

Wheat Research Progress Report

Project #: 3061-3923

Title: Control of Stripe Rust, Leaf Rust, and Stem Rust of Wheat

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The Goal and Objectives:

The goal of this project is to prevent major losses in wheat yield and quality caused by stripe rust, leaf rust, and stem rust and assure stable, sustainable wheat production. The specific objectives are the following: **1.** Monitor occurrence, distribution, and development of rusts; identify races of the wheat stripe rust; collaborate in race identification of leaf rust and stem rust; and determine population structures and factors involved in rust epidemics. **2.** Screen wheat germplasm and breeding lines for resistance to stripe rust, leaf rust, stem rust, and other foliar diseases; identify and develop new resistance sources for improving resistance to rusts. **3.** Determine genetics of resistance, identify new resistance genes, develop molecular markers for stripe rust resistance genes for marker-assisted selection; and determine mechanisms of rust resistance and wheat-rust interactions. **4.** Determine effectiveness and use of fungicides for rust control and develop strategies for integrated rust management.

Accomplishments:

Progress has been made for all of the four objectives as planned for the project in 2008. All planned studies have been completed on time. There is no any delay, failure, or problem so far for this project. The following lists the accomplishments made for each of the four objectives:

1. Monitor occurrence, distribution, and development of rusts; identify races of the wheat stripe rust; collaborate in race identification of leaf rust and stem rust; and determine population structures and factors involved in rust epidemics

In 2008, stripe rust, leaf rust, stem rust, and other foliar diseases of wheat were monitored throughout the Pacific Northwest (PNW) through field surveys and disease nurseries. Early prediction of wheat stripe rust epidemic was made using rust forecasting models based on temperatures in December 2007 and January 2008. Stripe rust forecast was reported to wheat researchers and growers as early as in late January 2008. The lower than normal (the 57 years average) temperatures in January reduced the survival of the pathogen, resulting in a late occurrence of stripe rust. From March, field survey was conducted periodically and rust updates on distributions and severities were provided to growers based on real-time rust situations. Wheat stripe rust was observed in the first week of April in the hard red winter wheat fields in the Horse Heaven Hill area. The timely application of fungicides in this region in April controlled the disease and prevented further spread of the disease to other areas. The low temperatures before mid June and the hot and dry weather conditions from mid June to late July also contributed to the general slow development and low severity of stripe rust in 2008. Therefore, yield losses caused by stripe rust were minimal (less than 1% across the state). However, stripe rust severity reached over 90% and yield losses were up to 30% on susceptible wheat cultivars in our experimental plots. A commercial spring wheat field in the Palouse region

had 100% stripe rust severity at the flowering stage and the field likely suffered over 30% yield loss. The data demonstrate that stripe rust could cause substantial yield losses if susceptible cultivars are grown without use of fungicides even under the relatively unfavorable weather conditions in the Pacific Northwest.

Leaf rust was substantial in western Washington, but very low in eastern Washington. Stem rust occurred as small hot spots in wheat fields in the Palouse region, but over 80% severity reached in some hot spots. In general the damage of wheat crops by leaf rust and stem rust was minimal. However, severe stem rust occurred in barley fields in Stevens County and yield losses in some fields were estimated as 30% or more.

Through cooperators in other states, stripe rusts of wheat and barley were monitored throughout the U.S. In 2008, a total of 330 stripe rust samples were obtained from 21 states, of which 139 (42%) from Washington State. 290 samples (88%) were from wheat and 40 (12%) from barley, triticale, and grasses. In addition, 15 stem rust samples were collected from wheat, barley, and barberry plants (the alternate host of wheat and barley stem rust). The stem rust samples were sent to the USDA-ARS Cereal Disease Laboratory in St. Paul for identifying races. The identification of stripe rust races were conducted in our program. Race identification of 85% of the samples was finished. From identified samples, 30 races of wheat stripe rust and 8 races of barley stripe rust were identified. Of 20 wheat stripe rust races detected in Washington, PST-114 was the most predominant (26.5%) followed by PST-100 (13.7%) and PST-116 (9.8%). The most virulent race PST-127 increased from 1 sample to 7 samples in Washington. So far, no races were identified with virulences on resistance genes *Yr5* and *Yr15*, which confers effective race-specific all-stage resistance and widely used in breeding programs for developing wheat cultivars with stripe rust resistance.

To determine population structures and variation mechanisms of the stripe rust pathogen at the molecular level, we have designed over 50 gene-specific PCR and EST-SSR primer pairs based on cDNA sequences obtained from our previous studies and used the primers to amplify DNA of stripe rust isolates from various regions in different years. Over 30 primer pairs showed polymorphism among different isolates. Currently, we are using the primers to study a large number of rust isolates to determine molecular variations. We have used sequences of seven important fungal genes to determine evolutionary relationships among some major races in the U.S. and obtained the first molecular evidence that heterokaryon (two different nuclei in a rust spore) is a major factor for making changes in the stripe rust fungus. Using the stem rust sequence as a reference, we constructed the first physical map or linkage group for over 3,000 genes of the stripe rust pathogen.

2. Screen wheat germplasms and breeding lines for resistance to stripe rust, leaf rust, stem rust, and other foliar diseases; identify and develop new resistance sources for improving resistance to rusts

In 2008, we evaluated more than 15,000 wheat and 5,000 barley entries for resistance to stripe rust and other foliar diseases. The entries included germplasms, breeding lines, rust monitoring nurseries, and genetic populations from various breeding and extension programs. All nurseries were planted and evaluated at both Pullman and Mt. Vernon locations under natural stripe rust infection. Some of the nurseries were also tested in Walla Walla and Lind, WA and various locations in other states in the U.S., Mexico, and Kenya. The wheat entries also were evaluated for resistance to leaf rust, stem rust, powdery mildew, and physiological leaf spot at the Mt. Vernon field plots and locations in other states and countries where these diseases occurred. Germplasms and breeding lines potentially for release also were tested in the greenhouse with selected races of stripe rust for further characterization of resistance. Disease data of regional nurseries were provided to all breeding and extension programs, while data of individual breeders' nurseries were provided to the individual breeders. We evaluated more entries for the Pacific Northwest, especially Washington breeding programs, than any other regions. Through these tests, susceptible breeding lines were eliminated, which should prevent risk of releasing susceptible cultivars and assisted breeding programs to release new cultivars of high yield and quality, good adaptation, and effective disease resistance. Through the germplasm screening, we

have established a collection of wheat germplasms with stripe rust resistance, which are valuable sources of stripe rust resistance for further characterization of resistance, identified new effective resistance genes, and for development of wheat cultivars with superior resistance.

High-temperature, adult-plant (HTAP) resistance to stripe rust is non-race specific and durable. However, because HTAP resistance is generally incomplete, cultivars with HTAP resistance may suffer some levels of yield losses, especially when the disease is severe. Spring wheat cultivars Alpowa and Express with HTAP resistance can suffer up to 15% yield losses. To improve the level of durable HTAP resistance, we made a cross between Alpowa and Express and selected recombinant inbred lines (RILs) from the cross with molecular markers developed for HTAP resistance genes in Alpowa and Express from our previous studies. In 2008, we tested 10 RILs together with Alpowa and Express in a randomized complete block field experiment with four replications. Five of the RILs had resistance higher than either Alpowa or Express and four of the lines were completely free of stripe rust infection. These lines were provided to breeding programs for developing wheat cultivars with durable and high level of HTAP resistance.

3. Determine genetics of resistance, identify new resistance genes, develop molecular markers for stripe rust resistance genes for marker-assisted selection; and determine mechanisms of rust resistance and wheat-rust interactions

We completed studies to determine the genetics and mapping the resistance genes in spring wheat cultivars IDO377s and Zak. Both cultivars each have a single dominant gene controlling the race-specific all-stage resistance to stripe rust. Both genes were mapped to the wheat chromosome arm 2BL, but at different loci and different from previously reported stripe rust resistance genes. Molecular markers identified for these genes were used to detect them in wheat cultivars and breeding lines.

We completed the study to identify and map a gene for HTAP resistance in wheat genotype Compair and the *Yr8* near-isogenic line. This novel HTAP resistance gene, together with *Yr8* for race-specific all-stage resistance, was mapped to wheat chromosomal arm 3DL. Molecular markers identified for both genes should be useful for incorporating the durable resistance gene to wheat cultivars. The identification of the HTAP resistance gene in the *Yr8* near-isogenic line also solved a problem of monitoring stripe rust virulence in the world. Working together with Kim Kidwell and Kim Campbell's programs, we have mapped another gene for HTAP resistance in spring wheat cultivar Louise on wheat chromosome arm 2BS. Molecular markers associated with this gene can be used in marker-assisted selection to transfer this source of HTAP resistance into regionally adapted wheat cultivars, thereby improving the durability of resistance.

To identify novel and effective genes for all-stage resistance to stripe rust, we made crosses of 200 resistant germplasms (113 common wheat and 87 durum wheat genotypes) selected based on our previous multiple race tests in the greenhouse and field tests to a single susceptible variety. In 2008, we tested the resistant parents in the greenhouse with race PST-127, the most virulent race identified first in 2007 and race PST-100, the most predominant race and in the fields under natural stripe rust infection. F₂ populations of 92 crosses were tested for resistance in an experimental field near Pullman, of which 70 crosses showed segregation. Based on the rust data, one resistance gene each was detected in 21 crosses, two genes each in 43 crosses, and three genes each in 6 crosses. In the greenhouse tests for 35 crosses, 10 crosses had a single dominant gene, 23 had two genes, and 2 had three genes for resistance. Of 26 crosses that were tested both in the field and greenhouse, 23 had the same results in both tests while 3 had different segregation ratios.

Screening the resistant parent, susceptible parent, resistant and susceptible bulks of F₂ of 38 common X common wheat crosses with resistance gene analog polymorphism (RGAP) markers showed very low genetic relatedness among the resistant parental genotypes. The data indicate that most of the genes for resistance to stripe rust in these genotypes are likely different from each other. Therefore, these wheat germplasms have numerous potentially novel genes for stripe rust resistance.

We have completed the study to identify and map a single dominant gene for resistance to stripe rust in wheat genotype PI 181434, originally from Afghanistan. PI 181434 was resistant to all nine tested stripe rust races including the most virulent race PST-127 and in all field tests. For these cross, 103 F₂ derived F₃ lines were tested with races PST-100 and PST-127 in the greenhouse to confirm the F₂ phenotypes in the field and to determine genotypes of F₂ plants. The F₃ data confirmed a single gene for resistance. A linkage map was constructed for this resistance gene with 8 RGAP and 2 SSR markers. The closest marker is 4.8 cM. This gene was mapped on the long arm of chromosome 3D. This gene is different from all previously reported stripe rust resistance genes by its resistance to all tested races and its unique chromosomal location. It should be useful for developing resistant wheat cultivars.

The common X durum wheat crosses were used to transfer novel and effective resistance genes from durum wheat. Because not many stripe rust genes have been reported from durum wheat, genes identified in durum wheat genotypes are likely to be novel. In the previous years we made 87 crosses between susceptible common (42 chromosomes) wheat Avocet S and resistant durum (28 chromosomes) wheat and obtained the F₃ seeds for 23 crosses. In 2008, we checked in average 30 plants for their number of chromosomes for each of 23 crosses. F₃ plants with 42 chromosomes were grown and tested in the greenhouse with race PST-127 at the boot stage. Five of the crosses showed one gene for resistance and 18 crosses showed two resistance genes.

We selected one cross, AVS X PI 480148, for mapping a single dominant gene for resistance in the resistant durum genotype originally from Ethiopia. We tested 150 F₄ plants from a heterozygous F₃ plant with 42 chromosomes with races PST-100 and PST-127 in the greenhouse. We have identified two RGAP markers and one of them is tightly linked to the gene within 3.0 cM. Studies are conducted to map the gene to a wheat chromosome for this cross and identify genes in other durum wheat genotypes.

To answer the questions why race-specific all-stage resistance is not durable and non-race specific HTAP resistance is durable, we have conducted studies to elucidate mechanisms of stripe rust resistance using the microarray technology. In previous studies, we identified genes regulated by *Yr5* for race-specific all-stage resistance and *Yr39* for non-race specific HTAP resistance using Affymetrix Wheat Genechips. In 2008, we made custom gene chips based on genes identified in the previous studies in order to determine common and unique genes regulated by various genes for either all-stage or HTAP resistance. Microarray experiments were completed and the data are being analyzed. We expect the studies to provide insights to the molecular mechanisms for durability of HTAP resistance compared to race-specific all-stage resistance. Through collaborating with Dr. Jorge Dubcovsky's program at UC Davis, we have cloned *Yr36*, a gene conferring HTAP resistance. This is the first officially cloned stripe rust resistance gene and it has a unique product structure different from all previously cloned plant resistance genes. The cloned resistance gene makes it more efficient to use the gene in breeding programs and understand the resistance mechanisms.

In 2008, we also made the first genechip for the stripe rust pathogen to study pathogen genes involved in infection. We have completed the microarray experiments and are currently analyzing the data. This is the first microarray study for the stripe rust pathogen in the world. We expect the results to lead to a better understanding of the plant-pathogen interaction.

4. Determine effectiveness and use of fungicides for rust control and develop strategies for integrated rust management

In 2008, we evaluated 18 fungicide treatments including different rates and application combinations of Topguard, BAS 556 01, and Evito for control of stripe rust in experimental fields near Pullman, WA. Susceptible winter wheat varieties 'PS 279' and 'Hatton', spring

wheat 'Lemhi', and spring barley 'Morex' were used in the studies. The tests were conducted as a randomized complete block design with four replications in each experiment. Fungicides were applied at different rates and stages of crop growth. Stripe rust severities were recorded three or four times during the rust season. Grains were harvested and weighted for each plot. Rusts and yield data were analyzed to determine the efficacy for each fungicide treatment. These fungicide treatments provided various levels of rust control and some significantly increased gain yield.

Results and Deliverables:

1. In 2008, stripe rust was accurately forecasted using predictive models based on weather data, monitoring data, and cultivar resistance. Rust updates and advises were provided on time to growers during the crop season. All of these efforts greatly reduced stripe rust damage and reduced unnecessary use of fungicides, which saved millions of dollars for growers.
2. We have identified 30 races of the wheat stripe rust pathogen and 20 of them occurred in Washington States in 2008. Frequencies of these races in the U.S. and Washington State were determined and predominant races were identified. In 2008, we have tested wheat cultivars and resistant stocks with the most virulent race PST-127 that was first identified in 2007 to determine potential impact of the new races to breeding programs and currently grown cultivars. We provided the race information to breeding programs to avoid use of or carefully use individual resistance genes overcome by the new and more virulent races.
3. Gene-specific and EST-SSR markers were developed for the stripe rust pathogen. Major isolates of stripe rust were studied with molecular markers and sequences of important rust genes. The preliminary study revealed mechanisms of rust evolution at the molecular level. We have made the first genechip for microarray studies to determine pathogen genes involved in infection and plant-pathogen interaction. We have constructed the first group of gene physic maps or linkage groups for the stripe rust pathogen using the sequence syntany with the stem rust pathogen. We have initiated the whole-genome sequencing and functional genomic studies for the stripe rust pathogen. These will provide great genetic resources and tools for better understanding stripe rust.
4. In 2008, more than 15,000 wheat and 5,000 barley entries were evaluated for resistance to stripe rust and other diseases. From the tests, new germplasms and advanced breeding lines with stripe rust resistance were identified. The data and information of stripe rust evaluation were provided to breeding programs for eliminating potential susceptible cultivars, and developing new cultivars with adequate resistance. Examples of new variety final releases and pre-releases in 2008 include 'Farnum' (WA 7975), 'Kelse' (WA 7954), 'Whit' (WA 8008), 'Scarlet 09' (WA 8034), 'JD' (WA 8047), 'Babe' (WA 8039), and 'Xerpha' (WA 7975).
5. Through the research of the past several years and collaborations with breeders, HTAP resistance has been characterized and widely used in breeding programs, which has led to the major wheat acreage planted with durable and adequate resistance to stripe rust in the PNW. HTAP resistance has continued to be the most effective type of stripe rust resistance. Without HTAP resistance in major wheat cultivars, the stripe rust epidemic would have been much more widely spread, much more fungicide application would have been used, and much greater yield losses growers would have suffered, as even demonstrated in our field experiments under the generally light rust conditions in 2008. To identify more genes or quantitative trait loci for HTAP resistance and make more efficient use of the durable type resistance in breeding programs, we published the *Yr39* genes in Alpowa and three genes in Express and their markers, and published the HTAP resistance genes in Stephens with Drs. Kidwell and Campbell's programs in 2008. In this year, we completed the studies to identify and mapping a gene in the *Yr8* near-isogenic line for HTAP resistance. Together with Drs. Kidwell and Campbell's programs, we identified another novel gene for HTAP resistance in Louise. All of these results

provide a good number of HTAP genes and molecular markers for developing wheat cultivars with durable resistance to stripe rust.

6. In 2008, we finished the identification of a dominant gene each in IDO377s and Zak for race-specific all-stage resistance. Although virulences to these genes have been detected, they can be used in combination with other genes, but more importantly, they are useful in studying stripe rust virulence. We tested F₂ populations of near 100 crosses in the field and F₃ populations of over 30 crosses in the greenhouse for resistance to stripe rust. Genetic analyses showed numerous potentially novel genes for effective all-stage resistance. We identified two new genes, one in an Afghanistan common spring wheat and one in an Ethiopia durum spring wheat. Markers were developed for these genes to be used in marker assisted selection for developing resistant cultivars.

7. In 2008, we tested 18 fungicide treatments for their efficacy to control stripe rust. Most of the treatments provided control of stripe rust and increased yields. The chemicals varied in their effectiveness. The data will be useful for registration of new fungicides for control of stripe rust. We also tested currently grown cultivars for response to fungicide application to develop integrated control strategies. The integrated control program combining resistant cultivars and effective fungicides reduces input cost and ensures sustainable wheat production.

Publications:

Scientific Journals:

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Stripe Rust Data and Updates posted on website <http://variety.wsu.edu/updates.htm> 2008:

- Stripe rust data - 2008 spring barley nurseries (posted 10/17/08)
- Stripe rust data - 2008 spring wheat nurseries (posted 10/07/08)

- Stripe rust data - 2008 winter wheat nurseries (posted 10/07/08)
- Stripe Rust Update, 7/02/08
- Wheat stripe rust races in 2007 (X. Chen) - Excel
- Barley stripe rust races in 2007 (X. Chen) - Excel
- Stripe Rust 2007 race summary and the 2008 race ID update (X. Chen)
- Stripe Rust Update, 6/17/08
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- Stripe Rust Update, 4/9/08

Presentations and Reports:

Xianming Chen. “Control of stripe rust, leaf rust, and stem rust of wheat”. Washington Wheat Commission Progress Reports. Pullman, WA. February 14, 2008. (Over 100 participants)

Xianming Chen. “Stripe rust in the US and stem rust in Washington in 2007” at the Cereal Rust Workshop, Fargo, ND. March 18, 2008. (Over 30 participants)

Xianming Chen. “Molecular mapping and manipulation of new stripe rust resistance genes” and “Current status of genomic research of the stripe rust pathogen”, Northwest A&F University, Yangling, Shaanxi, China. May 5-9, 2008. (Over 300 participants)

Xianming Chen. “Rust reports”. Western Wheat Workers, WERA-97, and NCERA Joint Meetings in Davis, California. May 12-15, 2008. (Over 50 participants)

Xianming Chen. “Stripe rust” La Cross Field day, June 10, 2008. (Over 30 participants)

Xianming Chen. “Advances in Stripe Rust Research”, All-Russia Research Institute of Phytopathology, Moscow, Russia. August 20, 2008. (Over 100 participants)