OSU WIT Research Proposal

OWRF FY2023-2024

Genetic improvement of winter wheat: Integrating classical and novel approaches

INVESTIGATORS:

OSU WHEAT IMPROVEMENT TEAM

Wheat Breeding and Variety Development

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Gene Discovery and Molecular Breeding

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Disease Resistance Breeding

Meriem Aoun Dep. of Entomology and Plant Pathology

Candidate Variety Assessment and Applied Wheat Physiology

Amanda de Oliveira Silva Dep. of Plant and Soil Sciences

Execution of a UAV Phenotyping Platform

Phil Alderman Dep. of Plant and Soil Sciences

Identification of Insect Resistance

Kris Giles (conducting work with other funding) Dep. of Entomology and Plant Pathology

Optimization of Grain Protein and Yield

Brian Arnall (conducting work with other funding) Dep. of Plant and Soil Sciences

WIT PARTNERS

Edralin Lucas, human health Dep. of Nutritional Sciences

EXTERNAL COLLABORATORS

Genotyping, molecular marker development and validation
  Guihua Bai, Paul St. Amand, USDA-ARS, Manhattan, KS

Testing and development of stripe rust resistant germplasm
  Kim Garland-Campbell, USDA-ARS, Pullman WA

Identification of Hessian fly resistant germplasm
  Ming Chen, USDA-ARS, Manhattan, KS

Testing and development of disease-resistant germplasm
  Robert Bowden, Mary Guttieri, USDA-ARS, Manhattan, KS

End-use quality evaluation – breeding lines
  Richard Chen, USDA-ARS, Manhattan, KS
    Prioritization of breeding line evaluation

Commercial validation of end-use quality; novel trait development
- Jayne Bock, Wheat Marketing Center
- Andrew Hoelscher, Forrest Hett; Farm Strategy
- Dave Katzke, Steve Eichten, General Mills
- Reuben McLean, Kathy Brower, Grain Craft
- Brady Sidwell, Chisholm Milling, Bellegarde Bakery
- Sean Finnie, Bay State Milling
- Vance Lamb, Ardent Milling

Milling and baking characterization of candidate varieties
- Members of the Hard Winter Wheat Quality Council

Glutenin subunit analysis
- Mike Tilley, USDA-ARS, Manhattan, KS

Hybrid wheat development; genome-assisted selection
- David Hessel, Corteva

Germplasm exchange and breeder line characterization
- Selected wheat breeders and pathologists throughout the USA

INTERNATIONAL GERMPLASM EXCHANGE (formal)
- Agricultural Research Institute, Martonvasar, Hungary (Gyula Vida)
- National Agric. Res. and Development Institute, Fundulea, Romania (Gabriela Serban)

FUNDING HISTORY:

Amount received, previous 8 years (**average, $365,620**):

FY1998	$200,000 (original award)
FY16	$361,200
FY17	$365,400
FY18	$377,900
FY19	$372,800
FY20	$335,679
FY21	$334,582
FY22	$384,156
FY23	$393,243

Amount requested FY24: **$330,493**

Why the WIT? Why a team approach to wheat improvement?

“Research shows that the climate of an organization influences an individual's contribution far more than the individual him/herself.” *W. Edwards Deming*

ABSTRACT:

OSU's Wheat Improvement Team's (WIT) mission is to discover and deliver genetic solutions that help grow Oklahoma's rural economy and bolster food security within and beyond our borders. Our focus is on the challenges and opportunities spanning the entire wheat supply chain in Oklahoma, from seed producer to consumer. Hence, our research continues to address traits relevant to sustainable wheat production in Oklahoma, particularly disease and insect resistance, adaptation to multiple management systems, and preferred functionality. This kind of research can be highly cyclic, as introgression of new germplasm to strengthen a certain targeted trait inadvertently introduces weaknesses in non-targeted traits.

Our proposal continues an altered strategy to justify and launch future varieties. The long-standing practice is to target the entire state of Oklahoma, with dual-purpose (graze plus grain) wheat management serving as the fulcrum. With programmatic maturity and a multi-branched pipeline that comes with maturity, we can offer more directed options to the entire wheat industry, such as contracted varieties with customer-specific quality attributes, High Plains varieties specifically selected in the panhandle for the panhandle, and varieties fit for late-planted systems which enable winter annual grassy weed control or which accommodate summer crop production.

Goals for the proposed work period include the following:

a) A first flush of 2-gene CoAXium <u>candidate</u> inbred lines worthy of qualification trials,
b) A first generation of commercial grain production from three HRS-like HRW (OX wheat) varieties that serve as an *ingredient* flour to displace vital wheat gluten and other additives in bread formulations,
c) Targeted seed distribution of a high-protein, strong-gluten, *soft red winter* wheat adapted to the central wheat corridor of Oklahoma,
d) OSU's first BYD-immune Doublestop CL Plus-derived candidate variety *without* the imazamox herbicide tolerance trait,
e) A selected panel of advanced lines possessing two genes indirectly conferring higher concentrations of *resistant starch* or digestible fiber in wheat flour,
f) Rapid infusion of multiple, novel leaf rust and powdery mildew resistance genes to the point the WIT can use and deploy them as mid-generation lines,
g) Continued development of advanced breeding lines featuring dual resistances to leaf rust and stripe rust,
h) Rendering of commercialization potential for OSU's first *Fhb1*-source of resistance to head scab in HRW wheat intended for eastern Oklahoma,
i) A first-time production of genomic-assisted breeding values that will enable WIT to better predict a maturity pattern that best fits the short-season ideotype,
j) A panel of short-season varietal candidate varieties (~10 lines) uniquely adapted to late planting regimes using Butler's Gold as the gold standard.

OBJECTIVES & PROCEDURES:

W I T
Wheat Breeding and Variety Development
Brett Carver
Plant and Soil Sciences

Deliverable

A winter wheat variety or varieties specifically adapted to Oklahoma and the southern Great Plains, with documentation approved by Oklahoma Ag Research to trigger a commercialization pathway led by OSU's Office of Technology Commercialization.

Advanced Line Pool

CoAX wheat

Background – finally, Finally, FINALLY!

WIT's variety development pipeline (VDP) is now loaded with AXium-traited germplasm. A jump to the end of the pipeline reveals the first likely commercial product will come from about 60 elite 2-gene (AB or AD) lines to be selected for i) statewide yield and quality testing in 2023-2024, and ii) two-year tolerance confirmation trials (TCTs) required for release. This germplasm is contained in an observation nursery at Lahoma and Stillwater (both with quizalofop application), Yukon, Chickasha, and Tipton comprising 124 doubled haploid (DH) lines with molecular marker confirmation of the AXium genotype: 28 AD and 96 AB lines. Thus the first order of business in summer 2023 will be to narrow this pool of 124 DH lines to 40-60 elite lines.

This first elite trial will be established in fall 2023 with two replicates per site at each of six sites. Up to three of the six sites will double as a TCT site. Eight TCT site-years are required to initiate the variety approval process according to the "CoAXium Executive Committee".

While WIT has ample but less advanced germplasm with the AXium trait upstream in the pipeline, this elite trial is where WIT will put its CoAXium marbles to play in 2023-2024. Lines with the pedigree, Incline AX/2*OK12621 (AD genotype), currently provide the best genetic marbles. OK12621 has been one of the most widely used parents in the WIT program for its stay-green potential, BYD immunity, leaf rust resistance, and Hessian fly resistance.

Yet to determine – First WIT must establish sufficient herbicide tolerance and adaptation to central Oklahoma among the 124 DHs. As of April 18, 2023, sufficient herbicide tolerance appears likely. This material has putative adaptation based upon pedigree and observed performance of highly related sib material advanced through the conventional inbreeding track. The conventional inbreds, expected to number approximately 250 after selection in spring and summer 2023, will be tested in an augmented, non-replicated field nursery assigned to 3-4 sites in 2023-2024.

Doublestop trait-free derivatives

Background –Six candidate lines, all with the pedigree OK12621/Doublestop CL Plus and <u>lacking</u> Beyond herbicide tolerance, remain under foundation seed increase in the current season.

This germplasm originated in 2015 as a molecular marker-assisted effort to introgress the *Bdv2* gene for BYD resistance (from intermediate wheatgrass) into Doublestop CL Plus. The principle source of *Bdv2* in WIT breeding populations has been OK12621. The original intent was to shore up BYD resistance in the <u>Clearfield</u> segment of the WIT VDP. However, this intensive breeding effort seemed more likely to have greater return on investment by developing and deploying this germplasm in both traited and trait-free versions. Hence, those six elite lines mentioned above are now in the final stages of selection and breeder-advancement.

A 5 to 10 bu/ac bump in statewide mean yield over Doublestop CL Plus is expected in this germplasm. Experimental lines having the root, OK16107133-, have repeatedly shown more likely to provide that yield bump, though their end-use quality is marginally acceptable at best. Disease resistance in this part of the pipeline is outstanding, as is acid-soil tolerance. Hessian fly resistance is frequently present due to the donor, OK12621, supplying the resistance from Duster.

Yet to determine – Of the six candidate lines, two have WIT's greatest attention: OK16107133-19HR-3 and OK16107133-19HR-4. Of those two, OK16107133-19HR-3 is the favorite for likely release in 2024, because it carries *Bdv2* (and thus is BYD-immune) at higher yield and test weight levels than OK16107133-19HR-4. Both candidates will be advanced to the 2023-2024 OET3, but only one will be released as a general commodity variety. If neither is released in 2024, the determining factor will have been to hold out for better quality in one of the other four candidates.

OX wheat

Background – A massive gene-introgression project was started in 2013 in cooperation with Dr. Guihua Bai, USDA-ARS, Manhattan, KS to introduce the over-expressed (oe) high-molecular-weight gluten subunit Bx7oe into four experimental lines or cultivars. This subunit, encoded by gene *Glu-B1al*, may provide an unprecedented level of dough strength with unique value to millers and bakers, and thus consumers, when introgression occurs within certain genetic backgrounds. WIT currently does not know the additional genetic factor, particularly in Gallagher, that magnifies dough strength, perhaps in concert with *Glu-1al*, beyond Canadian spring wheat levels. In other words, the positive effect of Bx7oe is not universal, and probably not even likely. Research is being conducted to better understand predictability of functionality associated with Bx7oe, but this will probably require a few years. Snowmass, a hard white variety developed by Colorado State University, was used as the *Glu-B1al* donor, and OK10130, Billings, Gallagher, and OK09634 were used as the original recipient parents. Nearly10 years later, and after round and round of yield and quality testing in cooperation with our milling industry partners, patent protection is currently under review for three lines.

Though commercial names are forthcoming before fall 2023, OK15MASBx7 ARS 8-20 and OK15MASBx7 ARS 8-29 are sister lines with the pedigree, Gallagher*3/Snowmass, whereas OK15DMASBx7 ARS 6-8 has the pedigree, OK10130*3/ Snowmass. All are products of the pedigree selection or DH pipelines, but their Gallagher and OK10130 backcrossed derivation makes them worthy of Graze*n*Grain® distinction. OK10130 is derived from grandparents KS93U206, Jagger, Tonkawa, Ponderosa, and KS92WGRC15. About 88% of the genetic background of these three candidate lines are accounted for by either Gallagher or OK10130. Snowmass accounts for the remainder.

All three candidates have now been extensively vetted in research labs and in commercial experimental labs across the country for several years. Environmental effects can moderate the dough strength of the strongest two candidates, OK15MASBx7 ARS 8-20 and OK15MASBx7 ARS 8-29, by about 10-15 minutes stability time, with an average stability of 60-65 minutes. However, human error in proper hydration and operation of the farinograph far surpasses the magnitude of any environmental effect. Either line can be mixed up to one hour with no appreciable breakdown in dough integrity, an amazing feature that does not exist in HRW wheat and perhaps not in HRS wheat. Consider a HRW wheat that functions like a HRS wheat and what that might mean to the value segment of our processing industry, and what the value potential might be to a producer who can segment their production of these Bx7oe derivatives into the supply chain. The greatest friction in adoption of the genetics may come from end-users who overvalue the status quo of their own existing supply chains, and understandably so.

In April 2023, OSU's Office of Technology Commercialization signed a collaboration agreement with Farm Strategy, led by Andrew Hoelscher, to execute the entire supply chain from grain production to flour milling of this germplasm. Seed production will still occur under the direction of an independent licensee, such as OGI.

Looking beyond this first panel of candidates, WIT will determine in 2023-2024 if a fourth line is worthy of patent protection and marketing from two current choices: OK15MASBx7 ARS 8-12-18HR-2 and OK15MASBx7 ARS 8-19-18HR-4. Both are descendents of Gallagher like OK15MASBx7 ARS 8-29 but may be higher yielding than OK15MASBx7 ARS 8-29 with similar functionality.

By year 2025, this novel level of functionality will occur in a wide assortment of other contemporary genetic backgrounds with about 10% frequency of the entire OSU WIT commercial-ready genetic stock. That much time remains to prepare for a non-commoditized market and thus return due value to those who create, preserve, and disseminate it.

Yet to determine – All that remains to be determined is outside of WIT's hands, and that is a pending market analysis by Farm Strategy. OSU is at the mercy of Farm Strategy's ability to create change in the marketplace. All five lines mentioned above remain under foundation seed increase and will be included in the 2023-2024 Oklahoma Elite Trial-3, or OET3.

OK16103083, with head scab resistance

Background – With the obvious need from eastern Oklahoma for additional Fusarium head blight, or scab, resistance, OK16103083 (Garrison*3/Wesley Fhb1-106) was pulled out of long-term storage in fall 2020 and re-tested in limited fashion near Morris, OK, where it performed exceptionally well for yield in 2021 under heavy stripe rust and scab infection and following a severe and prolonged spring freeze event in April 2021. Thus, this line was further tested in the 2022 OET3 across Oklahoma for more detailed monitoring of fitness and for scab protection. It ranked *highest* in scab resistance for the third year in a controlled, inoculated regionwide nursery at Rocky Ford, KS. Severe drought stress and accelerated crop development in 2022 hindered the yield competitiveness of this late maturing line, yet it still performed at the same yield level as Smith's Gold.

Yet to determine – OK16103083 provides a desirable yield-competitive option in scab-prone areas of Oklahoma, especially where a single fungicide treatment is part of the routine management plan to supplement genetic resistance to scab. This single shot of fungicide will be sufficient to protect OK16103083 from stripe rust. It remains highly effective against other common diseases present in Oklahoma, including leaf rust. Moving forward, OK16103083 will be evaluated for its 2023 performance, but only in the wheat variety trial conducted by WIT member Silva near Morris. This will allow WIT to gauge competitiveness in a broader varietal lineup than available in breeding nurseries. A release decision will likely be rendered in summer or early fall 2023, with ample foundation seed available for planting by seed producers in fall 2023.

Crossover SRW wheat

Background – Soft red winter (SRW) wheat is commonly used as a source of disease resistance, acid soil tolerance, and high yield potential in the WIT Graze*n*Grain® pipeline. Though obvious priority is given to HRW progenies, a SRW progeny with the combination of 12% wheat protein (or higher), good mixing tolerance, and acceptable to near-acceptable bread baking quality has merit in the Mexico soft wheat market, but again, OSU is dependent on Farm Strategy to flesh out this merit. This "crossover" in market class functionalities seemingly fits where HRW wheat is blended with SRW wheat to add gluten strength and body to the food product, but in that instance commodity SRW wheat would be blended with crossover SRW wheat.

We further understand the need to combine classical SRW wheat functionality with a harder endosperm for greater milling efficiency. This might be best achieved by creating a very soft HRW with soft wheat functionality (personal communication, Caleb Winsett, Shawnee Milling, 2023). Those genotypes do exist, but at a very low frequency unless created by designed crosses.

Yet to determine – WIT transitioned in 2022-2023 from field testing of OCW03S580S-8WF to strictly foundation seed supply maintenance and responding to functionality testing by the industry. The best use of this germplasm may emanate from the HRW-like protein *levels* shown by OCW03S580S-8WF. A higher-protein, soft-endosperm with good gluten extractability may be more suited to the vital wheat gluten manufacturing capacity growing in Kansas. This market fit remains under investigation with Farm Strategy. OCW03S580S-8WF is considered a strong-gluten soft wheat with a moderately high protein level, averaging above 12% wheat protein. It possesses the Dx5+Dy10 high molecular weight glutenin subunit pair often found in HRW wheat, but its *Pina-D1a + Pinb-D1a* genotype confers a soft endosperm. Genotype and phenotype altogether, OCW03S580S-8WF is representative of genetically soft, but functionally hard wheat.

Newer crossover SRW types will be tested in OET nurseries in 2023-2024, as these will likely make the cut in summer 2023:

OK20D88025F, with parentage from Virginia Tech, Fuller, CIMMYT, 2174, AgSeco 7853; and with high protein, strong gluten

OK21428F, 25R34 (Corteva)/OK12621, with high protein and test weight, and strong gluten

OK21412F, Kentucky experimental/Gallagher//Skydance, with high protein, strong gluten

OK20061CF, essentially a soft Strad CL Plus, with high protein and test weight, and strong gluten

The OSU VDP will be further screened after the 2023 harvest for the alternative crossover type, i.e., a softer HRW kernel with SRW-like functionality. The first filter in this search will be for genotypes with *Pina-D1a + Pinb-D1b*, which do exist at a rate of about 25% in the pipeline.

Doublestop CL+ derivatives

Background – Two highly contrasting CL Plus elite lines remain under foundation seed increase in the 2022-2023 season. OK198417C contains Doublestop CL Plus as 50% of its parentage, with a wall of disease resistance except for wheat soilborne mosaic. Test weight of OK198417C, at one lb/bu above Doublestop CL Plus, represents the test weight pinnacle of all elite lines currently tested in 2022-2023.

Second in priority will be OK16107125C-17HR-2, with pedigree OK12621/Doublestop CL Plus, as a near twin of OK16107133-19HR-3 mentioned above. Neither candidate, while outstanding for yield, lies in the same quality class as Doublestop CL Plus.

Yet to determine – WIT anticipates sufficient data will emerge in summer 2023 to make a final and single recommendation for release, but critical to that decision will be clear separation from Doublestop CL Plus and Strad CL Plus for statewide yield performance, enhanced disease protection, and acceptable functionality.

Remainder of elite pool

Other advanced genetics not mentioned here are summarized in Tables 1 and 2. Pending results in summer 2023, the selected lines will be evaluated in the 2023-2024 OET3 as candidate releases for the commodity market.

Overview and Procedures

Breeding pipelines. To best understand the varietal products forthcoming from WIT, it is essential to understand how the breeding program is structured. As for basic methodology, most of the OSU wheat breeding program employs a *modified bulk-pedigree* selection method, whereby early-generation populations are selected and advanced as bulk populations for three consecutive generations (F_2-F_4), purelines are derived from F_4 populations as headrows, and then advanced in subsequent generations through bulk-selfing. However, two of the four pipelines which now constitute the breeding program – the doubled haploid (DH) and pedigree selection (PS) pipelines – operate by very different breeding procedures. Essentially, the DH pipeline circumvents the time-consuming natural inbreeding process, whereas the PS pipeline relies heavily on cyclic family and within-family selection in the earlier inbreeding generations. The PS pipeline will often utilize molecular markers to identify lines for extensive field and quality testing.

A key overlay to the *modified bulk-pedigree* selection method is the Graze*n*Grain® breeding system, which incorporates a dual-purpose management selection environment at key points of the 10- to 13-year breeding cycle. This feature distinguishes the OSU breeding program as the only one in the world that *selects* for adaptation to dual-purpose conditions, rather than simply *testing* finished lines in a dual-purpose environment. Attributes improved by this selection method are stand establishment and canopy closure (time to "first bite"), tillering capacity, drought and cold tolerance, tolerance to barley yellow dwarf (BYD) and aphids which transmit BYD, resistance to Hessian fly (if present), strong winter dormancy retention, and stem carbohydrate remobilization. The latter may be induced by a reduction in flag leaf size after grazing.

This breeding system was installed in fall 1997 and has been operated continuously since that time. The critical F_2-F_4 generations of selection, during which cattle represent the primary selection agent, occurs in central Oklahoma near Okarche (Figure 1). The **Graze*n*Grain®** pipeline constitutes the *principle pipeline* for development of new OSU wheat varieties, but its relative size has diminished in the past two years due to significant furnishing of two newer pipelines, High Plains and DH pipelines.

A significant branch of the VDP was started in 2013 to address a growing need for germplasm specifically adapted to the Oklahoma panhandle region. This branch constitutes the ***High Plains pipeline***. Until 2022, we used *limited irrigation* at the OPREC in Goodwell, OK to establish a more reliable *dryland* nursery. Irrigation was used only to ensure crop establishment and a minimal yield potential of 30 bu/ac. Unfortunately, we could not achieve sufficient control of spatial variation in the field or sufficient yield across the field to allow selection in harvest years 2020, 2021, or 2022, which brought this pipeline to a stall or haul position. The dryland nursery was moved to a site near Balko for 2022-2023 and will continue in 2023-2024.

This pipeline relies on the same *modified bulk-pedigree* selection method used in the Graze*n*Grain® pipeline, except selection and population advancement in the earlier inbreeding generations (F_2-F_4) occurs in the panhandle without grazing. WIT continues to re-stock this pipeline, opening a critical door to experimental lines specifically targeted for the panhandle region, rather than relying on the "luck of the draw" from experimental lines developed downstate. Three candidate varieties with wheat streak mosaic/Triticum mosaic resistance were placed on preliminary breeder seed increase at Balko for the 2022-23 season, and one or more of those will be evaluated in elite nurseries at Balko for a final season in 2023-24.

On a completely separate breeding track, WIT will enter its 10th consecutive year of evaluating doubled-haploid (DH) lines produced primarily through fee-based service agreements with Heartland Plant Innovations, Inc. (HPI) in Manhattan, KS. The ***DH pipeline*** signifies an aggressive strategy to introduce key traits at rapid pace with negligible slippage in agronomic value. Other traits besides yield potential and quality performance that we will specifically target among DH lines are resistances to BYD, WSM, and head scab, modified starch composition (higher resistant starch), and other human health parameters.

The fourth and final leg of the VDP is populated with experimental lines intensively selected for yielding ability using a modified pedigree-selection breeding method, rather than a bulk-pedigree selection method. Instead of advancing populations of plants through the early inbreeding generations immediately following the cross, desirable families are selected for yield-determining characters, from which lines (sibs) within families are selected again the following generation. This repetitive process continues until highly inbred lines are selected with the highest yield potential, prior to replicated yield and quality testing. Up to about 10% of the total VDP is expected to travel the ***Pedigree Selection (PS) pipeline***. Moving the early inbreeding generations of the PS pipeline from Stillwater to Lahoma *could* provide the highest yielding germplasm in the VDP. Currently, the CoAXium germplasm occupies all headrow-plantable space at Lahoma. One candidate particularly stands out for possible release in 2023 as mentioned above ─ OK16103083, a Garrison derivative featuring *Fhb1*-mediated scab resistance with adaptation to eastern Oklahoma.

Figure 1. Breeding nursery sites anticipated in 2023-24.

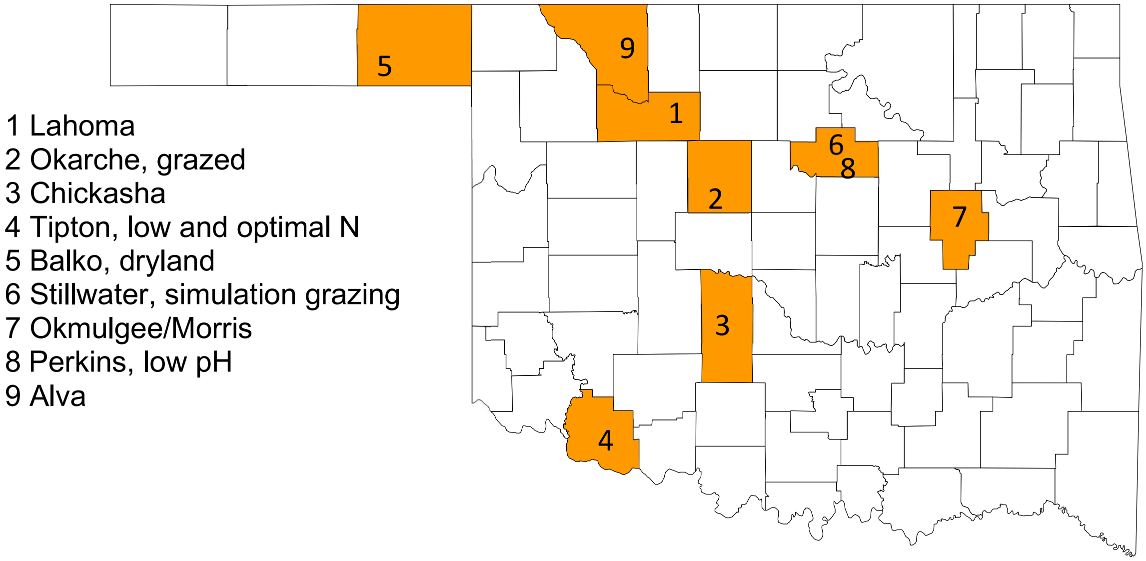

Table 1. OSU candidate varieties currently under seed increase by Oklahoma Foundation Seed Stocks, and number of years of Foundation seed production projected as of summer 2023. Most immediate candidates ready for release indicated in boldface.

Candidate	*Pedigree*	*OFSS*	*Featured traits*
OK18510 (rel. High Cotton)	TCI982345/OK05526-Hf//OK10415	2	Equally fitted for dual-purpose and intensive management systems
OK15MASBx7 ARS 8-20	Gallagher*3/Snowmass	3	Gallagher with HRS-like dough strength
OK15MASBx7 ARS 8-29	Gallagher*3/Snowmass	3	Gallagher with HRS-like dough strength, mod. resistance to WSM
OK15DMASBx7 ARS 6-8	OK10130*3/Snowmass	3	Highest yielding HRW with HRS-like dough strength; later & taller
OK15MASBx7 ARS 8-19-..	Gallagher*3/Snowmass	2	Agronomic improvement over OK15MASBx7 ARS 8-29?
OCW03S580S-8WF	G991502/BULK SELN 00F5-11-2	6	High yield, disease-resistant SRW with HRW-like quality
OK16107133-19HR-3	OK12621/Doublestop CL+	1-2	Doublestop CL+ with deep disease resistance package (*non*-CL+)
OK16107133-19HR-4	OK12621/Doublestop CL+	2	Like OK16107133-19HR-3 but lacks gene *Bdv2*; second choice
OK16103083	Garrison*3/Wesley Fhb1-106	2	Garrison with FHB resistance and high yield potential in E OK
OK16107125C-17HR-2	OK12621/Doublestop CL+	3	CL+; Doublestop CL+ with BYD immunity (like Uncharted)
OK198417C	OK10943C/OK09935C//Doublestop CL+	3	CL+; Yield exceeds Strad CL+; test wt exceeds Doublestop CL+
OK19225	KS06O3A~58-2/OK10408//Ruby Lee	2	4-yr top gun for yield among experimentals; IM-targeted with PGR
OKP17D101A666	HV9W07-1031/Gallagher//OK09429	1	Wall of disease resistance; earlier than Butler's Gold; short-season
OK18205-19HRBulk	Greer/OK09729	1	GrazenGrain for SW/central OK; novel leaf rust resistance
OK21227	MD01W233-06-1/Gallagher//OK13216	1	One of 4 beardless candidates to follow up OK Corral; better test weight

Table 2. Trait ratings (1-to-5 scale) for highest-priority candidate varieties currently under OFSS seed increase as of summer 2023.

	Trait category[a]											
Candidate	*DP*	*HF*	*YR*	*LR*	*TS*	*PM*	*V*	*AST*	*SS*	*BQ*	*TW*	*Weaknesses*
OK18510	1	5	2	1	3	3	1	3	1	2	2	Hessian fly
OK15MASBx7 ARS 8-20	2	1	1	1	3	1	1	4	3	1	1	Physiological leaf spotting
OK15MASBx7 ARS 8-29	2	1	1	2	3	1	5	3	3	1	2	Leaf spotting, WSBM-suscept.
OK15DMASBx7 ARS 6-8	2	5	2	3	3	3	1	3	2	1	2	Leaf rolling
OK15MASBx7ARS 8-19-	2	1	1	1	3	1	4	3	3	1	2	Leaf spotting, WSBM-suscept.
OCW03S580S-8WF	4	3	3	1	2	1	1	1	1	3	4	Minor shattering
OK16107133-19HR-3	1	2	1	3	4	1	1	2	2	4	2	Mixing tolerance
OK16107133-19HR-4	1	1	1	2	3	1	1	1	2	4	3	Mixing tolerance
OK16103083	1	5	4	1	1	1	1	1	2	3	2	Stripe rust, Hessian fly
OK16107125C-17HR-2	1	5	1	1	3	1	1	1	2	1	1	Tends mod. late and tall
OK198417C	1	2	1	1	2	1	4	1	1	2	1	Tends mod. late and tall
OK19225	2	5	3	2	3	4	5	3	3	2	1	Needs fungicide to max yield
OKP17D101A666	4	1	1	1	1	1	2	2	2	2	2	Needs no fungicide
OK18205-19HRBulk	1	5	2	1	4	2	1	1	1	3	2	Minor shattering tendency
OK21227	2	?	1	2	?	?	2	3	3	3	3	OK Corral - better low pH tol.

[a] Trait categories abbreviated as DP, grazeability (forage and grain combined); HF, Hessian fly; YR, stripe rust; LR, leaf rust; TS, tan spot; PM, powdery mildew; V, WSBM/WSSM complex; AST, acid-soil tolerance; SS, straw strength; BQ, baking quality; TW, test weight. Values ≤2 are considered very desirable; those ≥4 are undesirable. No value (--) indicates inconsistent or insufficient data for postulation.

W I T

Gene Discovery and Molecular Breeding (3 proposals)

Liuling Yan

Plant and Soil Sciences

Deliverables

1) Breeding lines independently carrying double-*AHAS* (Clearfield Plus) and triple-*ACCase* stacked mutants (CoAXium).
2) Breeding lines carrying *Wsm1* stacked (preferably) with *Cmc4*.
3) Breeding lines carrying double or triple *SBEIIa* mutants.
4) A platform for editing genes with specific value to the Oklahoma wheat industry.

Overview and Procedures

Deliverable 1. Molecular markers for mutations of two homoeologous genes, *AhasL-B1* and *AhasL-D1* on chromosomes 1B and 1D, were used to introduce herbicide resistance into WIT breeding lines for many years. These genes encoding acetohydroxyacid synthase (AHAS) inhibitors confer resistance to imazamox herbicide. More recently, molecular markers for the mutations of three homoeologous genes, *ACCase-A*, *ACCase-B*, and *ACCase-D* on chromosomes 2A, 2B, and 2D in hexaploid wheat, were also introduced into WIT breeding lines. These genes encoding acetyl-CoA carboxylase (ACCase) confer resistance to commercial herbicides like quizalofop.

In the new research period, we will use the available markers for *AHAS* and *ACCase* to stack them into separate breeding lines at a greater frequency. Patent rights prevent the combined stacking of both sets of mutations into a single genotype. The genotyping work will be performed using competitive allele-specific PCR (KASP) markers, enabling the bi-allelic scoring of mutations, including single nucleotide polymorphisms (SNPs), insertions, and deletions (indels) at specific loci in high-throughput genotyping of breeding lines.

The *AhasL* mutant genotypes will be identified using KASP assays developed by BASF and LGC Biosearch. Primers for *TaAHASL* genotyping will be directly ordered from LGC Genomics using the following catalog numbers: KBS-2300-112 (Clearfield wheat: TaAHASL1B-S653N; 2500 assays) and KBS-2300-113 (Clearfield wheat: TaAHASL1D-S653N; 2500 assays. LGC KASP mix (KASP-TF V4.0 2X with Std ROX) will be used in the new research period. The assays will be performed using a standard thermal cycler and fluorescent plate reader in a real-time PCR system. It is essential to use proper control genotypes in each plate to assess the validity of each run and to properly score data. Doublestop CL Plus will be used as a control for the *TaAHASL-1B* and *TaAHASL-1D* mutants, and Oakley will be used as a control for the *TaAHASL-1D* mutant alone. No *TaAHASL-1A* mutation is required to achieve the desirable level of herbicide tolerance. Assays will be conducted to either select for presence of the mutations in Clearfield Plus wheat or for their absence in wild-type genotypes derived from desirable Clearfield Plus germplasm.

In the past, we designed new primers and optimized KASP for detecting *ACCase* mutations. The *ACCase* mutant genotypes were identified using KASP assays developed initially by Colorado State University. The previous markers involved PCR amplification of each gene copy using high-fidelity Taq enzymes and the PCR products as templates in the KASP assay. This assay showed non-specific PCR products and low amplification efficiency because one sample was conducted with PCRs twice. Mutant control DNAs will include LCS Steel for AX-ab, CP72166 for AX-ab, AP18 for AX-bd, LCS Atomic for AX-bd, Crescent for AX-ad, and LCS Photon for AX-ad.

The *AhasL* and *ACCase* genotypic data will provide WIT with the rapid and accurate capability to select breeding lines for herbicide resistance (or lack thereof) for confirmation of lines entering candidacy status, as outlined in Carver's project.

Deliverable 2. Wheat curl mite (WCM) is a devastating insect that significantly affects grain yield and end-use quality of hard red winter (HRW) wheat in the Great Plains, particularly in the panhandle and western regions of Oklahoma. This insect also behaves as a vector to transmit *Wheat streak mosaic virus* (WSMV) to wheat plants, especially in drought-stressed areas. The resistance gene *Cmc4* was transferred via a fragment of the wild wheat relative *Ae. tauschii* into chromosome arm 6DS of common wheat and confers effective resistance to current WCM botypes. The resistance gene *Wsm1* resides on a recently shortened genome fragment of *Thinopyrum intermedium* (intermediate wheatgrass) in the form of a 4DL.4AgS translocation of chromosome arm 4DL in common wheat, and confers resistance to WSMV/TM. There are other resistance genes, such as *Cmc1-Cmc3* and *Wsm2-Wsm3*, but *Wsm1* and *Cmc4* were already introduced into WIT germplasm with OWRF funding in previous research cycles. In the new cycle, we will incorporate *Wsm1* and *Cmc4* simultaneously into the WIT variety development pipeline via marker-assisted selection among intermediate to advanced lines segregating for both genes.

The marker for *Wsm1* is available, and it works well because it represents the alien *T. intermedium* genomic fragment containing *Wsm1* that does not exist in the wheat genome. However, the previous PCR marker was dominant, which prevented us from distinguishing purebreeding (homozygous) genotypes from mere carriers (heterozygotes) of *Wsm1*. In the new research cycle, we will convert the gel-required marker for the *Wsm1* dominant allele to a KASP marker for bi-allelic scoring. Three WIT breeding lines, including OK168512 (Breakthrough), OK168517, and OK168513, that offer WSM protection via *Wsm1* will be used as positive controls for the presence of *Wsm1*.

The gene *Cmc4* was initially incorporated into Great Plains adapted germplasm via OK05312, an advanced hexaploid HRW wheat breeding line made available as a public release. *Cmc4* was mapped in a new RIL population from the cross, SD06165 x OK05312, using genotyping-by-sequencing (GBS)-based single nucleotide polymorphism (GBS-SNP) markers, and it was delimited within a 121.6 kb interval on the short arm of chromosome 6DS, where five putative genes are annotated based on the IWGSC Chinese Spring genome. Furthermore, KASP markers were developed for one candidate gene, *TraesCS6D01G005300.1*, which is annotated as an NBS-LRR-like resistance protein in the IWGSC reference genome. *TraesCS6D01G005300.1* was translocated from *Ae. tauschii* into the syntenic region of modern wheat cultivars. The KASP marker for *Cmc4* will be applied in molecular breeding with the pending KASP marker for *Wsm1*.

Genotypic data for the presence/absence of *Wsm1* and *Cmc4* will enable WIT to practice immediate selection in candidate breeding populations but also develop new crosses intended to combine the two genes into the same genetic background with minimal impact of yield-reducing genes linked to them. An effort will be made to enrich populations with *Wsm1/Cmc4*-based resistance before screening phenotypes. The expected results are needed for more precise selection for *Wsm1* and *Cmc4*, making the advanced breeding lines commercial-ready.

A second gene derived from Thinopyrum intermedium, called *Wsm3*, has already been introgressed to a limited degree into OSU germplasm via the translocation T7BS·7S#3L. *Wsm3* confers resistance to WSM at a higher temperature level than *Wsm1* and provides resistance to TM, but is often associated with linkage drag for yield. Marker strategies will be developed in 2023-2024 to facilitate a larger introgression effort, without losing emphasis on the aforementioned *Wsm1*/*Cmc4* stack.

Deliverable 3. The *SBEIIa* mutant genotypes conferring elevated amylose starch in the wheat endosperm will be identified using molecular markers we have developed to determine the presence of homoeologous mutant alleles, namely *SBEIIa-A* on chromosome 2A, *SBEIIa-B* on chromosome 2B, and *SBEIIa-D* on chromosome 2D in hexaploid wheat. It was reported elsewhere that amylose content increased by up to ~85% in a spring wheat breeding line fixed for all three *SBEII* mutant alleles, corresponding to increased resistant starch (dietary fiber) potential. These mutant alleles have been introgressed into a Gallagher background and will be further transferred into other Oklahoma-adapted backgrounds. The highest priority in 2023-2024 will be to confirm and validate the presence of double or triple *SBEII* mutants in advanced breeding lines (ca. 100 lines). Selected lines will represent the best SBE double mutants relative to field performance and will be subjected to resistant starch assessment in conjunction with an industry partner to move forward in the VDP for possible commercialization by 2025.

Diagnostic KASP markers for the three *SBEII* mutants have allowed WIT to convert Gallagher into single and double mutant variants, but the triple mutant remains mysteriously elusive. All three KASP markers have worked well, however, in different *SBEII* mutant populations with reproducible results. Original DNAs from individual mutants will be used as controls, T6-726 for *SBEIIa-A*, T6-111 for *SBEIIa-B*, and T6-630 for *SBEIIa-D*. The final selected lines will be confirmed with the PCR-digestion-based method used in previous studies.

Deliverable 4. The evolution and practical breeding of crops depends on genetic variation. The *CoAXium* wheat production system developed from SNPs is a good example. To accelerate crop improvement, clustered regularly interspaced palindromic repeat (CRISPR)-Cas9 systems will be used to engineer genome-targeted mutagenesis in Oklahoma wheat cultivars. The CRISPR-Cas9 system produces DNA double-strand breaks (DSBs) by site-specific cleavage, typically resulting in small indels. A platform will be established to generate a large number of point mutations in any susceptible genes or a repressor inhibiting development. An initiative for new wheat cultivars with edited genes (long-term) with specific value to Oklahoma wheat production should be pursued with key stakeholders in the Oklahoma wheat industry.

Charles Chen

Biochemistry and Molecular Biology

Deliverables

1) Genomic variants associated with developmental short-season characteristics relevant to Oklahoma.
2) Preliminary strategy to utilize genomic selection (GS) in predicting phenotypes with short-season adaptation from the mainstream variety development pipeline.

Background

Locally adapted winter wheat varieties require about nine-months to optimally complete their growth cycle. However, irregular extreme weather events such as excessive rains during the optimal planting window in October may prevent producers from planting in time to achieve maximum vegetative growth and tiller formation. This delayed planting will almost always reduce the subsequent yield of full-season wheat varieties. This is compounded by milder winter season projections, owing to a decrease in cumulative vernalization degree days over years. Thus, WIT aims to seek solutions to prepare the region by identifying the genetic basis for short-season wheat characteristics, which allow for an accelerated maturity pattern consistent with the harvest schedule of full-season varieties. Although WIT has previously conducted single-gene analysis of plant development, it is clear that the Butler's Gold short-season ideotype is not adequately described by previously studied genes in the flowering pathway (Table 1).

Using a whole-genome approach, we expect to go beyond single-gene screening and expose all genomic regions associated with the accelerated developmental pattern of short-season genotypes. Further, the development of these varieties has been primarily driven by conventional breeding methods that can be time-consuming and rely heavily on additional institutional capital for field testing. With the knowledge of genome-wide association for short-season ideotypes, WIT proposes to adopt a genomic selection (GS) approach, which uses genomic information to predict the breeding value of individuals, as a foundational approach to improve the efficiency and precision of selecting for short-season characteristics. *This is the current direction of global wheat improvement efforts, and the time has arrived for WIT to engage in this arena. The short-season adaptation pattern allows for a realistic and relevant trait target for WIT to engage with immediate outcomes.*

Table 1. Single-gene analysis conducted on seven winter wheat varieties and phenotypes from nine varieties.

Varieties	**vrn-A1**	**PPD-D1**	**vrn-D3**	**TaOGT**	**Dormancy release phenotype**	**Heading time**	**Physiological maturity**
Duster	NA	*not known*	b	a	Mid-late	Medium	Medium
Gallagher	b	b	b	a	Medium	Medium	Medium
Showdown	b2	b2	a	b	Mid-late	Medium	Medium
Green Hammer[1]	a	a	a	b	Early	Mid-early	Mid-late
Baker's Ann	b	b	b	b	Early	Early	Early
OK Corral	b	b	a	b	Medium	Medium	Medium
Butler's Gold[2]	b	b	a	b	Very Early	Early	Early
Skydance	*NA*	*NA*	*NA*	*NA*	Early	Mid-early	Mid-early
AM Cartwright	*NA*	*NA*	*NA*	*NA*	Medium	Medium	Medium
Bob Dole	*NA*	*NA*	*NA*	*NA*	Early	Medium	Medium

[1] Green Hammer represents the earliest haplotype based on the single-gene analysis.

[2] Butler's Gold represents a short-season ideotype based on its well-known developmental pattern phenotype.

Note: For *vrn-A1* and *vrn-D3*, allele *a* designates earliness (Jagger allele) and *b* = late (2174 allele), and *b2* = a new allele. For *PPD-D1*, allele *a* designates photoperiod sensitive (Jagger allele), *b* = photoperiod insensitive (2174 allele), and *b2* = a new allele. For *TaOGT*, allele *a* = late (Duster allele) and *b* = early (Billings allele). NA = information not available.

Procedures

Deliverable 1. To screen for genome-wide variants associated with short-season characteristics, WIT proposes to genotype and phenotype a winter wheat panel comprising approximately 140 advanced experimental lines and varieties specifically developed for the region. The panel is already known, and phenotyped accordingly but not exhaustively, to represent three divergent developmental patterns: very early, putative for short-season adaptation; intermediate, representing the vast majority of OSU released varieties; and very late, or diametric to the short-season ideotype. This panel provides WIT with the best opportunity to identify the genetic basis of short-season characteristics relevant to Oklahoma wheat production and highly relevant to the gene pool engaged by WIT.

Due to the impracticality of directly measuring arrival dates at key stages of development in the field, WIT has developed a relational scoring scheme, illustrated in Figure 1, to quantify key developmental junctures. The scoring scheme uses a maturity scale of 0 to 6, indicative of very early (0) to very late potential (6) based on a normal planting date. Our preliminary results showed that early dormancy release, a shortened period from the initiation of stem elongation to heading emergence, and accelerated grain-filling – all of which resemble the ideotype Butler's Gold – can be identified in the search for similar and future short-season phenotypes (Table 1, Figure 1).

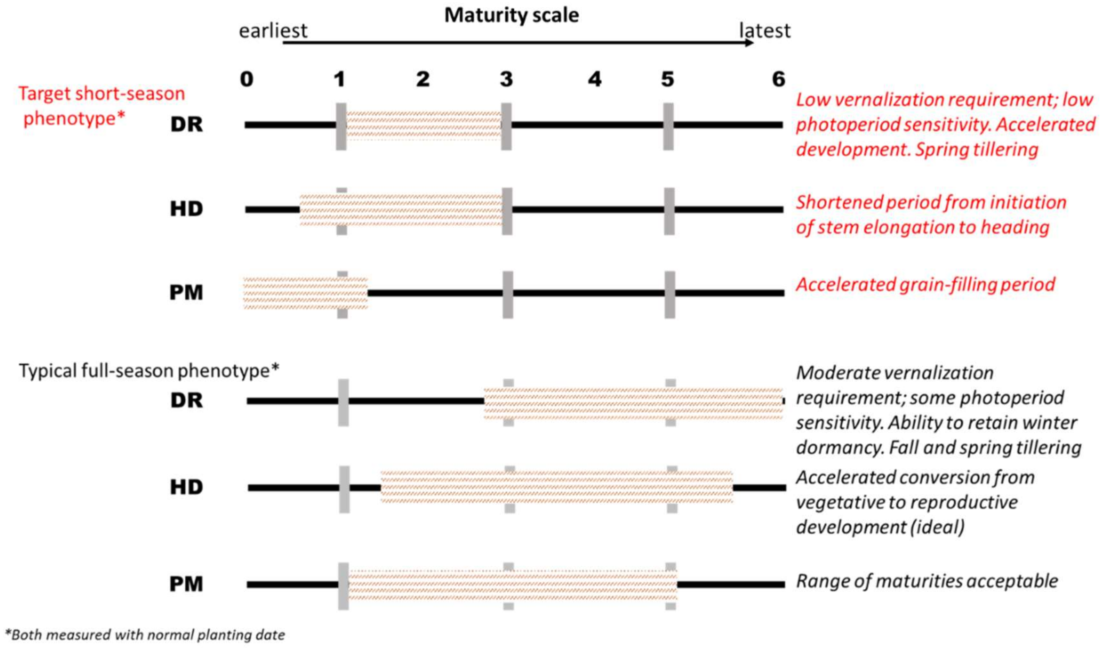

Figure 1. WIT's scoring system for short-season characterization. The system assigns a score 0 to indicate the earliest relative to the evaluated population, and a score of 6 for the latest. DR denotes dormancy release, which serves as a proxy for the initiation of stem elongation, while HD and PM, respectively, represent heading time and physiological maturity. The short-season system is typically planted from late November to mid-December and harvested from late May to mid-June (approximately 6 months), whereas the full-season system is typically planted from late September to late October and harvested from late May to mid-June (approximately 8-9 months).

To reveal the genomic regions responsible for short-season phenotypes, WIT will generate SNP profiles for the selected genotypes using a whole-genome GBS (genotyping-by-sequencing) approach to ensure the coverage required to identify novel associated variants. Furthermore, to mitigate potential issues of patent applications on Sequenced Based Genotyping technologies, WIT has identified sequencing service partners who can provide GBS services under license from Keygene N.V. (e.g., Next Generation Sequencing Core of the University of Wisconsin-Madison and Genomics Center of the University of Minnesota). The DNA sample preparation and library construction for sequencing will follow the protocol using the PstI

(CTGCAG) and MspI (CCGG) double-digest. Downstream sequencing read processing and SNP calling will be conducted using computational algorithms such as TASSEL and Stacks. WIT's previous work has demonstrated that this approach could efficiently generate over one million robust sequencing tags for SNP calling and for subsequent association mapping studies.

From the distribution of phenotypic variation that may represent simple Mendelian inheritance, WIT anticipates that, with a relatively simple ancestry shared among the selected genotypes, conventional association mapping methodologies should be adequate. To further mitigate the potential shortcomings in statistical power resulting from rare alleles, the SNP dataset will be filtered for minor allele frequency higher than which is normally conducted. In addition to guaranteeing the statistical power needed for discovery, this filtering can further alleviate issues related to false-positive SNP calling due to the complexity of the wheat genome.

For the discovery purpose of this research, WIT will begin with the standard single locus analysis with the relatedness matrix estimated using the VanRaden (2008) method. Additionally, when statistical power permits, we will evaluate conventional GWAS methods to better decipher genetic effects of short-season characteristics. Associations that are statistically robust and reproducible across different mapping algorithms will be subject to downstream investigation of the functional roles, like a priori candidate gene discovery with other known wheat genes.

Deliverable 2. Based on previous genetic screening (GS) results, WIT has decided to prioritize the evaluation of three algorithms: (i) rrBLUP, which stands for ridge regression Best Linear Unbiased Predictor, (ii) Bayesian LASSO, and (iii) RKHS, which stands for Reproducing Kernel Hilbert Space. These three algorithms were chosen based on their abilities. rrBLUP estimates the predicted phenotypic values by utilizing the entire SNP dataset without conducting significance tests, while the Bayesian LASSO performs variable selection and predicts the target phenotype using key SNP markers that are responsible for short-season characteristics identified in Deliverable (1). Finally, the RKHS computes phenotypic values via a kernel function that can model all degrees of genetic interactions.

The evaluation of GS algorithms will be conducted using the conventionally used 5-fold cross-validation method. Accuracy, which reflects the model's predictability, will be estimated by correlating genomic estimated breeding values (GEBVs) with actual phenotypes. The variability of predictability will be assessed by the standard deviation of 30 replicates of cross-validation. WIT expects to identify the most capable predictive algorithm to screen all varieties . We intend to expedite the identification of candidate genotypes for short-season wheat and to corroborate those candidates selected so far solely based on phenotype, e.g, OKP17D101A666 and others.

Xiangyang Xu

USDA-ARS, Stillwater, OK

Deliverables

1) Locally adapted progenies carrying newly identified pest and disease resistance genes.
2) Identities of leaf rust resistance genes in PI 351861, PI 621647, and PI 622129.
3) Identities of powdery mildew resistance genes in PI 578040 and PI 627871.

Procedures

Deliverable 1. With past support of OWRF, we used marker-assisted selection to transfer novel genes conferring resistance to powdery mildew (*Pm59*, *Pm63*, *Pm65*, *Pm223899*, *and PmBN418*), leaf rust (*Lr81*, *Lr622111*, and *QLr.stars-1BS*), and greenbug (*Gb8*) to WIT lines. Of these, *Pm59*, *Pm63*, *Pm223899*, *Lr622111* were identified in landraces, while *QLr.stars-1RS*, *Pm65*, *PmBN418*, and *Lr81* were found in exotic cultivars. We plan to continue introgression of these genes into WIT lines in 2023-2024, so that Oklahoma wheat producers can reap the benefits of these novel gene discoveries (Table 1).

In addition, a new powdery mildew resistance gene designated *Pm351817* was recently identified in PI 351817. Crosses will be made to transfer this gene into WIT lines in 2023-2024. Marker-assisted selection will be conducted at the seedling stage to retain progenies carrying the targeted gene in backcross or/and topcross populations in 2023-2024, which will be bulked for further tests and subsequent selection in the field by Carver (beyond this funding cycle).

Deliverable 2. PI 351861 is a wheat cultivar developed in the former Soviet Union, while both PI 621674 and PI 622129 are Iranian landraces. All three accessions exhibited superior resistance to leaf rust (*Pt*) races collected from Oklahoma. We have developed $F_{6:7}$ recombinant-inbred lines (RIL) populations from PI 351861× TAM110, PI 621674 × PI 626051, and PI 622129 × TAM110, respectively, to characterize the leaf rust resistance genes in each accession. We plan to evaluate the response of each RIL population to *Pt* race *Pt52-2* (race *MMPSD*) and genotype them using genotyping-by-sequencing (GBS). In brief, genomic DNA will be digested with *PstI* and *MspI* (New England Biolab address), ligated to barcoded adapters using T4 ligase, and PCR-amplified to construct GBS library. The libraries will be size-selected for 200–300 bp fragments, purified, quantified, and sequenced in an Ion Torrent Proton sequencer (Life Technologies, Inc.). Sequence reads will be processed as described by Mascher et al. (2013), and a reference-based SNP calling pipeline implemented in TASSEL 3 will be used to call SNPs. The QTL LciMapping V4.1 program will be used to map the leaf rust resistance genes, and SNP markers closely linked to the genes will be converted to KASP markers.

Deliverable 3. PI 578040 is a U.S. cultivar and PI 627871 is an Iranian landrace. Both accessions exhibited immune responses to powdery mildew (*Bgt*) isolates from Oklahoma. RIL populations from PI 578040 × CItr 11349 and PI 627871 × Jagalene will be genotyped using the GBS approach described above and evaluated for response to *Bgt* isolate *OKS(14)-B-3-1*. *OKS(14)-B-3-1* was avirulent to PI 578040 and PI 627871 but virulent to CItr 11349 and Jagalene. The GBS-SNPs will be used to map the powdery mildew resistance genes, and those flanking the resistance genes will be converted to KASP markers for tracking the resistance genes in breeding populations.

Table 1. Wheat accessions currently accessed in the WIT gene introgression program, their breeding status, target genes/QTL, and geographic origin.

Accession	**Breeding status**	**Target gene/QTL**	**Geographic origin**
PI 181356	Landrace	*Pm59*	Afghanistan
PI 628024	Landrace	*Pm63*	Iran
Xinmai 208	Cultivar	*Pm65*	China
PI 606247	Cultivar	*Pm606247*	Czech Republic
PI 223899	Landrace	*Pm223899*	Afghanistan
Bainong 418	Cultivar	*PmBN418* *QLr.stars-1RS*	China
PI 351817	Cultivar	*Pm351817*	Germany
PI 470121	Experimental line	*Lr81*	Croatia

PI 622121	Landrace	*Lr622111*	Iran
PI 697274	Reselection line	*Gb8*	USA

W I T

Disease Resistance Breeding

Meriem Aoun

Entomology and Plant Pathology

Deliverables

1) Disease reactions of OSU breeder lines to wheat soil-borne mosaic/wheat spindle streak mosaic (WSBM/WSSM), barley yellow dwarf, leaf rust, powdery mildew, and tan spot based on greenhouse and/or field testing.
2) Underlying genetics of leaf rust and stripe rust resistance in OSU advanced germplasm.
3) Identity of novel disease resistance genes in OSU's 'Big Country'.

Procedures

Deliverable 1. Continued evaluation of OSU breeding lines to fungal and viral diseases of importance to Oklahoma allow the advancement of resistant lines in the breeding pipeline. Disease evaluations will be conducted under field and greenhouse conditions to provide sufficient data for selection. In 2022-2023, 407 breeding lines were rated (seedling and adult-plant) in the greenhouse for leaf rust, powdery mildew, and tan spot. These greenhouse evaluations will be continued in 2023-2024.

In fall 2022, 1,421 experimental wheat lines were planted in a field nursery to evaluate their reactions to WSBM/WSSM. This nursery included 984 OSU breeding lines and 437 lines from USDA-ARS regional nurseries. Viral disease response was determined in spring 2023 based on visual symptoms. WSBM/WSSM testing will continue in 2023-2024, provided that WSBM/WSSM symptoms will be manifested in this nursery. If environmental conditions or other unknown factors limit or compromise expression throughout the nursery, we will collect DNA from the OSU breeding lines and use molecular markers to select for resistant lines. A diagnostic marker for the WSBM resistance gene *Sbm1* will be used for marker-assisted selection. No molecular marker exists for detection of WSSM resistance.

Another field nursery will be used to evaluate most advanced breeding lines to BYD and powdery mildew. In fall 2022, 136 advanced breeding lines were planted in this nursery with the susceptible variety 'Pete' in spreader rows to enhance disease pressure. Continuation of this nursery is planned for 2023-2024. Breeding lines in the BYD and WSBM/WSSM nurseries will also be evaluated for any other foliar diseases that appear from natural infection.

Deliverable 2. OSU wheat breeding lines carry known leaf rust resistance (*Lr*) and stripe rust resistance (*Yr*) genes including *Lr21*, *Lr24*, *Lr34/Yr18*, *Lr37*/*Yr17*, *Lr42*, *Lr46*, *Lr68* and *Lr77*. We hypothesized that additional resistance genes likely reside throughout the OSU wheat VDP. Genomics tools such as genome-wide association studies (GWAS) can be used to discover additional genes in OSU's elite lines. In 2022, we developed a germplasm collection of 584 OSU advanced breeding lines and cultivars. This germplasm was evaluated at the seedling stage in the greenhouse to the wheat leaf rust pathogen race MNPSD, the most dominant race in Oklahoma and Texas in the last 5 years. Additional seedling evaluations will continue in 2023-2024 using five leaf rust pathogen races. As adult-plant evaluations are ideally performed in the field, we planted this germplasm in fall 2022 in a leaf rust nursery in Stillwater. The susceptible wheat variety OK Bullet was used as spreader rows. The spreader rows were inoculated in spring 2023 using a mixture of isolates collected in Oklahoma in 2021 and 2022. This should create high disease pressure, which is needed to evaluate the breeding lines for their responses to leaf rust (results pending).

This OSU germplasm was also planted in fall 2022 in a stripe rust nursery in Chickasha. The susceptible variety Pete was planted as spreader rows. The spreader rows will be inoculated in spring 2023 with race Pstv-37, which is the most predominant race in Oklahoma and the U.S. The lines will then be evaluated in adult-plant stages in spring 2023 (results pending). Complementary stripe rust evaluations will be performed in 2023 in Pullman, Washington. Washington state has higher race diversity and a more conducive environment for stripe rust to produce high-quality stripe rust data for this project. In addition to field evaluations, we will conduct seedling evaluations in the growth chamber using the Oklahoma race Pstv-37 and four races from Washington state.

In 2023, we initiated genotyping-by-sequencing of this OSU germplasm at the USDA-ARS, Manhattan, KS. The generated phenotypic and genotypic data will be used to conduct GWAS that should identify tightly linked molecular markers to leaf rust and stripe rust resistance genes. User-friendly molecular markers, known as competitive allele specific PCR (KASP) markers, will then be developed for use in marker assisted breeding. It is important to note the distinction between this research and that of WIT member Xiangyang Xu. Here, novel resistance genes can be discovered and identified in WIT's own germplasm, while Xu's work in this proposal adds a uniquely critical dimension of access to more *distant* germplasm.

Deliverable 3. Big Country is a hard white wheat variety released in 2020 from the OSU breeding program (PV 202100243). Since its identification as a fixed experimental line in 2011, it remains the leader among all hard winter wheat varieties in providing blanket protection to leaf rust, stripe rust, bacterial leaf streak (BLS), and other diseases common to Oklahoma. Big Country has become a reliable donor in wheat breeding populations with commercial relevance, but breeders lack the critical molecular tools to resourcefully select and package these resistance traits. This project will map unknown disease resistance genes in this wheat cultivar and unlock this precious genetic resource for utilization throughout the southern Plains. This work will likely need to extend into future funding cycles.

In 2022, we started the development of a double haploid population Big Country × Jagalene. Jagelene is susceptible to multiple foliar diseases, making it a suitable parent in this bi-parental cross. A total of 160 double haploid (DH) lines will be available in fall 2023. For this funding cycle in 2023-2024, the DH lines will be genotyped using genotyping-by-sequencing and evaluated for reactions to leaf rust, stripe rust, and bacterial leaf streak. Rust evaluations will be similar to that described in Deliverable 2. BLS screening will be conducted in Morris, OK (high disease pressure in 2022) and in North Dakota where BLS is considered the top foliar wheat disease. Phenotypic data and marker data will then be used to map resistance genes in Big Country. To increase the mapping resolution of identified disease resistance genes, large $F_{2:3}$ families of Jagalene/Big Country (~ 2,000 individuals) will be developed in 2023-2024. DNA will be collected from ~ 2,000 F_2 plants before advancement to the F_3 generation.

WIT
Candidate Variety Assessment and Applied Wheat Physiology
Amanda de Oliveira Silva
Plant and Soil Sciences

Deliverables

1) Adaptability profiles of OSU candidate varieties for best positioning of new releases in Oklahoma.
2) Publicly available agronomic data and variety characteristics of new releases.
3) Single-year assessment (yr 2) of short-season winter wheat germplasm adapted to delayed planting with minimal harvest delay.
4) Single-year assessment (yr 2) of the physiological balance in maximizing yield and minimizing grain protein cost, under low and optimal nitrogen availability.

Procedures

Deliverable 1. Approximately 10-12 advanced experimental WIT lines will be evaluated as part of the OSU wheat variety trials in about 20 experiments across Oklahoma. Data collected from these trials will be used to measure and determine suitability of candidate varieties for production in Oklahoma. Environments will include conventionally tilled and no-till locations scattered throughout the wheat-production belt of Oklahoma and will be split approximately 60/40 between grain-only and dual-purpose trials. Including candidate varieties in the OSU variety trials provides side-by-side comparisons to wheat varieties that are commercially released for production in Oklahoma. This will serve as an integral part in the decision-making process for determining which candidates are advanced for commercial release. In addition, we will validate herbicide tolerance of 2-gene CoAXium experimental lines expected from the 2023 harvest, and refine the target-production region of the three OX candidates based on performance in the 2023 variety trials.

Deliverable 2. Variety comparisons represent the data most requested by Oklahoma wheat farmers. The inclusion of candidate variety grain and forage yield information in variety trial reports allows producers to make a better-informed shift to newer genetics. Similar to last year we will include protein concentration together with yield and test weight already being reported. This has allowed producers to simultaneously see varietal differences in producing both high yield and protein in a given environment, as for example, the OSU varieties Doublestop CL+ and Showdown (Figure 1). Data will be posted in near real-time at www.wheat.okstate.edu and advertised via email listservs and social media outlets. Hard copies will also be distributed via popular press throughout Oklahoma. Ultimately, we believe this bias-free reporting and outreach will allow Oklahoma wheat producers to better visualize his/her return to their checkoff dollars and their investment in the OSU Wheat Improvement Team.

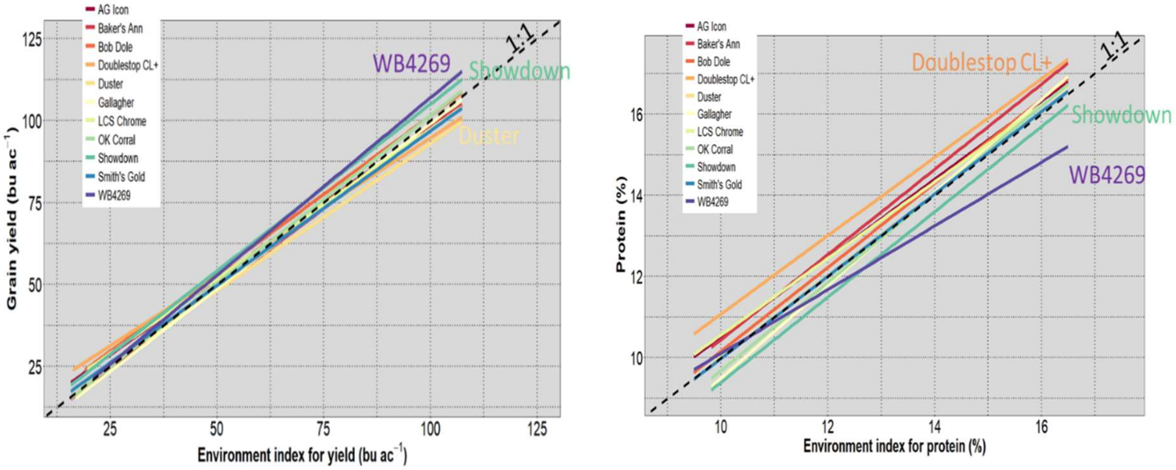

Figure 1. Environment responsiveness for grain yield (left) and wheat protein (right) of individual varieties, relative to the environment index computed as the mean of all varieties tested in each environment in the 2019 through 2022 Oklahoma Small Grains Variety Performance Tests. Analysis was conducted only for the 11 varieties planted in all 50 to 71 environments in those growing seasons.

Deliverable 3. We will continue collaborative research within and outside OSU's wheat improvement team, involving researchers from Texas, Spain, the UK, and Australia. Our overarching goal is to develop the concept hereby distinguished as *short-season* wheat varieties. The anticipated outcome will be hard *winter* wheat varieties adapted to late planting in November-December, featuring rapid early growth, reduced vernalization requirement, tolerance to heat and drought, and an accelerated maturity pattern consistent with the harvest time of current full-season varieties (Figure 2).

Figure 2. Full-season and short-season wheat varieties tested at optimal and late planting dates. The short-season variety Butler's Gold is pictured in the middle.

We are also applying for competitive grants to intensify evaluation on the genetics and physiological traits of diverse genetic materials, including experimental lines from an elite double-haploid nursery and varieties from the OSU wheat breeding program. In addition, we will continue to evaluate varieties currently being grown under severe drought and heat stress environments in Spain and Australia with low vernalization requirement, which could potentially be used as parental lines for future variety development by WIT. We will continue to evaluate the performance of current varieties when planted late in different environments. Field experiments will be installed in Stillwater, Lahoma, and possibly in Texas. Molecular markers created in the OSU WIT program will be used to identify unique plant development genotypes and phenotypes.

Deliverable 4. This research is intended to address a critical need to minimize the metabolic cost of protein deposition, i.e., breaking the yield-protein association. We will evaluate two pairs of OSU wheat varieties (Green Hammer and Doublestop CL+; Gallagher and Iba) with similar grain yield levels but different grain protein concentration and functionality. Our immediate objective is to examine how these contrasting varieties use nitrogen (N) to produce grain yield and protein at zero and high N rates, and how they partition N into different plant components during the growing season.

The novel approach for this study is to utilize labeled isotopic N (^{15}N) to evaluate N uptake dynamics potentially associated with greater yield and protein formation, and nitrogen use efficiency. Nitrogen has two stable isotopes (^{14}N and ^{15}N). The main difference between ^{14}N and ^{15}N is that ^{15}N is not as abundant in nature (~0.4% natural abundance) relative to ^{14}N (99.6% natural abundance). By utilizing ^{15}N as a phenotyping strategy we can better trace where the applied N goes within the plant and know how the more N-efficient varieties utilize the newly taken up N to produce grain.

We will use a method described as “multi-stage pulse labelling” with ^{15}N, which was developed to identify corn hybrids (De Oliveira Silva et al., 2017) with high NUE but can be applied to wheat. Therefore, labeled ^{15}N fertilizer in the form of $Ca(^{15}NO_3)_2$ containing 98% atom ^{15}N) will be applied at the jointing, boot, flowering, and soft dough stages. Then biomass samples will be collected 4 days after each application to monitor N recovery efficiency and partitioning of the N within the plant. This study will be conducted at Perkins and at the Lake Carl Blackwell research station in Stillwater. Other biomass samples will be collected for total N tissue analysis at soft dough and physiological maturity (Figure 3).

Figure 3. Wheat varieties planted at 0 and 120 lbs of N/acre. Flags symbolize areas where aboveground biomass samples are collected at different growth stages. Perkins, OK. Photo taken on March 26, 2021.

Analysis from the 2022 harvest season reveals the difference between varieties to maximize both grain yield and protein under limited and adequate N rates, but further investigation of the physiological traits driving that difference is needed. Doublestop CL+ and Green Hammer were able to maintain consistently higher protein than Gallagher and Iba, even when no nitrogen (0N) was applied (Figure 4). The greater grain N accumulation of Green Hammer relative to Iba (Figure 4) was explained by its greater N accumulation post flowering (Figure 5) in the leaf and stem components later in the growing season. Thus, further evaluation to understand the physiological traits associated with the tradeoff between yield and protein among varieties is needed, and this multi-stage pulse labelling ^{15}N approach could be a reasonable strategy to identify N allocation patterns associated with high yield, protein, and NUE.

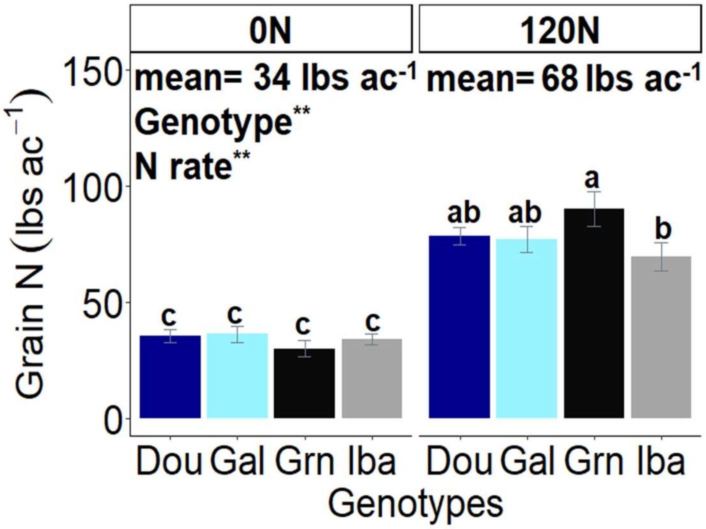

Figure 4. Grain nitrogen accumulation for four varieties tested under zero N rate and 120 lbs N per acre in the 2019-2020 and 2020-2021 growing seasons, averaged across two locations (Perkins and Stillwater). Varieties were abbreviated as Dou, Doublestop CL+; Gal, Gallagher; and Grn, Green Hammer.

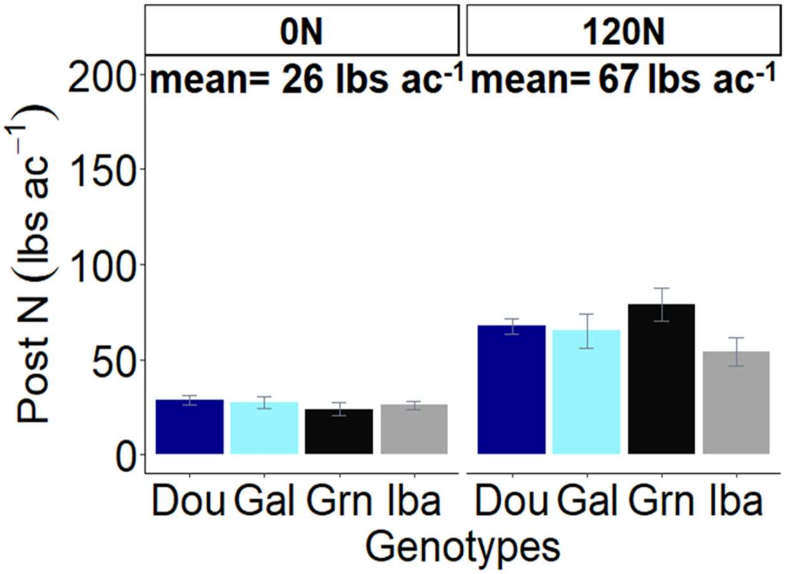

Figure 5. Leaf and stem nitrogen accumulation from flowering to maturity (Post N) for four varieties tested under zero N rate and 120 lbs N per acre in the 2019-2020 and 2020-2021 growing seasons, averaged across two locations (Perkins and Stillwater). Varieties were abbreviated as Dou, Doublestop CL+; Gal, Gallagher; and Grn, Green Hammer.

W I T

Execution of a UAV Phenotyping Platform

Phil Alderman

Plant and Soil Sciences

Successor to Dr. Gopal Kakani

Deliverables

1) Full-season imagery datasets (RGB, multispectral, hyperspectral, thermal, and LiDAR) on the Dual-Purpose Observation Nursery (DPON) at Stillwater, OK.
2) Automated post-processing workflows for each date of data collection for imagery (RGB, multispectral, hyperspectral, thermal and LiDAR).

Procedures

Deliverable 1. We will perform regular flights of the DPON at Stillwater, OK using a DJI Phantom 4 Multispectral drone and a DJI Matrice 600 equipped with Headwall Co-aligned VNIR/SWIR (hyperspectral) cameras, Velodyne LiDAR sensor, and FLIR thermal camera. The DPON is a 2,000+ breeding nursery central to the GrazenGrain breeding system, and represents the critical junction between inbred line development and line testing. The target frequency of flights will be once per two weeks during periods of active growth and once per month during dormancy. The data for each flight will be downloaded to a dedicated external drive and subsequently copied to a storage at the OSU High-Performance Computing Center (HPCC), where image post-processing operations will be performed.

Deliverable 2. We will develop customized computer scripts for downloading data from drones, for uploading and backing up imagery data to HPCC storage, and for performing post-processing (orthorectification, mosaicking and georeferencing) of the imagery. For RGB (redblue-green), multispectral, and thermal data, the open-source software Open Drone Map will be used. Hyperspectral and LiDAR data will be processed using the proprietary software (Hyperspec III) provided by the manufacturer. Open Drone Map and Hyperspec III are designed to be used interactively where each step of processing involves a user clicking within the graphical user interface. However, to automate this processing, we will write wrapper scripts to call the underlying software libraries that perform the actual image processing. The result will be a set of workflows customized to each data type that will allow a user to upload an imagery dataset and all the steps of processing will be performed automatically

W I T

Optimization of Grain Protein and Yield

Brian Arnall

Plant and Soil Sciences

Deliverables

1) Experimental lines with yield and quality stability across a range of N levels.
2) Understanding of nitrogen fertilizer management timing on two protein-divergent varieties, Gallagher and Green Hammer.
3) Determination of the varietal difference in optimum N application timing for maximizing yield and protein.

Procedures

Deliverable 1. WIT will continue using a low-N testing site at Tipton, OK to evaluate approximately 200 advanced lines in yield and quality trials at variable N levels. From this effort since 2015, WIT has uncovered wide diversity in apparent N-use efficiency (NUE) that previously went undetected.

New variety releases with consistently high yielding ability under N-challenged conditions *and* under optimal N supply are High Cotton, OK18205 (preliminary release consideration, SW OK), Green Hammer, Skydance, OK Corral, Strad CL Plus, Big Country, Smith's Gold, and the external check, Joe. Parameters currently guiding selection based on the yield data from Tipton are i) above-average yield in the optimal N treatment and ii) >65% of optimum-N yield in the N-challenged treatment, which now constitutes 40% and/or 60% of the optimal N rate in a given year. The novelty of this field site provides for no additional N available for plant uptake beyond what is applied. Application typically occurs in January to February each year.

Deliverable 2. Side by side trials each planted to a single variety, Gallagher or Green Hammer, will again be established at two locations. Each study will feature 13 fertility treatments replicated four times. The treatments will consist of a combination of N application timings (pre-plant, winter dormancy release, and jointing). All N may be applied at one time or spread across all three timings. Determination of yield and protein will enable understanding of protein stability in differing genotypes such as Gallagher and Green Hammer.

Deliverable 3. About 14 varieties will be evaluated under two unique N timings: i) all N preplant and green-up, and ii) all N applied post-jointing. Grain yield and wheat protein concentration will be determined, along with plant height, biomass, plant N content, head count, seed count, and kernel weight.

TIMELINES:

Current and forecasted pressures in the field and in the marketplace are what drive decisions and activities of the WIT. As those pressures are continuous or cyclic, so are the research endeavours described herein. Many of the research objectives identified in this proposal pertain to projects already in progress for a short-term duration of two to three years or for an indefinite period. Wheat variety development has been ongoing at OSU since the 1940s. ***The procedures described, however, should lead to the identified deliverables within one year.***

JUSTIFICATION:

In a variable climate that Oklahoma typically encounters, breeding efforts must be dedicated to both protecting yield potential and extending it, while at the same time preserving cherished gene complexes which provide a window of acceptable, if not desirable, grain functionality. Historically, that is indeed the mantra of wheat improvement programs across the globe, except perhaps to a lesser extent where the emphasis on yield per se may outweigh emphasis on yield protection. The argument could be made that genetic advances, at least in productivity of wheat, have come in the form of improved tolerance to stress, and largely, disease resistance. Such genetic advances may be largely transient.

Hence, it comes as no surprise that the WIT's mission focuses on protection against biotic stress factors, in which those effects are heightened even more by early-planting dates common in the southern Great Plains. WIT proposes to mitigate those stresses through an interactive, goal-oriented, product-directed, comprehensive research plan.

We are reminded, however, that buyers and consumers of Oklahoma wheat are indifferent to grain yield potential unless grain supply is at risk. Their desires, while unique and perhaps unfamiliar to the majority of Oklahoma producers, make our ultimate responsibility to develop not only that which can be produced but can be marketed and consumed. We relish this opportunity to strengthen every link in the supply chain – from producer to consumer.

RELATION TO OTHER RESEARCH:

The research proposed herein constitutes the majority of research efforts for WIT members Carver, Yan, and Aoun, whereas WIT members Xu, Arnall, Alderman, and Giles pursue other initiatives that are independently funded yet complementary to wheat improvement research, or they may work with other commodities. All team members assume other academic responsibilities in teaching and/or outreach.

REPORT OF ACCOMPLISHMENTS as of April 2023:

The Wheat Improvement Team gratefully acknowledges past continual support of the OWRF/OWC, without which these accomplishments would not have been achievable. Topping off WIT's accomplishments in 2022-2023, USDA-NASS data indicated that OSU-bred and OWRF-supported varieties accounted for the *top four most widely planted varieties* in Oklahoma for the 2022-2023 season.

- Published wheat variety trial reports in a practical format (i.e., print and online) to improve the public's accessibility of the information on variety characteristics. (*Silva*)

- Conducted second year of field experiments to measure in-season plant nitrogen fluctuation for a better understanding of the physiological traits associated with lower metabolic cost of protein deposition that will be required to break the yield-protein association. (*Silva*)
- Confirmed that some varieties were able to maintain consistently higher wheat protein than others, such as Doublestop CL+ and Green Hammer. The genetic basis for this superiority needs to be explored more deeply using physiological approaches. (*Silva*)
- Conducted second year of field experiments to compare winter wheat varieties for adaptation to a late planting date (in December). (*Silva*)
- Released OK18510 as ‘High Cotton’ with adaptability and disease resistance exceeding that of OSU’s most recent broad-utility varieties Showdown and Green Hammer. (*WIT*)
- Placed more than a dozen candidate lines under foundation seed increase which broadly feature i) novel end-use functionality worthy of additional value capture under contracted production (“OX” wheat), ii) improved Doublestop CL Plus derivatives that either possess or lack imazamox herbicide tolerance, iii) an improved Garrison derivative with proven head scab resistance worthy of production in eastern Oklahoma, and iv) short-season capability with finish maturity no later than Butler’s Gold. (*WIT*)
- Provided 10 wheat disease updates to wheat growers, consultants, extension educators and researchers, with secondary distribution by the Cereal Disease Lab (Minnesota), the OSU Plant Disease and Insect Diagnostic Lab, and the OSU Plant and Soil Sciences department. (*Aoun*)
- Among 984 OSU breeding lines, found a moderate to high frequency of effective resistance to one or more fungal and viral diseases common to Oklahoma. (*Aoun*)
- Evaluated 584 breeding lines from WIT and 790 hard winter wheat lines from the U.S. Great Plains for their reactions to leaf rust and stripe rust. (*Aoun*)
- Developed a double haploid population with pedigree Big County/Jagalene that will be used to identify novel disease resistance genes in Big Country. (*Aoun*)
- Confirmed the presence of double *SBEIIa* mutants in doubled haploid lines currently under statewide evaluation. (*Yan*)
- Modified KASP markers for detecting *ACCase* mutations in *ACCase-A*, *ACCase-B*, and *ACCase-D* on chromosomes 2A, 2B, and 2D in hexaploid wheat. (*Yan*)
- Identified Oklahoma winter wheat cultivars that can be used as host plants in future gene-editing and transformation. (*Yan*)
- Characterized novel leaf rust resistance gene *Lr622111*, and evaluated over 20 populations for leaf rust resistance to identify new resistance genes. (*Xu*)
- Identified a novel *Pm65* allele conferring a wide spectrum resistance to powdery mildew (designated *Pm351817*). (*Xu*)
- Discovered many greenbug resistant synthetic haploid wheat (SHW) lines that may enhance and diversify wheat greenbug resistance. (*Xu*)
- Coordinated purchasing and installation of Graphical Processing Units (GPUs) and increased Random Access Memory (RAM) upgrades to the TIGER system at the OSU HPCC for use with hyperspectral imagery processing. (*Alderman*)

Wheat Improvement Team
Budget, FY2023-2024

Principal Investigator (red means current)	BUDGET CATEGORY Wages & benefits (or salary if noted below)	Supplies & fees	Travel	Equipment and repair	Growth chamber & greenhouse rental	**TOTAL, $**
Carver	66,000	13,000	5,000	15,000		**99,000**
Carver – salary/benefits support*	37,463					**37,463**
Yan	30,000	23,000	3,000		6,000	**62,000**
Chen	13,000	18,000	2,000			**33,000**
Xu (sub-award)	4,730	23,000				**27,730**
Aoun	15,000	19,000	2,000		6,000	**42,000**
de Oliveira Silva	14,400	9,200*	3,000			**26,600**
Alderman	2,500*	200				**2,700**
Giles						**0**
Arnall						**0**
TOTAL						**330,493**

Carver

1. Wages and benefits support undergraduate employees.
2. Expendable supplies support field, greenhouse, and growth chamber activities.
3. Travel is dedicated predominately to in-state research activities, though up to 20% of Carver's travel may be outside Oklahoma to participate as an invited participant at conferences on demand.
4. Equipment and repair involve maintenance of large field equipment such as planters and combines or equipment housed in the wheat quality laboratory, and purchase of laboratory equipment on an as-needed basis for other WIT scientists.

*Salary/Benefit support for Carver accounts for scientist obligation (2 months or more) to department for Tina Johnson, Nathan Stepp, and Jason Ray. These are past salary+benefits expenditures for FY23. Salary for Melanie Bayles (50% support) is not budgeted here.

Yan

1. Personnel: Partial salary and benefits for Dr. Min Fan.
2. Supplies and fees: Mainly expendable supplies including standard laboratory chemicals and reagents, cloning kits, plasmid preparation kits, and gene expression kits to be used for molecular genetics research and genotyping, and

materials to grow plants in the greenhouse and growth chambers. Also included, gold particles and kits for gene-editing and producing transgenic plants. Fees include sequencing costs in the OSU Core Facility, and Eurofins.
3. Travel: Expenses for field work and professional conferences.
4. Greenhouse and growth chambers: rent and management fees.

Chen
1. Partial salary and benefits for a full-time quantitative genetics PhD graduate position is requested for genomic data analysis, including association mapping and genomic selection; the student is not expected to start until ca. midway through the funding cycle. The student who joins this project should has already been trained in Bayesian and genetic statistics.
2. Laboratory supplies for sequencing library preparation, including chemicals, devices, consumable plastics like tips and trays, are requested. Costs to purchase sequencing kits for ligation adaptors, Covaris g-tubes for fragmentation and size selection, PicoGreen to quantify DNA extracts, as well as sequencing devices like flow cells, are requested. Contractual costs are planned for DNA library preparation, genotyping, and the relevant data for varieties for prediction purposes.
3. Travel costs include Chen's participation in the 2023 Crop Science Society of America annual meeting.

Xu
1. Partial salary ($3819) and benefits ($912) (1 month) for a research associate.
2. Materials and supplies: sequencing, PCR primers (DNA oligos), PCR plates, centrifuge tubes, PCR reagents, chemicals, real-time PCR plates, deep plates, steel beads, and greenhouse supplies (soil, sand, growing trays, fertilizer, etc).

Aoun
1. Total for wages ($14,498) and benefits ($501) for undergraduate employees
2. Expendable supplies support field, greenhouse/growth chamber experiments: soil, fertilizer, pesticides, and plant growing trays. Laboratory supplies include DNA extraction plates, steel beads for grinding leaf tissue, chemicals for DNA extraction, KASP primers, real time PCR plates, PCR reagents, and supplies for inoculum preparation.
3. Travel is dedicated predominately to in-state research activities.
4. Fees for growth chamber ($2,046) and NRC greenhouse ($3,996)

Silva
1. Wages and benefit: 1440 hours at a rate of $10/hour requested for undergraduate labor assisting in seed weighing, planting, plot management, harvesting, sampling processing for nutrient analysis, and data collection.
2. Materials and supplies: A total of $1,520 is requested for supplies for plot management, chemicals, and syringes to inject ^{15}N into soil, metal box to apply water with the ^{15}N application, and data collection. A total of $7680 is requested to cover the costs of the total N tissue analysis, encompassing two locations, 4 reps, 2 N rates, 4 varieties, 4 growth stages at jointing; at flowering, whole plant; at soft dough, leaf+stem+head; and at final maturity, leaf+stem+grain+chaff.
3. Travel: Partially cover travel to trial locations for planting, plant growth and development assessments, disease ratings, treatment applications, biomass harvesting, data collection, and harvesting.

Alderman
1. Wages and benefits support undergraduate employees.
2. Materials and supplies: Dedicated external hard drive for primary storage of imagery

Arnall
Will use other funds in FY24 to continue ongoing OWRF projects with WIT

Giles
Will use prior-year funding and consider new avenues for WIT engagement.

SIGNATURE PAGE

Genetic Improvement of Winter Wheat: Integrating Classical and Novel Approaches

Submitted by:

OSU's Wheat Improvement Team

Proposed Funding Level: $ 330,493.00

Brett F. Carver

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Plant & Soil Sciences

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Genetic Improvement of Winter Wheat: Integrating Classical and Novel Approaches

Submitted by:

OSU's Wheat Improvement Team

Proposed Funding Level: $ 330,493.00

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