

**Research Proposal for OWRF/OWC  
FY2017-2018**

**Genetic improvement of winter wheat: Integrating classical and novel approaches**

**INVESTIGATORS:**

OSU WHEAT IMPROVEMENT TEAM

*Wheat Breeding and Variety Development*

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*Identification and Introgression of Disease Resistance*

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*Drought and Heat Tolerance Mechanisms*

**Gopal Kakani** (request not forwarded, FY2017-18)

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*Transforming N-Use Efficiency to End-Use Quality*

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*Information Exchange and Candidate Cultivar Assessment*

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Trait introgression by marker-assisted selection

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Forage systems breeding

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Development of Hessian fly resistant germplasm

Robert Bowden, USDA-ARS, Manhattan, KS

Mary Guttieri, USDA-ARS, Manhattan, KS

Development of disease-resistant germplasm

Richard Chen, USDA-ARS, Manhattan, KS

Becky Miller, KSU, Manhattan, KS

End-use quality evaluation – pan bread functionality

Mike Tilley, USDA-ARS, Manhattan, KS

Gliadin analysis and toxicity

Jon Rich, AgriPro-Syngenta

Bird cherry oat aphid tolerance and hybrid wheat development

Lora Kilgore-Norquest, Pioneer-DuPont

Germplasm exchange and hybrid wheat development

TBA

Introgression of novel herbicide resistance

Members of the Hard Winter Wheat Quality Council

Milling and baking characterization of candidate cultivars

Selected wheat breeders and pathologists throughout the USA

Germplasm exchange and breeder line characterization

International germplasm exchange:

Agricultural Research Institute, Martonvasar, Hungary (Laszlo Lang)

National Agric. Res. and Development Institute, Fundulea, Romania (Nickolae Saulescu)

**FUNDING HISTORY:**

Amount received, previous 5 years: \$200,000-\$365,400 per year; **\$365,400 in FY17**

Amount requested, 2017-2018: **\$377,900**

## **SUMMARY:**

The mission of OSU's Wheat Improvement Team (WIT) 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 wheat value chain in Oklahoma, from seed producer to consumer. Hence our research continues to address those traits relevant to Oklahoma, especially disease and insect resistance, dual-purpose adaptation, and multimodal functionality. This kind of research can be cyclic due to adjustments in the pathogen population following genetic alterations in the wheat plant, or because introgression of new germplasm to strengthen a certain targeted trait inadvertently introduces weaknesses in non-targeted traits.

Significant progress has been made in wheat genomics such that we can rapidly and effectively deploy specific genes of interest which control those traits, or in the case of genomic selection widely scattered blocks of genes. Our proposal capitalizes on genomic technology created within the WIT and by our USDA-ARS collaborators in the hard winter wheat region who enable access to a broader trait portfolio. One-third of the WIT now provides dedicated effort to utilizing advanced molecular breeding tools.

Development of improved hard red winter (HRW) or hard white (HW) wheat cultivars is central to our research mission, though other wheat classes (soft red winter or soft white) and other quality-based uses of wheat (organic, artisan, healthy) may come into focus as the genetic or market potential warrants. Our proposal is supported by the following seven research focus areas which ultimately place the multidisciplinary OSU wheat improvement program in a sustainable and unique position among regional or national breeding activities. Hence, research that sets OSU apart *and* which brings immediate or potential impact to Oklahoma are considered quintessential.

### **Focus Area:**

1. Characterization of traits within OSU germplasm that enables either a) determination of best adaptation zones and fitness for commercial use, or b) the unambiguous differentiation of experimental purelines,
2. Postulation or identification of genes conferring disease, insect (BCOA and Hessian fly, highest priorities), or general adaptation traits relevant to Oklahoma wheat production,
3. Development of parental genetic stocks or germplasm with known, or at least postulated, possession of specific genes for economic traits relevant to Oklahoma,
4. Development or execution of protocols which expedite variety development, such as rapid phenotyping assays and QTL, gene, or marker discoveries,
5. Elucidation of whole-plant, cellular, or molecular mechanisms that account for improved nutrient-use efficiency, or which confer optimal grain set and remobilization of stored carbohydrate under drought and/or heat stress,
6. Development of genomic estimated breeding values for grain yield and yield components, and eventually moving into the end-use quality spectrum (bread and tortilla quality).

## OBJECTIVES & PROCEDURES:

W I T

### **Wheat Breeding and Variety Development**

**Brett Carver**

**Plant and Soil Sciences**

#### **Deliverable**

Marketable winter wheat cultivar(s) specifically adapted to Oklahoma and the southern Great Plains, supported with documentation justifying OAES release and the associated know-how for optimum utilization.

#### **Overview and Procedures**

**Breeding materials summary** (as of 2017 harvest season). All phases of the variety-development pipeline (VDP) remain at full capacity relative to the number and expertise of permanent personnel on board capable of processing the germplasm. The VDP expanded significantly in 2017 as several thousand doubled-haploid (DH) lines entered from a formal partnership with Pioneer-DuPont that was established to develop hybrid varieties from OAES germplasm. WIT is not independently pursuing hybrid cultivar development now or in the foreseeable future; rather it is focused on pureline development for a broad spectrum of environments.

We intend to evaluate and select among and within early-generation bulk populations currently in the F<sub>2</sub>, F<sub>3</sub>, and F<sub>4</sub> generations, totalling **1,949** unique cross combinations. About 10% of those populations were additionally selected at the Oklahoma Panhandle Research and Extension Center (OPREC) at Goodwell, OK. Line selections will be made in 2017 among headrow nurseries containing **58,971** predominately F<sub>5</sub> lines, representing a 20% increase from 2016. Selections will be made among 500 additional headrows planted at Yuma, AZ to more rapidly purify two elite lines, one segregating for red and white kernel color (OK12716) and the other segregating for bearded and beardless plants (OK11208), as discussed in more detail below.

Additionally, we will complete field evaluation and greenhouse assays, conduct quality tests, incorporate molecular data for inbred line classification and selection, and invoke appropriate statistical methods to select among **6,129** predominately hard red winter (HRW) experimental purelines in the preliminary (F<sub>6</sub>), intermediate (F<sub>7</sub>), and advanced stages (F<sub>8</sub> and beyond). This number is noteworthy because *it constitutes a 43% increase over 2016 levels*, due to the infusion of more lines at the preliminary stages (F<sub>7</sub>) of line development, the one-time infusion of nearly 3,000 doubled haploids (DHs) from our cooperative effort with Pioneer, and one final evaluation of inbred lines produced in Dr. Art Klatt's pre-breeding program. In all WIT will evaluate **3,069** DH lines at various stages of yield and quality testing for accelerated release of highly anticipated elite germplasm, accounting for an unprecedented 50% of the total number of inbred lines in the VDP; by comparison 17% of all inbred lines in the VDP in 2016 were DH. This quantity of DH lines excludes several hundred more lines delivered by Heartland Plant Innovations, Inc. in March 2017 and now held in cold storage for crop year 2018 evaluation. Two DH elite lines were approved for release by the OAES in 2017: OK11D25056 and OK12DP22002-042. Our most advanced doubled haploid progeny (in terms of yield and quality testing) still feature Gallagher, Garrison, Duster, Billings, or a recent cultivar candidate, OK09634, as a parent. More importantly, all of these genetic backgrounds represent strength and

appeal among bread wheat millers and bakers.

The proportion of hard white (HW) experimental purelines will average **5%** across all generations from the F<sub>6</sub> generation and forward. This represents a sharp, but only temporary decline, from the 14% level in 2016, as more intensive selection was applied in 2016 for stripe rust resistance and resistance to pre-harvest sprouting (PHS). See below for more details.

We intend to produce and process about **1,000** unique crosses in summer 2017, of which 26% will lead to breeding populations segregating for progenies with red or white kernel color. This level constitutes a slight uptick as WIT slowly migrates toward a stronger emphasis on HW development. The proportion of crosses we will process that involves only HW parentage is **8%**. Hence we expect the frequency of hard white progeny from the current class of crosses to be about 12-18% in subsequent generations, a level sufficient to easily generate one legitimate HW candidate every two years.

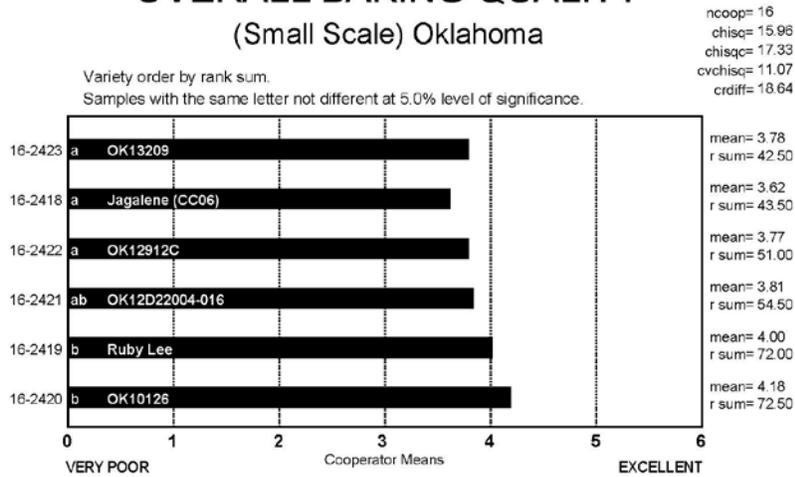
**Advanced Line Outlook and Candidate Assessment (Tables 1 & 2).** Three HRW candidates were released by OAES in early 2017 named **Spirit Rider** (OK10126), **Smith's Gold** (OK11D25056), and **Lonerider** (OK12DP22002-042), though the latter variety will not be publicized until conflicting end-use quality data received immediately after release can be further resolved, most likely by July 2017.

Spirit Rider satisfies an immediate need for better straw strength in higher yielding environments, which includes irrigated systems in the panhandle. The disease resistance package of Spirit Rider is exceptional, with the only weaknesses being intermediate to moderate resistance to wheat spindle streak mosaic, powdery mildew, and leaf rust. Smith's Gold satisfies an immediate need as a close relative to Gallagher with enhanced stripe rust protection in earlier reproductive stages relative to Gallagher and with superlative end-use quality, plus Hessian fly resistance stacked with greenbug resistance. Smith's Gold represents the first greenbug-resistant variety to be developed and released by OAES since the 1989 release, Century.

Moving forward in 2017-2018, several more HRW and HW candidates await disposition as potential releases. Our focus at this stage is to select i) one all-purpose HRW candidate that does not duplicate the positioning and intent of Spirit Rider and Smith's Gold, ii) one beardless HRW candidate that exceeds all available beardless options in yield potential, disease resistance, and end-use quality, and iii) one two-gene Clearfield candidate that my eventually replace Doublestop CL+.

The all-purpose HRW candidates are OK13209, OK12DP22004-016, OK13621, OK13625, OK12DP22007-077, and OK14319, and are further characterized in Tables 1 and 2. The first three listed in this group are favored at this time due to their broader utility and their proven yield and quality performance. OK13209 and OK12DP22004-016 were submitted to the 2016 evaluation program of the Wheat Quality Council, and results were disclosed in Feb. 2017. Both lines performed equal to or better than the industry standard, Jagalene (see chart below). OK13625 performed better than Jagalene in the 2015 WQC evaluation program. The other three candidates will be submitted as part of the 2017 evaluation program, pending field performance in 2017.

## OVERALL BAKING QUALITY (Small Scale) Oklahoma



Leading the beardless group of candidates—OK10430-2, OK11208, OK12206-127206-2, OK15115, plus two others not listed—is OK10430-2, simply because it has been tested the most extensively. It is an Endurance type, but with a highly aggressive early fall and vegetative growth pattern and earlier maturity. OK11208 is WIT’s best yielding and statewide adapted beardless candidate at this advanced stage of testing; however, OK12206-127206-2 owns the superior disease resistance package and end-use quality. OK15115 may demand equal billing with OK12206-127206-2, though with its greater sensitivity to wheat soil-borne mosaic/wheat spindle streak mosaic complex, it would be pushed further into western Oklahoma. Hence WIT chose OK12206-127206-2 for accelerated increase at Yuma, AZ in 2016-17, while at the same time re-purifying OK11208 to minimize awned-plant contamination. WIT is expected to forward a release recommendation from the beardless group of candidates during the proposed funding period.

Though several more are being tested in the BASF qualification trials, the two Clearfield Plus candidates chosen for foundation seed increase and purification are OK12912C-138407-2 and OK128084C. After final testing in breeder trials, OSU and KSU variety trials, and BASF qualification trials, a release recommendation will be forwarded to OAES for one of the two candidates during this funding period. OK12912C has already received a favorable valuation from the Wheat Quality Council.

**Hard white (HW) wheat outlook.** Though no HW candidate is currently under foundation seed production by OFSS, one line was placed under re-purification in Yuma, AZ (OK12716), whereas four others are currently on advanced breeder seed increase. Of those four, three are known to provide good to exceptional levels of pre-harvest sprout (PHS) tolerance, thus confirming our emphasis on this trait in recent years. OK12716 offers an exceptional level of yield potential. Hence in 2017 WIT will identify both HRW *and* HW sib lines with the desired marker genotypes for known PHS resistance genes and for glutenin subunits which confer the best dough strength (these subunits segregate currently in the original line). Of the 44 advanced lines on breeder seed increase at the OPREC in Goodwell, seven are HW and one is soft white. This proportion is certainly consistent with the expected output for white wheat as predicted above. The most advanced HW lines awaiting disposition in the proposed funding period are summarized below in Table 3. All exhibit very good straw strength, another constant source of selection pressure.

Table 1. OSU candidate varieties under seed increase as of Spring 2017 with Oklahoma Foundation Seed Stocks.

Candidate <sup>a</sup>	Pedigree	Increase status	Feature traits
OK11D25056	Gallagher/OK05511	Release	Greenbug + Hessian fly resistance; <b>GoldnGrain</b>
OK10126	OK Bullet/OK98680	Release	Lodging-resistant 3-year winner at Goodwell, OK
OK12DP22002-042	Billings/OK08328	Release	Lodging-resistant yield topper - 2016 SRPN; WSBM-S
OK12912C-138407-2	N91D2308-13/OK03926C//OK03928C	Year 1 or 2	Doublestop upgrade for 3 diseases, straw strength
OK128084C	N91D2308-13/OK04902C//OK05907C	Year 1	Like OK12912C but early; lacks PM/freeze tolerance
OK13209	OK Bullet/TX00D1390//Shocker	Year 1	Stout on stripe rust; central corridor & SW OK
OK12DP22004-016	Everest/OK08328//OK09634	Year 1	Very short and early; high yield and <b>GoldnGrain</b>
OK13621	TX00D1390/ Billings	Year 1	Broad disease package; <b>GoldnGrain</b>
OK13625	Billings/Fannin sib	Year 2	High N-use efficiency & very early; <b>GoldnGrain</b> /organic
OK12D22002-077	Billings/OK08328	Year 1	-042 sib but WSBM resistant; targeted downstate
OK14319	NE01533/OK02125//Duster	Year 1	Targeted strictly for NE OK; low pH/grazing tolerant
OK10430-2	CS+1V/2*Endurance sib	Pre-release	Awnletted, forage+grain ideotype; high early vigor
OK11208	Deliver/Santa Fe	Purification	Beardless with wide adaptation; early maturity
OK12206-127206-2	Y98-912/OK00611W//OK03716W	Year 1	All-around best beardless – agronomic & quality
OK15115	Pete/OK Bullet//TX03A0148	Year 1	Backup beardless; strong straw & disease package

Table 2. Trait ratings (1-to-5 scale) for OSU HRW candidates under foundation seed increase in 2016-17.

Candidate	Trait category <sup>a</sup>										Weaknesses
	DP	HF	YR	LR	TS	PM	V	AST	SS	BQ	
OK11D25056	2	1	2	3	4	1	1	2	2	1	
OK10126	3	5	2	3	3	4	2	2	1	2	April freeze
OK12DP22002-042	2	2	3	2	5	2	5	1	1	2	Pre-harvest sprouting
OK12912C-138407-2	2	2	2	2	3	2	1	1	1	1	
OK128084C	3	3	1	3	2	4	1	1	1	1	April freeze
OK13209	2	5	1	2	3	--	2	2	1	2	Shattering
OK12DP22004-016	4	2	3	3	2	2	1	1	1	1	Grazing recovery
OK13621	1	5	1	3	4	1	1	2	2	1	
OK13625	4	5	2	1	2	2	1	1	3	1	Winter injury
OK12D22002-077	3	1	1	3	--	2	1	2	1	2	
OK14319	2	1	2	1	2	--	1	1	1	1	April freeze
OK10430-2	1	5	3	1	4	2	2	1	3	4	Shattering
OK11208	2	5	2	2	2	--	1	1	1	2	April freeze
OK12206-127206-2	1	1	1	2	2	--	1	1	2	1	
OK15115	2	--	2	1	2	--	3	3	1	1	

<sup>a</sup>Trait categories are 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; and BQ, baking quality. Values  $\leq 2$  are considered very desirable; those  $\geq 4$  are undesirable. No value (--) indicates inconsistent or insufficient data for postulation.

Table 3. Most advanced HW lines awaiting disposition for release.

HW Candidate	Pedigree	Comments
OK12716	W99-194/OK Rising sib	Highest statewide yielding experimental line in the program (HRW and HW) since 2012 (average rank 2.2); selections with the more favorable 5+10 high-molecular weight glutenin subunit will be targeted to ensure adequate end-use quality
OK11755W-139129-3W	CIMMYT seln/Overley//Jagger-derivative	Extremely high yielding with strict adaptation to central Oklahoma (higher yield potential than Stardust); lacks leaf rust and BYD resistance but otherwise exceptional on other diseases and end-use quality
OK11709W-139122-1W	OK02523W/OK00608W//OK00611W	Very high PHS resistance and best fit for downstate Oklahoma; very good resistance to leaf rust and stripe rust; yielding ability needs extended assessment
OCW04S717T-6W	CIMMYT experimental/KSU experimental// KS91W047	Targeted strictly for NC and NE OK where its yield competitiveness is exceptional; <u>perhaps the highest level of PHS tolerance among all HW and most HRW experimental lines;</u> beardless
OK11999W-139144-6W	Westonia /2*OK Bullet	Earliest HW option currently available, but exhibits the lowest level of PHS tolerance

**Breeding methodology.** The OSU wheat breeding project typically employs a *modified bulk-pedigree* selection method, whereby early-generation populations are selected and advanced as bulk populations for three consecutive generations, purelines are derived from F<sub>4</sub> populations as headrows, subsequent generations are advanced primarily through bulk-selfing, and lines are reselected if

necessary to improve trait expression or genetic purity.

A key overlay to that 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 possibly the only one in the world which selects for adaptation to dual-purpose conditions, rather than simply testing finished lines, *ex post facto*, in a dual-purpose environment. Plant selection in a dual-purpose environment can improve genetic gain for yield in a grain-only environment, but the inverse does not hold; that is, plant selection in a grain-only environment does not necessarily lead to the highest genetic gain for yield in a dual-purpose environment. Attributes considered to be improved indirectly by this selection method are stand establishment and canopy closure, tillering capacity, drought and cold tolerance, resistance to barley yellow dwarf (BYD, or to its primary vector, bird cherry oat aphid) and to Hessian fly, strong winter dormancy retention, and stem carbohydrate remobilization. The latter may be induced by a reduction in flag leaf size after grazing.

A significant branch of the VDP was started in 2013 to address a growing need for germplasm and new cultivars better adapted or more specifically adapted to the Oklahoma panhandle region. Hence we now 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. Beginning in fall 2013, a portion of our early-generation materials – that is, those populations which offered the greatest likelihood of success in the High Plains – were sown under these simulated dryland conditions at OPREC. In spring 2017, WIT will complete its first-ever evaluation of headrow progeny lines at OPREC, capping off a 4-year dedicated process of population and line development. WIT will continue stocking this pipeline in 2017. This opens a critical door to select and further develop experimental lines which are specifically targeted for the panhandle region, rather than relying on the “luck of the draw” from experimental lines selected downstate. The bottom line is that we will attempt to conduct a smaller breeding program, one tailored for the panhandle, within the larger one that we normally conduct under the **GrazenGrain™** breeding system.

On a completely separate breeding track, WIT will enter its fifth consecutive year of evaluating doubled-haploid (DH) lines produced primarily through a service contract with Heartland Plant Innovations, Inc. (HPI) in Manhattan, KS. This effort signifies an aggressive strategy to introduce key traits into our research program at rapid pace and with negligible slippage in agronomic value. Other traits besides yield potential and quality performance that we intend to specifically target among DH lines are resistances to BYD, wheat streak mosaic, Hessian fly, and greenbug, in addition to enhanced gluten strength. This proposal calls for seed increase of more than 1000 new doubled-haploid lines from about 15 elite cross combinations, produced by HPI over the past 18 months (since Nov. 2015) and now held in cold storage for crop year 2018. Independent progenies of each cross combination will also cycle through the **GrazenGrain™** breeding system to ensure that we thoroughly mine the genetic potential of each cross.

In another breeding track initiated in FY2013-14, backcross derivatives were successfully produced completely outside of the **GrazenGrain™** breeding system with the assistance of Dr. Guihua Bai (USDA-ARS, Manhattan) and Dr. Liuling Yan. These derivatives will retain the high agronomic value of designated breeding stocks, such as experimental progenies derived from Gallagher, Iba, Billings, Ruby Lee, Garrison, and OK Rising, but conventionally inserted into these progenies will be key genes conferring curl mite and wheat streak mosaic resistance, resistance to multiple diseases including stripe rust, septoria tritici blotch, Fusarium head scab, and barley yellow dwarf, altered starch

composition, reduced polyphenol oxidase activity, and augmented dough strength. Backcross-derived progenies featuring a uniquely over-expressed glutenin protein have the potential for immediate commercial production (i.e., without the need for additional breeding) through a direct, contractual arrangement with processors.

Complementary to the numerous field and laboratory screens already in place will be a new series of gene marker assays based on the WIT's own genomic research under Dr. Liuling Yan's supervision and with past support of the OWRF. These DNA-based tests will be implemented at the intermediate to advanced stages of inbred line development as an inbred profiling tool; the resulting information will be used to aid and clarify interpretation of traditional screens currently used to assess maturity patterns, disease resistance, and one particular and effective source of Hessian fly resistance derived from Duster, which provides a more stable form of resistance than from 2174. Dormancy release is currently and indirectly characterized by genes present at the *VRN-A1* and *PPD-D1* loci, but we intend to utilize new research in Dr. Yan's lab involving other QTL that additionally confers later dormancy release in winter wheat, often quantified as first-hollow-stem date. Marker assays will be implemented during this proposal period to continue stacking resistance to wheat curl mite and wheat streak mosaic. The advantage to using this DNA-based selection approach is that we can greatly reduce errors encountered in field-based screening, or create selection opportunities that otherwise did not exist simply because we did not have access to or the 'luck' of targeted selection environments.

Finally, while the **GrazenGrain™** breeding system is the mainstay of the VDP, the outcomes from using this system are almost entirely convergent with desired outcomes for variety development in organic systems. For example, progeny of the **GrazenGrain™** breeding system are inherently exposed to transient (at minimum) episodes of nitrogen deficiency and inherently possess a vegetative growth habit and growth potential to compete well against weed pressures. They also feature a high level of disease resistance and the ability to produce acceptable end-use quality under non-excessive exposure to nitrogen. WIT will determine this degree of convergence over the course of several years, beginning with a small pilot test of one experimental line (OK13625 – Table 1) under actual certified organic management. Experimental lines already produced conventionally in the VDP from the F<sub>6</sub> generation forward will be sequestered and tagged for eventual organic testing simultaneous to, and not to distract from, mainstream breeding efforts. Testing of advanced experimental lines will continue at Tipton in cooperation with WIT member Brian Arnall to identify lines with unusually good nitrogen-use efficiency under a strict rate of N application equivalent to 25% of the optimum in-season level.

**Herbicide resistance introgression.** WIT continues to pursue, or will initiate, the introgression of resistance to key grass-controlling herbicides. These traits capitalize on artificially induced or rare naturally-caused mutation events. Negotiations remain in progress to access and incorporate novel resistance to quizalofop herbicide, an ACCase inhibitor with different activity from imazamox. The outcome of these negotiations will be fully disclosed when an agreement satisfactory to OSU can be executed.

The 2013 OAES release of Doublestop CL Plus marked a new beginning for OSU wheat improvement research as the first two-gene Clearfield cultivar to be licensed by the Oklahoma Agricultural Experiment Station (OAES). WIT will continue the same aggressive approach to identify new two-gene candidates with the high yield potential and exceptional quality characteristics of Doublestop CL Plus. Two-gene Clearfield® experimentals account for **4%** of all breeding lines currently in statewide yield testing. Five elite lines, including the two already mentioned above, are now in the

Qualification Trial stage required by BASF prior to commercialization, in cooperation with WIT member David Marburger.

**Trait emphasis.** The OSU wheat improvement program is dedicated to developing cultivars with dual adaptation to both early-planted, grazing systems and later-planted, grain-only systems, and now with an added dimension of adaptation to conservation tillage. Winter dormancy retention and reproductive development appropriate to Oklahoma environments are critical components of that adaptation complex. Acid-soil tolerance has been a focal point in OWRF-sponsored wheat genetic research since the mid-1980s, evidenced by more released varieties featuring this trait, Ruby Lee and Iba being the recent exceptions.

WIT will continue to pool vast amounts of tan spot data from greenhouse assays conducted by Dr. Hunger with more generalized or non-specific reactions to leaf spotting diseases collected in the field across Oklahoma. Progress is already evidenced in a section of this proposal contributed by Dr. Hunger for tan spot resistance. Septoria leaf blotch (SLB) resistance is WIT's next target for dedicated screening efforts. We still expect to achieve progress in tan spot resistance at a more rapid rate, simply because assays for SLB are compromised by environmental effects or difficulty in working with the SLB pathogen. Opportunistic ratings will be taken in the field for reaction to spot blotch, physiological leaf spot, and SLB.

In addition to those disease traits, we will maintain our traditional focus on wheat soil-borne mosaic (WSBM)/wheat spindle streak mosaic (WSSM) complex, BYD, and powdery mildew. Our focus on stripe rust depends on natural infections in Oklahoma (e.g., 2005, 2008, 2010, 2012, 2015, and 2016), but this effort is critically supplemented with artificial inoculation nurseries conducted by USDA-ARS near Manhattan, KS, and field nurseries under natural infection conducted by USDA-ARS in Washington.

Our own in-house molecular marker assays developed by Dr. Yan will be intensified to enable partial genetic selection for resistance to stripe rust, leaf rust, and/or powdery mildew, as the genetic background of a given population allows. Selection is partial, because markers have not been discovered (nor will they be very soon) for all targeted resistance genes. For example, molecular markers will be used to augment, if not corroborate, field selection for a specific *Pm3* allele that is likely frequent in our materials, two genes that provide adult-plant resistance to stripe rust, the adult-plant resistance gene *Lr34/Yr18* that provides durable resistance to leaf rust and stripe rust, and gene *Lr46* that also provides durable resistance to leaf rust (likely present in Duster). Molecular data also will enhance our ability to select adapted lines with the appropriate series of reproductive developmental traits controlled by genes on chromosomes 5A (first-hollow-stem emphasis), 2D (heading date emphasis), and 7D (physiological maturity emphasis), plus a newly discovered gene that interacts with the gene on chromosome 5A. Finally the work proposed by Dr. Charles Chen will enable genome-wide selection for molecular markers linked to yield and quality. This will be an area of intensive focus as WIT attempts to catch up to the genome selection capacity of its peer programs.

*W I T*  
**Molecular Breeding**  
**(2 proposals)**  
**Liuling Yan**  
**Plant and Soil Sciences**

## **Deliverables**

- (1) Validation of candidate genes for Hessian fly (Hf) resistance. (*Focus area 2*)
- (2) Identification and utilization of unique sequences within the grain yield QTL *QYld.osu-1BS*. (*Focus area 2*)
- (3) Deployment of favorable alleles from *TaVRN1*, *TaANR1*, and *TaHOX1* for increased grain yield. (*Focus area 5*)
- (4) Genotypes of current hard winter wheat varieties for beneficial alleles conferring adaptation and resistance to diseases and pests. (*Focus area 2*)

## **Background and Procedures**

*Deliverable 1.* In previous studies, we mapped the *TaHf-A1* gene, the first gene on chromosome 1AS for resistance against Hessian fly biotype GP, within approximately a 160-kb region in diploid *T. tauschii* and a 120-kb region in chromosome 1AS of hexaploid wheat cultivars Chinese Spring and 'LM15'. The latter is a hard red winter wheat cultivar used to construct a BAC library at Shandong Agricultural University. In recent studies, we have narrowed down the targeted region to a 43-kb region. Significant progress was made using the newly released International Wheat Genome Sequencing Consortium sequences of Chinese Spring. All of the markers used to clone the *TaHf-A1* gene indicated that Billings has the same sequences as Chinese Spring, but Duster has different sequences from Chinese Spring and Billings. Moreover, Duster showed unique sequences relative to diploid, tetraploid and hexaploid wheat, based on sequences available in public databases.

Therefore the next step of cloning the *TaHf-A1* gene will be to sequence the targeted region in Duster. We will design DNA probes specific to the targeted region and sequence the exon-captured fragments (i.e., of the exome) using a next-generation sequencer. The new markers will be mapped in two critical lines. One is the Billings/Duster doubled haploid line OK12D-Blgxst-DH212 and the other is a conventional F<sub>2:3</sub> progeny derived from Billings/Duster. We will also screen more F<sub>2:3</sub> progeny of Billings/Duster to find more crossover events in the targeted region. The phenotype and genotype of recombinant lines will be integrated to determine the location of *TaHf-A1*. Allelic variation in each of the candidate genes in the targeted region will eventually be identified to determine the final candidate for the *TaHf-A1* gene. Identification of this candidate is crucial to using marker-assisted platforms in forward breeding for Hessian fly resistance.

*Deliverable 2.* We recently discovered a major QTL for grain yield in the distal region of the short arm of chromosome 1BS, called *QYld.osu-1BS*. Based on field-based yield determinations, the Duster allele at the *QYld.osu-1BS* locus increased yield by approximately 20%, relative to the Billings allele. However, this yield difference was determined in a closed DH population derived from the cross of Billings x Duster. As one of the potentially largest QTL for grain yield discovered so far, *QYld.osu-1BS* provides a unique opportunity to clone the gene responsible for this QTL. However, *QYld.osu-1BS* was found to be in a region where no crossover events were observed with 42 GBS markers mapped among 280 Billings/Duster DH lines. It would largely benefit wheat breeding programs if this gene could be directly utilized before it is cloned.

Unique sequences representing *QYld.osu-1BS* locus will be identified using two strategies. The first is to use clustered GBS marker sequences to find scaffold sequences. The primers will be designed to amplify the genes specific to chromosome 1BS. Allelic variation between Duster and Billings will be determined. The second strategy will be to use an exome-capture platform to sequence the *QYld.osu-1BS* genomic region. SNPs in the genes will be converted into high-throughput KASP assays. Unique

sequences indicative of the Duster allele spanning *QYld.osu-1BS* will be used to ensure that the whole genomic region of the yield gene is introduced into novel lines and varieties. Heretofore we have found one of 18 sequenced genes to show large differences rather than SNPs. We predict that there might be 20 unique genes among the 360 genes possible within the 22 Mb spanning the *QYld.osu-1BS* region.

*Deliverable 3.* Wheat inherently exhibits low nitrogen-use efficiency. We found that a major QTL for N-related agronomic traits encompasses the vernalization gene *VRN-A1*. *VRN-A1* regulates NUE through development and its interaction with *TaANR1*, a protein encoded by a wheat ortholog of *Arabidopsis nitrata regulated 1 (ANR1)*. In wheat, *TaANR1* has been functionally characterized in a natural mutant with a 23-bp deletion in its gDNA sequence, which produced a transcript lacking the full 84-bp exon 6. *TaANR1* and *TaHOX1*, another protein affecting development in wheat, may directly bind with *VRN-A1*. Preliminary evidence indicates that the combination of favorable alleles from *VRN-A1*, *TaANR1*, and *TaHOX1* may increase grain yield by a moderate amount in the field.

Further work is needed before deploying this tri-locus model for NUE regulation in wheat breeding. In addition to the *TaANR1* marker for the 23-bp indel, SNPs in *VRN-A1* and *TaHOX1* will be converted into high-throughput KASP assays so that we can subsequently track the favorable alleles in breeding populations.

*Deliverable 4.* We have developed several gene markers for traits that bear significance to Oklahoma wheat production. In addition to *VRN-A1*, *TaANR1*, and *TaHOX1* for nitrogen-use efficiency as mentioned above, others include *VRN-A1*, *PPD-D1*, and *VRN-D3* for development, *Pm3* for powdery mildew resistance, *Lr34* and *TaXA21* for resistance to a complex of diseases, *OPR-A1* (from 2174) and *TaPI-A1* (from Duster) for Hessian fly resistance, and *TaALMT1* for acidic soils. Currently our strategy is to select for *TaPI-A1* as the candidate gene for *TaHf-A1*, though research described in Deliverable 1 is needed to proceed with that strategy. We have also developed molecular markers for translocated fragments that harbor genes for resistance, including *VPM1* for multiple diseases, *Wsm1* for wheat streak mosaic, and *Cmc4* for curl mite. In the new project year, we will use these markers to genotype a contemporary and complete hard winter wheat variety panel from the 2017 variety trial set, in collaboration with David Marburger.

### Charles Chen

#### Biochemistry and Molecular Biology

WIT will continue to pursue a still-emerging and exciting molecular technology called *genomic selection* (GS), having first evaluated the applicability of this breeding tool within the Billings x Duster DH population previously mentioned in Dr. Yan and Carver's sub-proposals. Our expectation will continue to be that lessons learned in this finite population may be extrapolated to a larger OSU germplasm base frequently populated by these two varieties and their progeny.

Broader application of genomic selection highly depends on the knowledge of genetic architecture of target traits and the interactions of genetic and environmental effects in determining trait expression. This interaction is too often expressed when the difference between two varieties (for example, in yield) changes dramatically from one location or year to another. To account for the environmental variability in the southern Great Plains and to simultaneously improve the economic value of wheat, we must be able to expand predictive capacity within the complexity of the breeding program.

Therefore, our main research aims are to *dissect* and *model* causative genetic polymorphisms in DNA sequence which govern multiple agronomic and end-use quality traits across environments. We intend to break this down in the form of three specific deliverables:

### **Deliverables**

- (1) Genomic predictions which ferret out genotype x environment interactions and contributions specifically from genic regions. (*Focus area 6*)
- (2) Improved knowledge base for genetic associations between grain yield and multiple indicators of end-use quality in hard red winter wheat. (*Focus area 6*)
- (3) Multiple-trait selection indices for grain yield and end-use quality parameters. (*Focus area 6*)

### **Background and Procedures**

*Deliverable 1.* Until now, GS has been mired in the test-drive stage of evaluating algorithm performance. Cross-validation, a popular technique used in assessing algorithm predictability, may be achieved by partitioning the same sample dataset into training and testing subsets and by quantifying the deviation between them. Using cross-validation, our Billings x Duster DH population generated an approximate 60% prediction accuracy for grain yield, averaged across all models applied from years 2014 to 2016. Unfortunately, such high predictive capability is often overly optimistic, because cross-validations generate more appealing results when the testing subset is drawn from the same population as the training dataset, but more importantly because predictive algorithms which neglect environmental variability are too simplistic.

To incorporate predictive algorithms in a more realistic design, we implemented a cross-year validation scheme and concluded that, for example, prediction of 2016 grain yield with training data taken from year 2015 suffered a significant decrease of 32% and 38%, respectively, for the additive linear model (GBLUP) and the non-parametric kernel methods (RKHS) model. Such a reduction of predictive capacity is mostly due to gene effects being modulated by environmental conditions, leading to a genotype (G) by environment interaction, known as G x E. A more comprehensive algorithm capable of considering G and G x E is therefore highly desired.

To account for highly inconsistent environmental conditions in the southern Great Plains, this research is proposed to establish a Bayesian hierarchical model that can simultaneously resolve both the whole genomic effect (the underlying genetic architecture for grain yield, for example) and the G x E interaction. This novel Bayesian model will be built directly on WIT's Billings x Duster DH population, with genomic information taken from a worthy set of 22,000 SNPs, encompassing inter-genic GBS SNPs and exome-capture SNPs in genic regions. Equipped with detailed genetic information anchored in both non-gene and gene regions, we also propose to decompose phenotypic variance into genetic components, including the genomic background composed of inter-genic regions, to evaluate the relative importance of functional genes to grain yield predictability.

*Deliverable 2.* About 50,000 genetic probes were designed using public databases from the D genome (*Aegilops tauschii*) and A genome (*Triticum uratu*) Agilent Wheat Microarray (MIPS v2.2) and from cDNA libraries from the NimbleGen SNP array, resulting in 709,063 SNPs in protein-coding regions for the Billings x Duster DH population. In January 2017, the International Wheat Genome Sequencing Consortium released the RefSeq v1.0 wheat genome assembly. In anticipation of a fully assembled and annotated bread wheat genome in the near future, WIT has migrated its own genomic resource, the

Buster GBS SNPv2014 build, together with the exome capture SNPs, for alignment with the RefSeq IWGSC v1.0 assembly.

Using this integrated resource, WIT conducted a preliminary genome-wide association study (GWAS) for grain yield. Three years (2014, 2015, and 2016) of field data were combined using a mixed linear model to remove year and plot effects, and using a high significance threshold of  $10^{-5}$ , 62 SNP variants were pinpointed to be associated with grain yield, including the previously identified QTL on the short arm of 1B genome (see more in the proposal by Dr. Yan).

From currently OWRP-funded research, RNA expression variation contributing to grain yield variation has also been investigated. Over 2,440 genes were considered to be highly expressed, *among which a protein-coding gene that co-localized with the grain yield associated SNP under the 1B5 yield QTL was discovered*. Based on a global search for functional homology, this gene encodes serine/threonine protein kinase, which plays a crucial role in signal transduction events under *drought stress*. For the proposed project period, WIT will continue *de novo* gene discovery to identify key genetic loci responsible for grain yield variation in Oklahoma environments, by integrating knowledge from public resources with DNaseq and RNAseq data derived from Billings x Duster DH population.

Considering most economically important traits such as yield and end-use quality involve a large number of genes, each subject to environmental effects, multi-trait associations allow us to address genetic pleiotropy, whereby a single QTL controls variation in more than one trait. Knowledge of pleiotropy provides essential clues about what genes to target so agronomic performance is maintained while targeting baking quality.

WIT has generated two years of milling and baking quality data for the Billings x Duster DH population, with a third year (2016) soon to be available. To remove environmental effects, best unbiased linear predictor (BLUP) values will be generated for all available traits; the BLUP model is “best” in the sense that experimental error is minimized, and “unbiased” in the sense that the average value of the estimates remains. We will conduct mixed linear GWAS for each trait individually, and for groups of traits to identify sources of pleiotropism. All association information will be mapped with the RefSeq IWGSC v1.0 assembly, and supported with transcriptional variation from RNASeq data derived from the Billings x Duster DH population. A filtered subset of SNPs will be used to construct linkage maps for QTL mapping. The comparison of GWAS and QTLs will further indicate to what degree chromosome rearrangements and genetic translocation are responsible for trait variation.

*Deliverable 3.* In practice, when selection is performed for crop traits of economic importance, selection is generally applied to several traits simultaneously. When breeders select for wheat yield, they are actually selecting *directly* for a myriad of genes which confer optimal adaptation under the available environmental conditions, and *indirectly* for traits conferred by other genes in linkage disequilibrium with those targeted directly or *indirectly* for traits which are genetically associated, or correlated, primarily via pleiotropism. Though widely accepted, genomic selection is currently still single-trait (STGS) centric, whereas MTGS, or multiple-trait genomic selection, is only explored in simulation studies. A few benefits resulting from simulations agree with theoretical advantages of indirect selection, that is, where prediction accuracies for a target trait with relatively low heritability can be substantially improved when a genetically correlated indicator trait with higher heritability is considered within the same GS model. Alternative scenarios of MTGS that consider secondary traits (for example, protein content) in low or negative correlation with a primary trait (for example, grain yield) can impede broad application of GS.

To maximize the benefit of GS for Oklahoma wheat breeding programs, we propose to investigate the efficiency of selection indices (SI) which are capable of manipulating multiple traits simultaneously in a favorable direction, such as improving grain yield with greater dough strength. Specific breeding targets such as improving grain yield while maintaining higher-tier protein levels (e.g., >12.5%), or such as improving grain yield while extending winter dormancy release, will too be explored using a restricted SI.

W I T

## ***Identification and Introgression of Disease Resistance***

**Bob Hunger**

**Entomology and Plant Pathology**

### ***Deliverables***

- (1) Reactions WIT experimental lines to the WSBM/WSSM complex, leaf rust, powdery mildew, tan spot, septoria, and BYD based on a combination of testing in the greenhouse and/or field. (*Focus area 1*)
- (2) Enhanced disease screening capability of WIT advanced lines via i) continued development of no-till leaf spot disease nurseries (tan spot and Septoria leaf blotch/Stagonospora glume blotch) and ii) assay for vernalized-plant reaction to leaf rust and powdery mildew, with comparison to ongoing assays for seedling reactions. (*Focus areas 1 and 4*)
- (3) Collection of contemporary foreign wheat germplasm with resistance to diseases of importance to Oklahoma, and development of first-generation hybrids with locally adapted parents. (*Focus area 3*)

### ***Procedures***

*Deliverable 1.* Reaction to the WSBM/WSSM complex is determined by visually rating symptoms expressed by lines growing in a field nursery and by testing foliage from these lines using the serologically-based test ELISA (enzyme-linked immunosorbent assay). Typically about 1,000 WIT lines are visually rated for virus symptoms, and symptomatic foliage from a subset of 600 to 800 lines is collected and tested for presence of *Soil-borne wheat mosaic virus* and *Wheat spindle streak mosaic virus* using ELISA. Combining results from these tests facilitates identification of lines resistant to both viruses, a key requisite for advancement through the VDP.

Reaction to leaf rust, powdery mildew, BYD, tan spot, and Septoria leaf blotch/Stagonospora glume blotch are determined either by rating seedlings inoculated in the greenhouse or by taking field ratings when and where available. To enhance field ratings, nurseries are used for powdery mildew, BYD, tan spot, and Septoria leaf blotch/Stagonospora glume blotch. The nurseries for powdery mildew and BYD rely on natural infection and contain spreader rows susceptible to each disease to facilitate infection and spread. The tan spot and Septoria/Stagonospora nurseries use infected wheat residue from the previous crop as a source of inoculum as well as supplemental inoculum placed into or on each nursery. For tan spot, this involves making and placing oat kernel inoculum in the nursery during the fall and winter that provides starter spores for the disease. For Septoria, spores of the fungus are produced in the lab and then sprayed onto plants in February during weather favorable for infection.

*Deliverable 2.* Both tan spot and Septoria leaf blotch field nurseries (no-till) have been established at the PLP Research Farm located just west of Stillwater. Advanced lines from the

Oklahoma Elite Trial (OET) are planted in both of these nurseries. To date, tan spot has not yet reached a level of severity sufficient to rate lines for reaction to tan spot. However, this no-till nursery will be maintained with the hope that continued inoculation will eventually result in establishment of tan spot. Supplemental irrigation will be used in the future to facilitate establishment of tan spot. Fortunately our testing procedure of seedlings in growth chambers provides us with critical information to gauge the reaction of experimental lines to tan spot. In 2017, approximately 277 lines have been assayed for seedling reaction to tan spot.

A similar nursery to test for reaction to leaf/glume blights caused by the fungi *Septoria* and *Stagonospora* also has been established and used in 2015 and 2016. In 2016, 145 lines in 4 advanced nurseries were assayed for reaction to these leaf spot diseases. As with the tan spot nursery, the *Septoria* leaf blight/*Stagonospora* glume blotch nursery will be continued and inoculation along with supplemental irrigation will be used in future years to enhance infection and disease development.

Seedling reactions to powdery mildew and leaf rust have been a consistent disease evaluation tool for WIT for many years. However, seedling reaction alone does not always provide the entire picture of reaction to powdery mildew and the wheat rusts because adult plant resistance (APR) also can be present. Hence, in 2016 and 2017 we have attempted to test seedlings and post-vernalized wheat plants of advance wheat lines for reaction to leaf rust and powdery mildew. If successful, a comparison of the seedling and post-vernalized plants can be made to see if APR is present. This was not successful with powdery mildew in 2015-2016, but appears to be on track with leaf rust for 2016-2017.

*Deliverable 3.* Winter wheat germplasm was exchanged with Hungary, Romania, and Turkey in 2016 for the 2017 growing season. Currently this germplasm is being crossed with wheat adapted to Oklahoma for transfer of disease resistance and other traits. For the coming 2017-2018 growing season, this germplasm exchange program is being expanded to exchange germplasm with selected wheat breeding institutions in China.

W I T

***Identification and Introgression of Aphid Resistance***  
**(2 proposals)**

**Xiangyang Xu**  
**USDA-ARS, Stillwater, OK**

Bird cherry-oat aphid (*Rhopalosiphum padi*; BCOA) poses a serious threat to wheat production in Oklahoma because of the heavy yield losses it causes each year, and its role in transmitting barley yellow dwarf virus (BYDV). Due to the lack of BCOA resistance sources, little progress has been made in breeding BCOA-resistant cultivars worldwide. In addition to locally developed germplasm identified in the next section (see section by Giles) that may harbor effective BCOA resistance, we have identified a few genetic stocks and landraces exhibiting BCOA resistance/tolerance. One that holds tremendous promise, TA3516, has been used in the ARS germplasm enhancement program. Genomic tools are urgently needed to move the BCOA resistance from TA3516 into WIT germplasm. Another feather in the cap of TA3516 is the greenbug resistance gene, *Gb5*. Greenbug is another vector of BYDV, and causes annual yield loss as much as \$250 million in the USA.

This proposal aims to reveal the genetic basis of BCOA resistance/tolerance in TA3516, develop genomic tools for introgression of BCOA and greenbug resistance, and select BC<sub>1</sub>F<sub>3</sub> plants carrying the

BCOA resistance QTL and *Gb5* via marker-assisted selection.

### **Deliverables**

- (1) Identity of BCOA resistance QTL in TA3516. (*Focus areas 2*)
- (2) KASP markers closely linked to the BCOA resistance QTL in TA3516 and to greenbug resistance gene *Gb5*. (*Focus area 4*)
- (3) BC<sub>1</sub>F<sub>3</sub> plants from crosses with WIT elite lines carrying desirable QTL for BCOA resistance and/or *Gb5* gene. (*Focus area 3*).

### **Procedures**

*Deliverable 1.* With the support of OWRF, we have tested a worldwide collection of wheat accessions and identified a few accessions that consistently provide moderate or high resistance to BCOA. One of them, TA3516, has been used in germplasm enhancement, and an F<sub>6</sub> recombinant inbred line (RIL) population derived from the cross TA3516 X Bainong418 was developed to map QTL for BCOA resistance in TA3516. A total of 248 RILs will be evaluated for BCOA resistance using a protocol established at the USDA-ARS Wheat, Peanut, and Other Field Crop Research Unit. A randomized complete block design with six replicates will be used, and fifteen plants of each RIL will be evaluated in each replicate. The interval between infestation and initiation of wilting (IIW) will be used to characterize the compound effects of antixenosis, antibiosis, and tolerance. The estimated broad sense heritability of IIW was 0.73 (unpublished data), indicating that IIW may offer greater breeding efficiency over antibiosis resistance, reported elsewhere to have a broad sense heritability of 0.16 (Crespo- Herrera et al.,2014). The RIL population will be also evaluated for greenbug resistance using a standard aphid evaluation protocol.

A WGR (whole genome resequencing)-based QTL mapping approach that achieves gene-level resolution will be adopted in this study. In brief, five DNA libraries will be constructed for multiplex Illumina sequencing of the 248 RILs and two parents. DNA from 50 samples will be pooled into a library. All libraries will be sequenced at a depth of 0.1X using a standard protocol on Illumina HiSeq2500, and 125 base pair-end reads will be generated. Additional sequences will be generated from the two parents to achieve >4X sequence depth.

A pipeline combining Burrows–Wheeler Aligner and Sequence Alignment/Map tools (SAMtools) will be used to call SNPs in RILs (Xu et al. 2013). The RIL sequences will be aligned against the Chinese Spring reference genome sequence (1.0 version) using the Burrows–Wheeler Aligner to identify SNPs. The “pileup” function of SAMtools will be used to merge the SNP dataset. Only biallelic SNPs will be kept in the dataset. Similarly, SNPs between two parents will be identified. Only SNPs identified in both RILs and parents are considered as candidate SNPs.

In order to use unique sequences to genotype the RIL population, all reads mapped to multiple locations of the reference genome will be excluded from analysis, and a  $\chi^2$  test will be conducted for each SNP with a null hypothesis that the two alleles at a locus segregate with a ratio of 1:1 in the RIL population. All SNPs that significantly deviate from this ratio ( $P < 0.01$ ) will be excluded from the SNP dataset, resulting in a set of “true” SNPs to genotype the RIL population. The  $\chi^2$  tests are expected to efficiently eliminate potentially false SNPs caused by misalignment of paralogous sequences, homeologous sequences, and sequence errors.

An approach based on the Hidden Markov Model will be adopted to genotype each RIL. A crucial task of the genotyping procedure is to locate recombination intervals (RI), the genomic regions

between two adjacent haplotype blocks (Figure 1). Based on genotyping results, RIs will be delineated and bins determined. Each bin harbors a number of SNPs and represents a haplotype, and thus can be used to construct the linkage map. The program R/QTL package will be used to construct a linkage map using bins as markers, and QTL for BCOA resistance will be mapped using the MQM method of the program MapQTL 5.0.

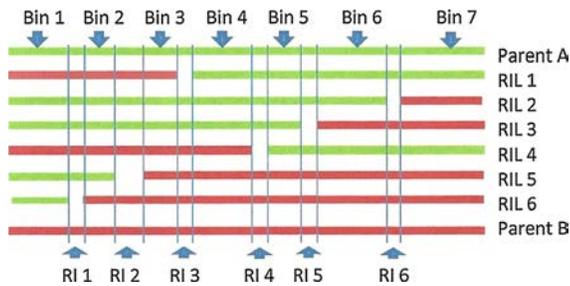


Figure 1. Construction of a bin map. The green represents parent A allele, and the red represents parent B allele. The transition regions, or blank regions, between two haplotype blocks are defined as recombination intervals (RI), and the genomic region between two adjacent RIs is defined as a bin. Each bin harbors a number of phased SNPs and represents a haplotype.

**Deliverable 2.** SNPs significantly associated with BCOA resistance, as well as the *Gb5* gene, in TA3516 will be converted to PCR-based Kompetitive Allele-Specific PCR (KASP) markers. The short SNP sequences will be used as queries to search the Chinese Spring reference sequence ([urgi.versailles.inra.fr/blast/blast.php](http://urgi.versailles.inra.fr/blast/blast.php)). For each query, three homeologous sequences from the A, B, and D genome, respectively, will be identified, and subsequently aligned using Genedoc 2.7 (<http://genedoc.software.informer.com/2.7/>). Based on alignment results, two allele-specific forward primers and one common reverse primer will be designed using the Primer3 program ([http://biotools.umassmed.edu/bioapps/primer3\\_www.cgi](http://biotools.umassmed.edu/bioapps/primer3_www.cgi)) to differentiate sequences on the target sub-genome from those sequences on the other two sub-genomes. A set of 40 RILs will be randomly selected to test KASP primers. KASP assays will be carried out on an ABI ViiA7 instrument using a protocol we described recently (Li et al., 2017).

**Deliverable 3.** The marker-assisted selection approach will be used to move BCOA resistance QTL into WIT elite lines. Two BC<sub>1</sub>F<sub>2</sub> populations already derived from OK13625/TA3516//OK13625 and Bentley/TA3516//Bentley are currently growing in the field. The KASP markers developed in this study will be used to screen about 1,000 BC<sub>1</sub>F<sub>3</sub> seedling progeny from each population. The plants carrying the resistance gene(s)/QTL will be vernalized, and grown in a greenhouse at the USDA-ARS Wheat, Peanut, and Other Field Crop Research Unit. Seeds harvested from these plants will be bulked and grown in the field for two more generations (beyond this proposal period), and homogeneous lines will be subsequently obtained and validated with KASP markers.

**Kris Giles**  
**Entomology and Plant Pathology**

**Deliverable**

Breeding populations enriched for resistance to BCOA infestation (*Focus area 3*).

## **Procedures**

Our continuing goal is to identify plants from segregating populations in the early-inbreeding generations in the WIT variety development pipeline which express resistance to BCOA infestations and feeding. During 2016, we utilized our reliable and sensitive phenotyping assay for BCOA on wheat seedlings and phenotyped entries from 23 segregating F<sub>5</sub> populations from the variety development pipeline that were chosen based on expected reactions to BYD infection. Most of these populations provided no resistant plants for selection, likely the result of an absence of resistance genes present in the parents. However, from six of these populations, we did find a useful level of resistance that would exceed a frequency associated with experimental error. We categorized 422 individual seedling plants as partially resistant to resistant. These plants were isolated for propagation and to date have been vernalized and are being grown for seed production as of April 2017. We expect that selfed progeny from these plants will be resistant to BCOA, but prior to advancement into the VDP, we believe it is essential that results are validated.

For the continuing study (year 3), we will phenotype a selected subset of seedlings representing selfed F<sub>5:6</sub> families from each of the six populations. Individual F<sub>5</sub> plants were chosen in fall 2016 based on resistance ratings during the 2016-2017 funding cycle. We will target at least 10 parent F<sub>5</sub> plants from each population for progeny testing and advancement. Six seeds will be collected from individual F<sub>5</sub> heads to constitute a family. If the original F<sub>5</sub> parent plant was homozygous for one resistance gene, the progeny should not segregate for resistance. We expect the rate of homozygosity to exceed 90% among the F<sub>5</sub> plants if resistance is conferred at a single locus. It is not known at this time the mode of inheritance for BCOA resistance in winter wheat. However, mistaken identity or phenotype in the F<sub>5</sub> parent population will be manifested as families with mostly susceptible progeny in this generation. Our purpose is to determine the accuracy of the assay conducted in the F<sub>5</sub> while advancing desirable (BCOA-resistant) selections for subsequent field testing in the VDP. All populations were chosen as worthy candidates for generating candidate cultivars.

Our BCOA assay is as follows: (i) infestation of emerging wheat seedlings with 22 aphids (14 hours photophase at 20°C), (ii) resistance classification within 6 days post-infestation based on development of a second leaf, and (iii) termination of assay 18 days post-infestation. Reactions are further classified based on leaf development and levels of chlorosis. In addition, we will measure aphid activity and reproduction on each plant in an effort to initially characterize the mechanism of resistance. To ensure consistency, assays will be conducted in environmental chambers at the CERL (Controlled Environment Research Lab) at OSU. Entries with validated resistance may be advanced in the variety development pipeline (subsequent to this funding period).

*W I T*

## ***Transforming N-Use Efficiency to End-Use Quality***

**Brian Arnall**

**Plant and Soil Sciences**

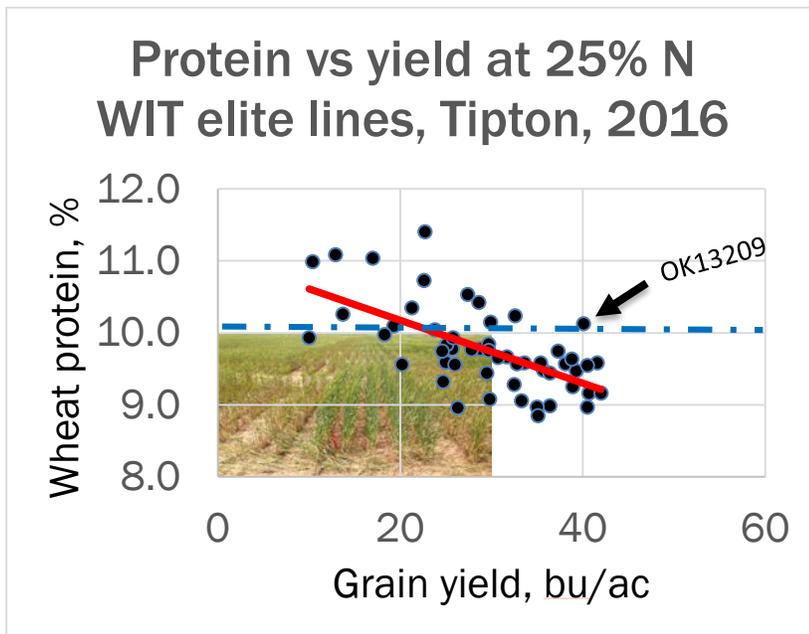
## **Deliverables**

- (1) Candidate lines with agronomic yield stability and end-use quality stability across a range of N levels. (*Focus area 3*)
- (2) Publicly available agronomic data and varietal NUE characteristics of new releases. (*Focus area 1*)

## **Procedures**

*Deliverable 1.* Advanced experimental lines previously identified by the WIT in the current funding cycle will be again evaluated in at least two environments across Oklahoma, using four nitrogen rates per environment. The range of nitrogen rates will represent the yield-goal nitrogen rate of the region and a series of rates below optimum. In year 1 (2016-2017) OK11D25056 (Smith's Gold), OK13209, OK12D220022-077, and Gallagher were planted at Lake Carl Blackwell and Lahoma. Each line was evaluated at 30, 60, 90, and 120 lbs of N per acre. Subjecting advanced lines to this series of rates will allow WIT to identify the environmental potential of each line and to assess their ability to utilize nitrogen and convert it efficiently to yield, protein, and targeted end use. During the growing season GreenSeeker, NDVI readings will be collected to document the growth pattern and biomass accumulation of each entry. At maturity grain will be collected and analyzed for yield and quality parameters including milling and baking characteristics. All location and treatments will be managed for maximum yields, with the exception of nitrogen. Preliminary evidence indicates OK13209 (also described in Dr. Carver's proposal) can maintain high yield at 25% of the optimum N-applied level while suffering minimal wheat protein loss. OK12D220022-077 may offer similar potential, though a second year is needed to confirm.

Supplemental to the activity described for this deliverable, we will continue to provide a low-N testing environment at Tipton, OK for evaluating approximately 150 WIT advanced lines in replicated yield and quality trials. *From this effort in 2015 and 2016 WIT has uncovered wide diversity in apparent N-use efficiency that previously went undetected.* Candidates with unusually and consistently high yielding ability under N-challenged conditions include OK11755W-139129-3W, OK12716, OK128084C, OK12912C, OK13625, OK13621, OK13209, and OK12D22002-077. These were further characterized in Carver's section of the proposal above. For unknown reason, a highly disproportionate number of Clearfield experimental lines excel in this low-N environment. The critical piece to this initiative is identifying market-suitable quality under depressed N conditions, the first indicator of which is wheat protein content. The figure below shows the wheat protein vs. yield relationship found among a subset of 60 advanced lines tested at Tipton in 2016. The negative association between wheat protein and yield typically does not exist at optimum N, but it will be critical moving forward to select lines with elevated yield at a wheat protein content exceeding 10% at this level of applied N. Essentially no additional N is available for plant uptake beyond what is applied at this site.



*Deliverable 2.* Data will be posted on the [www.npk.okstate.edu](http://www.npk.okstate.edu) website and presented at field days and extension meetings. The data will also be shared with the Small Grains Extension Specialist so that the information can be incorporated into variety trial reports along with any extension publications used to educate producers on currently available cultivars and those in the pipeline.

*W I T*

#### **Information Exchange and Candidate Cultivar Assessment**

**David Marburger**  
**Plant and Soil Sciences**

#### **Deliverables**

- (1) Adaptability profiles of OSU candidate cultivars for best positioning of new releases in Oklahoma. (*Focus area 1*)
- (2) Publicly available agronomic data and variety characteristics of new releases. (*Focus area 1*)

#### **Procedures**

*Deliverable 1.* Approximately 10 advanced experimental WIT lines will be evaluated as part of the OSU wheat variety trials in approximately 24 environments across Oklahoma. Data collected from these trials will be used to measure and determine suitability of candidate cultivars 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 70/30 between grain-only and dual-purpose trials. Including candidate cultivars in the OSU variety trials provides side-by-side comparisons to wheat cultivars that are commercially released for production in Oklahoma, and this will serve as an integral part in the decision-making process for determining which candidate cultivars should continue onward to commercial release.

In addition, the Oklahoma Elite Trial (approximately 30 entries) will be planted at Kingfisher and Cherokee, Oklahoma to add crucial sites to the database for elite line performance produced by Dr. Brett Carver.

*Deliverable 2.* Variety comparison statistics are the data most-requested by wheat farmers in the state of Oklahoma, and the inclusion of candidate cultivar yield information in variety trial reports allows producers to know what genetics are in the pipeline. Data will be posted in near real time at [www.wheat.okstate.edu](http://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 these efforts in the area of promotion will allow the Oklahoma wheat producer to better visualize his/her return to their checkoff dollars and their investment in the OSU wheat improvement team.

## 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 grain functionality. Historically, that is indeed the mantra of wheat improvement programs across the globe, except perhaps to a lesser extent in western Europe, where the emphasis on yield per may outweigh emphasis on yield protection. In fact, 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. The 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 also be marketed and consumed. We relish this opportunity to strengthen every link in the value chain – from producer to consumer.

### **RELATION TO OTHER RESEARCH:**

The research proposed herein constitutes the majority of research efforts for WIT members Carver, and Hunger, whereas WIT members Yan, Chen, Xu, Arnall, and Giles pursue other initiatives that are independently funded yet complementary to wheat improvement research, or they may even

work with other commodities. All team members assume other academic responsibilities in teaching and/or outreach.

## REPORT OF ACCOMPLISHMENTS:

The Wheat Improvement Team gratefully acknowledges past continual support of the OWRF/OWC, without which these accomplishments would not have been achievable.

- Released Spirit Rider HRW wheat in February 2017 after 7 years of statewide testing across Oklahoma, featuring a highly competitive yield and end-use quality record in OSU wheat variety trials and in the Wheat Quality Council. (WIT)
- Released Smith's Gold HRW wheat in February 2017, giving Oklahoma wheat producers downstate an improved option over Gallagher for insect resistance, stripe rust protection, and end-use quality. (WIT)
- Published wheat variety trial reports in a redesigned and branded format to improve viewing experience and comprehension for the reader. (Marburger)
- Provided 18 in-season wheat disease updates directly 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)
- Conducted more than 25 wheat variety tours across Oklahoma to provide stakeholders with current information on released and candidate varieties. (Marburger)
- Determined reaction specifically to the WSBM/WSSM complex for nearly 1,500 wheat experimental lines, most of which were developed by WIT. Determining reaction to the WSBM/WSSM complex has been a staple in the OSU program, and has nullified the impact of this virus disease complex since the mid-1980s. (Hunger)
- Determined reactions to leaf rust, tan spot, septoria, and barley yellow dwarf for about 385 WIT experimental lines. When considering replication of lines across all diseases, approximately 4,000 disease evaluations were made in 2016. These reactions contribute critical information used to make advancement decisions in variety development. (Hunger)
- Established no-till field nurseries to generate ratings among advanced WIT experimental lines for leaf spotting disease reactions, specifically tan spot and Septoria leaf blotch/Stagonospora glume blotch. During future years, presence of these diseases will be facilitated so that consistent and reliable reaction data can be procured. (Hunger)
- Exchanged germplasm with wheat research programs in Hungary, Romania, and Turkey for introgression of disease resistance and to enhance genetic diversity. Expansion to include China is planned for 2017-2018. (Hunger)
- Identified for the first time six experimental breeding populations expressing variable levels of resistance to bird cherry-oat aphid (BCOA) under artificial infestations. Progeny will be selected and advanced for assay validation and development of experimental lines as candidate cultivars. (Giles)
- Identified two more landraces exhibiting high resistance to BCOA, 'Osiris' and 'Ghund Hosa', collected from Egypt and Afghanistan, respectively. (Xu)
- Nearly completed the mapping and locating the chromosome position of a new greenbug resistance gene in the reselection line RSPI 595379 (expected summer 2017). (Xu)
- Delimited the *TaHf-A1* gene (resistance to Hessian fly biotype GP) within a 43 kb genomic region, greatly increasing WIT's chances of successfully cloning this gene in the coming year. (Yan)

- Discovered a natural mutant of *TaANR1*, a wheat ortholog of *Arabidopsis nitrate regulated 1* (*ANR1*), in winter wheat cultivar 2174, which has a 23-bp deletion partially including exon 6. (Yan)
- Released WITs first genotyping-by-sequencing (GBS) SNP build “BusterSNPv2014”, comprised of 165,654 SNPs anchored on IWGSC-WGA-v0.4 pseudo-molecules, averaging 7,888 SNPs per genome and varying from the highest (10,952 SNPs) on chromosome 3B to the lowest (4,802 SNPs) on chromosome 4D. (Chen)
- Merged exome-capture SNPs from genic regions with GBS SNPs to conduct genome-wide association study (GWAS), and identified 62 SNP variants in 21 genomic regions associated with grain yield, including the QTL in the 1BS genome discovered by Yan. (Chen)
- Identified the Billings x Duster DH line, OK12D-Blgs x Dst-DH236, with exceptional drought resistance characteristics and thus a worthy parental source for drought resistance. (Kakani)
- Confirmed the high NUE of OK13625 and OK13209, along with other candidates under preliminary or extended seed increase. Smith’s Gold, Sprit Rider, and Doublestop CL Plus were shown to have intermediate to moderately high yielding ability at 25% N supply, whereas Gallagher has performed poorly at reduced N. (Arnall)
- OSU-bred cultivars accounted for the top six cultivars planted in Oklahoma in fall 2017, and 66% of the acres reported and specified by known variety name. (WIT)
- Placed 11 additional winter wheat candidates under preliminary or extended seed increase by Oklahoma Foundation Seed Stocks (Carver, Marburger, Hunger):
 

OK12912C-138407-2	N91D2308-13/OK03926C//OK03928C
OK128084C	N91D2308-13/OK04902C//OK05907C
OK13209	OK Bullet/TX00D1390//Shocker
OK12DP22004-016	Everest/OK08328//OK09634
OK13621	TX00D1390/ Billings
OK13625	Billings/Fannin sib
OK12D22002-077	Billings/OK08328
OK14319	NE01533/OK02125//Duster
OK10430-2	CS+1V/2*Endurance sib
OK12206-127206-2	Y98-912/OK00611W//OK03716W
OK15115	Pete/OK Bullet//TX03A0148

**Wheat Improvement Team  
Budget, FY2017-2018**

Principal Investigator (C=continued project)	BUDGET CATEGORY					TOTAL, \$
	Wages & benefits (or salary if noted below)	Expendable supplies	Travel	Equipment and repair	Growth chamber & greenhouse rental	
<b>Carver (C)</b>	84,000	15,000	4,000	9,000		<b>112,000</b>
<b>Yan (C)</b>	41,000	34,000	4,000	5,000	5,000	<b>89,000</b>
<b>Chen (C)</b>	36,000	26,000	6,000			<b>68,000</b>
<b>Hunger (C)</b>	12,000	7,500	5,000		8,000	<b>32,500</b>
<b>Giles (C)</b>	2,100	2,500			4,800	<b>9,400</b>
<b>Xiangyang Xu (C)</b>		45,000				<b>45,000</b>
<b>Marburger (C)</b>	7,000 <sup>c</sup>	4,000	4,000			<b>15,000</b>
<b>Arnall (C)</b>	3,000	3,000	1,000			<b>7,000</b>
<b>TOTAL, \$</b>						<b>377,900</b>

Where not specified by scientist, wages and benefits support undergraduate temporary employees; expendable supplies support field, greenhouse, and growth chamber activities involving wheat; travel is dedicated predominately to in-state research activities, though up to 30% of Carver's travel may be outside Oklahoma to participate as an invited participant at conferences on demand; equipment and repair for Carver 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.

**Giles**

Two part-time (10h/wk/student) undergraduate research assistants are needed for 10 weeks at a pay rate of \$10 per hour. Students will assist with soil preparation, planting, colony maintenance, and ratings validation. Expendable materials and supplies include general controlled-environment items such as soil, sand, trays, and pots. To ensure viable aphid colonies and separate experimental areas, two environmental chambers (at \$400/chamber/month) will be rented from CERL (Controlled Environment Research Lab at OSU) for 6 months.

**Xu**

NGS sequencing: 561Gb x \$70/Gb = \$39,270; DNA extraction, development of KASP markers, and marker-assisted selection: \$5,730.

**Yan**

2-months salary for Carol Powers temporarily divested by PaSS, plus \$25,000 to supplement salary of a postdoc partly supported by a USDA-funded gene-editing project.

**Chen**

One-year support for a full time quantitative genetics PhD graduate position is requested for genomic prediction model development, subject to renewal. Partial support is requested for salary and benefits of research associate Dr. Karyn Willyerd. Expendable supplies include contractual service for 10 RNA libraries at \$160 per library preparation, and two flow cell lanes for Illumina HighSeq300 PE150 with each lane costing \$2,149. Laboratory supplies will be used for sequencing-library preparation, including chemicals, devices, consumable plastics like tips and trays. Also needed are 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. Travel funds will be used for Chen and Willyerd to attend a national conference such as the Plant and Animal Genome conference and to support a graduate student's involvement in the 2017 Joint Statistics meeting.

SIGNATURE PAGE

Genetic improvement of winter wheat: Integrating classical and novel approaches

Submitted by:

OSU's Wheat Improvement Team

Proposed funding level: \$377,900

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*Tom Coon*

Tom Coon  
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**SIGNATURE PAGE**

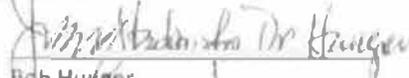
**Genetic improvement of winter wheat: Integrating classical and novel approaches**

**Submitted by:**

**OSU's Wheat Improvement Team**

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Brett Carver  
Plant & Soil Sciences

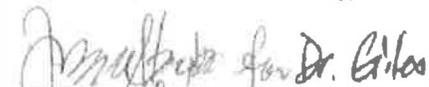
  
Bob Huirger  
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Liuling Yan  
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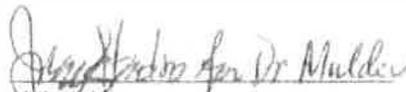
  
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