

Creation of a multiple-use recombinant inbred line population for the development of molecular markers in soft white winter wheat

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ABSTRACT

To develop molecular markers for soft white winter wheat in the most economical way possible, a community approach was taken to develop a recombinant inbred line (RIL) population for mapping traits in soft white winter wheat and to generate the molecular and phenotypic data necessary to identify useful molecular markers. Parental selection was done to maximize differences for multiple traits of interest instead of targeting one or two specific traits. Two commercially grown cultivars, Coda and Brundage, were selected based on differences in disease resistance, abiotic stress tolerance, agronomic traits and end-use quality to produce the population CB-2 composed of 268 F_{6,7} derived lines. Initial molecular analysis of the two parents identified 220 polymorphic SSR markers and 180 DArT markers. To economically produce the molecular and phenotypic data, different wheat breeding programs have taken the lead on phenotypic screening for specific traits and are pooling mapping data to minimize costs to any specific program and to rapidly map the population. To date, transgressive segregation has been observed in the population for resistance to *Puccinia striiformis*, tolerance to *Cephalosporium gramineum*, and height. The population has been used to identify new markers linked to the *Pch1* eyespot resistance gene and the *C* gene that controls spike compaction. Complete molecular mapping of the population is anticipated by the end of 2008. To maximize use of the population by other programs, all molecular marker data and agronomic data associated with the CB-2 RIL population will be publicly available.

INTRODUCTION

Molecular markers and marker assisted selection (MAS) offer to improve the efficiency of selection for traits that are either difficult or expensive to identify through phenotypic screening. The development of recombinant inbred line (RIL) populations has greatly facilitated the identification of useful molecular markers in wheat (*Triticum aestivum*). In the Pacific Northwest (PNW) region of the United States the development of resistant winter wheat cultivars to diseases such as *Puccinia striiformis* (yellow rust), *Pseudocercospora herpotrichoides* (Pseudocercospora foot rot or eyespot), and *Cephalosporium gramineum*

(Cephalosporium stripe) would be enhanced if molecular markers were available for genotypic selection. There are numerous challenges for the development of molecular markers for these traits. The first challenge is the lack of representative RIL populations for the predominant market class grown in the region, soft white winter wheat. Most RIL populations available involve other market classes or involve crosses between market classes or to synthetic wheat lines to increase the potential for polymorphism for a targeted trait. This limits the potential usefulness of these populations for the identification of markers for traits of importance in the PNW. A second challenge is that the development of a separate RIL population for each targeted trait would be cost prohibitive. The third challenge is covering the costs associated with developing a molecular map of the population for use in identifying potential molecular markers both in screening the individual RIL lines at the genotypic level and at the phenotypic level for each specific trait of interest.

To address the first two challenges it was decided that the parents of the population should differ for as many traits as possible while still using cultivars currently grown in the PNW. This approach maximizes the number of traits that could be studied while minimizing the amount of genotyping, since once a linkage map based on this population is available it can be used to identify various marker-trait associations. To maximize the level of variation a cross was made between the club wheat Coda (1) and Brundage (8). While both cultivars fall into the market class of soft white wheat, they differed for numerous agronomic traits (Table 1) and for *Puccinia striiformis* and *Pseudocercospora herpotrichoides* resistance. It was later determined that the parents differed for tolerance to *Cephalosporium gramineum*, demonstrating the advantage of using current cultivars in that they had already been phenotypically evaluated for resistance and/or tolerance to diseases as part of the regional testing during cultivar development. Variation was also observed in the population for some traits, such as height, that was not anticipated based on the parental phenotypes due to differences in the genes controlling the trait's expression in the parents.

Table 1. Trait differences observed in the Coda-Brundage (CB-2) RIL population.

Agronomic

Head type – common and club
Awn type – awned and awnletted
Heading date
Height – transgressive segregation
Straw strength
Straw composition – soluble sugars
Tiller number
Winter-hardiness
Yield

1000 kernel weight
End-use quality

Disease Resistance/Tolerance

P. herpotrichoides

C. gramineum – transgressive segregation

P. striiformis – transgressive segregation

To address the third challenge related to covering the costs associated with phenotypic and genotypic screening of the Coda-Brundage (CB-2) RIL population, a community approach was taken for both types of screening. The population was made available to wheat breeding and genetics programs in the PNW region (Idaho, Oregon, or Washington) with the most interest and expertise for a specific trait. In some cases this involved more than one state with each conducting one method of phenotypic evaluation. To develop the molecular map for the population, the programs in all three states will be screening the population with different markers and pooling the results in a common data file, thus minimizing the cost of genotyping the population for any single breeding program. In addition, the Oregon State University program submitted the CB-2 population for DArT marker analysis to increase the number of potential molecular markers for the population. The overall goal of the project is the development of a RIL population that could be used by any program to study a variety of traits with a molecular map publicly available to optimize the identification and deployment of useful molecular markers for wheat improvement.

MATERIALS AND METHODS

The development of the Coda-Brundage (CB-2) RIL population was done using single seed descent. After an initial cross between the two parents, the F₁ was allowed to self and 300 F₂ seeds were produced. From these seeds F₂ plants were produced and a single seed was selected from each plant and used to produce the F₃ generation. Single seed descent was followed for three additional generations to produce the F₆ generation that was subsequently selfed to increase the amount of seed.

Over the cycles of single seed descent 32 lines were lost resulting in a F₆ derived F₇ RIL population with 268 individual RIL lines.

The parents were evaluated at the Western Regional Genotyping Center (Pullman, Washington) and the University of Idaho using PCR to identify polymorphic markers. Two hundred twenty SSR markers were polymorphic between Coda and Brundage. To increase the number of available markers the CB-2 population was sent by Oregon State University for DArT analysis and 233 potential DArT markers were identified with 180 being used to develop an initial marker map.

Seed from the CB-2 population was made available for 1) development of an improved molecular marker for the *Pchl* gene for *Pseudocercospora herpotrichoides* resistance, 2) evaluation for *Cephalosporium gramineum* tolerance/resistance to identify markers for genotypic selection for resistance to this disease, and 3) evaluation for high temperature adult plant resistance (HTAP) to *Puccinia striiformis* to identify and validate markers for durable resistance to yellow rust.

RESULTS

***Pseudocercospora herpotrichoides* resistance:** The CB-2 RIL population was screened for the presence of the *Pchl* gene by whole plant screening in the growth chamber at the University of Idaho (5 with modifications) and using an endopeptidase assay for the *EpD1* locus (7). Coda was known to carry the *Pchl* gene and Brundage was known to not carry the gene and to be susceptible. The endopeptidase assay and whole plant data was made available to Oregon State University where it was used to identify sequence tagged site (STS) markers associated with *Pchl*. Three STS markers, *Xorw1*, *Xorw5*, and *Xorw6* showed complete linkage to *Pchl* improving the potential for genotypic screening for *Pchl*-derived resistance to *P. herpotrichoides* (4). Two of the STS markers, *Xorw1* and *Xorw5*, are currently being used by the breeding programs in the Pacific Northwest for early and mid-generation selection for the *Pchl* gene.

***Cephalosporium gramineum* tolerance/resistance:** The CB-2 RIL population was screened in the field in the 2006-2007 growing season in two locations in Oregon for tolerance/resistance to *Cephalosporium* stripe. Oat seeds pre-inoculated with *C. gramineum* were planted with each line to ensure uniform disease pressure. Coda was known to be tolerant to the disease (6) while Brundage was considered moderately susceptible. Disease response was evaluated phenotypically two to three weeks after heading and was based on the number of premature whiteheads (6). The CB-2 RIL population showed transgressive segregation for tolerance (Table 2). The population was planted again in Autumn, 2007

Table 2. Expression of disease resistance in the CB-2 RIL population and parents to *C. gramineum* at two locations (Moro and Pendleton, OR) in 2007. Resistance/tolerance to *C. gramineum* is expressed as the square root of the number of whiteheads (0–8).

<u>Moro</u>		
	mean	range
Coda	3.6	
Brundage	3.7	
CB-2 population	3.8	1.2 – 6.8
<u>Pendleton</u>		
	mean	range
Coda	2.2	
Brundage	5.1	
CB-2 population	3.7	0.4 – 7.9

for a second year of phenotypic screening at one location in 2008. Once the linkage map, based on the CB-2 population, is completed, quantitative trait analysis will be performed to identify the location, number, and effect of genetic factors underlying Cephalosporium stripe resistance. This exercise will also yield markers for use in MAS.

***Puccinia striiformis* HTAP resistance:** The CB-2 population was screened in the field in Pullman, and Mount Vernon, Washington for yellow rust resistance. Coda was found to show early resistance to yellow rust with some susceptibility appearing late in the growing season. Brundage showed early susceptibility to yellow rust with little or no decrease in level of susceptibility later in the growing season. Resistance notes based on reaction type (0-9) and leaf area covered by lesions (0-100%) were taken at two different plant growth stages. A decrease in scores from the first rating to the second rating is an indication of HTAP resistance (2). Transgressive segregation for resistance was observed among the RIL lines indicating the presence of resistance genes in both parents. The presence of transgressive segregation for HTAP resistance and prior knowledge that Coda carries a different source of HTAP resistance currently found in PNW wheat cultivars, indicates that new markers for durable, non-race specific resistance to yellow rust may be identified from the CB-2 population.

DISCUSSION

The development of a RIL population for the Pacific Northwest that differed for multiple traits has opened up the potential of mapping gene loci and identifying molecular markers for disease resistance and other agronomic traits. While in some cases the use of cultivars from a similar market class may lead to a lack of adequate polymorphism for fine mapping, the

availability of 220 SSR markers and 180 DArT markers should provide adequate coverage for initial identification of markers associated with the trait of interest. To date improved markers for the *Pchl* gene for *C. herpotrichoides* resistance (4) and flanking markers identified for the *compactum C* locus (3) were identified using the CB-2 population. With completion of the molecular map in Autumn, 2008, molecular markers associated with resistance to *P. striiformis* and *C. gramineum* should soon be identified. Differences in winter-hardiness, height, heading date, straw strength, straw composition, yield, 1000 kernel weight, and end-use quality among the RIL lines indicate that the CB-2 population has the potential for use in identification of useful markers for a number of additional traits. Once the map is complete it will be made publicly available via the web and the RIL population will be freely distributed so as to maximize the use of the CB-2 population for the identification of markers for wheat improvement.

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