Theor Appl Genet (2006) 113:611–618
DOI 10.1007/s00122-006-0325-z

ORIGINAL PAPER

Genetic characterization and molecular mapping of Hessian fly resistance genes derived from *Aegilops tauschii* in synthetic wheat

Tao Wang · Steven S. Xu · Marion O. Harris · Jinguo Hu · Liwang Liu · Xiwen Cai

Received: 22 January 2006 / Accepted: 9 May 2006 / Published online: 15 June 2006

Abstract Two synthetic hexaploid wheat lines (×*Aegilotriticum* spp., $2n = 6x = 42$, genomes AABBDD), SW8 and SW34, developed from the crosses of the durum wheat cultivar Langdon (*Triticum turgidum* L. var. *durum*, $2n = 4x = 28$, genomes AA BB) with two *Aegilops tauschii* Cosson accessions ($2n = 2x = 14$, genome DD), were determined to carry Hessian fly [*Mayetiola destructor* (Say)] resistance genes derived from the *Ae. tauschii* parents. SW8 was resistant to the Hessian fly biotype Great Plains (GP) and strain *vH13* (virulent to *H13*). SW34 was resistant to biotype GP, but susceptible to strain *vH13*. Allelism tests indicated that resistance genes in SW8 and SW34 may be allelic to *H26* and *H13* or correspond to paralogs at both loci, respectively. *H26* and *H13* were localized to chromosome 4D and 6D, respectively, in previous studies. Molecular mapping in the present study, however, assigned the *H26* locus to chromosome 3D rather than 4D. On the other hand, mapping of the resistance gene in SW34 verified the previous assignment of the *H13* locus to chromosome 6D. Linkage analysis and physical mapping positioned the *H26* locus to the chromosomal deletion bin 3DL3-0.81–1.00. A linkage map for each of these two resistance genes was constructed using simple sequence repeat (SSR) and target region amplification polymorphism (TRAP) markers.

Communicated by F. Ordon

T. Wang · L. Liu · X. Cai (✉)
Department of Plant Science, North Dakota State University, Fargo, ND 58105, USA
e-mail: xiwen.cai@ndsu.edu

S. S. Xu · J. Hu
USDA-ARS, Northern Crop Science Laboratory, P.O. Box 5677, Fargo, ND 58105, USA

M. O. Harris
Department of Entomology, North Dakota State University, Fargo, ND 58105, USA

Introduction

Hessian fly [*Mayetiola destructor* (Say) (Diptera: Cecidomyiidae)] is an important pest of wheat, including durum (*T. turgidum* L. var. *durum*) and common wheat (*T. aestivum* L.), in many of the wheat-producing regions around the world (Hatchett et al. 1987). Host resistance is considered the most effective management tactic for Hessian fly in wheat (Berzonsky et al. 2003). To date, over 30 major resistance genes, designated *H1* through *H32*, have been identified and assigned to eight different chromosomes in wheat genomes (McIntosh et al. 2003; Williams et al. 2003; Liu et al. 2005a; Sardesai et al. 2005). Among the Hessian fly resistance genes identified thus far, *H13*, *H22*, *H23*, *H24*, *H26*, and *H32* were derived from *Ae. tauschii*, the D-genome donor species of common wheat (Martin et al. 1982; Gill et al. 1986, 1991a, b; Raupp et al. 1993; Cox and Hatchett 1994; Ratcliffe and Hatchett 1997; Sardesai et al. 2005), and were localized to chromosome 6D, 1D, 6D, 3D, 4D, and 3D, respectively (Gill et al. 1987; Wilson et al. 1989; Sardesai et al. 2005).

Some of the Hessian fly resistance genes previously identified were mapped using molecular markers. Dweikat et al. (1994, 1997) identified 18 DNA marker loci linked to Hessian fly resistance genes *H3*, *H5*, *H6*, *H9*, *H10*, *H11*, *H12*, *H13*, *H14*, *H16*, and *H17* through

randomly amplified polymorphic DNA (RAPD) analysis in a set of near-isogenic wheat lines. *H9* and *H10* were localized to chromosome 5A in the previous studies by Carlson et al. (1978), Stebbins et al. (1982), and Ohm et al. (1995). Recently, these two resistance genes mapped to the short arm of chromosome 1A (1AS) by Liu et al. (2005b) and Kong et al. (2005). Another Hessian fly resistance gene derived from cultivated emmer wheat, designated *Hdic*, was also assigned to the same region as the *H9* and *H10* loci on 1AS (Liu et al. 2005a). Ma et al. (1993) assigned the Hessian fly resistance genes *H23* and *H24* to chromosome 6D and the long arm of 3D, respectively. A linkage map for each of these two genes was constructed using restriction fragment length polymorphism (RFLP) markers (Ma et al. 1993). The Hessian fly resistance gene *H6* was localized to chromosome 5A via monosomic analysis (Gallun and Patterson 1977). Dweikat et al. (2002) generated sequence tagged site (STS) markers linked to the *H6* locus. Williams et al. (2003) positioned *H31* to the short arm of chromosome 5B using amplified fragment length polymorphism (AFLP) and STS markers. Liu et al. (2005c) developed a linkage map of *H13* using SSR markers and assigned this resistance gene to a chromosomal region proximal to the breakpoint of del6DS-6 through deletion analysis. Recently, Sardesai et al. (2005) reported a new Hessian fly resistance gene designated *H32* and mapped this gene to the chromosomal deletion bin 3DL3-0.81–1.00 using molecular markers.

Two synthetic hexaploid wheat lines, SW8 [Langdon (LDN)/*Ae. tauschii* CIae 25] and SW34 (LDN/*Ae. tauschii* RL5544), were identified as resistant to the Hessian fly biotype Great Plains (GP) (Friesen et al. 2003; Xu et al. 2006). The durum wheat parent involved in SW8 and SW34, LDN, was identified as susceptible to biotype GP and strain *vH13* (Friesen et al. 2003; Xu et al. 2006). Thus, resistance in SW8 and SW34 should be derived from their *Ae. tauschii* parents CIae 25 and RL5544, respectively. The objectives of this study were to determine the allelism of the Hessian fly resistance genes in SW8 and SW34 with the previously identified resistance genes derived from *Ae. tauschii* and to precisely position the resistance genes in the synthetic hexaploid wheat lines using molecular markers.

Material and methods

Plants and insects

The three synthetic hexaploid wheat lines, SW8, SW34, and SW11, were derived from the crosses of LDN with three *Ae. tauschii* accessions, CIae 25, RL5544, and H-80–114–1, respectively. They were originally developed by L. R. Joppa (USDA-ARS, Fargo, ND) in the 1980s. SW8 and SW34 were identified as resistant and SW11 as susceptible to the Hessian fly biotype GP (Friesen et al. 2003; Xu et al. 2006). Two $F_{2:3}$ mapping populations were developed by hybridizing SW11 to SW8 and SW34, respectively. The four hexaploid wheat lines each carrying a Hessian fly resistance gene on a D-genome chromosome, including PI 562619 (*H13*, 6D), PI 535766 (*H23*, 6D), PI 535769 (*H24*, 3D), and PI 572542 (*H26*, 4D), were obtained from the National Small Grains Collection, Aberdeen, ID, USA.

Two colonies of Hessian fly, biotype GP and strain *vH13*, were initially obtained from the USDA-ARS Laboratory at Purdue University and maintained by the Department of Entomology, North Dakota State University, Fargo, ND. Hessian fly populations are characterized relative to the 'biotype' of individuals (Ratcliffe et al. 1994). The Hessian fly biotype GP is avirulent for most known resistance genes, including *H3*, *H5*, *H6*, *H7/H8*, *H9*, *H13* (Ratcliffe et al. 2000; Harris et al. 2006). The Hessian fly strain *vH13* is characterized as virulent to wheat genotypes having the *H13* resistance gene. The Hessian fly biotype GP and strain *vH13* used in this study were maintained on 'Reeder', a hard-red spring wheat cultivar susceptible to Hessian fly.

Evaluation of Hessian fly resistance

The hexaploid wheat lines, including SW8, SW34, SW11, PI 562619, PI 535766, PI 535769, and PI 572542, were evaluated for resistance to the Hessian fly biotype GP in a randomized complete block design with two replicates (7 plants per replicate) in the greenhouse. Reactions of SW8, SW34, SW11, PI 562619, and PI 572542 to the Hessian fly strain *vH13* also were evaluated. The F_1 hybrids and F_3 families derived from each of the F_2 individuals in the crosses of SW8 × SW11 and SW34 × SW11 were evaluated for resistance to biotype GP.

One plant was grown in each of the super-cell cones (Stuewe and Sons, Inc., Corvallis, OR) filled with Sunshine SB100 Mix (Sun Gro Horticulture Distribution Inc., Bellevue, WA), and fertilized with Osmocote Plus 15–19–12 (Scotts Sierra Horticultural Product Company, Marysville, OH). To infest plants with Hessian fly eggs, seedlings ($n = 100$) at the two-leaf stage were held for 24 h with mated Hessian fly females ($n = 40$–50) using a tent made from a blue cotton sheet. Three days after eggs were oviposited, plants were transferred to a high humidity chamber (60% RH, 25°C,

16:8 L:D) to ensure good survival rates during the migration of larvae from egg-laying sites on the leaf blade to feeding sites at the base of the plant. After this migration was complete, plants were transferred to the greenhouse at 25°C, with a photoperiod of 16:8 (L:D) h and 30% RH. Twenty days after infestation, plants were scored 'resistant' or 'susceptible' as described by Cartwright and LaHue (1944). Susceptible plants were stunted and dark blue-green in color with broad second or third leaves. In addition, live larvae or pupae were found at the base of the leaf sheath in the susceptible plants. Resistant plants showed normal growth with dead first-instar larvae found at the base of leaf sheath.

Allelism tests

The hexaploid wheat lines, PI 562619 and PI 572542, were hybridized with SW8, and PI 562619 was hybridized with SW34 in the greenhouse. Two co-dominant SSR markers, *Xgwm484–2D* and *Xgwm337–1D*, were used to select true F_1 hybrids from the allelism test crosses. The F_2 individuals derived from true F_1 hybrid plants were evaluated for reactions to the Hessian fly biotype GP. Chi-square (χ^2) analysis was conducted to determine the goodness-of-fit to Mendelian segregation ratios.

Molecular markers and linkage analysis

Genomic DNA of the parents, F_1's, and F_2 populations were extracted as described by Dellaporta et al. (1983). Primer sequences of the SSR markers assigned to the D-genome chromosomes were obtained from the GrainGenes Database (http://www.wheat.pw.usda.gov/GG2/index.shtml). Bulked segregant analysis was performed to identify SSR marker loci linked to the Hessian fly resistance genes. Two bulks of DNA were prepared by pooling equal amounts of DNA from five homozygous resistant and five homozygous susceptible F_2 individuals, respectively. The SSR loci were amplified as described by Röder et al. (1998). PCR products were separated on 6% non-denaturing polyacrylamide gels in 1 × TBE buffer at 300 V for 1.5 h. The gels were visualized by staining with ethidium bromide or scanned with a Typhoon 9410 variable mode imager (Molecular Dynamics, Ithaca, NY) after staining with SYBR Green II (Sigma, St. Louis, MO).

Target region amplified polymorphism (TRAP) marker analysis was conducted following the procedures of Hu and Vick (2003). One fixed primer in combination with two random primers labeled by different dyes (IR-700 and IR-800, Li-Cor Biosciences, Lincoln, NE) was used to amplify TRAP loci via the polymerase chain reaction (PCR). The fixed primer was designed based on a conserved telomeric repeat (Hu and Vick 2006) and the random primers were designed as described by Hu and Vick (2003). The PCR products were visualized on a Li-Cor Global DNA Sequencer 4300. Electrophoresis was conducted at 2,000 V, 40 mA, 50 W for 2.5 h.

Linkage of molecular markers with the Hessian fly-resistance genes in the two mapping populations was analyzed using MAPMAKER 2.0 (Lander et al. 1987) for Macintosh at LOD > 3.0 with Kosambi mapping function (Kosambi 1944).

Results

Reactions to Hessian fly infestation and resistance genes

Both SW8 and SW34 exhibited resistance to the Hessian fly biotype GP, whereas SW11 showed susceptibility. All the F_1's of SW8 and SW34 with SW11 were also resistant to biotype GP (Table 1). A total of 97 F_2 individuals from SW8 × SW11 and 111 from SW34 × SW11 were self-pollinated to produce F_3 families. About 16–30 plants from each of the F_3 families were evaluated for resistance to biotype GP. No segregation was found in the F_3 families derived from homozygous dominant and recessive F_2 plants. A 3:1 segregation ratio of resistant to susceptible plants was observed in most of the F_3 families derived from the heterozygous F_2 plants. A few F_3 families derived from the heterozygous F_2 individuals exhibited a segregation ratio deviated from 3:1. This might result from the small sample size of the F_3 families. Three categories of F_2 individuals, including homozygous resistant, heterozygous resistant, and homozygous susceptible, were identified based on the evaluation data of the F_3 families. A segregation ratio of 1:2:1 (homozygous resistant:heterozygous resistant:homozygous susceptible) was obtained in both F_2 populations (Table 2). These results indicated that Hessian fly resistance in SW8 and SW34 was conditioned by a single dominant gene.

PI 562619 (*H13*), PI 572542 (*H26*), PI 535766 (*H23*), SW8, and SW34 all exhibited resistance to biotype GP, whereas PI 535769 (*H24*) was susceptible. Therefore, resistance genes in SW8 and SW34 were different from *H24* in PI 535769. PI 562619 (*H13*) and SW34 were susceptible to the Hessian fly strain *vH13*. SW8 and PI 572542 (*H26*) showed resistance to *vH13*. These results suggested that SW34 may have a resistance gene allelic to or the same as *H13*, and the resistance gene in SW8 is different from *H13* (Table 1).

Table 1 Reactions to the Hessian fly biotype Great Plains (GP) and strain *vH13*

Genotypes	Resistance genes	Replicates	Number of plants evaluated			
			GP		*vH13*	
			Resistant	Susceptible	Resistant	Susceptible
SW11		1	0	7	0	7
		2	0	7	0	7
SW8		1	7	0	7	0
		2	7	0	7	0
SW34		1	7	0	0	7
		2	7	0	0	7
(SW8 × SW11)F_1		1	7	0	–	–
(SW34 × SW11)F_1		1	7	0	–	–
PI 562619	*H13*	1	7	0	0	7
		2	7	0	–	–
PI 535766	*H23*	1	7	0	–	–
		2	7	0	–	–
PI 535769	*H24*	1	0	7	–	–
		2	0	7	–	–
PI 572542	*H26*	1	7	0	7	0
		2	7	0	–	–

Table 2 Segregation of Hessian fly resistance in two F_2 populations

Crosses	Number of F_2 plants			Total	$\chi^{2*}_{1:2:1}$
	Homozygous resistant	Heterozygous resistant	Homozygous susceptible		
SW8 × SW11	19	53	25	97	1.58
SW34 × SW11	22	61	28	111	1.74

* χ^2 ($df = 2$, $P = 0.05$) = 5.99

Allelism tests

The two hexaploid wheat lines showing resistance to biotype GP, PI 562619 and PI 572542, were included in the allelism tests with SW8 and SW34. PI 535769 was not included in the allelism tests because it was susceptible to biotype GP (Table 1). Over 90 F_2 individuals were evaluated for resistance to biotype GP in each of the allelism test crosses. A 15:1 segregation ratio of resistant to susceptible plants was obtained in the F_2 generation of the cross SW8 × PI 562619. One stunted plant was observed among the 405 F_2 plants derived from the cross SW8 × PI 572542. A live larva or pupa, however, was not found at the base of this stunted plant (Table 3). These results indicated that the resistance gene in SW8 is independent of *H13* in PI 562619, but may be allelic to *H26* in PI 572542 or correspond to paralogs at this locus. All the 217 F_2 plants of the cross PI 562619 × SW34 were resistant to biotype GP and no segregation was observed in this cross. These results demonstrated that the resistance gene in SW34 may be allelic to *H13* in PI 562619 or correspond to paralogs at this locus.

Table 3 Segregation of Hessian fly resistance in the allelism tests

Crosses	Number of F_2 plants		Total	$\chi^{2**}_{15:1}$
	Resistant	Susceptible		
SW8 × PI 562619	85	8	93	0.88
SW8 × PI 572542	404	1[a]	405	24.91*
PI 562619 × SW34	217	0	217	14.47*

[a] Stunted plant without a live larva or pupa found

*Significant different from a 15:1 ratio at $P < 0.05$

** χ^2 ($df = 1$, $P = 0.05$) = 3.84

Molecular mapping of the Hessian fly resistance genes in SW8 and SW34

SW8 was determined to have a Hessian fly resistance gene at the *H26* locus based on the allelism test. This gene locus was assigned to chromosome 4D based on the monosomic analyses for chromosome 1D, 2D, 4D, 5D, 6D, and 7D in PI 572542 (*H26*) in a previous study by Cox and Hatchett (1994). Molecular mapping, however, was not previously conducted with this gene locus. The present study attempted to precisely

position this resistance gene via genetic and physical mapping. Since monosomic analysis for chromosome 3D in PI 572542 was not included in the study by Cox and Hatchett (1994), we decided to verify the chromosomal location of this gene locus using bulked segregant analysis prior to molecular mapping. A total of 216 SSR marker loci assigned to the D-genome chromosomes, including 42 BARCs (Song et al. 2005), 73 GWMs (Röder et al. 1998), 20 GDMs (Pestsova et al. 2000), 24 WMCs (Gupta et al. 2002), 55 CFDs (Sourdille et al. 2004), and 2 CFAs (Sourdille et al. 2003), were surveyed for polymorphisms between the parents (SW8 and SW11) of the mapping population. Sixty-five of them were polymorphic between SW8 and SW11. Bulked segregant analysis with these polymorphic markers identified eight SSR markers (*Xbarc71*, *Xgwm3*, *Xcfd211*, *Xcfd223*, *Xgwm314*, *Xwmc656*, *Xbarc42*, and *Xgwm664*) linked with the Hessian fly resistance gene in SW8 (Fig. 1). These eight SSR markers were mapped previously to the long arm of chromosome 3D (3DL) (Sourdille et al. 2004; Somers et al. 2004; Song et al. 2005). In addition, we conducted bulked segregant analysis with seven SSR markers assigned to the long and short arm of chromosome 4D. Linkage was not detected between the marker loci on 4D and the resistance gene in SW8 (Fig. 1). Therefore, the Hessian fly resistance gene locus *H26* should reside on chromosome 3D instead of 4D.

A linkage map of the resistance gene locus *H26* was constructed with those eight SSR and one TRAP markers in the population of 97 F_2 individuals derived from the cross SW8 × SW11 (Fig. 2). Comparative analysis of this linkage map and the consensus genetic map of 3DL (Somers et al. 2004) is illustrated in Fig. 2. The three marker loci, *Xgwm314*, *Xcfd223*, and *Xgwm3*, were found at a different location on the consensus map than on the genetic map constructed in the present study (Fig. 2; Somers et al. 2004). The molecular markers proximal (*Xbarc42, Xgwm314, Xcfd223*, and *Xcfd211*) and distal (*Xgwm3* and *Xbarc71*) to the *H26* locus were assigned to the chromosome bin 3DL3-0.81–1.00 (Fig. 2, Sardesai et al. 2005; Song et al. 2005; http://www.wheat.pw.usda.gov/GG2/index.shtml). Therefore, the *H26* locus should reside within this chromosome bin. Since this gene locus is localized in the last distal bin of 3DL, we tried to generate a molecular marker within the telomeric region of 3DL to define the physical end of the linkage map using the TRAP marker technique. Amplification with a fixed primer (5′-CCCTAAACCCTAAACCCTAAAA-3′) designed based on the conserved telomeric repeat, and a random primer (5′-CTATCTCTCGGGACCAAAC-3′) identified a TRAP marker locus, *Xwgc7330–3D*, linked to the resistance gene. This TRAP marker, however, mapped 4.0 cM proximal to *Xgwm3* rather than within the telomeric region (Fig. 2).

Reactions of SW34 and PI 562619 to the Hessian fly strain *vH13* and the allelism test between SW34 and PI 562619 revealed allelism of the resistance gene in SW34 with *H13* in PI 562619. The *H13* locus was

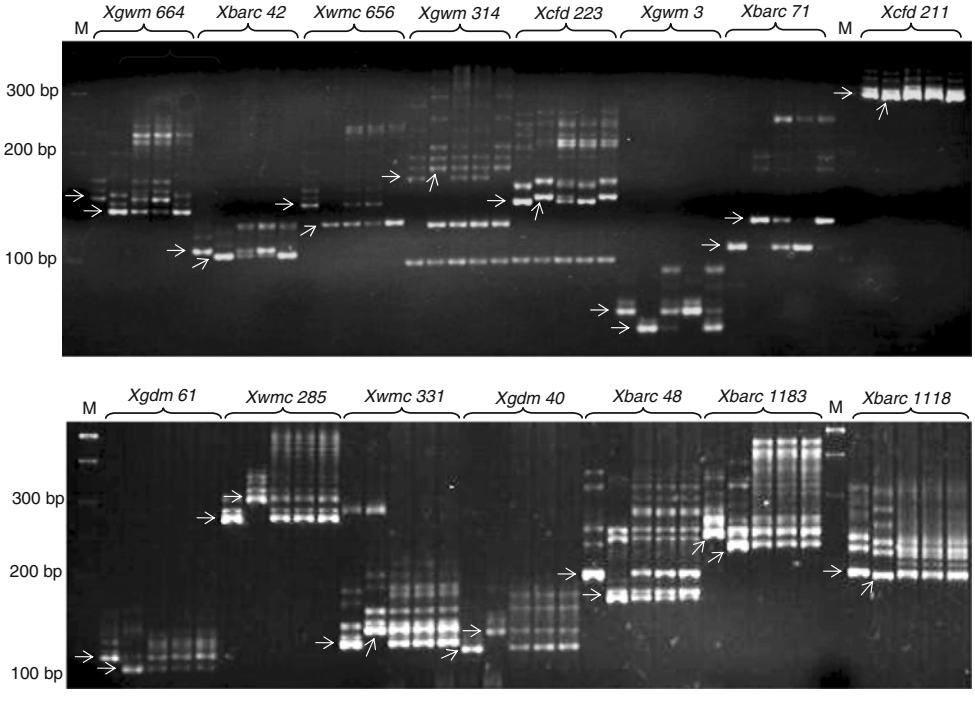

Fig. 1 Bulked segregant analysis to determine chromosomal location of *H26*. Polyacrylamide gels show polymorphic DNA fragments amplified in SW8 (*Lane 1 from left*), SW11 (*Lane 2*), (SW8 × SW11) F_1 (*Lane 3*), resistant bulk (*Lane 4*), and susceptible bulk (*Lane 5*) at each of the SSR marker loci on chromosome 3D (*upper*) and 4D (*lower*). The SSR marker loci are indicated at the *top of the gels*. The Lane M is 100-bp DNA ladder. *Arrows* point to the DNA fragments amplified from each of the SSR loci in the parents

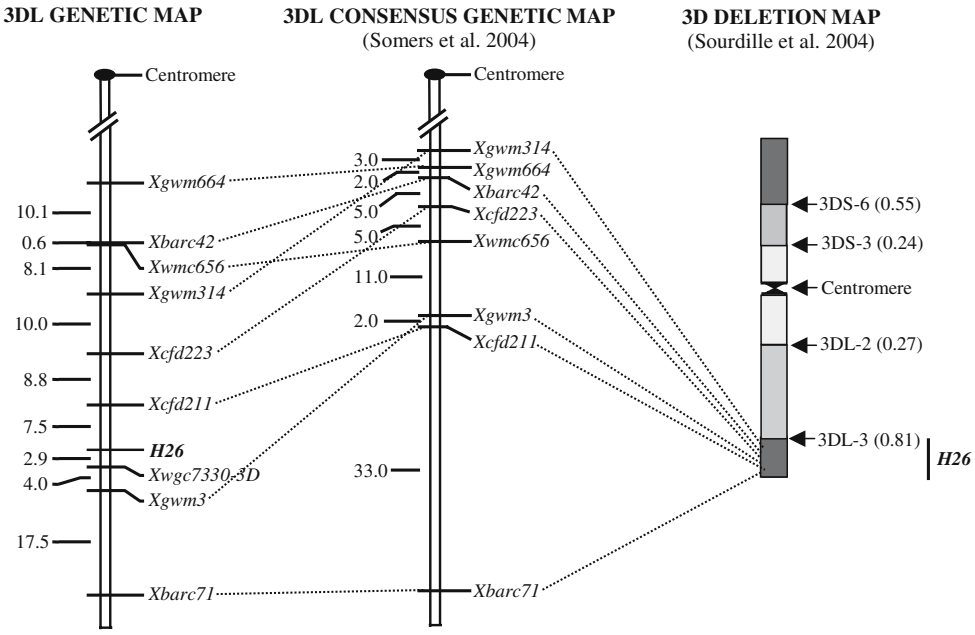

Fig. 2 Genetic and physical mapping of *H26*. *Left* Genetic map of *H26* on 3DL. *Middle* Consensus genetic map of the chromosomal region harboring the *H26* locus on 3DL. *Right* Deletion map of chromosome 3D (*right*). Marker loci are listed to the right and centiMorgan (cM) distances are shown to the left. The fraction length of the deletions (*within parentheses*), the breakpoints, and the centromere are indicated to the *right of the deletion map*

mapped recently to 6DS by Liu et al. (2005c). We used the SSR marker *Xcfd132* co-segregating with *H13* and two other co-segregating SSR markers *Xcfd75* and *Xgdm132* that mapped 14.4 cM distal to *H13* (Liu et al. 2005c) to verify the allelism of the resistance gene in SW34 with *H13*. Linkage analysis indicated that the resistance gene in SW34 was 3.7 cM from *Xcfd132*. This analysis also demonstrated that *Xcfd75* and *Xgdm132* were 0.6 cM apart and mapped distal to *H13*. A linkage map of the resistance gene in SW34 was constructed with these three SSR markers in the population of 111 F_2 individuals derived from the cross SW34 × SW11 (Fig. 3). The order of the loci and the map distance among them in this map are consistent with those in the linkage map of *H13* constructed by Liu et al. (2005c). These results confirmed the allelism of the resistance gene in SW34 with *H13*.

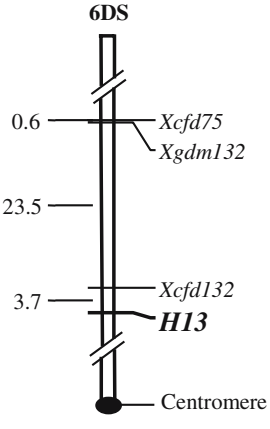

Fig. 3 Linkage map of *H13* on 6DS. Marker loci are listed to the right and centiMorgan (cM) distances are shown to the *left*

Discussion

Hessian fly resistance derived from *Ae. tauschii* was identified in synthetic hexaploid wheat lines and their derivatives (Hatchett et al. 1981; Xu et al. 2006). We screened a number of synthetic hexaploid wheat lines for resistance to the Hessian fly biotype GP and strain *vH13* and discovered resistance in some of them (data not shown). The synthetic hexaploid wheat lines SW8 and SW34 were included in this study because they exhibited different reactions to strain *vH13*, although both were resistant to biotype GP.

Allelism tests with the resistance gene derived from *Ae. tauschii*, including *H13*, *H22*, *H23*, *H24*, and *H26* (Hatchett and Gill 1981; Gill and Raupp 1987; Ratcliffe and Hatchett 1997), revealed allelism of the resistance gene in SW8 and SW34 with *H26* in PI 572542 and *H13* in PI 562619, respectively. One stunted plant was observed among the 405 F_2 individuals derived from the cross SW8 × PI 572542, but a live larva or pupa was not found in the stunted plant. Stunting of this plant, therefore, may have been caused by some physiological factors.

SW8 carries a Hessian fly resistance allele at the *H26* locus. The *H26* locus was previously localized to chromosome 4D through monosomic analyses (Cox and Hatchett 1994). This resistance gene locus, however, mapped to chromosome 3D based on allelism tests and molecular mapping in the present study. Linkage analysis and physical mapping of the molecular markers near the *H26* locus positioned this resistance gene locus within the chromosome bin 3DL3-0.81–1.00. A

recently discovered Hessian fly resistance gene, designated *H32*, mapped within the same chromosome bin as *H26* (Sardesai et al. 2005). The two SSR markers flanking *H32*, *Xgwm3* and *Xcfd223*, mapped near *H26* although their orders were opposite on these two maps (Sardesai et al. 2005; Fig. 2). It appears these two Hessian fly resistance gene loci map close to each other. Allelism between *H26* and *H32* has not been tested. Sardesai et al. (2005), however, reported that the synthetic wheat line having *H32* was susceptible to the Hessian fly biotype GP. This could differentiate *H32* from *H26* because *H26* confers resistance to biotype GP.

Another Hessian fly resistance gene, *H24*, was also mapped to 3DL (Ma et al. 1993). Sardesai et al. (2005) indicated that *H24* resides distal to *H32* within the chromosome bin 3DL3-0.81–1.00. In the present study, PI 535769, carrying *H24*, exhibited susceptibility to biotype GP; indicating *H26* and *H24* were two different Hessian fly resistance genes. These results demonstrate that *H24* is a distinct Hessian fly resistance gene from *H32* and *H26*, although all three of them are localized within the same chromosome bin.

To date, over 30 Hessian fly resistance genes have been identified in various sources. Accordingly, 16 Hessian fly biotypes, designated GP and A through O, were identified (Ratcliffe and Hatchett 1997). Additional sources of resistance are continually needed to manage this insect in wheat (Berzonsky et al. 2003). It is widely accepted that gene stacking is an important strategy in the development of new cultivars with durable resistance to different biotypes of an insect pest, including Hessian fly. However, evaluation of Hessian fly resistance is a labor and time-consuming process and leads to the loss of plants evaluated. These factors limit progress of breeding for Hessian fly resistance in wheat. The identification of molecular markers linked to the Hessian fly resistance genes could facilitate efficient utilization of the resistance gene in breeding and minimize screening during the breeding process. In the present study, we positioned the *H26* locus in the distal region of 3DL and identified a SSR marker (*Xcfd211*) 7.5 cM proximal to *H26* and a TRAP marker 2.9 cM distal to *H26*. These markers should prove useful in breeding to assist selection of the Hessian fly resistance conferred by *H26* and to pyramid *H26* and other Hessian fly resistance genes in wheat.

Acknowledgments The authors thank Kirk Anderson and Karin Anderson (Department of Entomology, North Dakota State University, Fargo, ND) for their help in maintaining and providing Hessian flies to this research.

References

Berzonsky WA, Ding H, Haley SD, Harris MO, Lamb RJ, Mckenzie RIH, Ohm HW, Patterson FL, Peairs F, Porter DR, Ratcliffe RH, Shanower TG (2003) Breeding wheat for resistance to insects. Plant Breed Rev 22:221–296

Carlson SK, Patterson FL, Gallun RL (1978) Inheritance of resistance to Hessian fly derived from *Triticium turgidum* L. Crop Sci 18:1011–1014

Cartwright WB, LaHue DW (1944) Testing wheats in the greenhouse for Hessian fly resistance. J Econ Entomol 37:385–387

Cox TS, Hatchett JH (1994) Hessian fly resistance gene *H26* transferred from *Triticum tauschii* to common wheat. Crop Sci 34:958–960

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation. Version II. Plant Mol Biol Rep 1:19–21

Dweikat I, Zhang W, Ohm H (2002) Development of STS markers linked to Hessian fly resistance gene *H6* in wheat. Theor Appl Genet 105:766–770

Dweikat I, Ohm H, Patterson F, Cambron S (1997) Identification of RAPD markers for 11 Hessian fly resistance genes in wheat. Theor Appl Genet 94:419–423

Dweikat I, Ohm H, Mackenzie S, Patterson F, Cambron S, Ratcliffe R (1994) Association of a DNA marker with Hessian fly resistance gene *H9* in wheat. Theor Appl Genet 89:964–968

Friesen TL, Xu S, Faris JD, Rasmussen JB, Miller JD, Harris MO (2003) Evaluation of *Langdon durum-Aegilops tauschii* synthetic hexaploid wheats for reaction to tan spot, *Stagonospora* leaf blotch, leaf rust, stem rust, and Hessian fly. pp 1133–1135. In: Pogna NE, Romanò M, Pogna EA, Galterio G (eds) Proceedings of the 10th international wheat genetics symposium, Pasetum, Italy. 1–6 Sept. 2003. Istituto Sperimentale per la Cerealicoltura, Rome, Italy

Gallun RL, Patterson FL (1977) Monosomic analysis of wheat for resistance to Hessian fly. J Hered 68:223–236

Gill BS, Wilson DL, Raupp WJ, Hatchett JH, Cox TS, Amri S, Sears RG (1991a) Registration of KS89WGRC3 and KS89WGRC6 Hessian fly-resistant hard red winter wheat germplasm. Crop Sci 31:245

Gill BS, Wilson DL, Raupp WJ, Hatchett JH, Cox TS, Amri S, Sears RG (1991b) Registration of KS89WGRC4 hard red winter wheat germplasm with resistance to Hessian fly, greenbug, and soil-borne mosaic virus. Crop Sci 31:246

Gill BS, Raupp WJ (1987) Direct genetic transfer from *Aegilops squarrosa* L. to hexaploid wheat. Crop Sci 27:445–450

Gill BS, Hatchett HJ, Raupp WJ (1987) Chromosomal mapping of Hessian fly resistant gene *H13* in the D genome of wheat. J Hered 78:97–100

Gill BS, Hatchett JH, Raupp WJ, Sears RG, Martin TJ (1986) Registration of KS85WGRC01 Hessian fly resistant hard red winter wheat germplasm. Crop Sci 26:1266–1267

Gupta PK, Balyan HS, Edwards KJ, Isaac P, Korzun V, Roder M, Gautier MF, Joudrier P, Schlatter AR, Dubcovsky J, De la Pena RC, Khairallah M, Penner G, Sharp P, Keller B, Wang RCC, Hardouin JP, Jack P, Leroy P (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105:413–422

Harris, MO, Anderson KG, Anderson KM, Kanno H (2006) Proximate cues for reduced oviposition on wheat plants attacked by conspecific larvae. Environ Entomol (in press)

Hatchett JH, Starks KJ, Webster JA (1987) Insect and mite pests of wheat. In: Heyneg EG (eds) Wheat and wheat improvement. 2nd edn. Am Soc Agron, Madison, WI pp 626–675

Hatchett JH, Gill BS (1981) D-genome sources of resistance in *Triticum tauschii* to Hessian fly. J Hered 72:126–127

Hatchett JH, Martin TJ, Livers RW (1981) Expression and inheritance of resistance to Hessian fly in synthetic hexaploid wheats derived from *Triticum tauschii* (Coss) Schmal. Crop Sci 21:731–734

Hu J, Vick BA (2006) Integration of the conserved telomere sequence-derived TRAP markers onto an existing sunflower SSR linkage map. Plant Genome XIV. January 14–18, San Diego, CA

Hu J, Vick BA (2003) TRAP (target region amplification polymorphism), a novel marker technique for plant genotyping. Plant Mol Biol Rep 21:289–294

Kong L, Ohm HW, Cambron SE, Williams CE (2005) Molecular mapping determines that Hessian fly resistant gene *H9* is located on chromosome 1A of wheat. Plant Breed 124:525–531

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander E, Abrahamson J, Barlow A, Daly M, Lincoln S, Newburg L, Green P (1987) Mapmaker: a computer package for constructing genetic-linkage maps. Cytogenet Cell Genet 46:642

Liu XM, Brown-Guedira GL, Hatchett JH, Owuoche JO, Chen MS (2005a) Genetic characterization and molecular mapping of a Hessian fly-resistance gene transferred from *T. turgidum* ssp. *dicoccum* to common wheat. Theor Appl Genet 111:1308–1315

Liu XM, Fritz AK, Reese JC, Wilde GE, Gill BS, Chen MS (2005b) *H9*, *H10*, and *H11* compose a cluster of Hessian fly-resistance genes in the distal gene-rich region of wheat chromosome 1AS. Theor Appl Genet 110:143–1480

Liu XM, Gill BS, Chen MS (2005c) Hessian fly resistance gene *H13* is mapped to a distal cluster of resistance genes in chromosome 6DS of wheat. Theor Appl Genet 111:243–249

Ma ZQ, Gill BS, Sorrells ME, Tanksley SD (1993) RFLP markers linked to Hessian fly-resistance genes in wheat (*Triticum aestivum* L.) from *Triticum tauschii* (Coss.) Schmal. Theor Appl Genet 85:750–754

Martin TJ, Harvey TL, Hatchett JH (1982) Registration of greenbug and Hessian fly resistance wheat germplasm. Crop Sci 22:1089

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers J, Appels R (2003) Catalogue of gene symbols for wheat. MacGene 2003 (http://www.grs.nig.ac.jp/wheat/komugi/genes/download.jsp, verified 1 Nov. 2005)

Ohm HW, Sharma HC, Patterson FL, Ratcliffe RH, Obanni M (1995) Linkage relationships among genes on wheat chromosome 5A that condition resistance to Hessian fly. Crop Sci 35:1603–1607

Pestsova E, Ganal MW, Roder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Ratcliffe RH, Cambron SE, Flanders KL, Bosque-Perez NA, Clement SL, Ohm HW (2000) Biotype composition of Hessian fly (Diptera: Cecidomyiidae) populations from the southeastern, mid-western, and northwestern United States and virulence to resistance genes in wheat. J Econ Entomol 94:1319–1328

Ratcliffe RH, Safranski GG, Patterson FL, Ohm HW, Taylor PL (1994) Biotype status of Hessian fly (Diptera: Cecidomyiidae) populations from the Eastern United States and their response to 14 Hessian fly resistance genes. J Econ Entomol 87:1113–1121

Ratcliffe RH, Hatchett JH (1997) Biology and genetics of the Hessian fly and resistance in wheat. In: Bondari K (eds) New developments in entomology. Research Signpost Publ., Scientific Information Guild, Trivandrum, India pp 47–56

Raupp WJ, Amri A, Hatchett JH, Gill BS, Wilson DL, Cox TS (1993) Chromosomal location of Hessian fly resistance genes *H22*, *H23*, and *H24* derived from *Triticum tauschii* in the D genome of wheat. J Hered 84:142–145

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Sardesai N, Nemacheck JA, Subramanyam S, Williams CE (2005) Identification and mapping of *H32*, a new wheat gene conferring resistance to Hessian fly. Theor Appl Genet 111:1167–1173

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Sourdille P, Cadalen T, Guyomarc'h H, Snape JW, Perretant MR, Charmet G, Boeuf C, Bernard S, Bernard M (2003) An update of the Courtot-Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor Appl Genet 106:530–538

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi LL, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genomics 4:12–25

Stebbins NB, Patterson FL, Gallun RL (1982) Interrelationships among wheat genes *H3*, *H6*, *H9*, and *H10* for Hessian fly resistance. Crop Sci 22:1029–1032

Xu SS, Cai X, Wang T, Harris MO, Friesen,TL (2006) Registration of two synthetic hexaploid wheat germplasms resistant to Hessian Fly. Crop Sci (in press)

Williams CE, Collier CC, Sardesai N, Ohm HW, Cambron SE (2003) Phenotypic assessment and mapped markers for *H31*, a new wheat gene conferring resistance to Hessian fly (Diptera: Cecidomyiidae). Theor Appl Genet 107:1516–1523

Wilson DL, Gill BS, Raupp WJ, Hatchett JH (1989) Chromosomal mapping of three new Hessian fly resistance genes in common wheat, derived from *Aegilops squarrosa*. Agron Abstr ASA. Madison, WI p 106