘Tatanka’ is a hard red winter wheat variety released by Kansas State University. PVP application No. 201700131, submitted 2017-03-20.

9. 'Jasper' Soft white winter wheat released by Washington State University. PVP 20160029, application submitted 2016-11-28.
10. 'Sequoia' is a hard red winter wheat released by Washington State University. PVP 201600315, application submitted 2016-07-27.

### ***Pending PVP***

11. 'Smith's Gold' (PVP application pending) is a hard red winter wheat cultivar developed by the Oklahoma Agricultural Experimental Station and designated as a potential replacement for 'Gallagher' with improved stripe rust resistance and baking quality. PVP pending.
12. 'Spirit Rider' (PVP application pending) is a hard red winter wheat cultivar developed by the Oklahoma Agricultural Experimental Station with elevated total dietary fiber in the grain and was selected with the aid of DNA marker assays for *Lr34* and *Rht8*. It was also found to carry the *Wx-B1b* allele, and it apparently has gene(s) conferring strong acid-soil tolerance different from *ALMT1*. PVP pending.
13. 'Lonerider' (PVP application pending) is a hard red winter wheat cultivar developed by the Oklahoma Agricultural Experimental Station with unusually broad adaptation to the southern and central Plains. Critical to its release was using marker assays to select for the absence of IRS and presence of *Glu-D1d* in a genetic background prone to weaker gluten. PVP pending.
14. 'Tiburon HP' is a Dessert Durum variety developed in collaboration between the UCD and Arizona Grain. Tiburon HP is a backcross derivative of Tiburon with the introgression of the high grain protein content gene and the *Yr36* stripe rust resistance gene.
15. 'Bob Dole', is a winter wheat varieties released by Kansas State University in 2017 pending PVP.
16. 'AG Icon' are hard red winter wheat varieties released by Kansas State University in 2017 pending PVP.
17. 'Thompson' is a HRW wheat released by South Dakota State University that has an excellent combination of high yield and disease resistance. Thompson has medium height and medium maturity with average protein content, test weight and grain quality. It is moderately resistant to leaf rust, stem rust and FHB.
18. 'Lang-MN' (PVP application in progress) is a hard red spring variety released by the MN wheat-breeding program in 2017. Lang-MN has competitive grain yields, high grain protein. Lang-MN has good resistance to Fusarium head blight, leaf rust, stripe rust, and bacterial leaf streak. PVP pending.

### **Public release**

19. 'Earl' hard white winter wheat released by Washington State University. Published in the Journal of Plant Registration. 'Jasper' Soft white winter wheat released by Washington State University. Public release Washington State University.

### ***2017 Germplasm releases***

1. Two lines derived from the D genome NAM population fixed from *Pm58*. Developed by E. L. Olson at Michigan State University.
2. ‘Yecora Rojo 515’ is a backcross derivative of Yecora Rojo combining *Puccinia striiformis* resistance genes *Yr5* and *Yr15*. It was developed by the University of California Wheat Breeding program.

### **2017 Cloned wheat genes**

*Sr13*. From *T. turgidum* ssp. *dicoccoides*. Confers resistance to the wheat stem rust UG99.

*Sr21*. From *T. monococcum*. Confers resistance to the wheat stem rust UG99-race group.

### **APPENDIX 2. Peer reviewed publications. <https://www.triticeacap.org/publications-2017/>**

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2. Arruda, M.P., P. Brown, G. Brown-Guedira, A.M. Krill, C. Thurber, K.R. Merrill, B.J. Foresman, F.L. Kolb. 2016. Genome-wide association mapping of Fusarium head blight resistance in wheat using genotyping-by-sequencing. *Plant Genome* 9(1) *doi: 10.3835/plantgenome2015.04.0028*
3. Assanga, S.O., G. Zhang, C.-T. Tan, , J.C. Rudd, A. Ibrahim, Q. Xue, S. Chao, M.P. Fuentealba, S.Y. Liu. 2016. Saturated genetic map of wheat streak mosaic virus resistance gene *wsm2* in wheat. *Crop Sci*. 57:332-339.
4. Babiker, E.M., T.C. Gordon, S. Chao, M.N. Rouse, R. Wanyera, M. Newcomb, G. Brown-Guedira, Z.A. Pretorius, J.M. Bonman. 2016. Genetic mapping of resistance to the Ug99 race group of *Puccinia graminis* f. sp. *tritici* in a spring wheat landrace CItr 4311. *Theor Appl Genet* 2016:1-10.
5. Babiker, E.M., T.C. Gordon, S. Chao, M.N. Rouse, R. Wanyera, M. Acevedo, G. Brown-Guedira, M. Bonman. 2016. Molecular mapping of stem rust resistance loci effective against the Ug99 race group of the stem rust pathogen and validation of a single nucleotide polymorphism marker linked to stem rust resistance gene *Sr28*. *Phytopathology* 107:208-215.
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7. Carter A.H., S.S. Jones, K.A. Balow, G.B. Shelton, A. Burke, S.R. Lyon, R.W. Higginbotham, X.M. Chen, D.A. Engle, T.D. Murray, C.F. Morris. 2017. Registration of ‘Jasper’ soft white winter wheat. *J. Plant Reg.* 10.3198/jpr2016.09.0051crc.

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9. Carter A.H., K.K. Kidwell, A. Burke, G.B. Shelton, R.W. Higginbotham, V. DeMacon, M.J. Lewien, X.M. Chen, D.A. Engle, C.F. Morris. 2017. Registration of ‘Earl’ hard white winter wheat. *Journal of Plant Registrations* 11:275-280.
10. Cai J., S. Wang, T Li, G. Bai. 2016. Multiple minor QTLs are responsible for Fusarium head blight resistance in Chinese wheat landrace Haiyanzhong. *PLoS ONE* 11:e0163292
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21. Jernigan, K.L., C. F. Morris, R. Zemetra, J. Chen, K. Garland-Campbell, A.H. Carter. 2017. Genetic mapping of quantitative trait loci for end-use quality traits in a soft wheat RIL population. *Journal of Cereal Science*. 76:148-156
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26. Lan, C., I. Lowe Hale, S.A. Herrera-Foessel, B.R. Basnet, M.S. Randhawa, J. Huerta-Espino, J. Dubcovsky and R.P. Singh. 2017. Characterization and mapping of leaf rust and stripe rust resistance loci in hexaploid wheat lines UC1110 and PI610750 under Mexican environments. *Frontiers in Plant Science* doi: [10.3389/fpls.2017.01450](https://doi.org/10.3389/fpls.2017.01450)
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45. Turner, M.K., J.A. Kolmer, M.O. Pumphrey, P. Bulli, S. Chao, and J.A. Anderson. 2016. Association mapping of leaf rust resistance loci in a spring wheat core collection. *Theor . Appl. Genet.* 130:345–361.
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### **APPENDIX 3. Community resources generated**

#### ***2017 Public databases***

1. T3 database <https://triticeaetoolbox.org/wheat/>
2. Sequenced mutant populations [https://dubcovskylab.ucdavis.edu/wheat\\_blast](https://dubcovskylab.ucdavis.edu/wheat_blast)

#### ***2017 Mapping Populations***

1. Eric Olson from Michigan State University developed a D genome nested association mapping population (DNAM).

2. Dr. Jianli Chen from the University of Idaho developed a DH population UI Platinum x LCS Star (UIP-Star) and genotyped it with the 90K SNP platform.
3. Dr. Daolin Fu from the University of Idaho generated an EMS population of UI Platinum, the parental line contributing the SNS 5AL QTL in the CAP project.
4. Dr. Dubcovsky from UC Davis developed a TILLING population of 1,535 lines from the tetraploid wheat Kronos. All lines were sequenced by Exome Capture.
5. Dr. Sunish Sehgal developed an EMS Tilling population from the variety Overland (recurrent parent of winter wheat NAM population).

#### APPENDIX 4. Graduate students.

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

Institution	Last Name	First Name	Degree	Gender	Start Date
University of Arkansas	Dylan	Larkin	PhD	M	9/1/2017
	Winn	Zachary	MS	M	8/21/2017
University of California-Davis	Kuzay	Saarah	PhD	F	9/1/2016
	Glenn	Priscilla	PhD	F	9/1/2017
	Zhang	Junli	Post Doc	M	12/1/2016
Colorado State University	Katz	Andrew	PhD	M	8/21/2017
University of Idaho	Isham	Kyle	MS	M	1/1/2018
University of Idaho postdoc	Wang	Rui	Post Doc	M	12/1/2016
University of Idaho	Katrina	Johnson	MS	F	8/1/2017
USDA-Kansas State University	Xu	Yuzhou	PhD	M	8/1/2017
Kansas State University	Qianli	Pan	MS	F	9/1/2016
	Adhikari	Elina	PhD	F	9/1/2017
Michigan State University	John	John	PhD	M	5/22/2017
University of Minnesota	Fraser	Max	PhD	M	7/1/2017
Montana State University	Brewer	Brittaney	PhD	F	9/1/2016
USDA-N. Carolina State Univ.	Lauer	Eddie	MS	M	12/1/2016
	Recruitment	in progress	PhD	NA	1/1/2018
North Dakota State University	Peters	Amanda	MS/PhD	F	6/1/2017
Cornell University	Taagen	Ellie	PhD	F	6/12/2017
Oklahoma State University	Kan	Forrest	PhD	M	9/1/2016
	Xiaoyu	Zhang	PhD	M	7/1/2017
South Dakota State University	Halder	Jyotiymoy	PhD	F	8/22/2017
Texas A&M	Dhokal	Smit	PhD	M	9/1/2014
Washington State University	Recruitment	in progress	PhD	NA	1/1/2018

## **D. Concluding remarks**

The new wheat varieties and germplasm developed by the WheatCAP are more productive, resistant to pathogens and have improved quality. The increased productivity of these varieties is essential to maintain the competitiveness of the USA wheat industry, whereas their improved resistance to pathogens reduces the applications of fungicides benefiting directly the population and the environment. The improved quality of these varieties benefit consumers directly. The genomic tools and databases generated by the WheatCAP accelerate the pace of wheat improvement and therefore, the adaptability of the US wheat to a changing environment. The coordination among all the major wheat breeding and research programs eliminates unnecessary duplication and generates positive collaborations that would not have been possible without these integrated Collaborative Agricultural Projects. Finally, the training of the new generation of plant breeders guarantees the continuity of the US agricultural enterprise into the future.