Development of Isogenic Lines for Resistance to Septoria Tritici Blotch in Wheat

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Abstract: Septoria tritici blotch (STB), caused by the fungus *Mycosphaerella graminicola* (asexual stage: *Septoria tritici*), is one of the most economically important diseases of wheat worldwide. During the past decade 13 genes for resistance to STB have been identified and several molecular markers have been developed. However, analysis of resistance gene expression and utility for plant improvement programs would be increased if the resistance genes were isolated in a common susceptible background. To address this problem, a program was begun to backcross resistance genes *Stb1–8* into two susceptible wheat cultivars. Work with genes *Stb2*, *Stb3*, *Stb6* and *Stb8* has proceeded the farthest. Resistance gene *Stb3* is dominant, while *Stb2* may be recessive. This will be the first report of recessive resistance to STB if confirmed. Molecular markers linked to the resistance genes are being validated in the backcross progeny and should provide the materials for efficient introgression of these genes into elite germplasm for future wheat improvement.

Keywords: marker-assisted selection; molecular markers; *Mycosphaerella graminicola*; STB

Septoria tritici blotch (STB), caused by the ascomycete fungus *Mycosphaerella graminicola* (asexual stage: *Septoria tritici*), is one of the most economically important diseases of wheat worldwide (Eyal *et al*. 1987). Control of the disease is by fungicides or cultural practices and, when possible, by resistant cultivars. Breeding for resistance has been hampered by disagreement about whether resistance was qualitative or quantitative and by a lack of rapid methods for phenotypic characterization (Goodwin 2007).

Despite the high economic importance of this disease, as of 2000 only four resistance genes had been identified (Rillo & Caldwell 1966; Somasco *et al*. 1996; Wilson 1985), none had been mapped and no molecular markers were available for marker-assisted selection (Goodwin 2007). Fortunately, this situation has changed rapidly. During the past decade 13 genes for resistance to STB have been identified and mapped in the wheat genome (Adhikari *et al*. 2003, 2004b, c, d; Arraiano & Brown 2006; Arraiano *et al*. 2001, 2007; Brading *et al*. 2002; Chartrain *et al*. 2005a, b, 2009; McCartney *et al*. 2003) and several molecular markers have been developed that can be used for marker-assisted selection. Additional resistances have been identified recently (Tabib Ghaffary *et al*. 2010) which should soon bring the catalog of mapped *Stb* genes to at least seventeen.

Transferring these genes into elite wheat germplasm has been complicated by the presence of multiple resistance genes within some cultivars. Furthermore, expression of genes for resistance varies over time and so far almost nothing is known about the mechanism of resistance. An unusual aspect of the interaction between *M. graminicola*

and wheat is the presence of two peaks of gene expression in resistant cultivars, one within the first 2–3 days after inoculation and the second after 14–16 days (Adhikari *et al*. 2007). The second peak occurs just prior to symptom expression and corresponds to the time when rapid growth of the pathogen is observed in susceptible interactions (Adhikari *et al*. 2004a). Analysis of resistance gene expression and utility for plant improvement programs would be improved if the resistance genes were isolated in a common susceptible background.

The goal of this work was to develop improved methods for phenotypic analysis and use them to develop isogenic lines for mapped *Stb* genes in two susceptible backgrounds of wheat. These lines will be useful for analyzing the responses of individual resistance genes, quantifying their effects, validating molecular markers linked to the genes and for differentiating genetic variation within populations of the pathogen. Furthermore, they can be used as donors of single resistance genes for future wheat improvement.

MATERIALS AND METHODS

Phenotypic evaluation. Several methods were used to evaluate the resistance or susceptibility of wheat parents and progeny. Most of the testing was by spray inoculating wheat plants with blastospores of a single isolate in a greenhouse as described by Adhikari *et al*. (2003). After inoculation, plants were kept in a plastic tent for three days to maintain near 100% humidity and then placed on a greenhouse bench for 21–28 days until symptoms could be scored. Indiana isolate T48 was used for most inoculations; other isolates were used occasionally if necessary.

Because the spray-inoculation technique does not always give good results and biotechnological approaches were too cumbersome and expensive (Adhikari *et al*. 2004a), alternatives to the usual inoculation technique were explored. One of these involved injection of spores directly into the growing whorl of leaves and was used for testing many of the backcross progeny.

Parental lines. Resistant parents included the cultivars containing resistance genes *Stb1–8*. Two susceptible wheat cultivars were chosen as recurrent parents. Taichung 29 was chosen because it was the most highly susceptible spring wheat identified in previous testing. However, it is late and tall so is not ideal for greenhouse work. To ameliorate those problems and to have a very different genetic background in case of fertility problems between Taichung 29 and the other wheat cultivars, the rapid-cycling cultivar Apogee was chosen to be the other recurrent parent. Apogee can flower within 3–4 weeks of planting so has a much shorter generation time compared to other wheat cultivars. It is susceptible to STB but not to the same degree as Taichung 29 so tester isolates must be chosen carefully, and it has smaller leaves so there is less tissue available for fungal colonization and laboratory analyses. The program was initiated with all eight resistance genes, but problems with the markers and phenotypic testing has slowed progress for some genes.

Gene introgression. To develop isogenic lines, the resistance genes *Stb1–8* were backcrossed into the two susceptible backgrounds. The initial plan was to perform four backcrosses, then self pollinate to generate homozygous lines. However, due to the unexpected result of a resistance gene that appears to be recessive, BC plants were selfed and homozygous resistant BCF_2 lines were used for the next backcrossing. Furthermore, due to a large number of putative small quantitative trait loci (QTL) for resistance in some lines, it was decided to extend the program to seven backcrosses before self pollinating to make sure that the resulting lines contain only a single resistance gene without modifying QTL.

RESULTS AND DISCUSSION

Through the efforts of the Goodwin lab and others around the world there are now 13 *Stb* genes that have been mapped in the wheat genome (Goodwin 2007). These genes occur on each of the wheat genomes and on all seven homoeologous chromosome groups (Table 1). Therefore, each of the three diploid ancestors of hexaploid wheat probably had its own complement of *Stb* genes that was different from those in the other species.

Apogee appears to have the gene *Stb6* and the small size of its leaves can complicate testing. To address this problem, a new method of inoculation was developed that avoids the need for high humidity for infection and gives better results than spray inoculation of Apogee. The stem injection method provided an excellent alternative to the

Table 1. Genome and chromosome group of the 13 genes for resistance to Septoria tritici blotch (STB) that have been mapped to date in wheat

Genome	Homoeologous chromosome group						
	1	2	3	4	5	6	7
A			*Stb6*	*Stb7*; *Stb12*		*Stb15*	***Stb3***[a]
B	*Stb11*	*Stb9*	***Stb2***		*Stb1*		***Stb8***
D	*Stb10*						***Stb4***; *Stb5*

[a]*Stb3* was mapped incorrectly by Adhikari *et al.* (2004c); the correct location is on chromosome 7A, not 6D as published; bold indicates genes that were mapped in the Goodwin lab

usual spray inoculation technique and helped with classifying the progeny lines.

The best progress has been made with *Stb2*, *Stb3*, *Stb6* and *Stb8*; most lines are now at the BC_3-BC_4 stage. An erosion of resistance was noted for most lines during the backcrossing process, i.e., it decreased with increasing numbers of backcross generations. This most likely reflects small QTL for resistance present in the original donor cultivars that were gradually left behind in the backcross progeny. To ensure that the final lines have only one resistance gene each it was decided to extend the backcrossing program to 6 or 7 generations in total. This will slow down generation of the lines but will provide a more stable final result.

An unexpected result was that the resistance in Veranopolis appears to be recessive rather than dominant. *Stb2* was mapped originally in a doubled-haploid population (Adhikari *et al*. 2004c) so dominance was not tested but was assumed. However, in the current project F_2 populations segregated 1:3 for resistance:susceptibility and F_1 plants were susceptible indicating recessive resistance. This is quite interesting because it has not been reported previously for an *Stb* gene. Recessive resistance could indicate segregation for a dominant toxin sensitivity gene rather than a gene for resistance in the usual sense. In contrast, resistance gene *Stb3* was clearly dominant and held up the best through backcrosses with little erosion of its effectiveness. If the *Stb2* results are confirmed it will be the first report of recessive resistance to STB.

Apogee appeared to be genetically incompatible with the cultivars containing some of the resistance genes; F_1 plants turned yellow and died before producing viable seed. Therefore, it will not be possible to make isogenic lines in both susceptible backgrounds for all resistance genes.

CONCLUSIONS

Dramatic progress on mapping of *Stb* genes has occurred during the past 15 years (Table 1). We have gone from zero mapped genes to 13 with several others on the way so our ability to analyze the genetics of resistance to *M. graminicola* in wheat is increasing rapidly. Molecular markers linked to the resistance genes are being validated in the backcross progeny and large recombinant-inbred populations are being developed for *Stb2* and *Stb3*. All of these materials will be publicly available once completed and verified and should provide the materials for precise analyses of resistance gene expression, pathogen virulence differentiation and efficient introgression of these genes into elite germplasm for future wheat improvement.

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