

## **CONTROL OF STRIPE RUSTS OF WHEAT AND BARLEY**

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**ABSTRACT:** Rusts of wheat and barley were monitored throughout the Pacific Northwest (PNW) using trap plots and through field surveys during the 2006 growing season. Through collaborators in other states, stripe rusts of wheat and barley were monitored throughout the US. In 2006, wheat stripe rust occurred in more than 20 states, but the disease was generally much less than in 2005 due to the weather conditions unfavorable to stripe rust. However, the disease caused 15% yield losses in California and up to 28% yield losses on susceptible winter and spring wheat cultivars in our experimental plots. Barley stripe rust occurred in Arizona, California, Idaho, Oregon, and Washington, but the damage was low. Leaf rust and stem rust were either light or absent in the PNW. A total of 18 wheat stripe rust races and 11 barley stripe rust races were identified, of which five wheat stripe rust and two barley stripe rust races were new. In 2006, we evaluated more than 16,000 wheat and 5,000 barley entries for resistance to stripe rust and other diseases. We have mapped four genes in wheat and one gene in barley for durable high-temperature, adult-plant resistance and four genes in wheat and two genes in barley for race-specific all-stage resistance to stripe rust. We identified genes regulated by the *Yr5* resistance using the microarray technique. To study functional genomics of the stripe rust and further characterize stripe rust populations, we have constructed the first bacterial artificial chromosome (BAC) library and full-length cDNA library for the wheat stripe rust pathogen. From the 196 cDNA clones sequenced, we identified 51 different genes with functions in the fungal growth, defense, and virulence/infection. In 2006, we evaluated nine foliar fungicide treatments and eight seed treatments for control of stripe rust. Better chemicals, formulations, and application schedules were identified.

### **1. Monitor occurrence, distribution, and severity of rusts; identify races of the wheat stripe rust; and determine population structures and factors involved in rust epidemics**

In 2006, 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 2005 and January 2006. The later planting of winter wheat in the south central area of Washington State reduced the fall infection and the colder-than-normal weather in December 2005 reduced survival of the stripe rust fungus, resulting in a late start and slow development of stripe rust in the PNW. Stripe rust forecast was reported to wheat researchers and growers as early as in February 2006. From March, field survey was conducted periodically and rust updates on rust distributions and severities were provided to growers based on real-time rust situations. The low temperatures in March and April and the dry weather conditions in May slowed stripe rust development, resulting in the low level of rust inoculum and light rust in commercial fields. However, in our experiment field plots, susceptible winter wheat had more than 90% rust severity at the flowering stage and susceptible spring wheat had over 90% severity at soft dough stage, resulting in up to 30% and 25% yield losses on susceptible winter and spring wheat cultivars, respectively. The estimated state average of yield losses due to stripe rust is below 1% for both winter and spring wheat crops, the lowest since 2002. The amount of fungicides used to control stripe rust in 2006 also was the lowest in the last five years. The growth of resistant and moderately resistant cultivars in most of the wheat fields contributed to the much low yield damage in commercial fields because susceptible cultivars had potential of up to 30% yield losses under the less favorable weather conditions during the 2005-2006 growing season.

Through cooperators in other states, wheat stripe rust was monitored throughout the United States. In 2006, wheat stripe rust occurred in 20 states and was not as widely distributed as in 2005. Severe epidemics occurred in California with 15% yield losses and few locations in other

states. The early detected stripe rust in Minnesota indicated that the fungus over-wintered there, which does not occur very often. Similar to the stripe rust situation in the PNW, stripe rust was generally much less severe in 2006 than in 2005, which was due to the widespread and long period of drought conditions during the wheat growing season in the Great Plains.

In 2006, Barley stripe rust occurred in Arizona, California, Idaho, Oregon, and Washington and the yield loss was below one million bushels. Wheat leaf rust was moderately severe in our experimental plots in western Washington and light in some locations in eastern Washington that did not cause noticeable damage. Wheat stem rust was basically absent in Washington.

In 2006, we collected and received 250 stripe rust samples from wheat, barley, and grasses, of which 88% were from the western US and 36% from Washington. The stripe rust samples were tested on a set of 20 wheat differential cultivars to identify races of the wheat stripe rust pathogen and on a set of 12 barley differential cultivars to identify races of the barley stripe rust pathogen. From the samples, we have determined a total of 18 wheat stripe rust races and 11 barley stripe rust races, of which five wheat stripe rust races and two barley stripe rust races were new. The most predominant and widespread wheat stripe rust race was PST-100 (virulent on Lemhi, Heines VII, Produra, Yamhill, Stephens, Lee, Fielder, Express, Yr8-AVS, Yr9-AVS, Clement, and Compair) with a frequency of 29.4%. Other predominant PST races were PST-114 (20.3%) only in the Pacific Northwest, PST-101 (16.8%) almost restricted in California, and PST-102 (11.9%) throughout the U.S., which all have the PST-100 virulences plus those on Moro and Tres, Chinese 166, and Tres, respectively. The most predominant barley stripe rust race was PSH-71 (Virulent on Topper, Emir, Hiproly, Varunda, Abed Binder 12, Trumpf, Mazurka, Bigo, and Bancroft) with a frequency of 25%. The changes in race frequencies were reflected in reaction changes of some wheat cultivars and lines in our germplasm tests. The results should be useful in choosing resistance genes for breeding programs.

To study functional genomics of the stripe rust and further characterize stripe rust populations, we have constructed a full-length cDNA library from genomic DNA of the wheat stripe rust pathogen. Of the 196 cDNA clones sequenced, 74 (37.8%) had significant homology to the sequences of genes with characterized functions, 33 clones (16.8%) had significant homology to hypothetical proteins, 38 clones (19.4%) had some homology with genes, and the remaining 51 clones (26.0%) did not have significant homology with any genes in other fungi. A total of 51 genes producing different proteins were identified and they are involved in growth, defense, and virulence/infection. In order to develop specific primers to differentiate rust species, formae speciales, and isolates, we designed 28 gene-specific primer pairs based on sequences of some of the identified genes. Using these primers, we have identified polymorphic markers to distinguish stripe rust from stem rust and leaf rust of wheat and barley, and also markers to separate wheat stripe rust from stripe rusts of barley, bluegrass, and orchard grass. Some of the markers can be used to differentiate races of wheat stripe rust. We are going to use these markers to detect stripe rust in plant tissue before rust showing up, study population structures, and determine mechanisms of virulence changes in the stripe rust population.

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

In 2006, we evaluated more than 7,900 winter wheat, 8,000 spring wheat, and 5000 spring 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. Based on different sources and purposes, the wheat materials formed 55 nurseries with entries ranging from 20 to 2,000. All the nurseries were planted and evaluated at both Pullman and Mt. Vernon locations under natural stripe rust infection. The wheat entries also were evaluated for resistance to leaf rust, powdery mildew, and physiological leaf spot at the Mount Vernon field plots, where these diseases occurred. Some of the nurseries were also tested in Walla Walla and Lind, WA and various locations in other states. Germplasms and breeding lines potentially for release were also 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 of that region, while data of individual breeders' nurseries were provided to the individual breeders. Through these tests, susceptible breeding lines were eliminated, which have prevented risk of releasing potential susceptible cultivars and assisted breeding programs to release new cultivars of high yield and quality, good adaptation, and adequate 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.

### **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 plant-pathogen interactions.**

The spring wheat cultivars 'Alpowa' and 'Express' have both race-specific all-stage resistance (also called seedling resistance) and non-race specific high-temperature adult-plant (HTAP) resistance to stripe rust. We have completed genetic and molecular studies to identify and mapping the resistance genes in Alpowa and are close to finish the studies for the genes in Express. The all-stage resistance gene we named *YrAlp* was mapped on the short arm of wheat chromosome 1B. Although this gene is not very useful in breeding for resistant cultivars because it is ineffective to most of tested races, it is useful in understanding plant-pathogen interactions because it is different from any previously reported genes. Using both classic genetics and quantitative trait locus (QTL) mapping approaches, we identified and named a new gene, *Yr39*, for the durable HTAP resistance in Alpowa. This gene explained 54.8% of the total variation based on rust severity data and 48.9% of the total variation of infection type data. Its high contribution makes this gene relatively easy to transfer to other cultivars. We mapped *Yr39* on the long arm of wheat chromosome 7B. The information on chromosomal locations and molecular markers obtained from this research make *Yr39* valuable in breeding programs for developing wheat cultivars with durable resistance to stripe rust.

To identify and map the stripe rust resistance genes in Express, Express was crossed with 'Avocet Susceptible' (AVS). The parents and F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, and F<sub>5</sub> progenies were evaluated at the seedling stage under the controlled greenhouse conditions for all-stage resistance with four selected races. A total of 146 F<sub>5</sub> and their F<sub>6</sub> lines were evaluated for HTAP resistance in field plots in 2005 and 2006, respectively, under natural infection of the stripe rust populations virulent on seedlings of Express. The resistance gene analog polymorphism (RGAP) and simple sequence repeat (SSR) techniques were used to identify markers for mapping the resistance genes for all-stage resistance and quantitative trait loci (QTL) for HTAP resistance. Genetic analysis showed that Express has two dominant genes for all-stage resistance. The gene for resistance only to races PST-1 and 21 was mapped on chromosome 1BL and the gene resistant to PST-1, 21, 43, and 45 was mapped on chromosome 5BL. Three QTL for HTAP resistance were mapped on chromosomes 6AS, 3BL, and 1BL. These genes (QTL) are distinct from previously report genes and should be useful in developing cultivars with durable resistance.

We identified two genes for race specific all-stage resistance and three QTL for non-race specific HTAP resistance in Express. One of the all-stage resistance genes was mapped on wheat chromosome 1B and the other is under determination. The three QTL were mapped on the short arm of chromosome 6A, the long arm of chromosome 3B, and the long arm of chromosome 1B, respectively. The QTL on chromosomes 6A and 3B each explained over 30% of the total phenotypic variation while the QTL on chromosome 1B explained only 14% of the variation. Molecular markers flanking the QTL on chromosome 6A and 3B also were amplified in cultivar ‘Expresso’ that was developed from Express, but not in other wheat cultivars tested. The results indicate that the markers are useful in marker-assisted selection to transfer the durable resistance to other cultivars.

The AVS/6\*Yr8 wheat line has *Yr8* for race-specific all-stage resistance and was identified to have non-race specific high-temperature adult-plant (HTAP) resistance to stripe rust. To identify molecular markers for the resistance genes, 116 BC<sub>7</sub>:F<sub>3</sub> lines from the ‘Avocet Susceptible’ (AVS)/‘Compair’ cross were evaluated at the seedling stage with races PST-17, PST-43, and PST-45 under controlled greenhouse conditions and at the adult-plant stages in the fields under natural infection of the rust population virulent on AVS/6\*Yr8. The rust evaluations of the mapping population confirmed that the AVS/6\*Yr8 lines has a single gene for all-stage resistance. The resistance gene analog polymorphism (RGAP) and simple sequence repeat (SSR) techniques were used to identify markers linked to the resistance genes. The results confirmed *Yr8* on the long arm of chromosome 2D and mapped the HTAP resistance gene also on 2DL. The two genes were closely linked to each other with a genetic distance of 1.3 cM. Using molecular markers, we determined that the HTAP resistance gene is neither *Yr16* nor *Yr18*, and it should be a previously not described gene. Wheat lines with separate *Yr8* and the HTAP resistance gene developed from the crosses are single gene lines for better differentiating races of the pathogen and for breeding for resistance. The molecular markers should be useful in accelerating the introgression of the HTAP resistance gene into wheat cultivars and pyramiding these genes with other genes for durable resistance.

Bancroft was one of the first barley cultivars developed in the US with resistance to stripe rust, and has been identified to have high-temperature, adult-plant resistance (HTAP) that has been characterized as quantitative inheritance. To determine genetics and map genes for HTAP resistance in Bancroft, crosses were made of Bancroft with ‘Harrington’ and ‘Baronesse’ in greenhouse. F<sub>4</sub> and F<sub>5</sub> progeny of the cross were evaluated in the field in three locations by artificial inoculation in 2004 and 2005, respectively. Infection type (IT) and disease severity were recorded three times during the growing season. Area under disease progress curve (AUDPC) was calculated based on the severity data. The genetic analysis of the IT data showed a single gene controlling the difference in IT. The AUDPC data showed a continuous variation indicating the involvement of QTL. The disease severity was fairly correlated with IT in all three locations indicating that IT and disease severity may be controlled by the same gene. A total of 19 RGAP markers have been developed for the resistance gene using 119 F<sub>6</sub> recombinant lines, of which eight was significantly associated with the HTAP resistance. Infection type, disease severity, and AUDPC were controlled by one single QTL with different effects, 95%, 76% and 71%, respectively, through Mapmaker/QTL analyses. Three chromosome-specific simple sequence repeat (SSR) markers mapped the QTL on the long arm of barley chromosome 3H. The eight RGAP markers and three SSR markers were mapped in the same linkage group with the smallest gap 0.4 cM and the largest gap 21.7 cM. A total of 26 barley cultivars were tested with the closest RGAP markers for the resistant allele. It detected polymorphism in 88% of the genotypes, indicating that the markers are useful to incorporate the HTAP resistance into most of

these cultivars, and may be useful to incorporate the HTAP resistance into most other barley cultivars to obtain superior and durable resistance to barley stripe rust.

To understand mechanisms of stripe rust resistance, the wheat GeneChip® was used to profile the changes occurring in two wheat isolines that differed for the presence of the *Yr5* gene after inoculation with the stripe rust pathogen. *Yr5* represents a single *R* gene that confers all-stage resistance to all currently known wheat stripe rust races in the US. This time-course study identified numerous defense-related transcripts that were important for *R*-gene-mediated race-specific resistance as well as basal defense, and supported a model that links *P. s. tritici* recognition to the expression of plant defense responses. The presence of *Yr5* resulted in a rapid and amplified resistance response involving signaling pathways and defense-related transcripts known to occur during *R*-gene-mediated responses. Basal defense also involved substantial induction of many defense-related transcripts but the lack of *R*-gene signaling resulted in a delayed and weaker response, most likely due to the absence of increased cellular signaling and nitric oxide production that were able to confer an effective hypersensitive response in the presence of *Yr5*. The data generated provides novel insights into the cellular mechanisms of wheat defense to an economically important pathogen, and the findings will be useful for the development of durable resistant cultivars.

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

In 2006, we evaluated nine fungicide treatments for control of stripe rust in experimental fields near Pullman, WA. Susceptible winter wheat cultivar 'PS 279' was planted on 11 October 2005 and susceptible spring wheat cultivar 'Fielder' was planted on 3 May 2006 using a completely randomized block design with four replications. The fungicide treatments were compared with non-treatment check. Severities of stripe rust were recorded five times after fungicide application. Area under disease progress curve (AUDPC) was calculated for each treatment from the multiple sets of rust severity data, and also used for comparison of rust severities over the time period. Grain test weight and yield were recorded for each plot at the time of harvesting. All fungicide treatments significantly reduced rust severity and increased yield compared to the untreated check, except the treatment of 7.0 fl oz Quilt applied at the time of herbicide application on the winter crop because the too-early application did not protect crop long enough from the late rust development. Similarly, all treatments also significantly increased test weight of the winter crop, except the Quilt spray at the time of herbicide application. All treatments also increased test weight of the spring crop, but only the data of treatments of 14 fl oz Quilt at the flag leaf stage, two applications of Tilt, Topguard, and Stratego were significantly different from the non-treated control, and also all fungicide treatments were not significantly different from each other. These treatments varied in duration of effectiveness in rust control. Generally, one application at the flag leaf stage was adequate under the stripe rust situation in 2006.

Yield losses due to stripe rust and benefit of fungicide application were studied in experimental plots. We evaluated yield reduction by stripe rust and yield increase by fungicide application with 24 winter wheat and 16 spring wheat cultivars in field experiments of a randomized split-block design with 4 replications. Yield losses were up to 30% on highly susceptible winter wheat cultivars such as 'PS 279' and 25% on highly susceptible spring wheat cultivars such as 'Fielder'. Moderately resistant cultivars like 'Eltan' and 'Tubbs' winter wheat and 'Alpowa' and 'Louise' spring wheat had 0 to 6% yield reductions, which was often not statistically significant, suggesting that the moderate level of resistance was generally adequate. Foliar spray of Tilt increased yield by 42% on susceptible winter wheat and 33% on susceptible spring wheat, but

only increased yield by 0 to 5% on moderately resistant winter and spring wheat cultivars, indicating that fungicide spray was generally not necessary on such resistant cultivars.

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