The Hessian fly [Mayetiola destructor (Say)] is one of the most significant insect pests of wheat (Triticum aestivum L.) in the United States. Host resistance has been the preferred approach to provide crop protection against this pest but the extensive deployment of a single gene in a large area causes the fly population to adapt and overcome resistance.

Major genes transferred from diploid and tetraploid wheat relatives have been an important factor in Hessian fly resistance. Among them, the diploid Aegilops tauschii Coss. (2n = 2x = 14; genome DD) has been the donor of resistance genes H13, H22, H23, H24, H26, and H32. H13, identified in a synthetic hexaploid, was originally mapped to chromosome 6DL using monosomic and telocentric analysis (Gill et al., 1987). More recently, Liu et al. (2005) developed a microsatellite linkage map for H13 and physically mapped the linked markers to the short arm of chromosome 6D. Ratcliffe et al. (2000) found H13 effective against several Hessian fly populations from the southeastern United States.


**ABSTRACT**

Hessian fly [Mayetiola destructor (Say)] is a major threat to wheat (Triticum aestivum L.) production in the eastern United States. Culti- 

var releases containing major Hessian fly resistance genes have proven effective in minimizing losses caused by this pest. Nevertheless, the ephemeral nature of major gene resistance necessitates the identification of novel sources of resistance. Hessian fly resistance from Aegilops tauschii Coss. (2n = 2x = 14; genome DD) was introgressed into the genetic background of the soft red winter wheat cultivar Saluda during the development of the germplasm line NC09MDD14. Our genetic characterization and linkage mapping studies showed that resistance to Hessian Fly biotype L in NC09MDD14 was monogenic and the most likely order of the linked microsatellite markers was: Xcfd13-4.2 cm- Xcfd42-1.8 cm- Xgdm141-4.9 cm- Xgdm36-1.5 cm- NC09MDD14 Hf gene/Xcfd132-13.4 cm- Xcfd19. This linkage map situated the NCD-09MDD14 Hf gene on the short arm of chromosome 6D, within the same deletion bin as the named gene H13. No recombinants between H13 and the NCD09MDD14 Hf gene were found in an allelism study that included 170 F2 individuals from the cross between NCD09MDD14 and Molly (H13). The Hessian fly resistance gene present in the germplasm line NC09MDD14 could be an allele of H13, but unlike H13, the gene in NC09MDD14 provides resistance against biotype vrH13.

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**Abbreviations:** LOD, likelihood of odds; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeat.

The Hessian fly [Mayetiola destructor (Say)] is one of the most significant insect pests of wheat (Triticum aestivum L.) in the United States. Host resistance has been the preferred approach to provide crop protection against this pest but the extensive deployment of a single gene in a large area causes the fly population to adapt and overcome resistance.

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H13, identified in a synthetic hexaploid, was originally mapped to chromosome 6DL using monosomic and telocentric analysis (Gill et al., 1987). More recently, Liu et al. (2005) developed a microsatellite linkage map for H13 and physically mapped the linked markers to the short arm of chromosome 6D. Ratcliffe et al. (2000) found H13 effective against several Hessian fly populations from the southeastern United States.
The genes H22, H23, and H24 were assigned to chromosomes 1D, 6D, and 3D, respectively, using monosomic analysis (Raupp et al., 1993). Ma et al. (1993) identified restriction fragment length polymorphism (RFLP) markers linked to H23 and H24 and mapped the genes to chromosomes 6DS and 3DL, respectively. Despite being on the same chromosome arm, a genetic distance of approximately 25 cM was estimated between H23 and H13 (Gill et al., 1991a).

H26 originated from the A. tauschii accession TA 2473 and was transferred into the hard red winter wheat cultivar Karl to develop the germplasm line KS92WGR26 (Cox and Hatchett, 1994). Wang et al. (2006) developed a microsatellite linkage map for H26 and positioned this gene within the chromosome bin 3DL3-0.81-1. H26 could be allelic to H32, another A. tauschii–derived gene also mapped to the same deletion bin (Sardesai et al., 2005). However, H26 is resistant to biotype GP while H32 is susceptible. The relationship between these two loci and H24 also needs to be clarified.

In addition to the six loci with a formal gene designation, several other A. tauschii–derived Hessian fly resistance genes have been identified that are either allelic to a known gene or have not been assigned to a particular chromosome (Wang et al., 2006; Gill et al., 1991b, 1991c; El Bouhssini et al., 2008). These genes represent a very valuable source of resistance to a wide range of biotypes, including the Syrian Hessian fly biotype which is reported to be the most virulent worldwide (El Bouhssini et al., 2008).

In this study, we present the genetic characterization, linkage mapping, and allelism test for the A. tauschii–derived Hessian fly resistance gene present in the soft red winter wheat germplasm line NC09MDD14.

MATERIALS AND METHODS

Plant and Insect Material

NC09MDD14 (PI 656395) is a Hessian fly biotype L resistant soft red winter wheat germplasm line with the pedigree: Saluda*2//TA 2492//Saluda*2/TA2377. TA 2492 and TA2377 (PI 603250) are A. tauschii accessions collected in Iran and kindly provided by the Wheat Genetic and Genomic Resources Center, Kansas State University. These A. tauschii accessions are resistant to biotype D (W.J. Raupp, personal communication, 2009). Saluda is a Hessian fly biotype L susceptible soft red winter wheat cultivar developed by the Virginia Polytechnic Institute and State University (Starling et al., 1986). Based on its pedigree, it can be concluded that biotype L resistance in NC09MDD14 is derived from one or both of the A. tauschii parents. NC09MDD14 was crossed to the susceptible breeding line NC00-15332 and the F1 hybrid was selfed to produce F2 seed. F2 plants were grown in the greenhouse without selection and harvested on an individual plant basis to produce the F2:3 families used in all the phenotypic evaluations against Hessian fly biotype L.

Biotype L is predominant in the southeastern United States (Ratcliffe et al., 2000) and is virulent to the wheat genes H7H8, H3, H5, and H6 (Ratcliffe et al., 1994) but is avirulent to H13.

The two parents, along with the germplasm lines Molly (Patterson et al., 1994), KS89WGRC03 (Gill et al., 1991a), and KS89WGRC04 (Gill et al., 1991b) were also tested against biotype vH13 which is virulent to H13 (Rider et al., 2002). Molly (PI 562619) contains H13 and has the pedigree Newton–207*/7/3/KU221-19/Eagle//KS806. KS89WGRC03 and KS89WGRC04 are hard red winter wheat Hessian fly resistant germplasm lines containing H23 and an unknown gene allelic to H13, respectively (Raupp et al., 1993; Liu et al., 2005).

Hessian Fly Resistance Evaluations

Hessian fly resistance in 143 F2:3 families was evaluated in two independent tests conducted by the University of Georgia (UGA) and USDA-ARS, West Lafayette, IN.

UGA Evaluation

Conetainers (RLC–3; Stuewe and Sons, Corvallis, OR) with a top cylinder of 2.5 cm and a length of 12 cm were filled with Sunshine SB400 (Sun Gro Horticultural Distribution Inc., Marysville, OH) and supplemented with 10–10–10 (N–P–K) fertilizer and Micromax (Scotts Inc., Marysville, OH). Approximately 14 to 21 seeds per F2:3 family were sown (one seed per conetainer), lightly watered, and placed in cold storage at 4°C for 10 d to promote uniform germination. Seeds from NC09MDD14 and NC00-15332 were included in all Conetainer racks as resistant and susceptible controls. Conetainers were then transferred to a growth chamber maintained at 18 ± 1°C and 12:12 h (light/dark) photoperiod. Plastic trays containing wheat material infested with Hessian fly biotype L pupae were placed next to each rack. A high relative humidity during infestation was maintained by daily watering. Three weeks after infestation, individual plants were scored as resistant or susceptible. Susceptible plants were stunted, with dark green leaves and live larvae or pupae present at the base of the leaf sheath. Resistant plants showed normal growth and absence of live larvae or pupae.

USDA-ARS Evaluation

Hessian fly evaluations in Indiana were conducted following the procedure described by Kong et al. (2008). Approximately 20 to 40 seedlings per F2:3 family plus the parents were evaluated for their reaction against biotype L, using the same families included in the UGA evaluation. The screening of germplasm lines against biotype vH13 was conducted only at this location.

Allelism Test

NC09MDD14 was crossed to Molly. F1 seed from this cross was selfed to obtain the F2 progeny used to test for allelism between H13 and the NC09MDD14 Hessian fly resistance gene. One hundred seventy F2 plants from a single F1 seed were tested for their reaction to Hessian fly infestation using the same procedure described for the UGA evaluation. Twenty randomly sampled plants were genotyped with microsatellite markers that were polymorphic between the parents to confirm that the individuals evaluated were derived from a true F1 hybrid.

Microsatellite Markers Analysis

Genomic DNA was extracted from leaf tissue of F2 seedlings following the C-TAB procedure described by Stein et al. (2001). Wheat microsatellite (simple sequence repeat [SSR]) primers evenly distributed across the D genome were synthesized according to the sequences published in the GrainGenes database (http://wheat.pw.usda.gov), modifying all forward primers evenly distributed across the D genome.
primers to include the M13 sequence (CAGCGACGTTGTA-AAACGAC-) at the 5’ end for labeling purposes (Schuelke 2000; Rampling et al., 2001). Polymerase chain reaction (PCR) protocols, separation of PCR products, and fragment size calling were as described by Miranda et al. (2006).

Primer pairs that were polymorphic between resistant and susceptible parents were used for bulked segregant analysis (Michelmore et al., 1991). Resistant and susceptible bulks were made by pooling equal amounts of DNA from 10 lines phenotypically scored as homozygous resistant and 10 lines phenotypically scored as homozygous susceptible.

**Data Analysis**

Deviations of observed data from theoretically expected segregation ratios were tested using chi-squared ($\chi^2$) tests for goodness-of-fit. Linkage analysis was performed using MAPMAKER/Exp version 3.0b (Lincoln et al., 1993). Map distances were determined using the Kosambi mapping function (Kosambi, 1944) and loci were ordered using the “sequence” and “compare” commands, with an LOD threshold score $\geq$3.0.

**RESULTS**

**Reaction of the Mapping Population to Hessian Fly Biotype L**

All NC09MDD14 plants exhibited resistance to Hessian fly biotype L and all NC00–15332 plants showed susceptibility. There was very good agreement in the results from the UGA and USDA-ARS evaluations. Each family was given a phenotypic class by adding the number of resistant and/or susceptible plants from both tests. Families were classified as resistant or susceptible only if all plants within a family belonged to either class exclusively. The $F_{2,3}$ families that fitted a 3:1 (resistant to susceptible) ratio were classified as segregating. Six families showed resistant and susceptible plants but had an unusual segregation ratio (probably due to escapes and/or seed mixtures). Although these six lines did not significantly change the results, they were not considered in the analysis to avoid potential errors in the construction of the linkage map.

The observed segregation ratio for the population was 33 homozygous resistant, 71 segregating, and 31 homozygous susceptible, fitting the expected 1:2:1 ($\chi^2 = 0.18, P = 0.91$) segregation ratio for a monogenically controlled resistance.

**Reaction of the Germplasm Lines to Hessian Fly Biotype vH13**

NC00–15332 and Molly (H13) exhibited susceptibility to biotype vH13. NC09MDD14, KS89WGRC04, and KS89WGRC03 (H23) were resistant to this biotype.

**Allelism Test**

All 170 $F_2$ plants from the cross between NC09MDD14 and Molly (H13) were resistant to Hessian fly biotype L. These results indicated that the Hessian fly resistance gene in NC09MDD14 is either allelic or closely linked to H13.

**Microsatellite Linkage Mapping**

Among the 120 primer pairs tested, CFD42 and GDM141 yielded amplification products that were polymorphic between the parents as well as the resistant and susceptible bulks. Because these two markers mapped to chromosome 6DS (http://wheat.pw.usda.gov), 12 additional 6DS primer pairs were screened among the parents. Five proved to be polymorphic and were tested in the population. Seven codominant SSR markers linked to the Hessian fly resistance gene in NC09MDD14 were identified. These markers were Xcfd13, Xcfd19, Xcfd42, Xcfd75, Xcfd132, Xgdm36, and Xgdm141 (Table 1). Primer pairs CFD13, CFD19, and GDM36 amplified more than one locus in either one or both parents but the differences in band sizes were large enough to unequivocally determine the target allele for each marker (Table 2). A genetic linkage map for the NC09MDD14 Hessian fly resistance gene and these seven linked SSR markers was constructed using MAPMAKER (Fig. 1a).

The SSR marker Xcfd132 cosegregated with the NC09MDD14 Hessian fly resistance gene. The 169-bp band associated with the NC09MDD14 allele was present in all resistant individuals, the 135-bp band associated with the NC00–15332 allele was present in all susceptible individuals, and all heterozygous individuals showed both bands (Fig. 2). This marker was also reported to show no recombination with H13 (Liu et al., 2005) (Fig. 1b).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Observed segregation Ratio</th>
<th>$\chi^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xcfd75</td>
<td>33:75:29</td>
<td>1.47</td>
<td>0.48</td>
</tr>
<tr>
<td>Xcfd13</td>
<td>37:67:31</td>
<td>0.54</td>
<td>0.76</td>
</tr>
<tr>
<td>Xcfd42</td>
<td>38:66:32</td>
<td>0.65</td>
<td>0.72</td>
</tr>
<tr>
<td>Xgdm141</td>
<td>33:72:32</td>
<td>0.37</td>
<td>0.83</td>
</tr>
<tr>
<td>Xgdm36</td>
<td>32:70:34</td>
<td>0.18</td>
<td>0.76</td>
</tr>
<tr>
<td>Xcfd132</td>
<td>33:71:33</td>
<td>0.18</td>
<td>0.91</td>
</tr>
<tr>
<td>Xcfd19</td>
<td>41:62:31</td>
<td>2.23</td>
<td>0.33</td>
</tr>
</tbody>
</table>

$^{1}$A = Homozygous for the NC09MDD14 allele, H = heterozygous, B = Homozygous for the NC00–15332.

<table>
<thead>
<tr>
<th>Primer pair</th>
<th>NC09MDD14</th>
<th>NC00–15332</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFD13</td>
<td>289</td>
<td>248, 326</td>
</tr>
<tr>
<td>CFD19</td>
<td>291,326, 332</td>
<td>308, 326, 342</td>
</tr>
<tr>
<td>CFD42</td>
<td>208</td>
<td>225</td>
</tr>
<tr>
<td>CFD75</td>
<td>316</td>
<td>321</td>
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<tr>
<td>CFD132</td>
<td>169</td>
<td>135</td>
</tr>
<tr>
<td>GDM36</td>
<td>189, 137</td>
<td>147,137</td>
</tr>
<tr>
<td>GDM141</td>
<td>154</td>
<td>132</td>
</tr>
</tbody>
</table>

$^{1}$Numbers in italics indicate the 6DS locus when more than one allele is present.
DISCUSSION

One gene for resistance to Hessian fly biotype L was identified in the germplasm line NC09MDD14. The resistance gene was derived from an accession of diploid A. tauschii and introgressed into a hexaploid winter wheat background adapted to the southeastern United States.

The Hessian fly resistance gene present in the germplasm line NC09MDD14 may be an allele of H13 (Fig. 1a, 1b), but the two genes differ in their reaction to Hessian fly biotype vH13. Virulence to H13 is controlled by a single recessive sex-linked gene (Zantoko and Shukle, 1997) and this gene (vrH13) corresponded to the same locus in Hessian fly populations from four different states: Georgia, Maryland, Virginia, and Washington (Rider et al., 2002). Unlike H13, the gene in NC09MDD14 triggers an incompatible (resistant) reaction when it interacts with the Hessian fly virulence gene vH13.

Two other A. tauschii–derived Hessian fly resistance genes have been reported for the short arm of chromosome 6D: H23, present in the germplasm line KS89W-GRC3 (Gill et al., 1991a) and H_{WGRGC4}, in KS89WGRGC4 (Gill et al., 1991b). In an allelism test between H23 and H13, Raupp et al. (1993) established a genetic distance of 25 cM. However, this genetic distance has not been completely corroborated by genetic and physical mapping.

Ma et al. (1993) reported two RFLP markers, XksuH4 and XksuG48, linked to H23 with distances of 6.9 and 15.6 cM, respectively. The wheat composite linkage map (http://wheat.pw.usda.gov) and the D genome linkage map developed by Pestova et al. (2000) located XksuG48 distal from the SSR marker Xgdm141 (linked to H13 and NC09MDD14), but the position of H23 with respect to XksuG48 is unknown because no flanking markers were found in the study by Ma et al. (1993).

Liu et al. (2005) used SSR markers in an effort to determine the size of the A. tauschii–derived segment in H13, H23, and H_{WGRGC4}, but these segments overlapped and independent deletion bins could not be established. They also reported no recombination between H23 and H_{WGRGC4}.

Gene clusters have been reported before for Hessian fly resistance (Kong et al., 2008), but in contrast with disease resistance genes (McIntosh et al., 2008), no allelic series have been identified before for Hessian fly genes. Corroboration of the presence of allelic series for disease resistance genes in wheat was facilitated by cloning the powdery mildew [caused by Blumeria graminis (DC.) E.O. Speer f. sp. tritici] resistance gene Pm3b (Yahiaoui et al., 2004). Soon after, Srichumpa et al. (2005) sequenced several Pm3 alleles and found small sequence variations that provide different disease specificities.

In this study, the NC09MDD14 gene, H23, and H_{WGRGC4} were all resistant to biotype vH13; therefore, they are different from H13, but additional studies are needed to determine whether they represent a cluster of closely linked genes or they are a true allelic series.

NC09MDD14 has shown consistent resistance over several seasons in field evaluations in North Carolina and

![Figure 1](image1.png)

**Figure 1.** Comparative view of the linkage maps for (a) the NC09MDD14 Hf gene and (b) H13 (Liu et al., 2005).

![Figure 2](image2.png)

**Figure 2.** Genotyping of the NC09MDD14 × NC00-15332 F2 population using microsatellite marker Xcdf132. F2 progeny labeled according to the reaction of their F2–derived family: R, resistant; H, segregating; S, susceptible; B, NC0015332; A, NC09MDD14.

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Georgia (data not shown). Virulence to H13 has been observed in the southeast (G.D. Buntin, personal communication, 2008), and NC09MDD14 has become a popular parent in cultivar development programs. The identification of flanking markers for this gene greatly enhances breeders’ capability to increase the frequency of this resistance gene in segregating populations.

REFERENCES


