

CONTROL OF STRIPE RUSTS OF WHEAT AND BARLEY

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ABSTRACT: Stripe rusts of wheat and barley were accurately forecasted in 2004. Wheat stripe rust was severe while barley stripe rust was generally light. Fungicide application was implemented to control stripe rust on both winter and spring wheat crops, which prevented major losses. In Washington State, yield losses were reduced to 1.5% for winter wheat and 3% for spring wheat. High-temperature, adult-plant (HTAP) resistance to stripe rust, which is in most winter wheat and the major spring wheat and barley cultivars, continued to be the most effective and durable type of stripe rust resistance. In 2004, 28 races of the wheat stripe rust pathogen and 15 races of the barley stripe rust pathogen were detected, of which six and three races were new for the wheat and barley stripe rust pathogens, respectively. PST-100 was the most predominant race of the wheat stripe rust pathogen throughout the country. More than 13,000 wheat and 5,000 barley entries were evaluated for stripe rust resistance, from which new germplasm and advanced breeding lines with stripe rust resistance were identified. The information was provided to breeding programs for developing and releasing new cultivars with adequate resistance. To more efficiently incorporate stripe rust resistance into commercial cultivars and to understand mechanisms of resistance, crosses were made to identify genes, develop molecular markers for genes, and use the markers to transfer genes for resistance. Molecular markers were identified for several genes in wheat and barley for resistance to stripe rust and other diseases. A bacterial artificial chromosomal (BAC) library was constructed for wheat to clone rust resistance genes. BAC and cDNA libraries were constructed for the wheat stripe rust pathogen to study its genome and functional genomics. More than 30 genes of the rust fungus were identified and primers were designed based on selected genes to study populations of the stripe rust and to determine relationships of the wheat stripe rust to other rusts. Foliar applications of Folicur, Tilt, Quadris, Quilt, Headline, and Stratego were effective for controlling stripe rust when sprayed at the right time. Profitability of fungicide application on various cultivars of wheat and barley without and with different level of stripe rust resistance was determined.

1. Monitoring rust development, predicting rust epidemics, assessing crop losses, determining prevalent races, and identifying new races

In 2004, stripe rust and other foliar diseases of wheat and barley were closely monitored throughout the Pacific Northwest (PNW) through field survey and disease nurseries. Stripe rust was accurately predicted for the PNW using monitoring data and predictive models based on environmental factors such as temperature, precipitation, and resistance of wheat cultivars. Through cooperators in many other states, stripe rust was monitored throughout the U.S. In 2004, wheat stripe rust occurred in more than 20 states. However, severe epidemic mainly occurred in the Pacific West (California, Oregon, Idaho, and Washington). Severe stripe rust of barley occurred in western Washington, western Oregon, and California. Susceptible barley varieties had up to 100% stripe rust in experimental plots near Pullman and Mt Vernon. In the major barley growing regions of eastern Washington, trace stripe rust occurred in commercial fields. Leaf rust occurred in some areas of the PNW and the severity levels were low except at Mt Vernon. Stem rust was not found in Washington in 2004.

Stripe rust started appearing in late April in central Washington, which is normal for the region but much later than 2003 because of the cold weather in January, 2004. The wet conditions in May speeded the rust development and spread, which was threatening wheat crops, especially the spring wheat crop because of the considerable acreage grown with susceptible and moderately susceptible cultivars. Stripe rust alerts were sent to growers through e-mails and news releases to implement control with fungicide applications. As a result, most of the fields grown with susceptible or moderately susceptible cultivars were appropriately sprayed with fungicides. The on-time application of fungicides prevented major losses of multimillion dollars.

The epidemic impact and benefit of fungicide control were assessed based on our experimental data and disease survey throughout the state. In 2004, 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 caused by stripe rust were up to 44% on susceptible winter wheat and up to 49% on susceptible spring wheat. Fungicide spray increased yield up to 42 bushels per acre for susceptible winter cultivars like Hatton and 12 bushels per acre for susceptible spring wheat cultivars like Zak. Yield losses and fungicide benefits should be greater than these figures in large-scale commercial fields because stripe rust should develop much faster in large fields of a single cultivar than our small experimental plots consisting of both resistant and susceptible cultivars. Yield differences between the sprayed and non-sprayed plots were not statistically significant for resistant and moderately resistant cultivars, showing effectiveness of stripe rust resistance in major cultivars.

In 2004, a total of 313 stripe rust samples were received throughout the US, from which 247 isolates of the wheat stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*, PST) and 42 isolates of the barley stripe rust pathogen (*P. striiformis* f. sp. *hordei*, PSH) were obtained. Wheat stripe rust samples were obtained from 18 states (Alabama, Arkansas, California, Colorado, Idaho, Indiana, Louisiana, Minnesota, Missouri, Nebraska, Ohio, Oklahoma, Oregon, South Dakota, Texas, Washington, and Wisconsin), while barley stripe rust samples were obtained from 4 states (California, Oregon, Idaho, and Washington). The isolates of the wheat stripe rust were tested on 20 wheat genotypes and those of the barley stripe rust were tested on 12 barley genotypes for identifying races. Six of 28 PST races and three of 15 PSH races were new. One of the new PSH race (PSH-72) is virulent on all 12 barley differential genotypes. The new PST races have various combinations of previously existing virulences. More than 90% of the 2004 PST isolates belong to the group of races (exemplified by PST-78, PST-98, and PST-100) with virulence on *Yr8*, *Yr9* and other resistance genes, which have caused widespread of stripe rust epidemics since 2000. Race PST-100 (virulent on Lemhi, Heines VII, Produra, Yamhill, Stephens, Lee, Fielder, Express, *Yr8*, *Yr9*, Clement and Compair) accounted for 50% of the isolates and distributed in all states where wheat stripe rust occurred. Five of the six new PST races are in this group. New races add virulences to Moro (*Yr10* and *YrMor*) and Paha (*YrPa1*, *YrPa2*, and *YrPa3*) to the race group, rendering the resistance genes no longer effective against the race group. We also test a numerous isolates on Summit that has recently been grown in California and suddenly became susceptible in 2003. Isolates virulent on Summit also were virulent on Chinese 166 (*Yr1*), confirmed the presence of *Yr1* in Summit postulated based on its pedigree. The “breakdown” of the resistance in Summit was caused by new races with addition of the *Yr1*-virulence to the *Yr8*-*Yr9* race group. Although wheat genotypes with *Yr5* have been tested in field nurseries for many years, the *Yr5* line was first included in the differential set in 2004. It was resistant to all isolates in 2004. Concerning the fast appearance of new races, cultivars with a single or few effective genes can not keep resistance very long. High-temperature, adult-plant (HTAP) resistance, which is non-race specific, is much durable. Through collaborating with Milus in the University of Arkansas, we clearly separated the new group of races (races identified since the year of 2000) from the old races (races identified before 2000 using molecular techniques).

To understand mechanisms of resistance and interactions between wheat and stripe rust, we have initiated genomic and functional genomic studies of the wheat stripe rust pathogen. For physical mapping and gene isolation, we have constructed a BAC library of the fungal pathogen. The BAC library that consists of 22,272 clones with an average insert size of 60 kb covers about 10x of the fungal genome. To study functional genomics, especially genes involved in pathogenicity and genes of biological importance, we also constructed a full-length cDNA library of the pathogen. The full-length cDNA library consists of 42,240 clones, and 99% of the clones reached full-length with the average cDNA insert of 1.5kb. We sequenced 180 cDNA clones and identified genes with functions similar in other fungi and also genes unique to stripe rust. The libraries were the first genomic resources for this important pathogen in the world. The identified sequences and genes were the first for this pathogen. These genetic resources will serve as genomic platforms for further research towards a better understanding of the pathogen.

2. Test germplasms and breeding lines of wheat and barley for rust resistance

In 2004, we evaluated more than 13,000 wheat and 5,000 barley entries for resistance to stripe rust and other foliar diseases. The entries included germplasm, genetic populations, and breeding lines from the National Germplasm Collection Center, and public and private breeding programs. All 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 in field sites where these diseases occurred naturally. Some of the nurseries 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 our testing, Kidwell's program released spring wheat varieties 'Louis' and 'Otis', Jone's program released winter wheat 'Masami', 'WA7936' and 'WA7939', and several other varieties were released by the breeding programs in Idaho, Oregon, and California. These new varieties have adequate resistance to stripe rust.

Through the germplasm screening, we have established a core collection of wheat germplasms with stripe rust resistance. The current collection has more than 4,000 entries, which will be valuable sources of stripe rust resistance for further characterization of resistance and for development of wheat cultivars with superior resistance.

3. Determine genetics of resistance and develop molecular markers for genes resistance to stripe rust

To identify genes for resistance and develop molecular markers for the resistance genes, we made crosses among Alpowa, Express, IDO377s, and Zak and Avocet Susceptible (AVS). In 2004, F₃ progeny and parents of these crosses were evaluated in the field for resistance to stripe rust. Seed of the F₄ progeny were harvested from the field and grown in the greenhouse for the F₅ generation. The mapping populations will be evaluated in the field in 2005 for determine the genetics of resistance, identify genes, and develop molecular markers.

Through collaborations with Kidwell, Campbell, and Gill, markers of *Yr5* and *Yr15* were used to incorporate these effective genes into elite cultivars such as 'Zak', 'Scarlet', and 'Eden'. Through collaboration with Souza, we identified genes for high-temperature, adult-plant resistance (HTAP) resistance to stripe rust in winter wheat 'IDO444'. Through collaborations with Campbell and Kidwell, we identified a gene on chromosome 6B having a major effect on HTAP resistance in 'Stephens' wheat and identified microsatellite markers for the gene. Through collaboration with Dubcovsky, we identified a new gene and named it *Yr36* from *Triticum dicoccoides* controlling HTAP resistance, and initiated the cloning of *Yr36* and the gene in Stephens.

To clone stripe rust resistance genes, we have constructed a bacterial artificial chromosome (BAC) library for common wheat. The BAC library consists of 410,000 clones with an average insert size of 130 kb, and covers approximately 3.3x wheat genome equivalents. Using the BAC library, we identified 12 positive BAC clones containing molecular markers for *Yr5*, which is effective against all the races identified so far in the US and many parts of the world. Sub-cloning of the positive clones is underway. We also constructed a full-length cDNA library consists of 42,000 clones with an average cDNA length of 1.5kb. Using the cDNA library, we are studying the expression of the resistance gene. The wheat BAC library and the full-length cDNA library will be very valuable resources for wheat research. It will be used to clone not only genes for resistance to stripe rust, but also genes for other important traits, as well as study the wheat genome structure. For example, Skinner's program is using the BAC library to clone genes involved in wheat cold hardness.

Most barley cultivars are resistant to stripe rust of wheat that is caused by *Puccinia striiformis* f. sp. *tritici* (PST). The barley cultivar Steptoe is susceptible to all identified races of *P. striiformis* f. sp. *hordei* (PSH), the barley stripe rust pathogen, but is resistant to most PST races. To determine inheritance of the Steptoe resistance to PST, a cross was made between Steptoe and Russell, a barley variety susceptible to some PST races and all tested PSH races.

Seedlings of parents and F₁, BC₁, F₂, and F₃ progeny from the barley cross were tested with races PST-41 and PST-45 under controlled greenhouse conditions. Genetic analysis of infection type data showed that Steptoe had one dominant gene and one recessive gene (provisionally designated as *RpstS1* and *rpstS2*, respectively) for resistance to races PST-41 and PST-45. Genomic DNA was extracted from the parents and 150 F₂ plants that were tested for rust reaction and grown for seed of F₃ lines. The infection type data and polymorphic markers identified using the resistance gene analog polymorphism (RGAP) technique were analyzed with the Mapmaker computer program to map the resistance genes. The dominant resistance gene in Steptoe for resistance to PST races was mapped on barley chromosome 4H using a linked microsatellite marker, HVM68. A linkage group for the dominant gene was constructed with 12 RGAP markers and the microsatellite marker. The results show that resistance in barley to the wheat stripe rust pathogen is qualitatively inherited. Similarly, we have determined a single dominant gene in the wheat cultivar Lemhi conferring resistance to the barley stripe rust pathogen and mapped the gene on wheat chromosome 1B. These genes might provide useful resistance to stripe rust when introgressed from wheat to barley or from barley to wheat.

To map the resistance genes conferring all-stage resistance in barley genotypes GZ and BBA 2890, 200 F₈ RILs from each of Steptoe X GZ and Steptoe X BBA 2890 were phenotyped by inoculating with races PSH-14 and PSH-54. Genomic DNAs were obtained from 182 F₈ lines of Steptoe X GZ and from 200 F₈ RILs from the Steptoe X BBA 2890. The RGAP technique was used to identify markers linked to the resistance genes. For the Steptoe X GZ cross, 73 primer pairs were screened in the bulk segregant analysis. Of 15 repeatable polymorphic markers that have been tested with 96 F₈ lines, six were linked to the resistant allele and nine were linked to the susceptible allele. A linkage group for the recessive resistance gene, *rpsGZ*, was constructed with 14 RGAP markers. Two of the markers were co-segregating with the resistance allele in repulsion. The closest marker linked to the resistance allele in coupling is 13 cM away. Currently, these markers are tested with the remaining RIL lines and chromosome-specific SSR markers are used to determine chromosomal location of the resistance gene. For the Steptoe X BBA 2890 cross, DNAs of the bulks of resistant and susceptible F₈ RILs and the parents were amplified with 50 combinations of RGA primers and three markers were identified. These markers are to be tested with the 200 RILs.

To determine the genetics of HTAP resistance in barley cultivars, we have made all possible crosses (including reciprocals) among Bancroft, Baronesse, Harrington, and Steptoe, except the crosses between Harrington and Steptoe. F₁ and F₂ seed were obtained for all crosses. Seed of 200 F₃ lines were obtained from the greenhouse for each of crosses Harrington X Baronesse, Harrington X Bancroft, and Baronesse X Bancroft. In addition, backcrosses were made and seed of BC₁ were obtained for crosses of Steptoe X Bancroft, Steptoe X Baronesse, Harrington X Bancroft, Harrington X Baronesse, and Baronesse X Bancroft for incorporating HTAP resistance or improving HTAP resistance in the background of Steptoe, Harrington, and Baronesse. In 2004, F₄ plants from crosses Harrington X Baronesse, Harrington X Bancroft, and Baronesse X Bancroft were grown in the field to obtain seed of F₅ RILs and to determine stripe rust reactions of F₄ lines. The nursery was inoculated with a mixture of four races. Infection type and severity were recorded for each plant three times with one week in between during the growing season. Area under disease progress curve (AUDPC) was calculated for each plant using the severity data. Mean AUDPC were calculated for each line. The infection type data of 197 F₄ lines derived from the Harrington X Bancroft cross best fit the one-gene model ($P = 0.88$). The area under disease progress curve (AUDPC) data showed continuous variation (Fig.3), indicating the involvement of QTL. Thus, the preliminary results indicated one QTL with major effect and one or more QTL with smaller effects on the HTAP resistance. The

transgressive segregating indicated gene interactions among the QTL. The F₅ progeny will be planted in two locations in eastern and western Washington for further phenotyping their stripe rust reactions. The data will be used to identify molecular markers.

4. Determine effectiveness and use of foliar fungicides for rust control

Fungicides were evaluated for controlling stripe rust in experimental fields near Pullman, WA. Susceptible winter wheat cultivars 'Hatton' and 'PS 279' were planted on 22 October 2003 and spring wheat 'Lemhi' and spring barley 'Russell' were planted on 22 April 2004 using a completely randomized block design with four replications. Six fungicide treatments were tested with non-treatment as a check. Fungicides were sprayed on 6 June in the winter plots when the plants were at the boot stage with 1% stripe rust, and on 19 June in the spring plots when the plants also were at the boot stage with 10% stripe rust. A second spray was only used for one of the treatments with Quilt. Severities of stripe rust were recorded three times after fungicide application. Test weight and yield were recorded for each plot at the time of harvesting. All the fungicide treatments effectively reduced stripe rust severity. Stripe rust started re-developing about one month after the application and therefore the fungicides kept effective for about one month. The treatments increased yield by 30 to 45 bu/A for 'PS 279', 36 to 42 bu/A for 'Hatton', 7 to 18 bu/A for 'Lemhi', and 8 to 33 bu/A for 'Russell'. The two-application of Quilt best controlled stripe rust, but did not significantly increase yield compared to the one-application of Quilt and some other fungicides.

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