OSU WIT Research Proposal OWRF FY2022-2023 Genetic improvement of winter wheat: Integrating classical and novel approaches

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OSU WHEAT IMPROVEMENT TEAM	
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Identification of Insect Resistance	
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Candidate Variety Assessment and Applied Wheat Phys	siology
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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 currently under revocation by ARS

Commercial validation of end-use quality Jayne Bock, Wheat Marketing Center Sean Finnie, Bay State Milling Dave Katzke, Steve Eichten, General Mills Reuben McLean, Kathy Brower, Grain Craft Scott Baker, Vance Lamb, Ardent Milling Brady Sidwell, Graison Gill, Chisholm Milling, Bellegarde Bakery

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

Gliadin analysis and toxicity; 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

Agricultural Research Institute, Martonvasar, Hungary (Gyula Vida) National Agric. Res. and Development Institute, Fundulea, Romania (Nickolae Saulescu)

FUNDING HISTORY:

Amount received, previous 7 years (average, \$361,674):

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

Amount requested FY23: **\$393,243** (+\$31,569 above 7-yr average)

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 longstanding 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.

Projected goals for the proposed work period include the following:

- a) Final development and initial seed distribution of a functionally unique but yieldcompetitive HRW variety (ies) that generate not only flour but a flour *ingredient* otherwise present as processed vital wheat gluten or as native hard red spring wheat,
- b) Targeted seed distribution of a high-protein, strong-gluten, *soft red winter* wheat adapted to the central wheat corridor of Oklahoma,
- c) Identification of advanced lines with a stacked combination of yield enhancing genes discovered by WIT scientists,
- d) First wave of BYD-immune Doublestop CL Plus derivatives with or *without the imazamox* herbicide tolerance trait,
- e) First public rendering 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 actually 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) An adapted candidate line that may be commercialized as the first *Fhb1*-source of resistance to head scab in HRW wheat intended for *eastern* Oklahoma,
- i) First report card on variety availability for, and prospects for developing *short-season* genotypes with, adaptation to late planting regimes, and
- j) Development of preliminary inbred lines featuring 2-gene herbicide tolerance fit for the CoAXium system.

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.

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, <u>most</u> 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 these lines are 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 **GrazenGrain**® 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 F₂-F₄ generations of selection, during which cattle represent the primary selection agent, occurs in central Oklahoma near Okarche (Figure 1), although the drought of 2021-22 disallowed cattle grazing in those breeding populations. The **GrazenGrain® 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 <u>Oklahoma panhandle region</u>. This branch constitutes the *High Plains pipeline*. We use *limited irrigation* at the OPREC in Goodwell, OK to establish a more reliable *dryland* nursery. Irrigation is used only to ensure crop establishment and a minimal yield potential of 30 bu/ac. Unfortunately, we could not achieve an average of 25-30 bu/ac in neither 2020 nor 2021, which brought this pipeline to a stall position.

This pipeline relies on the same *modified bulk-pedigree* selection method used in the **GrazenGrain®** pipeline, except selection and population advancement in the earlier inbreeding generations (F_2 - F_4) occurs at OPREC (Goodwell, OK). 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. Previous candidate, OK19P825 was discontinued following the 2021 harvest, due to an elevated risk to infection by stripe rust or leaf rust. Candidate OK19P808 (CO050337-2/OK10218//OK10132) overtook the pole position in this pipeline, leading to a decision forthcoming in summer 2022 to place it under foundation seed increase.

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. Central to this pipeline in fall 2022 will be our first round of AXigen 2-gene lines (n=139) capable of the CoAXium production system. Confidence remains high that one or more of these AXigen experimental lines will qualify for release by the end of the 2023-2024 crop season, marking a highly accelerated path to commercialization.

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 <u>populations</u> of plants through the early inbreeding generations immediately following the cross, desirable <u>families</u> are selected for yield-determining characters, from which lines (sibs) <u>within families</u> 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 pipeline*. One candidate particularly stands out for possible release in 2022 – OK16103083, a Garrison derivative featuring *Fhb1*-mediated scab resistance with adaptation to eastern Oklahoma, and discussed further below.

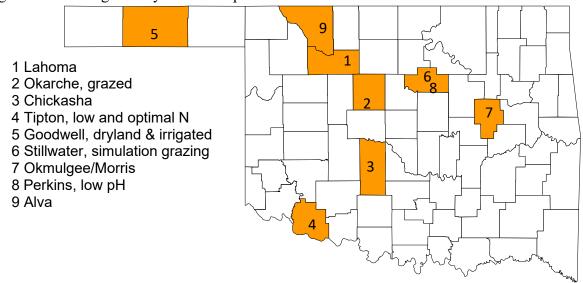


Figure 1. Breeding nursery sites anticipated in 2022-23.

Additional Advanced Line Outlook (Tables 1-2). OK15MASBx7 ARS 8-20, OK15MASBx7 ARS 8-29, or OK15DMASBx7 ARS 6-8

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*, provides an unprecedented level of dough strength with unique value to millers and bakers, and thus consumers, when introgression occurs within certain genetic backgrounds. In other words, the positive effect of Bx7oe is not universal, nor is it readily predictable. Snowmass, a hard white cultivar developed by Colorado State University, was used as the *Glu-B1al* donor, and OK10130, Billings, Gallagher, and OK09634 were used as the recipient parents. Nearly10 years later, and after round and round of yield and quality testing in cooperation with our milling industry partners, three surviving progeny remain as named above in the section header.

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 <u>backcrossed</u> derivation makes them worthy of **GrazenGrain**® distinction. OK10130 lost to Bentley in the decision to release in 2015, and 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 were evaluated by the Wheat Marketing Center in Portland, OR to assess their relative gluten and dough strength and value to the grain supply chain (report available through the Oklahoma Wheat Commission). All candidates were as strong as, or even stronger than, Canadian Western Extra Strong wheat; yet, baking quality, including water absorption, met or exceeded HRW expectations. 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 Bx70e derivatives into the supply chain.

OSU's Office of Technology Commercialization is currently in negotiations with the company, Farm Strategy, led by Andrew Hoelscher, to execute the entire supply chain from grain production to flour milling using this unique germplasm. Seed production will still occur under the direction of an independent licensee, such as OGI. As of April 2022, it is not known which of these three candidates, if not all of them, will be dedicated to contract production. Commercialization of all three candidates will more likely satisfy the projected level of demand for this kind of functionality, Two large U.S. milling companies are currently working with the germplasm to internally investigate optimum usage in displacement of flour additives, vital wheat gluten, or perhaps hard red spring wheat (Canadian or U.S.) in their own formulations.

Looking beyond this panel of candidates, reselected progenies of these and other Bx70e derivatives will be available for release consideration in the next 1-3 years. In the next 4-5 years (by year 2025), this level of functionality will occur in a wide assortment of other contemporary genetic backgrounds with frequency <u>exceeding 15%</u> (a conservative estimate) 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 – Which one, if not all three lines, will be launched? Agronomic and functionality assessments are generally satisfied. This remains to be determined pending market analysis by Farm Strategy, which should be completed by summer 2022. Among the three lines, 225 acres of foundation seed increase are on schedule for harvest in summer 2022.

OCW03S580S-8WF

Background – Soft red winter (SRW) wheat is commonly used as a source of disease resistance, acid-soil tolerance, and high yield potential in the **GrazenGrain**® pipeline. OK Corral and Uncharted are WIT's most recent HRW releases featuring 25% SRW parentage. During the breeding process, WIT will consider both SRW and HRW progeny, but with obvious priority to HRW descendants. When considering a SRW progeny, preference is given to HRW-like functionality; hence, emphasis is placed on the combination of 12% wheat protein or higher, good mixing tolerance, and acceptable breadbaking quality.

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, or what WIT refers to as a SRW wheat with crossover functionality. In 2019 it was evaluated at the Wheat Marketing Center for potential use in cracker or bread applications. Higher than acceptable formula water requirements would render its preferred use in a cracker flour blend with <u>soft wheat</u>. Bread loaf volume and crumb structure may be fitting for a bread flour, though bake absorption and tolerance to over-mixing may be moderately low.

OCW03S580S-8WF was selected as an F_5 -derived line from the single cross, G991502/KSU Bulk seln 00F5-11-2, by Dr. Art Klatt. OCW03S580S-8WF is adapted to the traditional HRW central corridor, extending from the Texas Rolling Plains through Oklahoma into south central Kansas. It exhibits high levels of acid-soil tolerance and recovery from suboptimal nitrogen supply, and effective resistance to the predominant fungal and viral diseases common to Oklahoma. The weakest link in this disease package may be stripe rust resistance, depending on the level of *Yr17* virulence present in the pathogen population in a given year. From 2015 to 2020, OCW03S580S-8F exceeded the mean yield of Gallagher, the most common HRW cultivar planted in Oklahoma, by 7 bu/ac, but the test weight of OCW03S580S-8F was 1.8 lb/bu lower. The SRW section of the Wheat Quality Council evaluation confirmed in 2021 that this line would provide a desirable flour for cracker products.

Yet to determine – Contrary to opinion held within WIT until 2022, a viable market for contracted sourcing of OCW03S580S-8WF may not come from mills that routinely run soft and hard wheat blends. The best use of this germplasm may emanate, instead, from the HRW-like protein *levels* shown by OCW03S580S-8WF. A <u>higher</u>-protein, <u>soft</u>-endosperm with good gluten <u>extractability</u> may be the more likely driver of adoption of this germplasm by the vital wheat gluten manufacturing facilities growing in Kansas. This market fit remains under investigation with Farm Strategy, while ample foundation seed has already been produced.

OK16103083

Background – OK16103083 (Garrison*3/Wesley Fhb1-106) had everything going for it up until 2019, when it was removed from the WIT VDP due to undesirable stripe rust reaction in north central Oklahoma. With the need voiced from eastern Oklahoma for additional Fusarium head blight, or scab, resistance, this line was pulled out of long-term storage in fall 2020 and retested 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. Thus, this line is being further tested primarily in eastern OK for more detailed monitoring of scab protection, with DON analysis expected in 2022. *Yet to determine* – From a breeding perspective, OK16103083 may provide 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 2022 performance, particularly in the wheat variety trial conducted by Silva near Morris. This will allow WIT to gauge competitiveness in a broader varietal lineup than available in breeding nurseries. OK16103083 is under preliminary foundation seed increase in 2022.

Doublestop trait-free derivatives (five, non-CL+)

Background – This germplasm now under foundation seed increase as five sister lines 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, elite lines are now in the final stages of selection and breeder-advancement.

A 5 to 10 bu/ac bump in statewide mean yield is expected in this germplasm. Experimental lines having the root, OK16107133-, appear more likely to provide that yield bump, though results from 2022 are sorely needed to confirm. Disease resistance in this part of the pipeline is outstanding, as is acid-soil tolerance, and Hessian fly resistance is frequently present due to the donor, OK12621, supplying the resistance from Duster.

Yet to determine – This first flush of five advanced lines will be differentiated in spring and summer 2022 for yield, test weight, disease resistance, acid-soil tolerance, straw strength, desirable end-use quality, and the absence of herbicide tolerance genetics. From that process, one or more will be chosen in summer 2022 for further advancement and accelerated foundation seed production.

Doublestop CL+ derivatives (four)

Background – Two lineages represent these four CL+ lines, all with Doublestop CL+ as 50% of their parentage. One lineage with two lines originated with the *Bdv2*-introgression program mentioned in the previous section. The second lineage, also with two lines, features unique parentage on the other side of Doublestop CL+, and of those two, OK198417C is the early favorite over OK198438C due to its much broader disease resistance package. Both have been outstanding for yield, yet about average for end-use quality and certainly not in the same class as Doublestop CL+.

Yet to determine – Critical to the remainder of the 2022 crop season will be to examine all four Doublestop CL+ derivatives under the same roof, while all undergo foundation seed increase. WIT anticipates sufficient data will emerge in summer 2022 to make a single recommendation for release, but critical to that decision will be clear separation from Doublestop CL+ for statewide yield performance, enhanced disease protection, and acceptable functionality.

OK18510

Background – WIT has had minimal success in perpetuating the Ruby Lee combination of yield and quality, until OK18510 (TCI982345/Ruby Lee//OK10415). This HRW candidate

was the highest yield-ranking experimental in elite trials during the past four years, with a mean yield rank of 2.8 of about 40 entries. OK18510 exhibits good GrazenGrain fitness, wide adaptation, and it can easily go west into the panhandle. Results from 2022 and beyond must show yield superiority to Showdown, Green Hammer, and Smith's Gold, though OK18510 has shown susceptibility to Hessian fly (greenhouse tests only), BYD, and powdery mildew.

Yet to determine – Performance data in USDA regional performance nurseries and in the OSU wheat variety trials are needed to ascertain the best region of adaptation. Industry-partner assessment of functionality is needed to confirm our own lab findings.

Dating back to Gallagher, which was released in 2012, 15 HRW varieties from OSU are now circulating throughout Oklahoma and parts of Texas and Kansas. All are profiled in Table 3 with the newest information, showing the more obvious strengths and weaknesses as a backdrop for the next wave of advanced lines.

Candidate	Pedigree	OFSS	Featured traits
OK15MASBx7 ARS 8-20	Gallagher*3/Snowmass	2	Gallagher with HRS-like dough strength
OK15MASBx7 ARS 8-29	Gallagher*3/Snowmass	2	Gallagher with HRS-like dough strength, mod. resistance to WSM
OK15DMASBx7 ARS 6-8	OK10130*3/Snowmass	2	Highest yielding HRW with HRS-like dough strength; later & taller
OCW03S580S-8WF	G991502/BULK SELN 00F5-11-2	5	High yield, disease-resistant SRW with HRW-like quality
OK16107123 thru -133 (5 candidates)	OK12621/Doublestop CL+	1	Doublestop CL+ with deep disease resistance packages (non-CL+)
OK16103083	Garrison*3/Wesley Fhb1-106	1	Garrison with FHB resistance and high yield potential in E OK
OK18510	TCI982345/Ruby Lee//OK10415	1	Highest yield-rank experimental past 4 yrs; intended for IWM systems
OK16107125-18HR-4C	OK12621/Doublestop CL+	1	Doublestop CL+ with BYD immunity (like Uncharted); CL+
OK16107125C-17HR-2	OK12621/Doublestop CL+	2	Doublestop CL+ with BYD immunity (like Uncharted); CL+
OK198417C	OK10943C/OK09935C//Doublestop CL+	2	Yield exceeds Strad CL Plus, but lower quality; broad disease resistance; CL+
OK198438C	OK12911C/Doublestop CL+	2	Yield exceeds Strad CL Plus with same quality; CL+

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 2022. High-priority candidates indicated by orange highlight; lower priority, no highlight.

	Trait category ^a									_		
Candidate	DP	HF	YR	LR	TS	PM	V	AST	SS	BQ	TW	Weaknesses
OK15MASBx7 ARS 8-20	1	1	1	1	3	1	1	4	3	1	1	Physiological leaf spotting
OK15MASBx7 ARS 8-29	1	1	1	2	3	1	1	3	3	1	2	Leaf spotting
OK15DMASBx7 ARS 6-8	1	5	2	3	3	2	1	3	2	1	2	Leaf rolling
OCW03S580S-8WF	3	3	2	1	2	1	1	1	1	3	4	Minor shattering
OK16103083	1	5	4	1	1	1	1	1	2	3	2	Stripe rust, Hessian fly
OK18510	2	5	2	1	3	3	1	3	1	2	2	Hessian fly
OK16107133-18HR-4	1	1	2	1	1	1	1	1	1	1	3	Minor shattering possible, late
OK16107125-18HR-4C	1	5	1	1	3	1	1	1	2	1	1	Minor shattering possible, late
OK198417C	1	2	1	1	-	1	4	1	1	2	1	Tends mod. late and tall

Table 2. Trait ratings (1-to-5 scale) for highest-priority candidate varieties placed under seed increase in fall 2021 with Oklahoma Foundation Seed Stocks.

^aTrait categories abbreviated as DP, dual-purpose capability (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.

Table 3. Essential characteristics of all OSU HRW wheat varieties dating back to Gallagher.

	Stripe rust	Leaf rust	Barley yellow dwarf	Powdery mildew	Hessian fly	Low pH	Grazing	Test weight	Protein
<u>L</u>	Field (adult plant) resistance	Field (adult plant) resistance	Resistance equivalent to Duster (intermed. to moderate resistance)	Field (adult plant) resistance, equivalent to Duster	Resistance to Great Plains biotype	Tolerance sufficient to reach normal grain yield at pH <u>4.8</u>	Recommended for dual-purpose systems at conventional stocking rates	Should exceed 60 lb/bu during normal harvest window	Should exceed 12.0% wheat protein with recommended N fertilization
Gallagher	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Doublestop CL+	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Bentley	No	No	Yes	No	No	Yes	Yes	IC	Yes
Smith's Gold	Yes	No	No	Yes	Yes	No	Yes	Yes	Yes
Lonerider	No	No	No	Yes	Yes	Yes	No	Yes	Yes
Green Hammer	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes
Baker's Ann	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes
Showdown	Yes	No	No	Yes	Yes	No	Yes	Yes	Yes
Skydance	Yes	Yes		Yes	No	Yes	No	Yes	Yes
OK Corral	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes
Uncharted	Yes	Yes	Immune	Yes	No	Yes	Yes	Yes	Yes
Big Country	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes
Butler's Gold	Yes	Yes	No	No	No	Yes	No	Yes	Yes
Strad CL Plus	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes
Breakthrough	No	No	No	No	No	No	Yes	Yes	Yes

WIT Gene Discovery and Molecular Breeding (2 proposals) Liuling Yan Plant and Soil Sciences

Deliverables

- 1) Identification of *TaCol-B5* and *QYld.osu-1BS* gene stacks for higher grain yield in WIT germplasm.
- 2) Gene-edited *Rht1* plants from 'Skydance' and other yet-named OSU varieties.
- 3) Pyramid of genetic loci for resistance against insects and viruses.
- 4) *AHASL*-marker selected or confirmed genotypes (wild type or resistance alleles for imazamox resistance in the CL Plus system), triple *SBE* mutants with high amylose phenotype, and an improved marker assay for *ACCase* mutations in the CoAXium system.

Overview and Procedures

Deliverable 1. Previous studies on yield genes were financially supported by OWRF funds, leveraged by other related USDA and OCAST grants. We cloned a major gene on chromosome arm 7BL that increases the number of spikelets, or meshes, per spike. By association, this extends the length of the spike and enhances grain yield. We originally reported this gene as *SBY1* and *WSA1* in previous reports to OWRF. The effects of the over-expressed gene on grain yield in transgenic wheat plants were confirmed with one more year of funding. Finally, this gene was named *TaCol-B5* to be more consistent with its orthologue (*CONSTANS-like 5*) in other plant species. In the last field experiment conducted outside of Oklahoma, *TaCOL-B5* was found to promote tillerering and thereby increase the number of spikes per plant, generate more spikelets and thereby increase the number of grains per spike, and extend spike length and thereby decrease spikelet density. Ultimately, *TaCol-B5* enhanced field-based grain yield by 11.9%. We published this work in *Science* on April 7, 2022

(<u>https://www.science.org/doi/10.1126/science.abm0717</u>). *Science* magazine also published a perspective paper on our gene in the same issue

(https://www.science.org/doi/10.1126/science.abo7429), which stated "The discovery of *TaCol-B5* is a milestone toward enhancing yield in cereals because it improves our understanding of molecular mechanisms that control yield related architectural traits". Further, "the identification of *TaCol-B5* offers a new route to maximize yield in wheat", as commented by Dr. Wilma van Esse, who wrote the perspective paper for *Science*.

In transgenic field and greenhouse experiments, TaCol-B5 increased the number of kernels per spike while not decreasing kernel weight. The trade-off between three individual yield components is a major bottleneck for further yield improvement. More kernels produced by TaCol-B5 will be located in the first and second floret positions in additional spikelets, whereas more kernels gained in the absence of increased spikelet number must set in the third, fourth, or higher seed positions. The higher the position a kernel resides in the spikelet, the less it will likely weigh, thus illustrating the trade-off between the number of kernels and kernel weight. TaCol-B5 can be used to remove some of this trade-off by extending the size of the "sink" to accommodate more spikelets and thus improve productivity, provided the photosynthetic source nourishing this sink is up to the task under allowable environmental conditions.

Another critical finding in our *Science* paper is that the *TaCol-B5* allele for higher grain yield is found in ancestral emmer wheat, but only 2% of an international modern wheat variety panel in that study possessed the same *TaCol-B5* allele as the original source, CItr17600 from CIMMYT (33 of 1657 varieties). Among 479 North American varieties we genotyped, only 15 of them were found to have the *TaCol-B5* allele, including 'Sioux', 'Quanah', 'Caddo', 'Sturdy', 'Gent', 'Newana', 'Kitt', 'Coronation', 'Renown', 'Pembina', 'Marberg', 'Hi-Line', 'AC Reed', 'AC Phil', and 'Iona'. Among 49 U.S. wheat accessions, only three varieties, Billings, McNeal, and RSI5 (PI584453), were found to have the *TaCol-B5* allele. Among 57 U.S. HRW varieties from the Great Plains, only the OSU variety 'Baker's Ann' was found to have the *TaCol-B5* allele. Interestingly, its closely related half-sib, 'Skydance', did not possess this gene. We have extensively utilized Billings and Baker's Ann and their descendants as parental lines in WIT breeding populations.

The story on *TaCol-B5* is not over yet. In the new funding year, we will test how *TaCol-B5* functions in various genetic backgrounds in winter wheat varieties (research in progress by Carver). We will use molecular markers for tracking *TaCol-B5* and effectively select for lines that carry the *TaCol-B5* allele. In addition, we will use molecular markers to track another major gene for grain yield at the *QYld.osu-1BS* locus on chromosome arm 1BS that was found in 'Duster' in our previous studies. The *QYld.osu-1BS* allele is unique among 100 genetically diverse hexaploid wheat varieties or experimental lines. We are aiming to stack *QYld.osu-1BS* and *TaCOL-B5* in WIT germplasm to enhance yield potential.

Deliverable 2. The TaCol-B5 allele has low frequency in the common wheat gene pool, perhaps due to its phenotypic linkage with increased plant height. This observation would implore that TaCol-B5, as a spontaneous mutation (ancestral form), be targeted for improvement in spikelet production and associated grain yield while simultaneously targeting reduced height genes for reduction in height. While many alternative height-reduction genes have already been discovered such as *Rht8* (in Spirit Rider) and *Rht18*, one other option is to edit the more common semi-dwarfing genes already present in Oklahoma wheat.

We have successfully identified a sgRNA editing site in the primary height-reduction gene, *Rht1*, and effectively edited this gene in a spring wheat variety. The edited *Rht1* proteins interacted with *TaGID1* in the presence and *not* the absence of GA₃, indicating sensitivity to gibberellic acid. This discovery was important because these *Rht1*-edited GA-sensitive wheat plants showed semidwarfism (confidential information), suggesting a potential *decoupling* of reduced plant height and reduced coleoptile elongation.

Additionally, the GA-sensitive *Rht8* was recently cloned, driven by its lack of negative effects on coleoptile length in the presence of semidwarfism. We will use the same sgRNA to edit the *Rht1* gene in 'Skydance', which has shown regeneration ability from tissue culture in our preliminary experiments. It is expected that the edited *Rht1* in OSU wheat varieties would not have any negative effect on coleoptile elongation in moisture-deprived environments where deeper sowing is needed for successful plant establishment. This study may bring forth a second revolution of the Green Revolution genes in wheat.

Deliverable 3. As reported in the previous funding year, we identified candidate genes for *TaHf-A1* on chromosome arm 1AS and used transgenic approaches to validate the functions of the candidate genes for Hessian fly resistance. In addition, we developed KASP markers for rapid deployment of *TaHf-A1* in breeding populations. In the new funding year, we will improve our understanding of the responsible locus or loci for Hessian fly resistance in variety 'OK Corral'. This variety was produced in a completely different genetic track than Duster or 2174,

both which feature two distinct genes on the same chromosome arm 1AS. Given its pedigree, Hessian fly resistance in OK Corral could have originated in soft red winter wheat.

Another effort on insect resistance genes is utilization of *Cmc4* in WIT breeding populations. Two KASP markers (SDOKSNP6314 and SDOKSNP9699) flanking the *Cmc4* gene were developed in Dr. Guihua Bai's lab (USDA-ARS, Manhattan, KS) using the highly resistant experimental line, OK05312. However, when we tested OK05312 from our own seed stock, it was heterozygous for the first KASP marker and susceptible for the second one. To cross-check the marker results, 'KS Western Star' a new variety known to have *Cmc4* from a different genetic origin, produced the expected marker outcomes. Out of 144 genotypes, only two lines were positive for both markers. The first marker (SDOKSNP6314) worked well; all but the two positive lines produced wildtype marker alleles. The second marker (SDOKSNP9699), however, worked only on the same two lines, but failed for all others. The KASP PCR conditions were used per published procedures. We will continue to determine sources of the *Cmc4* gene, while at the same time, stacking this source of curl mite resistance with one or more sources of wheat streak mosaic virus (WSM) resistance (genes *Wsm1*, *Wsm2*, and *Wsm3*) currently in the WIT variety development pipeline.

The presence of resistance against Hf, WCM, and WSM throughout the WIT pipeline has become an all-time high. Several breeding lines have *Wsm1* or *Wsm2*, some have *Wsm3*, and OK05312 has proven curl mite resistance. We will use the previous markers to incorporate resistance against these insects and viruses into the variety development pipeline, with the ultimate goal that *Hf*, *Wsm1* (or *Wsm2* or *Wsm3*), and *Cmc4* are combined in the same genetic background.

Deliverable 4. As reported in the previous funding years, KASP assays developed by BASF were successfully used to identify mutants conferring imazamox resistance in Clearfield wheat in multiple breeding populations, or in genetic stocks with commercialization potential, or parental stocks deployed in the WIT hybridization program. This work will continue to effectively validate phenotypes either lacking or possessing the necessary mutations for imazamox resistance.

On the other hand, KASP markers for ACCase mutations developed by Limagrain Cereal Seeds did not perform as expected. Initial results of these markers on varieties known to have the mutations showed the expected genotype for ACCase-A and ACCase-D genes but not for the ACCase-B gene. The marker for ACCase-B gene identified wildtype alleles in Smith's Gold as expected but produced an inappropriate heterozygous reaction for three other varieties (Crescent AX, LCS-Fusion AX, and LCS-Photon AX) possessing mutations for ACCase-A and D genes. The original hypothesis was that heterogeneity existed at this locus within these three varieties for ACCase-B, as we pooled leaf samples from at least three different plants. However, when we tested six individual leaf samples from another CoAXium-confirmed variety, it was clear that the ACCase-B marker was not specific in the background of other ACCase mutants and produced false positive results. We concluded that the presence of SNPs in the primer binding sequences may suggest the appearance of intra-varietal segregation when it truly does not exist. We will optimize the reaction with annealing different temperatures or obtain mutant sequences for designing new KASP markers to resolve this issue.

In previous OWRF-funded research, we developed gene markers for mutations that reduce starch branching enzyme II (*SBEII*) activity, which by default increases the proportion of resistant starch (amylose) in wheat flour. We have already produced single or double mutants, and segregating lines that could produce triple-mutant progeny, in previous breeding cycles. In

the new funding year, we will determine DH lines homozyogous for all three mutations by PCR genotyping (*SBEII-A*, *SBEII-B* and *SBEII-D*) to explore a commercialization opportunity, in conjunction with starch phenotyping assays.

Xiangyang Xu USDA-ARS, Stillwater, OK

Deliverables

- 1) Locally adapted progenies carrying newly identified pest and disease resistance genes.
- Further characterization of leaf rust (LR) resistance gene *Lr622121*, and identities of LR resistance genes found in PI 578040 and PI 565358, with KASP markers closely linked to these genes.
- 3) Recombinant inbred line (RIL) populations enabling genome-wide mapping of stripe rust resistance genes in PI 57348, PI 627871, and PI 470121.
- 4) Identities of powdery mildew (PM) resistance genes in PI 606247, PI 57348, and PI 627871.

Procedures

Deliverable 1. With past support of OWRF, we identified a few genes conferring resistance to powdery mildew (*Pm59*, *Pm63*, *Pm65*, *Pm223899*, and *PmBN418*), leaf rust (*Lr81*, *Lr622111*, and *QLr.stars-1BS*), and greenbug (*Gb8*). Of these, *Pm59*, *Pm63*, *Pm223899*, and *Lr622111* were identified in landraces, while *QLr.stars-1RS*, *Pm65*, *PmBN418*, and *Lr81* were found in exotic varieties. Crosses or backcrosses have been made to introgress them into a set of WIT breeding lines. We will continue this introgression in 2022-2023, so that Oklahoma wheat producers can reap the benefits of these novel gene discoveries (Table 4).

In addition, *Gb8* was identified in a U.S. reselection line PI 697274 (formerly PI 595379-1). Crosses have been made to combine *Gb8* with the Hessian fly resistance present in Gallagher and the barley yellow dwarf resistance present in Uncharted, using WIT lines Uncharted, OK16107131 and OK16107155. Marker-assisted selection will be conducted in the seedling stage to select progenies carrying the target genes in backcross or/and topcross populations in 2022-2023, which will be bulked for further tests and selection in the field by Carver.

Deliverable 2. PI 622111 exhibited near-immune resistance to a mixture of LR pathotypes collected in Oklahoma, and we mapped the LR resistance gene in PI 622111, designated Lr622121, in the terminal region of chromosome 5BS and developed KASP markers for its introgression into WIT lines. In 2021, we conducted an allelism test to determine its relationship with Lr52, which also mapped on 5BS but responded differently to LR races collected from Oklahoma. However, the allelism test result was not conclusive due to poor seed germination. We produced new seeds from the cross PI 622111 × CSTR 442 (Lr52) in 2021-2022, and we plan to conduct another allelism test and characterize Lr622111 using LR races from different regions of the USA in 2022-2023.

PI 578040 is a breeding line developed in Florida, and PI 565358 is a landrace collected from Uzbekistan. Both PI 578040 and PI 565358 exhibited superior resistance to LR races collected from Oklahoma. We have developed $F_{6:7}$ RIL populations from PI578040 × CItr 11349 and PI 565358 × TAM 110 to characterize the LR resistance genes in each accession according to their response to LR race *Pt52-2* (race *MMPSD*). We intend to genotype the two F₇ RIL populations using genotyping-by-sequencing. In brief, genomic DNA will be digested with *PstI* and *MspI* (New England Biolab), ligated to barcoded adapters using T4 ligase, and PCR- amplified to construct the 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 LR resistance genes, and SNP markers closely linked to the genes will be converted to KASP markers.

Deliverable 3. PI 57348 and PI 627871 exhibit excellent resistance to both stripe rust and powdery mildew, and PI 57348 also confers resistance to Hessian fly. We have developed RIL populations from crosses Jagalene × PI 57348 and PI 627871 × Jagalene using the single seed descent approach. Jagalene was chosen as the opposite parent for its susceptible reaction to most diseases and pests. However, seeds are insufficient for testing multiple traits in multiple environments. Similarly, PI 470121 carries the LR resistance gene *Lr81* and confers adult plant resistance to stripe rust. We have developed a RIL population from the cross PI 470121 × Stardust, with limited seeds for each line. We plan to increase seeds for these populations in 2022-2023.

Deliverable 4. We have evaluated the response of an $F_{2:3}$ population derived from PI 606247 × Jagalene and genotyped a selected set of F_2 plants using the genotyping-by-sequencing (GBS) approach. Bulked-segregate analysis indicated that a single gene on chromosome 5D conferred resistance to PM isolates from Oklahoma. We will convert about 30 GBS-SNPs to KASP markers to construct a linkage map. Primers will be designed using the PolyMarker program, and a protocol based on the OT2-Pippetting Robot and ABI ViiA7 Real-time PCR system (Thermo Fisher Scientific, MA) will be followed to genotype the F_2 population.

The RIL populations developed from crosses Jagalene × PI 57348 and PI 627871 × Jagalene will be genotyped using the GBS approach described above, and evaluated for response to PM isolate OKS(14)-B-3-1, which was avirulent to PI 57348 and PI 627871 but virulent to Jagalene. The GBS-SNPs will be used to map the powdery mildew resistance genes, and the GBS-SNPs flanking the resistance genes will be converted to KASP markers.

In addition, we plan to evaluate \sim 150 WIT breeding lines for resistance to greenbug biotype E and Russian wheat aphid (RWA) biotype 2. The resistant lines will be further evaluated for responses to other RWA or greenbug biotypes.

Accession	Breeding status	Target gene/QTL	Geographic origin		
PI 181356	Landrace	Pm59	Afghanistan		
PI 628024	Landrace	Pm63	Iran		
Xinmai 208	Variety	Pm65	China		
PI 606247	Variety	Pm606247	Czech Republic		
PI 223899	Landrace	Pm223899	Afghanistan		
Bainong 418	Variety	PmBN418 QLr.stars-1RS	China		
PI 470121	Experimental line	Lr81	Croatia		
PI 622121	Landrace	Lr622111	Iran		
PI 697274	Reselection line	Gb8	USA		

Table 4. Wheat accessions currently accessed in the WIT gene introgression program, their	•
breeding status, target genes/QTL, and geographic origin.	

W I T Enhancement of Disease Resistance Meriem Aoun Entomology and Plant Pathology

Deliverables

- (1) Disease reactions of OSU breeder lines to the wheat soil-borne mosaic/wheat spindle streak mosaic (WSBM/WSSM) complex, leaf rust, powdery mildew, tan spot, spot blotch, and BYD based on a combination of testing in the greenhouse and/or field.
- (2) Preliminary genotypic/phenotypic profile of an association mapping population from WIT breeding lines and released cultivars.
- (3) Preliminary evaluation of the US Great Plains association mapping panel for resistance to leaf rust and stripe rust, as a first step toward genome-wide association analysis.
- (4) Biparental populations for indispensable discovery of disease resistance genes in OSU's 'Big Country'.

Methods

Deliverable 1. OSU breeding lines are constantly evaluated each year to multiple fungal and viral diseases of importance to Oklahoma and the southern Great Plains. This will ensure the advancement of resistant lines in OSU's wheat variety development program (VDP). These evaluations are conducted under field and greenhouse conditions to provide confidence in breeder line advancement. In 2021-22, nearly 28,000 single-plant (seedling and adult-plant) ratings were made in the greenhouse for leaf rust, powdery mildew, tan spot, and spot blotch. These greenhouse evaluations will be continued in 2022-2023.

As adult-plant evaluations are ideally performed in the field, we will develop for the first time a leaf rust nursery at the Stillwater Agronomy Research Station. OSU breeding lines will be planted in fall 2022 with spreader rows of leaf rust susceptible varieties. The spreader rows will be artificially inoculated in spring 2023 with a mixture of leaf rust pathogen races collected from Oklahoma in 2021 and 2022. This should create high disease pressure which is needed to evaluate adult plant reactions. This will enhance the advancement of leaf rust adult-plant resistance sources, even in years when natural infection in the field is below-threshold for spontaneous selection.

In fall 2021, we planted 1,092 experimental wheat lines in a field nursery to evaluate their reactions to the WSBM/WSSM complex. This nursery included 660 OSU breeding lines, 431 lines from USDA-ARS regional nurseries, and 6 lines from Eastern Europe. Evaluations were based on visual symptoms and enzyme-linked immunosorbent assay (ELISA). In March 2022, we observed symptoms of WSBM/WSSM on susceptible checks in this nursery, and thus, ratings were performed for the 1,092 wheat lines. Evaluation for WSBM/WSSM will continue in 2022-2023. However, based on historical data, WSBM/WSSM symptoms can disappear due to environmental conditions and other unknown factors. Therefore, in the event visual evaluation is not possible, we will collect DNA from the breeding lines and conduct marker-assisted selection of resistant breeding lines. As WSBM is a much more important disease than WSSM, we will prioritize marker-assisted selection for the gene *Sbm1*, which confers resistance to WSBM.

A separate field nursery is used to evaluate most advanced breeding lines to powdery mildew and BYD. In fall 2021, 170 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 2022-2023. As stripe rust has reached severe levels in Oklahoma in four of the past six years, we will also evaluate the breeding lines to stripe rust and any other foliar diseases that may appear in spring 2022 and spring 2023.

Deliverable 2. Over the years, WIT has been able to advance resistant sources to multiple diseases. More recent examples of commercial launches include Big Country, Uncharted, Strad CL Plus, and Skydance. Some known leaf rust (*Lr*), stripe rust (*Yr*), and powdery mildew (*Pm*) resistance genes were identified in OSU breeding lines and cultivars, some of which confer multiple resistances, such as *Lr21*, *Lr24-Sr24*, *Lr34-Yr18-Sr57-Pm38*, *Lr37-Sr38-Yr17*, *Lr42*, *Lr46*, *Lr67-Yr46-Sr55-Pm46*, *Lr68*, *Lr77*, *Yr5*, *Yr36*, *Sr36-Pm6*, and *SrTmp*. However, the underlying genetics of disease resistance in OSU wheat lines remains largely unknown. Therefore, additional 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.

We selected a collection of ~ 600 OSU breeding lines and cultivars for genetic studies. The OSU breeding lines were from 14 doubled haploid (DH) populations involving elite and contemporary parentage. This collection will be profiled using a genotype-by-sequencing (GBS) approach, which is based on single nucleotide polymorphism (SNP) markers. In addition, diagnostic markers associated with the above-listed resistance genes will be used in the genotyping. Evaluations to multiple diseases will be conducted under greenhouse and field conditions in 2022-23 and two or three years beyond. The generated phenotypic and genotypic data will be used to conduct a genome-wide association study (GWAS). GWAS provides high resolution mapping, and therefore, SNP markers tightly linked to the resistance genes can be identified and converted into kompetitive allele specific PCR (KASP) markers for use in marker-assisted selection in the OSU wheat breeding program.

For this funding cycle, we will conduct evaluation at the seedling stage in the greenhouse/growth chamber to prevalent races of leaf rust and stripe rust pathogens in Oklahoma. This collection will also be planted in the leaf rust nursery (described in deliverable1) for evaluation in adult-plant stages. For stripe rust evaluation, we had high natural infections of stripe rust in 2021 in some locations in Oklahoma. Therefore, we will plant this wheat collection in Stillwater and Chickasha. Evaluations for stripe rust will be complemented by field evaluations in Washington State where a stripe rust conducive environment and high pathogen virulence diversity is known to occur. OSU breeding lines are already evaluated in Washington annually, but for the purpose of line selection and advancement and not for GWAS. In fall of 2022, we will perform the genotyping of the OSU GWAS panel with Carver.

Deliverable 3. In 2022, a hard winter wheat association mapping panel (HWWAMP) was acquired from the USDA-ARS Small Grains and Potato Germplasm Research facility in Aberdeen, ID. This panel is comprised of 299 accessions from breeding programs in the US Great Plains region including Oklahoma, Colorado, Kansas, Montana, Nebraska, North Dakota, South Dakota, and Texas. This panel was developed and genotyped using the Illumina 90K iSelect wheat array under the USDA Triticeae Coordinated Agricultural Project (TCAP). The genotypic data for this panel is publicly available from the T3 Toolbox. We are currently increasing the HWWAMP to acquire sufficient seed for evaluation to multiple diseases. In 2022-2023, we will evaluate the HWWAMP to multiple races of leaf rust and stripe rust pathogens at seedling stage in the growth chamber/greenhouse. Field evaluations at adult-plant stages will be performed for both diseases. Both phenotypic and genotypic data will be used for GWAS to

identify associated molecular markers with leaf rust and stripe rust responses specifically relevant to Oklahoma.

Deliverable 4. The OSU hard white winter wheat cultivar Big Country is resistant to multiple diseases including leaf rust, stripe rust, tan spot, powdery mildew, Septoria leaf blotch, and the WSBM/WSSM complex. However, the genes underlying resistance in Big Country to these diseases are unknown. To identify the resistance genes in Big Country, we will use biparental population mapping. In fall 2022, we will plant the F₅ generation of F-Gen14/Big Country to generate recombinant inbred lines in 2023. We will also develop DH populations from the cross Lonerider/Big Country and/or OK Bullet/Big Country. Based on historical data, Lonerider, OK Bullet, and F-Gen14 are susceptible to multiple diseases; therefore, we will be able to map the resistance genes in Big Country using either of these bi-parental populations. We are currently evaluating Lonerider, OK Bullet, F-Gen14, and Big Country to multiple diseases to confirm their reactions and the best population will be selected for mapping.

W I T 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 of short-season winter wheat germplasm adapted to delayed planting with minimal harvest delay.
- 4) Single-year assessment 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, and this will serve as an integral part in the decision-making process for determining which candidates are advanced for commercial release.

Deliverable 2. Variety comparisons represent the data most requested by wheat farmers in Oklahoma, and the inclusion of candidate variety grain and forage yield information in variety trial reports allows producers to make an 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 the capability for both high yield and

protein in a given environment for certain varieties (e.g., Big Country and Strad CL+, Fig. 2). 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.

Deliverable 3. Continue to conduct collaborative research within and outside OSU's wheat improvement team, involving researchers from Texas, Spain, and Australia. Our overarching goal is to develop the new 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. We will evaluate the genetics and physiological traits of diverse genetic materials. These include experimental lines from a WIT double-haploid, elite-line nursery, varieties from the OSU wheat breeding program, and varieties currently being grown under severe drought and heat stress environments in Spain and Australia with low vernalization requirements, which could potentially be used as parental lines for future variety development. Field experiments will be installed in Stillwater, Lahoma, and possibly in Texas. Genome-wide molecular markers, either outsourced or produced at OSU, will be used to construct genetic profiles uniquely associated with the short-season maturity pattern.

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. We will examine how 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 experiments in 2022-2023 is to utilize labeled isotopic N (¹⁵N) to evaluate N uptake dynamics potentially associated with greater yield and protein formation, and nitrogen use efficiency. Nitrogen has two stable isotopes (¹⁴N and ¹⁵N). The main difference between ¹⁴N and ¹⁵N is that ¹⁵N is not as abundant in nature (~0.4% natural abundance) relative to ¹⁴N (99.6% natural abundance). By utilizing ¹⁵N as a phenotyping strategy we can precisely trace where the current applied N goes within the plant and how efficient varieties utilize the newly taken up N to produce grain.

We will use a method described as "multi-stage pulse labelling", which was developed to identify corn hybrids (<u>De Oliveira Silva et al 2017</u>) with high NUE and can be applied to wheat. Therefore, labeled ¹⁵N fertilizer in the form of Ca(¹⁵NO₃)₂ containing 98% atom ¹⁵N (donated by Dr. Tony Vyn, Purdue University) will be applied at jointing, flag leaf emergence, and at heading/flowering 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 Lake Carl Blackwell in Stillwater. Other biomass samples will be collected for total N tissue analysis at soft dough and physiological maturity (Fig. 3).

Preliminary analysis from the 2021 harvest season shows the different ability of varieties to maximize both grain yield and protein under limited and adequate N rates; however, physiological traits driving that difference remains uncertain. Doublestop CL+ and Green Hammer were able to maintain consistently higher protein than Gallagher and Iba, even when no nitrogen (0N) was applied (Fig. 4, 5). 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" ¹⁵N approach could be a strategy to identify N allocation patterns associated with high yield, protein, and NUE.

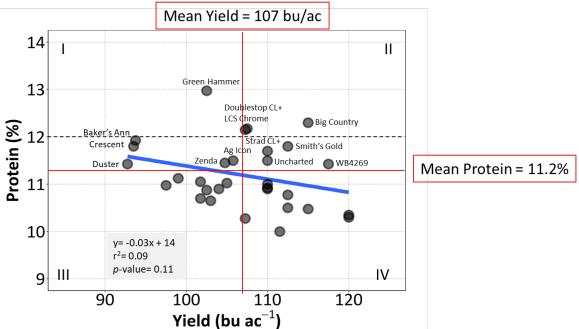


Figure 2. Relationship between wheat protein concentration and yield in the 2020 Homestead variety trial. For some varieties, high protein did not come at the expense of high yield (e.g. Quadrant II).



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.

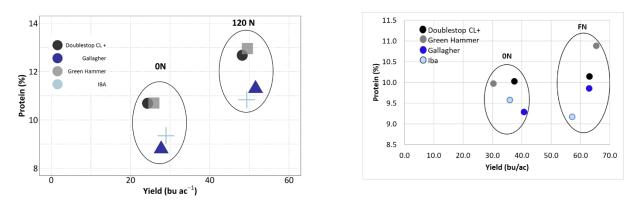


Figure 4 (left). Wheat protein concentration and yield for four varieties tested under zero and 120 lbs N per acre in the 2019-2020 growing season, averaged across two locations, Perkins and Stillwater, OK.

Figure 5 (right). Similar to Figure 4, but for the 2020-2021 growing season. Protein concentration was potentially capped in 2021 by a fall N application, without a second topdress application.

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 Green Hammer, Skydance, OK Corral, Strad CL Plus, Big Country, Uncharted, 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.

In fall 2021, we modified the treatment structure to use a 40% N rate (instead of 25%) for all genotypes, and additional 60% and 100% N rates were applied to a subset of 75 lines. The novelty of this field site provides for no additional N available for plant uptake beyond what is applied.

Deliverable 2. Side by side trials each planted to a single variety, Gallagher or Green

Hammer, will 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 (preplant, 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. Fourteen 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.

WIT OSU CoAXium Variety Development Misha Manuchehri Plant and Soil Sciences

Deliverable

Herbicide tolerance assessment of OSU AXigen (doubled haploid) lines.

Procedures

Since 2016, the OSU Small Grains Weed Science lab has evaluated crop tolerance of commercially available CoAXium varieties. In year two (2017-2018), crop tolerance concerns were detected and have been an issue in our research trials and in Oklahoma grower fields since. Currently, MS student Caitlyn Carnahan, is studying crop tolerance of six CoAXium varieties (AP18 AX, Atomic AX, Crescent AX, Helix AX, Fusion AX, and Photon AX) at two locations in Oklahoma and one location in Kansas. Data from year one revealed that yield decreased following 1x spring applications at two of the three sites. In year two, which is currently being conducted, a variety by herbicide rate interaction for crop visual injury was observed in Kansas where all varieties were at least four times more sensitive to the 2x quizalofop (Aggressor) rate than the 1x rate. Qualifying trials for CoAXium lines will require that future varieties tolerate a 2x rate. More importantly, overlap of Aggressor herbicide will occur in fields as our guidance systems are not flawless.

As WIT continues to develop CoAXium lines, it is critical that we have robust tolerance to Aggressor herbicide. It is also important to understand why tolerance issues may occur. During the 2022-2023 season, the weed science team will investigate tolerance of OSU CoAXium lines under development. Lines will represent unique pedigrees and two genomic sources of Aggressor tolerance (genome sources AD or AB) that confer 2-gene tolerance to Aggressor herbicide. Treatments will include various Aggressor rates, surfactants, and environmental conditions after application. Visual ratings, end-of-season wheat biomass, and grain yield will be collected. This research will overlay and facilitate selection among the "first round of AXigen 2-gene lines (n=139) capable of the CoAXium production system", as mentioned in Carver's proposal.

W I T Execution of a UAV Phenotyping Platform Gopal Kakani Plant and Soil Sciences *Year 2 of project*

Deliverables

- 1) UAV-based HLT (Hyperspectral-LiDAR-Thermal) sensor data and groundtruth phenotypes at biweekly intervals from emergence to harvest.
- 2) Spectral algorithms for Oklahoma-relevant wheat traits which contribute to biomass production and grain yield.

Overview and procedures

Our goal is to continue development of a rapid, phenotypic protocol for predicting vegetative biomass and grain yield through a UAV-based HLT (Hyperspectral-LiDAR-Thermal) sensor system. The most logical plant material for which the stated deliverables may be generated is the dual-purpose observation nursery (DPON), a large collection of WIT experimental lines (>2,000) tested in one nursery with multiple series of repeated check entries or common varieties. The size of this collection provides the necessary genetic diversity to adequately data-mine the phenotypic variability. The repeated check plots provide a basis for error estimation and validation of the phenotyping platform. The DPON is one of the earliest nurseries planted in the breeding program, allowing for thorough and representative trait monitoring and measurement. This nursery is located at Stillwater in a convenient position for UAV work. See Figure 6 for one type of product that can be derived from this work to enable selection in near real-time. This output of normalized difference vegetation index (NDVI) was generated March 25, 2022.

With support of OWRF, a graduate research assistant (GRA) was hired to collect, process, and analyze the UAV sensor data in 2021-2022; this research will continue in 2022-2023. Funds are requested for a postdoc to develop a machine learning/artificial intelligence (ML/AI)-based data processing pipeline. The GRA and postdoc will be supervised by Dr. Kakani. The postdoc and GRA will collaborate to develop phenotyping data products for WIT. The GRA will coordinate collection and processing the raw data. The processed image data and groundtruth data will be used to develop spectral algorithms for yield and related traits. The algorithms developed will eventually be used to develop predictive models specifically for grain yield and biomass (beyond the timeframe of this project). The GRA will participate in developing project reports and publications and will be actively engaged in disseminating the research results at NIFA-CAP, regional, national, and international meetings.

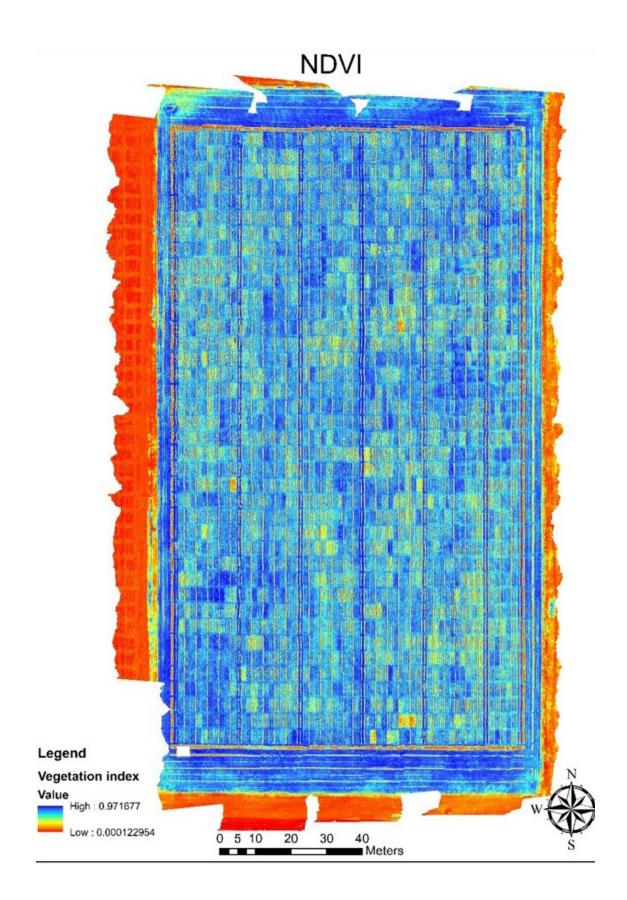


Figure 6. NDVI rendering from UAV sensor data collected 25 March 2021 at Stillwater, in which NDVI reflects N status, chlorophyll content of the whole canopy, and biomass. Each rectangle represents a single line evaluated in the 2022 Dual-Purpose Observation Nursery (DPON), with 2, 418 plots.

W I T

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, Manuchehri, Kakani, 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 2022:

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, USDA-NASS data indicated that OSU-bred and OWRF-supported varieties accounted for the *top eight most widely planted varieties* in Oklahoma for the 2021-22 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. This was the second year in which protein data were reported together with yields in near real-time. (*Silva*)
- Installed first field experiments to measure in-season plant nitrogen fluctuation and improve our understanding of the physiological basis for breaking the negative 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, even when no nitrogen was applied. The genetic basis for this superiority needs to be explored using physiological approaches. (*Silva/Arnall*)
- Found a significant difference in nitrogen uptake patterns between two low-protein varieties and two high-protein varieties. This finding will help lead to improved nitrogen management strategies for both types. (*Arnall, Silva*)
- Discovered significant varietal differences in the magnitude of N- deficiency symptom expression according to when N was applied. At jointing a few notable cultivars showed very little evidence of N deficiency while others expressed significant chlorosis and reduced biomass. (*Arnall*)
- Installed first field experiments to compare winter wheat varieties for adaptation to a late planting date (in December). (*Silva*)
- 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, 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) adaptability and disease resistance exceeding that of OSU's most recent broad-utility varieties Showdown and Green Hammer. (*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. (*Hunger/Aoun*)
- Determined the reaction to the WSBM/WSSM complex for more than 1,000 experimental wheat lines, including 660 lines from WIT, 431 lines from USDA-ARS regional nurseries, and 6 lines from Eastern Europe. (*Hunger/Aoun*)
- Ascertained resistance among <u>302</u> advanced WIT breeding lines to leaf rust, powdery mildew, tan spot, and spot blotch, encompassing nearly 28,000 single-plant evaluations of greenhouse seedlings and adult plants. (*Hunger/Aoun*)
- Discovered and cloned *TaCol-B5* that modifies spike architecture by increasing the number of spikelets per spike, and by association increases spike length, and which enhances grain yield by increasing the number of spikes per plant and kernels per spike. (*Yan*)

- Showed how *TaCol-B5* increases the number of kernels per spike without decreasing kernel weight, thereby potentially uncoupling those yield components. (*Yan*)
- Fine-mapped and won international approval for naming the novel leaf rust resistance gene, *Lr81*, originally discovered in a Croatian breeding line (formerly *Lr470121*) and developed KASP markers to continue its introgression into locally adapted varieties. (*Xu*)
- Created F₇ lines stacked with leaf rust resistance genes *Lr81*, *Lr34*, *Lr37*, and *Lr39* using marker-assisted selection, which will be further pyramided with newly discovered powdery mildew and pest resistance genes. (*Xu*)
- Discovered a novel greenbug resistance gene on chromosome 3D of *Ae. tauschii*, which can be transferred to bread wheat from a synthetic wheat. (*Xu*)
- Ranked five experimental WIT lines for their competitive ability against feral rye, Italian ryegrass, and henbit. (*Manuchehri*)
- Identified 5 imazamox-*susceptible* advanced lines but similar in genetic expectations to Doublestop CL+, from which one line will be chosen in June 2022 for foundation seed advancement. (*Manuchehri, Carver*)
- Implemented a drone platform with a hyperspectral camera funded by OAES, upgraded with LiDAR and thermal camera funded by OWC and OGI, for first-time characterization and selection among breeding lines. (*Kakani*)
- Initiated biweekly data collection Dec. 2021, totaling 5 UAV data collection campaigns synced with simulated grazing treatments in the DPON trial at Stillwater, OK (first campaign ever). (*Kakani*)
- Established virtual machines (VMs) on OSU high-performance computing center (HPPC) for processing raw hyperspectral data (~50GB per campaign) generated by drone platform to create orthomosaics and to extract phenotype data. (*Kakani*)

Wheat Improvement Team Budget, FY2022-2023

		BUDO	GET CAT	EGORY		
Principal	Wages & benefits				Growth chamber &	
Investigator (red	(or salary if noted	Supplies &		Equipment	greenhouse	TOTAL,
means current)	below)	fees	Travel	and repair	rental	\$
Carver	65,000	13,000	5,000	15,000		98,000
Carver – salary/benefits support*	37,463					37,463
Yan	36,930	28,000	4,000		7,000	75,930
Xu (sub-award)	19,460	28,040				47,500
Aoun	15,000	25,000	2,000		5,250	47,250
Giles						0
de Oliveira Silva	12,600	10,000	4,000			26,600
Arnall		6,500	1,000			7,500
Kakani	47,000	4,000	2,000			53,000
Manuchehri	<u> </u>					0
TOTAL, \$393,243						

Carver

1. Wages and benefits (\$65,000) 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 projected salary+benefits expenditures for FY23. Salary for Melanie Bayles (50% support) is not budgeted here.

Yan

- 1. Personnel: Dr. Ragupathi Nagarajan is expected to contribute 70% of his appointment to the proposed OWRF project. Dr. Nagarajan has an annual salary of \$39,459 plus benefits (37%). 70% of \$52,757 for the annual salary and benefits equals \$36,930 (not to exceed).
- 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 (\$20,000 total). Gold particles and kits for geneediting and producing transgenic plants (\$4,000) is budgeted. Fees include sequencing costs in the OSU Core Facility, and Eurofins. \$2,000 is requested for sequencing 200 samples (\$5 per sample at the OSU Core Facility) via internal Service vendor, and \$2,000 is requested for sequencing 1000 samples (\$2 per sample at Eurofins) via external service vendor

- 3. Travel: Expenses for field work and professional conferences.
- 4. Greenhouse and growth chambers: rent and management fees.

Xu

- 1. Four months' salary (\$15,710) and benefits (\$3,750) 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 and benefits for undergraduate employees
- 2. Expendable supplies support field, greenhouse/growth chamber, and laboratory experiments: inoculum preparation/increase, inoculation supplies, DNA extraction plates, steel beads for grinding leaf tissue, soltrol, chemicals for DNA extraction, KASP primers, PCR and real-time PCR plates/reagents, greenhouse supplies (soil, growing trays, fertilizer, pesticide, etc), Biochemistry Core Facility services
- 3. Travel is dedicated predominately to in-state research activities, though a small component may occur in Kansas.
- 4. Fees for growth chamber (\$3,000) and NRC greenhouse (\$2,250)

Silva

- 1. Wages and benefit: 1400 hours at a rate of \$9/hour are requested for undergraduate labor assisting in seed weighting, planting, plot management, harvesting, sampling processing for nutrient analysis, and data collection.
- 2. Materials and supplies: A total of \$3000 is requested for supplies for plot management, chemicals, syringes to inject the ¹⁵N in the soil, pvc pipes to apply water with the ¹⁵N application, data collection. A total of \$7000 is requested to cover the costs of the total N and ¹⁵N tissue analysis. Two locations, 4 reps, 2 N rates, 4 varieties, 5 growth stages [jointing; whole plant, flag leaf emergence; leaf+stem, anthesis; leaf+stem+head, soft dough; leaf+stem+grain+chaff, maturity; leaf+stem+grain+chaff].
- 3. Travel: Partially cover travel to trial locations for planting, plant growth and development assessments, treatment applications, biomass harvesting, data collection, and harvesting.

Arnall

- 1. Materials and supplies: Support for field experiments (\$2,500) and soil/plant analyses (\$4,000)
- 2. Travel: Partially cover travel to in-state trial locations

Kakani

- One-time, half-time support including benefits (<u>not to exceed</u> \$23,000, remainder linked to CAP funding) for postdoctoral position (6 months to develop data processing pipeline based on ML/AI) – salary to be absorbed by CAP-UAS in subsequent funding cycles; single-year support including benefits for M.S. student (<u>not to exceed</u> \$21,000) to collect field drone and ground truth data; undergraduate wages (\$3,000) to process field samples.
- 2. Expendable supplies support field research and the UAV system (sensor calibration, software upgrade, batteries, parts).
- 3. Travel dedicated to workshops for dissemination of research results

Manuchehri

Prior year funding will support the research plan with no further request here, and with pending assistance from Wheat Genetics Chair endowment as needed.

Giles

Will use prior-year funding to terminate research, and consider new avenues for WIT engagement in the near future

SIGNATURE PAGE

Genetic improvement of winter wheat: Integrating classical and novel approaches

Submitted by:

OSU's Wheat Improvement Team Proposed funding level: \$393,243

Brett Carver

Brett Carver Plant & Soil Sciences Meriem (Apr 30, 2022 09:16 CDT)

Meriem Aoun Entomology & Plant Pathology

Amanda De Oliveira Silva

Amanda de Oliveira Silva Plant & Soil Sciences

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Gopal Kakani Co-PI and Head, Plant and Soil Sciences

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Brian Arnall Plant and Soil Sciences

Xiangyang Xu USDA-ARS HWW Genetics Research Unit

Justin Talley

Justin Talley Head, Entomology & Plant Pathology Kelly Chamberlin Research Leader, USDA-ARS, Stillwater

Gol Z. Ofn -- (May 2, 2022 09:43 CDT)

Tom Coon Vice-President, Dean and Director OSU Agricultural Programs

SIGNATURE PAGE

Genetic improvement of winter wheat: Integrating classical and novel approaches

Submitted by: OSU's Wheat Improvement Team Proposed funding level: \$393,243

Brett Carver Plant & Soil Sciences Meriem Aoun Entomology & Plant Pathology

Amanda de Oliveira Silva Plant & Soil Sciences Liuling Yan Plant and Soil Sciences

Gopal Kakani Co-PI and Head, Plant and Soil Sciences

XIANGYANG XU Digitally signed by XIANGYANG XU Date: 2022.04.28 08:41:39 -05'00'

Xiangyang Xu USDA-ARS HWW Genetics Research Unit Brian Arnall Plant and Soil Sciences

KELLY CHAMBERLIN Digitally signed by KELLY CHAMBERLIN Date: 2022.04.28 09:24:52 -05'00'

Kelly Chamberlin Research Leader, USDA-ARS, Stillwater

Justin Talley Head, Entomology & Plant Pathology Tom Coon Vice-President, Dean and Director OSU Agricultural Programs