ANNUAL REPORT TO THE ARKANSAS WHEAT PROMOTION BOARD
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TITLE: Arkansas Rust Initiative

INVESTIGATOR: Gene Milus

COOPERATORS: Scott Monfort and Jason Kelley, Cooperative Extension Service
Dave Marshall, USDA, ARS, Raleigh, NC
Jim Kolmer, USDA, ARS, St. Paul, MN
Dave Long, USDA, ARS, St. Paul, MN
Gina Brown-Guedira, USDA, ARS, Raleigh, NC

OBJECTIVES:

To characterize adult-plant resistance to stripe rust in soft red winter wheat and to incorporate resistance genes *Lr34/Yr18* and *Lr46/Yr29* into adapted lines.

To characterize the seasonal population diversity of the wheat leaf rust fungus in southern Arkansas.

To determine the life cycle of the wheat stripe rust fungus in eastern North America.

ABSTRACT

Resistant varieties are the most cost-effective means of managing stripe rust and leaf rust, but new races of the pathogens can overcome some types of resistance. Little is known about the genes for stripe rust and leaf rust resistance in contemporary soft red winter wheat cultivars. The two most widely studied genes (*Lr34/Yr18* and Lr46/Yr29) for adult-plant resistance to all tested races of leaf rust and stripe rust are not found in contemporary varieties and would be valuable additions to current resistance genes. Although leaf rust is usually found in the field during the fall and survives the winter, leaf rust epidemics usually do not develop until late in the season even though the environment appears favorable for leaf rust during the spring. The goal of this project was to learn more about the resistance genes protecting contemporary cultivars from leaf rust and stripe rust and about the variability within contemporary populations of the leaf rust and stripe rust pathogens in order to understand how best to provide effective long-term protection against these diseases.

A master's-level graduate student was recruited to work on the characterization of adult-plant resistance to stripe rust. Thirty lines from the USDA Eastern Stripe Rust Nursery that had low to moderate levels of stripe rust across five locations and the 20 varieties in the 2008 Disease Management Plots were evaluated for resistance in the seedling and heading stages to old and new races of the stripe rust fungus. Most of these lines were susceptible to one or both races at the seedling stage but expressed various levels of resistance at the heading stage, indicating that they had some type of adult-plant resistance. A diverse subset of 20 lines was selected for research in growth chambers and in the field at two locations to further characterize the resistance and attempt to determine which lines have different genes for adult-plant resistance (a necessary preliminary step to actually identify the genes). Six stripe rust collections from across

the state were identified as the same race that was first identified here in 2000, indicating that this race replaced the old race but has not yet evolved to overcome additional resistance genes.

Suspected sources of genes *Lr34/Yr18* and *Lr46/Yr29* were obtained, and individual plants of these lines were evaluated for molecular markers linked to these genes. Only some of the suspected sources and some of the plants within a source had the appropriate markers for the genes, and these plants were grown in growth chambers to transfer the genes to locally-adapted varieties by crossing. Plants from backcross populations will be evaluated for molecular markers linked to these genes to identify plants with one or both of these genes. This is the first project in Arkansas to use molecular markers for facilitating the transfer of genes in wheat and may open the door for additional uses of this technology.

The race designations of 39, 23, and 48 isolates were identified from leaf rust collections from four plots of each of five varieties in the Disease Management Plot in Chicot County in December, March and April, respectively. Twenty-two races with ability to overcome 18 of the 20 evaluated resistance genes were found. Only 8 of 48 isolates collected in April matched races that were identified in December, indicating that new races blew in during the spring and caused most of the disease. Ten named and one or more unnamed race-specific resistance genes for all-stage leaf rust resistance were identified among the 20 varieties in the Disease Management Plot. Only gene Lr 37 in Terral LA 841 and genes Lr 9 + 24 in Hornbeck 3266 provided complete protection against all of the races found in the plot, indicating that unnamed genes are likely protecting the varieties from leaf rust. Furthermore, none of the varieties appeared fully susceptible to leaf rust in the field, indicating that most of the varieties have adult-plant resistance conferred by unidentified resistance genes.

As the Board was informed in March, I have not been able to find a Mexican scientist willing to collaborate on the objective to determine the life cycle of the wheat stripe rust fungus in eastern North America, so this is no longer part of the project.

INTRODUCTION

Leaf rust and stripe rust are among the most important wheat diseases in Arkansas, and resistant varieties are the most cost-effective means of preventing losses from these diseases. Many varieties of soft red winter wheat are susceptible to stripe rust and/or leaf rust as seedlings but have moderate to high levels of resistance as adult plants. This type of resistance is known as adult-plant resistance and can be race specific (effective against some races but not others) or race nonspecific (effective against all tested races). Recent evaluations using molecular markers indicated that the most widely studied adult-plant, race nonspecific resistance genes, *Lr34/Yr18* and *Lr46/Yr29*, are not present in contemporary soft red winter wheat varieties. If the molecular data are correct, then there are several valuable resistance genes waiting to be discovered in adapted varieties. Furthermore, incorporating *Lr34/Yr18* and *Lr46/Yr29* along with some of the resistance genes already present should provide a high level of durable resistance to both leaf and stripe rusts.

Leaf rust is commonly found in southern Arkansas from fall through mid March and frequently at high severities. Although conditions appear favorable for an early leaf rust epidemic, the epidemic usually does not occur until late in the season. At least two hypotheses could explain these observations: 1) the rust population that overwinters is not capable of causing an epidemic, and a different population arrives later to cause a late-season epidemic, or 2) the type of resistance in our varieties delays the epidemic until late in the season. The results of this research should determine which hypothesis is correct.

In 2000, a new strain and race of the stripe rust fungus first appeared in the US and has replaced the old strain and races in the eastern US. Before 2000, stripe rust occurred infrequently in Arkansas, Louisiana, and Texas. Since 2000, stripe rust has overwintered every year in these states and caused moderate to severe epidemics across the Great Plains and the lower Mississippi River Valley whenever spring weather was favorable. The new strain appears to be more fit than the old strain to survive over summer because the incidence fall-infected wheat has been higher across the south-central US since 2000. Spores of the stripe rust fungus are short lived, require cool temperatures for infection, and can be produced only on living plants of wheat and various wild grasses. However, relatively few spores are produced on wild grasses compared to wheat, so wheat with stripe rust during October and November is the most likely source of spores that infect wheat in the south-central US. Apparently, spring wheat is grown at high elevations in Mexico during this time of year and may be connected to stripe rust epidemics in the south-central US. More information is needed about wheat production and stripe rust development in Mexico in order to piece together the life cycle of the stripe rust fungus in eastern North America. Once the life cycle is understood, various options for managing stripe rust in both the US and Mexico could be evaluated.

MATERIALS AND METHODS

Characterization of stripe rust resistance and races. Thirty soft red winter wheat lines with diverse parentage and low levels of stripe rust across five field locations were obtained from Dave Marshall who coordinates the USDA Soft Red Winter Wheat Stripe Rust Nursery. The 20 varieties identified as the most widely grown in Arkansas also were included. Seedlings and adult plants of these lines were grown in growth chambers and a greenhouse and then inoculated with old and new isolates of the stripe rust fungus. Experiments with adult plants were conducted at both high and low temperature regimes because some adult-plant resistances are known to be temperature sensitive. Infection types were recorded as a measure of resistance.

Seven collections of the stripe rust fungus were made from locations across Arkansas and were evaluated on the US set of 20 differential wheat lines to determine race.

Transferring genes *Lr34/Yr18* and Lr46/Yr29. Five plants of each line suspected to have resistance gene *Lr34/Yr18* or *Lr46/Yr29* and their respective molecular markers were vernalized and grown in growth chambers and a greenhouse. Small pieces of leaf tissue from each plant were assayed for markers linked to these genes, and plants with the appropriate markers were selected for crossing with adapted varieties to transfer these genes.

Seasonal diversity of the leaf rust fungus and identification of genes for leaf rust resistance. Collections of the leaf rust fungus were made in December, March and April from four plots of each of five varieties grown in Disease Management Plots in Chico County. Single-pustule isolates were obtained from as many collections as possible and inoculated onto sets of 20 differential lines to identify races.

Seedlings of the 20 varieties in the Disease Management Plots were inoculated with ten races of leaf rust to determine which genes for all-stage resistance likely are present in the varieties.

Life cycle of the stripe rust fungus. Attempts were made to collaborate with Eduardo Espitia and his colleagues in Mexico, but they do not appear to be interested in working on this project..

RESULTS AND DISCUSSION

Characterization of stripe rust resistance and races.

In the seedling stage, most of the 50 wheat lines evaluated had high infection types to isolates of the stripe rust fungus representative of strains and races before 2000 (old isolates) and since 2000 (new isolates) (Table 1) and had low to moderate infection types on flag leaves of adult plants and low to moderate levels of stripe rust in the field (data not shown). These results indicate that these lines likely have genes for adult-plant resistance. The identity of these genes is unknown. Because some genes for adult-plant resistance are only effective against certain races whereas other genes are effective against all races tested, the long-term effectiveness of the resistance in contemporary lines is unknown.

Of the seven collections of the stripe rust fungus, one was lost due to contamination with leaf rust and six had virulence similar to the race that was first identified in 2000. These results indicate that the new strain and race replaced the old strain and races that were here before 2000 and that the new strain and race has not yet evolved virulence to overcome additional resistance genes. However, a new race with additional virulence on *Yr17* in the new strain background has caused epidemics in Mexico and western United States, and this new race likely will overcome the all-stage resistance in Terral LA 841, AGS 2060 and other varieties with *Yr17*.

Transferring genes *Lr34/Yr18* and Lr46/Yr29.

Based on the presence of molecular markers linked to these resistance genes, plants of Pioneer 25R18 have been identified as sources of gene *Lr34/Yr18*, and plants of Kansas breeding lines KS06O3A-50 and KS06O3A-58 have been identified as sources of gene *Lr46/Yr29* (data not shown).

Seasonal diversity of the leaf rust fungus and identification of genes for leaf rust resistance.

Eight, 8, and 12 races were found from collections made in December, March and April, respectively (Table 3), indicating that the contemporary population of the leaf rust fungus is very diverse. Furthermore, only two races were found at all three sampling times, indicating that new races likely blew in to the field throughout the season.

Ten race specific genes for all-stage resistance were identified among the 20 varieties in the Disease Management Plots (Table 4), but only gene *Lr37* in Terral LA 841 and genes *Lr9 + 24* in Hornbeck 3266 provided complete protection against all races in the plot. The low to moderate levels of leaf rust among the remaining 18 varieties (Table 4) indicates that these varieties likely have genes for adult-plant resistance. The identity of these genes is unknown, so it is not possible to predict if these genes are likely to be overcome by new races.

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Leaf rust race identification	Dave Long, USDA Cereal Disease Lab, St. Paul, MN
Leaf rust gene identification	Dr. Jim Kolmer, USDA Cereal Disease Lab, St. Paul, MN
Molecular marker tests	Dr. Gina Brown-Guedira, USDA Genotyping Lab, Raleigh, NC
Seed of wheat lines	Dr. Dave Marshall, USDA Plant Science Unit, Raleigh, NC
	Dr. Alan Fritz, wheat breeder, Kansas State University

Table 1. Stripe rust infection types (1-3 = low, 4-6 = intermediate, and 7-9 = high) on seedlings of wheat lines after inoculation with isolates AR90-01 and AR97-01 (representative of old isolates) and isolates AR00-05 and AR03-33 (representative of new isolates).

Line	AR90-01	AR97-01	AR00-05	AR03-33
AgriPro/Coker 9553	7	5,7	7	4
AgriPro/Coker Beretta	8	5/8	7	.
AgriPro/Coker Magnolia	7	7	7	8
AGS 2000	0	2	6/8	7
AGS2050	8	7	8	8
Armor 5110	8	6/8	9	6
Croplan Genetics 554w	9	7/9	6/8	7
Croplan Genetics 8302	8	7	8	8
Delta Grow 1600	8	7	9	7
Delta Grow 4500	8	8,4	6/8	9
Delta King 7710	9	6/8	9	9
Dixie 989	8	6	8	8
Hornbeck 3266	8	8	8	8
Pat	8	8	8	8
Pioneer 26R15	5	6	9	.
Pioneer 26R22	8	7	8	7
Pioneer 26R87	7	4/7	7	8
Progeny 166	8	8	8	8
Terral 8558	8	8	8	8
Terral LA 841	7	6	7	7
MO 040117	9	8	8,6	9
LA9415D104-5-2-B-3-B	2	2	7	.
NC97BGTD7	8,4	8	8	8
LA978UC-36-1-1-B	8	8	7	8
LA01112D-20-B	0	0,2	8	7
LA98005D3-1-C	9	9	7/9	8
LA98113D-41-1-C	9	8	8	8
LA98133D-160-3-C	8	8	8	7
LA98149BUB-3-4-B	8	8	8	.
LA98214D-14-1-2-B	2/8	2/7	8	8
GA991336-6E9	6	2/5	7/8	7
ARS03-5929	8	8	7/8	6/8
ARS05-1034	2	7,2	7	8
KS03HW72	5	6	8	5
VA05W-65	8	9	8	.
MD01W233-06-21	8	8	8	.
D03*9603	8	7	7	6/8
MO 021532	8	8	8	8
MO 040152	8	8	8	8
IL87-2834-1	8	8	8	8
IL99-26442	9	8	8	8
IL02-10754	8	8	8	8
99840C4-8-4	2	0,1	8	7
LA02-923	8	8	8	8
E5015	8	7	7	6/8
E5025	8	7	6/8	8
Pioneer 26R61	0	0,2	6/8	6/8
Sturdy 2K	8	7	8	8
Croplan Genetics 514	8	8	8	8

Table 2. Stripe rust infection types (1-3 = low, 4-6 = intermediate, and 7-9 = high) produced by collections made in Arkansas during 2008 on seedlings of lines used to differentiate races stripe rust.

Differential	**Yr gene**	**08-001**	**08-003**	**08-004**	**08-005**	**08-006**	**08-007**
Lemhi	21	8	8	8	9	8	8
Chinese 166	1	1	1	1	1	0	0
Heines VII	2, HVII	4	5	3	6	6	4
Moro	10, Mor	2	2	2	2	2	2
Paha	Pa1, Pa2, Pa3	2	3	2	2	2	2
Druchamp	3a, D, Dru	2	2	2	2	2	2
Yr 5	5	1	1	1	1	1	1
Produra	Pr1, Pr2	4	4	4	4	3	4
Yamhill	2, 4a, Yam	3	3	2	5	4	4
Stephens	3a, S, Ste	5	5	5	6	5	7,4
Lee	7, 22, 23	6	6	5	6	6	6
Fielder	6, 20	9	8	8	8	8	8
Tyee	Tye	1	1	1	1	0	0
Tres	Tr1, Tr2	2	2	2	2	2	2
Hyak	17	2	2	2	3	2	2
Express	?	8	8	8	8	8	7
Yr 8	8	5	.	.	.	6	6
Yr 9	9	8	7	7	8	8	8
Clement	9, Cle	7	7	4,2	7	7	7
Compair	8, 19	7	7	4	7	7,2	6

Collection	Cultivar	Location	Date	
08-001	Crop. Gen. 554	Lonoke	2-Apr	
08-002	Terral TV 8558	Lonoke	2-Apr	(lost)
08-003	VA04W-628	Kibler	7-Apr	
08-004	Beretta	Altheimer	7-Apr	
08-005	Pioneer 26R87	Stuttgart	10-Apr	
08-006	AGS 2000	Lewisville	23-Apr	
08-007	LW 303	Chico Co.	23-Apr	

Table 3. The number of isolates of various races of the leaf rust fungus identified from collections made from five of the most susceptible varieties in the Disease Management Plots in Chicot County in December, March and April and the resistance genes that each race can overcome.

	Number of isolates identified							
Race	15-Dec-07	24-Mar-08	23-Apr-08	Virulence for Lr genes				
MBPSB	3	0	0	1,3		3ka,17a,30	B,10,14a	
MFPSC	4	6	4	1,3	24,26	3ka,17a,30	B,10,14a	42
MLDSD	1	0	0	1,3	9	17a	B,10,14a	41
SCPBB	2	0	0	1,2a,2c	26	3ka,17a,30		
TCJSB	5	0	0	1,2a,2c,3	26	11,17a	B,10,14a	
TDBGH	13	5	4	1,2a,2c,3	24		10	28,42
TDBJH	7	0	0	1,2a,2c,3	24		10,14a	28,42
TFBGH	4	0	0	1,2a,2c,3	24,26		10	28,42
TBRKG	0	2	3	1,2a,2c,3		3ka,11,30	10,14a,18	28
TCRJG	0	2	0	1,2a,2c,3	26	3ka,11,30	10,14a	28
TFRJG	0	2	3	1,2a,2c,3	24,26	3ka,11,30	10,14a	28
TCSBB	0	2	0	1,2a,2c,3	26	3ka,11,17a		
MBSNB	0	2	0	1,3		3ka,11,17a	B,14a	
MCGJG	0	2	0	1,3	26	11	10,14a	28
MBBJG	0	0	2	1,3			10,14a	28
MBDSC	0	0	1	1,3		17a	B,10,14a	42
MBGJG	0	0	2	1,3		11	10,14a	28
MCDSB	0	0	2	1,3	26	17a	B,10,14a	
MCTSB	0	0	3	1,3	26	3ka,11,17a,30	B,10,14a	
MFRJH	0	0	2	1,,3	24,26	3ka,11,30	10,14a	28,42
TCRKG	0	0	19	1,2a,2c,3	24	3ka,11,30	10,14a,18	28
TBSSB	0	0	3	1,2a,2c,3		3ka,11,17a	B,10,14a	

Table 4. Leaf rust resistance genes present in the 20 varieties evaluated in Disease Management Plots during 2008 based on seedling tests with ten races conducted and the average leaf rust severity across four replicated plots in Chico County in December, March and April.

		% leaf area with leaf rust		
Variety	Lr genes present	15-Dec-07	24-Mar-08	23-Apr-08
AgriPro/Coker 9553	11	0	0.3	4.5
AgriPro/Coker Beretta	14a	0.1	0	0.5
AgriPro/Coker Magnolia	11	0.1	0.3	6.5
AGS 2000	14b, 26	0	0	0.5
AGS2050	14a	0.3	0	4.8
Armor 5110	1	0.6	0.8	3.3
Croplan Genetics 554w	1	0.1	2	7.3
Croplan Genetics 8302	11	0.1	0.8	11.3
Delta Grow 1600	2a, 11	0.1	1	7.8
Delta Grow 4500	1	0.6	1	4.5
Delta King 7710	10, 14a	0.1	1.3	5.8
Dixie 989	2a, 11	0.1	0.8	7
Hornbeck 3266	9,24,+*	0	0	0
Pat	11	0	0.8	1.5
Pioneer 26R15	11	0	0.6	2.8
Pioneer 26R22	11	0.1	1.8	20.5
Pioneer 26R87	+	0	0.1	6.5
Progeny 166	1	1	1.3	5.8
Terral 8558	2a, +	0.1	1	20.5
Terral LA 841	37	0	0	0

* "+" indicates an unknown gene for resistance