

# Registration of 'Becker'/'Massey' Wheat Recombinant Inbred Line Mapping Population

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## ABSTRACT

'Becker'/'Massey' (Reg. No. MP-5, NSL 479730 MAP), is a soft red winter wheat (*Triticum aestivum* L.) recombinant inbred line (RIL) population developed by Virginia Polytechnic Institute and State University. This mapping population is composed of 152  $F_{7:14}$  RILs that were developed using single-seed descent in earlier generations ( $F_{2:3}$ – $F_{7:8}$ ) and then advanced and tested in headrows in the field in later generations. The population has been used to map quantitative trait loci (QTL) associated with adult plant resistance to powdery mildew [APR-PM; caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal (syn. *Erysiphe graminis* f. sp. *tritici*)] and Fusarium head blight (FHB), primarily caused by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schw. (Petch)]. It also has been used to study the effects of dwarfing and photoperiod-sensitivity genes on expression of FHB resistance. Genetic marker data collected on this population includes 96 simple sequence repeat and 740 Diversity Arrays Technology (Yarralumla, ACT, Australia) markers. Chromosome linkage maps of this population were constructed, except for chromosomes 3D and 6D, and used for QTL analyses. Unique and major QTLs were identified for APR-PM on chromosomes 1BL, 2AL, and 2BL and for FHB resistance on 3BL, 4BS, and 4DS of Massey, which can be incorporated into cultivar development programs via enrichment of favorable alleles.

In the soft red winter wheat (*Triticum aestivum* L.) regions of the United States, powdery mildew [caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal (syn. *Erysiphe graminis* f. sp. *tritici*)] and Fusarium head blight (FHB; caused primarily by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schw. (Petch)]) are two major diseases that decrease the grain yield and quality of wheat. There are many race-specific resistance genes that have been

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**Abbreviations:** APR-PM, adult-plant resistance to powdery mildew; B/M, 'Becker'/'Massey'; DArT, Diversity Arrays Technology; FHB, Fusarium head blight; MOSN, Missouri scab nursery; QTL, quantitative trait loci; RIL, recombinant inbred line; SSR, simple sequence repeat; VAFLD, Virginia field test; VASN, Virginia scab nursery.

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mapped and a few of them have been cloned (Huang and Röder, 2004; Yahiaoui et al., 2004). However, adult-plant resistance to powdery mildew (APR-PM) is more important and effective to prevent reduction in wheat yield because they are not race specific (Liu et al., 2001). Sources resistant to FHB have been studied worldwide, and some major sources of resistance, such as Sumai 3, have been used in many wheat breeding programs (Buerstmayr et al., 2009; Liu et al., 2009). But these unadapted sources were not very effective in the U.S. soft red winter wheat regions because linkage drag often occurred. On the other hand, several cultivars with native intermediate resistance to FHB have been released, such as 'Ernie' (PI 584525; McKendry et al., 1995), 'Truman' (PI 634824; McKendry et al., 2005), and 'Bess' (PI 642794; McKendry et al., 2007), 'Roane' (PI 612958; Griffey et al., 2001), 'Tribute' (PI 632689; Griffey et al., 2005), and 'Jamestown' (PI 653731; Griffey et al., 2010). These cultivars and other advanced breeding lines are major sources with native FHB resistance and have been used in most soft red winter wheat programs in the United States. The quantitative trait loci (QTL) for APR-PM resistance were mapped by Liu et al. (2001), but the FHB resistance in Massey has not yet been located on chromosomes. Quantitative trait loci for FHB resistance in Ernie were mapped based on data from greenhouse point inoculation (Liu et al., 2005, 2007; Abate et al., 2008).

The 'Becker'/'Massey' (B/M) recombinant inbred line (RIL) mapping population (Reg. No. MP-5, NSL 479730 MAP) is a soft red winter wheat population developed by Virginia Polytechnic Institute and State University. The initial cross was made in 1991 (Liu et al., 2001). A set of 180 lines was tested for APR-PM as  $F_{2:3}$  lines in 1994 and as  $F_{5:6}$ ,  $F_{6:7}$ ,  $F_{7:8}$

headrows in 1997, 1998, and 1999 (Liu et al., 2001). A set of 152 F<sub>7:14</sub> RILs were tested for FHB resistance, in greenhouse experiments in 2008 and in mist-irrigated and inoculated FHB field nurseries in 2008 and 2009. In addition, the dwarf genes *Rht1*, *Rht2*, *Rht8* and photoperiod-sensitivity gene Ppd-D1 were found to be segregating in B/M RILs (Liu et al., 2011). The development of B/M RILs can facilitate the molecular characterization of APR-PM and FHB resistance in Massey. The resulting knowledge may help breeders in soft red winter wheat regions to better understand and utilize these sources of resistance to APR-PM and FHB.

## Materials and Methods

### Characteristics of the Parents

Massey (Reg. No. CV-689, CItr17953; Starling et al., 1984), tested as VA76-52-12 and released in 1981, was derived from the cross of 'Blueboy' (CItr 14031; Murphy, 1967)/'Knox 62' (CItr 13701; Patterson et al., 1978). The dwarfing gene *Rht1* in Blueboy was derived from 'Norin 10' (Murphy, 1967). Massey has some resistance to stem rust (caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn.) and good field resistance to powdery mildew and *Wheat spindle streak mosaic virus*. Its resistance to Hessian fly [*Mayetiola destructor* (Say)] was derived from the parent Knox 62. Massey has *Lr1* resistance gene, but it is susceptible to leaf rust (caused by *Puccinia triticina* Eriks.).

Becker (Reg. No. CV-726, PI 494524), tested as OH 234 and released by Ohio State University in 1985 (Lafever, 1988), was derived from the cross of 'Hart' (CItr 17426; Sechler et al., 1977)/VA66-54-10 (CItr 15293). It was released on the basis of superior yield, excellent milling and baking quality, and excellent winter hardiness. Becker is tolerant to acid soil and resistant to *Wheat spindle streak mosaic virus*. It has the *H3* gene, which confers resistance to Hessian fly races GP, A, C, and F. At the time of its release, Becker was highly resistant to loose smut [caused by *Ustilago tritici* (Pers.) Rostr.] and moderately resistant to leaf rust. Becker is highly susceptible to powdery mildew.

### Phenotyping for Powdery Mildew and Fusarium Head Blight

The severity of powdery mildew on the parents and B/M RILs was assessed with the James disease assessment key, which ranges from 0 to 50% (James, 1971). A score of 50% was given when the leaf being scored had maximum disease coverage (Liu et al., 2001; Tucker et al., 2006, 2007).

Parents and B/M RILs were screened for FHB in multiple environments (Table 1). These included inoculated and mist-irrigated experiments in the Virginia scab nurseries (VASN) at Blacksburg from 2008 to 2009, the Missouri scab nursery (MOSN) at Columbia in 2008, and Virginia

greenhouse in 2008. An experiment under natural field conditions was conducted in Virginia (VAFLD) at Warsaw in 2009, and in the latter, grain spawn inoculum was applied to the plots. Single 1.2-m headrows with 0.304-m spacing between the rows were planted in randomized complete block designs with two replications for all tests except the VAFLD test, which had one replication of standard-size yield plots composed of seven 2.74-m rows spaced 0.15 m apart. The greenhouse experiments were arranged in a randomized complete block designs with parents and RILs planted in pots and evaluated according to the procedures described in Liu et al. (2007). Two replications with four plants per replication for each RIL were screened. The most aggressive *Fusarium graminearum* isolates from Virginia or Missouri were used for point inoculation ( $5 \times 10^4$  conidia spores mL<sup>-1</sup> in the greenhouse) or for spray inoculations ( $1 \times 10^4$  conidia spores mL<sup>-1</sup> in the field FHB nurseries) at flowering. For field experiments, the percentage of the incidence and severity of FHB were assessed for each plot in the field based on visual estimation. The FHB index was calculated according to the formula (incidence  $\times$  severity)/100. Plots or head samples were harvested and threshed with minimal or no air to assure that diseased kernels were retained during sample harvest and cleaning. Seed samples from the VASN and VAFLD were used to assess Fusarium-damaged kernels visually by the same person in 2008 and 2009. The thousand-kernel weight was calculated based on the weight of 100 cleaned random seeds. The concentration of deoxynivalenol was measured with gas chromatography/mass spectrometry (Tacke and Casper, 1996; D.G. Schmale, personal communication, 2009). Flowering time (days from planting to 50% flowering) and plant height were recorded in the VASN and VAFLD tests in 2009.

### Genotyping of RILs and Mapping QTLs

Sample DNA from each parent and the RILs of B/M was extracted according to the protocol from Diversity Arrays Technology (DArT; Yarralumla, ACT, Australia) and sent to Australia for whole-genome DArT analysis (Akbari et al., 2006). A total of 740 DArT markers were scored, and 589 polymorphic markers were used to construct the genetic maps. A set of 199 simple sequence repeat (SSR) markers were screened for polymorphism between the parents. Ninety-six of them were used to screen all RILs for the

**Table 1. Fusarium head blight screening in the greenhouse and field for 'Becker'/'Massey' recombinant inbred wheat lines in 2008 and 2009.**

Location <sup>†</sup>	Years	Inoculation method	Traits measured <sup>‡</sup>
Field			
Blacksburg, VA (VASN)	2008, 2009	Conidial spore spray	INC, SEV, IND, FDK, TKW, DON, FT, HT
Columbia, MO (MOSN)	2008	Conidia spore spray	INC, SEV, IND
Warsaw, VA (VAFLD)	2009	Natural	INC, SEV, IND, FDK, TKW, DON, HD, HT
Greenhouse			
Blacksburg, VA	2008	Conidial spore injection	SEV

<sup>†</sup>VASN, Virginia scab nursery; MOSN, Missouri scab nursery; VAFLD, Virginia field test.

<sup>‡</sup>INC, incidence (%); SEV, severity (%); IND, index (0–100); FDK, Fusarium-damaged kernels (%); TKW, thousand-kernel weight (g); DON, deoxynivalenol (mg kg<sup>-1</sup>); FT, flowering time (d from 1 Jan.); HD, heading date (d from 1 Jan.); HT, plant height (cm).

**Table 2. Means of wheat parents and ranges of 'Becker'/'Massey' recombinant inbred lines (RILs) for Fusarium head blight-related traits in different environments in 2008 and 2009.**

Trait <sup>†</sup>	Massey	Becker	RILs mean	SD	Minimum	Maximum	Ernie <sup>‡</sup>	Coker 9835 <sup>§</sup>
2008 Greenhouse								
SEV	17.7	87.5	63.3	22.6	10.4	100.0	—	—
2008 Virginia Scab Nursery								
INC	42.5	80.0	61.3	20.4	22.5	100.0	50.0	90.0
SEV	22.3	23.7	29.4	10.2	12.5	66.0	19.0	68.6
IND	9.5	19.0	19.4	11.8	4.0	66.0	9.8	61.9
FDK	35.0	52.0	36.1	16.5	7.5	79.0	—	—
TKW	25.0	22.0	24.5	3.8	17.0	36.5	—	—
DON	5.0	6.1	5.2	4.3	0.0	20.3	1.8	2.7
FT	139.0	142.5	138.0	6.9	90.0	148.0	—	—
2008 Missouri Scab Nursery								
INC	100.0	100.0	93.8	7.3	70.0	100.0	—	—
SEV	16.5	39.0	25.4	12.3	9.5	71.5	—	—
IND	16.5	39.0	24.3	12.5	8.1	71.5	—	—
2009 Virginia Scab Nursery								
INC	25.0	40.0	27.8	14.6	5.0	80.0	13.0	57.0
SEV	15.0	25.0	15.7	6.9	7.5	55.0	24.0	76.0
IND	3.8	10.0	5.2	5.8	0.5	44.5	3.0	41.9
FDK	36.5	68.5	39.3	16.8	10.0	89.5	—	—
TKW	23.0	21.0	24.4	4.0	14.0	35.0	—	—
DON	3.6	5.3	4.0	2.3	0.9	12.9	1.6	4.0
FT	129.0	137.0	133.8	2.9	129.0	143.0	—	—
HT	95.2	115.6	107.8	13.5	52.5	146.3	—	—
2009 Virginia Field Test								
INC	10.0	15.0	9.4	4.3	5.0	30.0	—	—
SEV	10.0	25.0	12.5	5.2	5.0	40.0	—	—
IND	1.0	3.7	1.4	1.6	0.3	13.5	—	—
FDK	9.0	21.0	24.3	12.3	0.0	62.0	—	—
TKW	35.0	24.0	32.5	5.5	20.0	47.0	—	—
HD	120.0	129.0	122.5	4.6	116.0	141.0	—	—
HT	100.3	105.4	99.9	12.2	58.8	136.3	—	—

<sup>†</sup>INC, incidence (%); SEV, severity (%); IND, index (0–100); FDK, Fusarium-damaged kernels (%); TKW, thousand-kernel weight (g); DON, deoxynivalenol (mg kg<sup>-1</sup>); FT, flowering time (d from 1 Jan.); HD, heading date (d from 1 Jan.); HT, plant height (cm).

<sup>‡</sup>Ernie, resistant check.

<sup>§</sup>Coker 9835, susceptible check.

target chromosome regions determined after preliminary QTL analysis. The single nucleotide polymorphism markers for the *Rht1* and *Rht2* dwarfing genes were analyzed by the USDA-ARS Eastern Regional Small Grain Genotyping laboratory at Raleigh, NC (J. Smith and G. Brown-Guedira, personal communications, 2010). The SSR marker Xgwm-261 was used to screen for *Rht8* (Worland et al., 2001) and three gene-specific primer pairs were used for the *Ppd-D1* gene (Yang et al., 2009).

Liu et al. (2011) constructed a new map with the DArT and SSR markers. Molecular markers were analyzed using Joinmap 3.0 (Van Ooijen and Voorrips, 2001) and combined with MapMaker 3.0 (Lander et al., 1987) with the Kosambi mapping function (Kosambi, 1944). Generated map and phenotypic data were analyzed with QTL Cartographer 2.5 (Wang et al., 2010), and the QTL regions were identified through composite interval mapping.

## Results

### Agronomic Characteristics

Massey is susceptible to powdery mildew in the seedling stage but resistant in the adult-plant stage (Starling et al., 1984). Becker is very susceptible in both stages. The powdery mildew severity of the F<sub>2:3</sub> lines and F<sub>5</sub>–F<sub>7</sub> RILs was assessed in field tests conducted in Virginia (Liu et al., 2001).

Parental means and ranges among RILs for eight traits from tests conducted in four environments in 2008 and 2009 are shown in Table 2. Transgressive segregants were identified among RILs for all traits except for FHB incidence in the 2008 MOSN, which was due to high disease pressure. Plants inoculated in the FHB nurseries VASN and MOSN had high infection levels with maximum incidence and/or severity up to 100.0% (Table 2). Overall, the FHB data from the VASN in 2008 and 2009 were close to a normal distribution. The FHB incidence ranged from 20.4 to

100.0%, with Massey at 42.5% and Becker at 80.0%, which were comparable with the resistant check Ernie (50.0%) and the susceptible check Coker 9835 (90.0%) at the VASN in 2008, while FHB incidence ranged from 5.0 to 80.0%, with Massey at 25.0% and Becker at 40.0% in the VASN in 2009, and the FHB incidence in Ernie was 13.0% and that in Coker 9835 was 57.0% (Table 2).

### QTL Mapping for APR-PM and FHB Resistance

Three QTLs for APR-PM—*Qpm.vt-1B*, *Qpm.vt-2A*, and *Qpm.vt-2B*—were identified with bulk segregant analysis of 213 restriction fragment length polymorphism and 139 SSR markers (Liu et al., 2001). These three QTL explained 50% of the variation among  $F_{2:3}$  lines for APR-PM (Liu et al., 2001). Fourteen new SSR markers were mapped to the QTL regions by Tucker et al. (2007) using the same 180  $F_{2:3}$  RILs. The three QTL mapped in the B/M population were confirmed by Tucker et al. (2006) using an RIL population (NSL 465777 MAP; Hall et al., 2010) derived from the cross of ‘USG3209’/‘Jaypee’, with the female parent being a derivative of Massey.

Based on the new map that included the DArT and SSR markers, consistent QTL were detected for FHB resistance on chromosomes 2BL, 2DS, 3BL, 4BS, 4DS, and 5AS across multiple environments. The individual QTL of FHB resistance and other traits explained a maximum of the phenotypic variation in FHB incidence (13.3%), severity (17.8%), index (13.2%), Fusarium-damaged kernels (22.1%), 1000-kernel weight (22.4%), concentration of deoxynivalenol (21.3%), heading date (24.5%), flowering time (31.6%), and plant height (36.5%) based on trait data collected from the VASN in 2008 and 2009 (Liu et al., 2011). Some QTL for FHB resistance were located close to the dwarfing genes *Rht1*, *Rht2*, *Rht8* and photoperiod-insensitivity-gene allele *Ppd-D1a*. Together these genes explained up to 32.0%, 20.5%, 32.9%, 37.9%, 29.0%, 6.8%, and 30.1% of the variation in the above mentioned nine traits, respectively, observed in the 2009 VASN (Liu et al., 2011).

### Availability

Seed of the B/M RIL mapping population and its parents Becker and Massey is maintained by the Virginia Polytechnic Institute and State University Small Grains Breeding Program, Blacksburg, VA, 24061. A 25-g seed sample of each RIL and a 200-g seed sample of both parents were deposited with the National Plant Germplasm System. Seed of the parents is currently available from National Plant Germplasm System, and small quantities of seed of the RILs for research purposes may be obtained initially from the corresponding author and subsequently from National Plant Germplasm System 2 yr after the date of this publication or when seed is available for distribution. Appropriate recognition of the source should be noted if the population contributes to research on APR-PM or FHB or to the development of new genetic stocks, molecular tools, germplasm, or cultivars.

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