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Marker-assisted selection for Fusarium head blight resistance in wheat

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Abstract

The cultivation of wheat varieties resistant to Fusarium head blight (FHB) is recognized as one of the most important components to diminish losses due to this disease. Although there is no known immunity to this disease in wheat germplasm, considerable improvements in genetic resistance have been achieved by concentrated breeding efforts that have relied primarily upon repeated field and greenhouse-based screening. DNA markers are a relatively new technology that can be used to increase breeding progress, especially for traits such as FHB that are difficult to select for under field conditions and that are controlled by multiple genes. Marker-assisted selection (MAS) uses markers to select for particular DNA segments that are genetically linked to genes that provide incremental resistance to FHB. One particular gene, designated *Fhb1*, provides a 20–25% average reduction in FHB symptoms. This gene and its associated markers have been validated in numerous breeding programs and is widely used to more precisely breed for resistance. About a dozen other genes affecting FHB reaction have been identified, but they have smaller and more inconsistent effects compared with *Fhb1*. Nevertheless, breeders are discovering which of these markers can be combined with *Fhb1* in their genetic backgrounds to enhance resistance. The establishment of the USDA-ARS Regional Small Grains Genotyping Centers and similar facilities around the world have increased the capacity for wheat breeders to utilize this powerful technology. More efficient DNA extraction technologies and marker platforms will allow breeders to more fully implement MAS in the future.

Keywords: Wheat; *Triticum aestivum*; Fusarium head blight; Scab; Disease resistance; Marker-assisted selection; Quantitative trait loci

1. Fusarium head blight in wheat

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae*) is an important disease in most wheat-growing regions of the world where rainfall frequently occurs during flowering time through early grain fill. The hard red spring wheat producing areas of the United States and Canada are vulnerable and have experienced periodic epidemics of this disease (Wilcoxson et al., 1992). Epidemics of FHB from 1993 to 1997 resulted in devastating economic losses to the wheat industry of the region, with 1993 estimates alone surpassing $1 billion (McMullen et al., 1997). Epidemic levels of FHB were present in the region in 2005 and revealed that even the best varieties available today do not offer enough protection against this disease. Fusarium head blight causes both severe yield reduction and decreases grain quality (Bai and Shaner, 1994). Infected grain may contain harmful levels of mycotoxins that prevent its use for human consumption or feed (Bai and Shaner, 1994). Control of FHB has been difficult due to the ubiquitous nature and wide host range of the pathogen and dependence of the disease upon unpredictable climatic conditions (Paulitz, 1996; Wiersma et al., 1996). Cultural control methods are ineffective and/or not feasible (Bai and Shaner, 1994). Fungicide use has become popular during this decade, but this practice adds to grower costs and is only partially effective, especially under heavy inoculum pressure. The ability of the pathogen to cause significant damage when appropriate climatic conditions are present makes rapid incorporation of durable resistance into new varieties a priority for the entire wheat industry.

2. Breeding for FHB resistance

Prior to the 1990's, few wheat breeding programs in the U.S. bred specifically for FHB resistance. Although it was recognized as a potentially devastating disease, its occurrence was sporadic and instrumentation for measuring DON levels was not in widespread use. Fortunately, there is genetic resistance available for this disease, albeit it in varieties of eastern Asian origin. These varieties require significant breeding effort and multiple crossing cycles to recover progenies that combine the necessary agronomic and quality traits with FHB resistance. Available resistance to FHB in wheat is quantitatively inherited with a continuous distribution among progeny (Snijders and van Eeuwijk, 1991; Bai and Shaner, 1994; Snijders, 1994; Waldron et al., 1999; Anderson et al., 2001,

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Table 1
Flow of materials through the University of Minnesota wheat breeding program

Year	Generation (No.)	Plot type	Location(s)
1	Crossing/F_1 (300)	Single plant	Greenhouse
	F_2 (300 × 1000)	Single plant	MN 1 location
2	F_3 (20,000)	Single plant	New Zealand
	F_4 (16,000)	Headrow	MN 1 location
3	F_5 (1400)	Single plant	New Zealand
	F_6 (1200)	Headrow	MN 2 locations
4	600	Headrow	New Zealand increase
	350	Prelim. yield trial	MN 3 locations
5–6	150	Adv. yield trial	MN 3 locations
7–9	10	MN variety trial	MN 7+ locations/regional

Buerstmayr et al., 2002). Although resistance to FHB can be manifested in many ways (e.g. resistance to initial infection, resistance to spread of infection within the spike, low DON, etc.), screening for resistance to spread of infection within the spike (Schroeder and Christensen, 1963) can be effectively accomplished under greenhouse (Stack, 1989) or field conditions (Dill-Macky, 2003). Field screening for resistance to this disease is both time and resource-intensive, and results are often confounded by environmental factors, and needs to be repeated over environments (Campbell and Lipps, 1998; Groth et al., 1999; Fuentes-Granados et al., 2005). Resistance genes from multiple sources can enhance the level of resistance, and also reduce genetic vulnerability, should the resistance genes not be durable. Evidence to date suggests that genetic vulnerability with these resistance genes should not be a concern (Snijders and van Eeuwijk, 1991), but with the large genetic variability known to exist in *Fusarium* spp. (Bowden and Leslie, 1999), the introduction of at least a few different genes would be a wise approach.

The cultivation of wheat varieties resistant to Fusarium head blight (FHB) is recognized as one of the most important components to diminish losses due to this disease. Developing a new wheat variety typically takes up to 10 years from when a cross is first made until release (Table 1). In our breeding program, materials from the F_6 generation on are evaluated for FHB reaction in one to three inoculated, misted nurseries each year. Materials are grown in single, 1 meter rows with up to 3 replications at each location and are assessed for disease symptoms approximately 3 weeks after flowering. A portion of each row is harvested, carefully threshed to preserve all the grain (including Fusarium-damaged kernels), and assessed for its degree of visually scabby kernels, test weight, and total amount of seed from a fixed number of spikes. This represents an extremely laborious and time-consuming activity, but one that I consider essential in breeding for resistance to this disease. Our project screens an average of 5000 to 8000 rows per year for FHB reaction in the field. Substantial progress has been made in breeding for FHB resistant wheat varieties through the use of new resistance sources and screening under carefully controlled field conditions.

3. Marker-assisted selection

Molecular markers can help breeders select for genes that enhance FHB resistance. The basic concept is that naturally occurring differences in the DNA sequence of wheat varieties are identified as being genetically linked to a gene that confers greater resistance to FHB. These DNA differences could be due to a difference in the number of repeat units of a sequence (e.g. microsatellite or simple sequence repeat (SSR); an insertion or deletion of a DNA segment; or a single base pair difference (single nucleotide polymorphism = SNP). Most markers used today in wheat are PCR-based SSRs. Dozens of QTLs have been identified for FHB resistance (Table 2); the most widely used ones in breeding programs are located on chromosomes 3BS and 5AS. Other QTLs listed in Table 2 (and many not in this table or not yet published) are used within a single breeding program or region. The strongest effect of the best FHB resistance gene identified to date, *Fhb1*, can reduce disease by as much as 50%, but on average about 20–25% depending on the genetic background (Pumphrey et al., 2007). Other QTLs listed in Table 2 show considerably smaller effects. However, there is evidence that combining major QTLs increases FHB resistance levels (Chen et al., 2006; Miedaner et al., 2006).

One advantage of marker technology is that it can be used to rapidly select for combinations of multiple marked genes in a population. For example, if a breeder wished to combine FHB resistance genes on 3BS and 5AS, markers could be used to preferentially select the portion of F_2 progeny (1/4 AA × 1/4 BB = 1/16 AABB) that would be expected to be homozygous for each gene. This subset would be selected in later generations for other disease resistance, agronomic and quality traits, in addition to FHB resistance. In contrast, field-based selection alone of homozygous lines from the same population would be burdened by the need to screen a 4× larger population to identify (imprecisely) those lines carrying both genes (AABB). Therefore, marker-assisted selection (MAS) can greatly enrich populations for desired types, leaving breeders to concentrate their resources on materials that have a better chance of resulting in improved varieties.

I consider three broad, practical criteria that must be satisfied for MAS to be effectively implemented to improve FHB resistance: 1) efficiency/gain compared to phenotypic selection; 2) usefulness of

Table 2
Quantitative trait loci and associated markers for FHB resistance in wheat

Name	Chromosome	QTL donor	Marker(s)	Reference(s)
Fhb1	3BS	Sumai 3	barc133	Liu et al. (2006)
Qfhs.ifa-5A	5AS	Sumai 3, Wuhan 3	barc180, barc186	Buerstmayr et al. (2002); McGowan (2002), Somers et al. (2003)
Qfhs.ndsu-3AS	3AS	*Triticum dicoccoides*	gwm2	Otto et al. (2002); Hartel et al. (2004)
	1B	F201R	barc8	Shen et al. (2003)
	2A	Freedom	gwm296	Sneller et al. (2004)
	2B	Goldfield	barc200	Gilsinger et al. (2005)
	3A	F201R	gwm674	Shen et al. (2003)
	3AL	Frontana	wms 720	Miedaner et al. (2006)
	3BSc	Nyu Bai	gwm566	Somers et al. (2003)
	5BL	Fujian 5114	barc004, barc089	Bowen (2002)
	6B	Sumai 3	barc101	Anderson et al. (2001)

markers in breeding-relevant populations; and 3) the cost, throughput, and expertise required. If the markers (and associated genes) do not increase FHB resistance more efficiently than conventional screening based in the greenhouse or field nurseries, it does not make sense to invest time and resources into this technology. The sizable effects of the QTLs discovered to date suggests that they should be useful for tools for selection. The markers must be useful in breeding populations, meaning that the DNA marker shows differences between the two parents and the QTL of interest is known to be present in one of the parents. This requires diagnostic markers that have marker alleles that are unique to the QTL (Liu and Anderson, 2003).

Efficient implementation of MAS demands the use of high-throughput equipment and trained personnel. Although MAS is becoming a new capability in many wheat breeding programs, its implementation is limited by the cost to support trained personnel and purchase equipment and reagents. Backcrossing with markers and parental characterization for key genes are cost effective ways of utilizing this technology on a small scale (Dubcovsky, 2004). Establishment of the USDA-ARS Regional Small Grains Genotyping Centers in the U.S. has dramatically increased the capabilities of breeders to apply MAS by providing access to high throughput DNA extraction and genotyping equipment. With such facilities, MAS activities have expanded to include early generation (e.g. F_2 and F_3) populations. However, only a fraction of genotypes potentially segregating for important genes can be accommodated, even with this equipment and technology (Bonnett et al., 2005). More efficient DNA extraction technologies and marker platforms [e.g. single nucleotide polymorphisms (SNPs)] will allow more complete implementation of MAS in wheat breeding programs in the future.

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