Theor Appl Genet (2003) 107:1516–1523
DOI 10.1007/s00122-003-1393-y

ORIGINAL PAPER

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Phenotypic assessment and mapped markers for *H31*, a new wheat gene conferring resistance to Hessian fly (Diptera: Cecidomyiidae)

Received: 6 January 2003 / Accepted: 30 June 2003 / Published online: 20 August 2003

Abstract A new source of resistance to the highly virulent and widespread biotype L of the Hessian fly, *Mayetiola destructor* (Say), was identified in an accession of tetraploid durum wheat, *Triticum turgidum* Desf., and was introgressed into hexaploid common wheat, *Triticum aestivum* L. Genetic analysis and deletion mapping revealed that the common wheat line contained a single locus for resistance, *H31*, residing at the terminus of chromosome 5BS. *H31* is the first Hessian fly-resistance gene to be placed on 5BS, making it unique from all previously reported sources of resistance. AFLP analysis identified two markers linked to the resistance locus. These markers were converted to highly specific sequence-tagged site markers. The markers are being applied to the development of cultivars carrying multiple genes for resistance to Hessian fly biotype L in order to test gene pyramiding as a strategy for extending the durability of deployed resistance.

Keywords Gene pyramiding · Deletion mapping · Sequence-tagged site · *H31* · Chromosome 5BS

Communicated by J. Dvorak

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Introduction

Host-plant resistance has proven to be highly effective in protecting wheat from devastation by the Hessian fly. The use of resistant cultivars in the U.S. can effectively maintain the loss of wheat yield due to Hessian fly damage at about 1% (Maxwell et al. 1972). By comparison, wheat-growing regions in Morocco, which lacked resistant cultivars until recently, averaged an estimated 36% crop loss due to the insect (ICARDA News 1999, http://www.icarda.cgiar.org/News/OldNews/2Aug99.htm). Similarly, breakdown of host-plant resistance can have a substantial economic impact in the US; losses in the state of Georgia alone exceeded $28 million dollars in a single year, due to Hessian fly damage (Hudson et al. 1988, 1991).

Currently, 30 genes conferring resistance to the Hessian fly (genes; *H1–H30*) have been identified in wheat and its wild relatives (Delibes et al. 1997; Ratcliffe and Hatchett 1997; Cebert 1998). Of these named resistance genes, only *H1–H3, H5–H8, H13, H18, H21* and *H25* have been deployed in commercial cultivars. Although an occasional cultivar has exhibited multigenic resistance, in general, Hessian fly-resistance genes have been deployed individually. This strategy has provided protection for the crop for at least five decades. However the use, over a large acreage, of wheat containing a single dominant resistance gene results in strong selection that favors biotype development (Gallun 1977). Consequently, many Hessian fly resistance genes are no longer effective after 8 to 10 years of deployment (Patterson et al. 1990). The result of this type of selection was clearly demonstrated by the change in predominant biotype frequency that occurred in Indiana during the 13 years following the 1955 introduction of the *H3*-containing cultivar 'Dual' (Hatchett and Gallun 1968). Before the introduction of the *H3* resistance gene, Hessian fly biotype A (avirulent to the *H3* resistance gene) was the most prevalent (Gallun et al. 1961). By 1968, five *H3*-containing wheat cultivars were the most common types grown in eight Indiana counties. By that time biotype A was predominant only in

fields of wheat lacking the *H3* resistance gene, and the *H3*-virulent biotype B had become the most abundant biotype in Indiana fields of wheat cultivars with *H3* (Hatchett and Gallun 1968).

Surveys of Hessian fly populations throughout the soft winter-wheat regions of the Eastern U.S. revealed that biotype L, virulent to deployed resistance genes *H3, H5, H6* and *H7/8*, is widespread and predominant in Alabama, Arkansas, Georgia, Illinois, Indiana, Mississippi, North Carolina and Virginia (Ratcliffe et al. 1997, 2000). In addition, pockets of biotype L have been identified as far away as Idaho and Washington. However, the biotype composition of most populations is a mixture of genotypes resulting from the segregation of virulence and avirulence alleles at only a few loci. Thus, segregation for virulence to wheat resistance genes *H3, H5, H6* and *H7/8*, which involves only four Hessian fly genes, results in up to 16 biotypes in a population, depending on allelic frequency. The emergence of a new Hessian fly biotype is generally a response to widespread use of a new resistance gene. However, individuals carrying virulence to undeployed genes (*H9–H15* and *H19*) are present in low abundance and are expected to become more prevalent if exposed to heavy selection pressure (Ratcliffe et al. 1994, 1996). For this reason, it is important to carefully manage the use of future resistance genes to maximize their utility and longevity.

Gould (1986) predicted that a pyramided cultivar containing multiple genes for resistance to a single biotype of the Hessian fly could increase field resistance for up to 20-times longer (over 400 fly generations) than single-gene cultivars. In addition to predicted gains in durable resistance, pyramiding has been shown to increase the breadth of resistance. Researchers combining bacterial blight resistance genes *Xa-4* and *xa-13* in rice, found that the pyramided lines gained resistance to an additional race of the blight-causing pathogen, *Xanthomonas oryzae* pv *oryzae*, against which neither gene individually was effective (Huang et al. 1997). The phenotypes of plants containing either a single resistance gene or a pyramid of genes conferring resistance to biotype L are indistinguishable since expression of one gene effectively masks the presence of additional genes. Efficient pyramiding can be achieved only by employing molecular markers that confirm the presence of each resistance gene. Before the advent of molecular-marker technologies, the predicted gains from a line with multiple Hessian fly resistance genes were not high enough to justify the added time and labor of verification through genetic analysis (Foster et al. 1991).

The objectives of this research were to characterize the phenotypic expression of a new source of resistance to Hessian fly biotype L, to design molecular markers for use in constructing a highly resistant cultivar and to determine the genomic location of the gene. The gene described here, *H31*, confers a resistance phenotype with minimal variation due to the environment. It is being incorporated into a resistance gene-pyramid along with other genes that confer resistance to Hessian fly biotype L.

Materials and methods

Hessian fly stocks

Hessian fly biotype L (virulent to resistance genes *H3*, *H5*, *H6* and the *H7H8* combination) is maintained by the USDA-ARS Crop Production and Pest Control Research Unit, Purdue University, in a 4°C cold room as a purified laboratory stock. Each year, the fly population is increased (Cartwright and LaHue 1944) on 'Magnum' (*H5*) wheat, which is resistant to all other biotypes maintained in the laboratory. During all experiments utilizing biotype L, four differential cultivars are included to confirm the genotype of the stock.

Construction of resistant wheat line P921696

An accession of *Triticum turgidum* Desf. (durum wheat, 2n=4*x*=28), CI3984, was determined to likely have three genes that are effective against biotype L of the Hessian fly (Cambron et al. 1995). This accession was obtained from the USDA-ARS National Plant Germplasm Collection (Aberdeen, Idaho). Infestation of CI3984 resulted in plants that were unstunted and harbored dead first-instar larvae. One of the resistance genes of CI3984 was transferred to common wheat (*Triticum aestivum* L., 2n=6*x*=42), resulting in the line P921696A1-15-2-1. Parentage of P921696A1-15-2-1 is: 'Cardinal' *3//'Knox'/D6647/CI3984. This crossing scheme was followed by six generations of self-pollination with progeny testing to verify resistance to biotype L of individuals in the F_2, F_4 and F_6 generations, in the pedigree breeding method. The resulting germplasm line, P921696A1-15-2-1-6 (referred to as P921696 for simplicity), was homozygous for resistance to Hessian fly biotype L.

Hessian fly bioassay

Hessian fly bioassays were conducted in the manner described by Maas et al. (1987). Seedlings were grown in flats (flat dimensions were 54×36×8 cm with each row containing 30 seeds from one head—nine test rows per flat) to the single-leaf stage in growth chambers that were maintained at 18°C with 14 h light. To determine the resistance genotype of each individual plant, its progeny were tested. In order to verify that the Hessian fly stock used in the bioassay was biotype L (virulent to *H3, H5, H6* and *H7H8*), each flat also contained one half-row of each of the four differential cultivars, Monon (CI13278; homozygous for resistance gene *H3*), Magnum (PI477285; *H5*), Caldwell (CI17897; *H6*) and Seneca (CI12529; *H7* and *H8*), plus one half-row of the homozygous resistant (P921696) and the homozygous susceptible (Cardinal) parental lines. One flat of plant material (Magnum *H5*), with several thousand emerging adult biotype L Hessian flies of both sexes, was placed under cheesecloth tents with groups of nine test flats. Mating and oviposition proceeded for 24 h before all adult flies were removed. Three weeks after infestation, the plants were classified as resistant, susceptible or escapes (uninfested) and the level of infestation was estimated (the average was about ten larvae per seedling, data not shown). Susceptible plants appeared stunted with dark green leaves inhabited by living white larvae, whereas resistant plants appeared normal in height with dead first-instar red larvae present. Any unstunted experimental plants or susceptible control plants were dissected, and dead first-instar red larvae were noted. Thus, resistant plants could be distinguished from uninfested escapes.

Progeny testing

Throughout this paper, the P921696 inbred line will be referred to as the resistant parental line. Populations for genetic analysis that were constructed through crosses of the resistant parental line with susceptible parental lines (F_2 or BC_1) will be referred to as test plants. All Hessian fly-resistance screens were conducted as progeny tests. Thus, approximately 30 progeny of each test plant were scored for resistance (as described above) in order to determine the resistance genotype of that test plant. Progeny testing results in more reliable phenotypes than if the test plants were scored directly. The evaluation of just 18 progeny plants reduces to less than 1% probability of misclassifying the genotype of the parental test plant (Allard 1956). In cases where the progeny of a test plant included both phenotypically resistant and susceptible individuals, the unstunted plants were dissected to distinguish uninfested escapes from resistant plants by verifying the presence of dead first-instar larvae. The presence of at least one verified resistant progeny (unstunted with dead red first-instar larvae present) among susceptible siblings resulted in classification of the parental test plant as a heterozygous resistant plant. The resistance-phenotype data and marker-segregation data from the test plants were used to define the phenotype of *H31* and to construct genetic maps linking DNA markers to the resistance gene.

Backcrosses to evaluate the number of Hessian fly-resistance loci

The resistant parental line, P921696, was crossed to the susceptible cultivar Cardinal, and the resulting F_1 plants were backcrossed once to Cardinal to produce a BC_1 test plant population of 90 individuals. Each BC_1 test plant was self-pollinated and approximately 30 seedlings from each BC_1 F_2 family were tested in a Hessian fly bioassay. The expected ratio of BC_1 test plants was one resistant to one susceptible, given that the P921696 resistant parental line carried a single gene for resistance.

Backcrosses to compare the degree of dominance to other resistant lines

The percentage of phenotypically resistant individuals was calculated, based on data from the Cardinal*2/P921696 BC_1 F_2 families that were derived from heterozygous BC_1 test plants. The expectation was that a fully dominant single resistance gene would segregate to yield a progeny generation with a ratio of 75% resistant to 25% susceptible plants, whereas a recessive resistance gene would result in only 25% of the progeny being resistant.

The P921696 F_2 population for AFLP marker identification

An F_2 population of 88 individuals was derived from a cross between the P921696 resistant parental line and the Hessian fly susceptible-cultivar 'Ning 7840' (an accession from China; H.W.O., unpublished). F_3 families containing approximately 30 seedlings were scored for resistance to Hessian fly biotype L to determine the resistance genotype of each F_2 plant. DNA from these F_2 plants was used in AFLP mapping experiments.

DNA isolation

DNA was isolated by the method of Saghai-Maroof et al. (1984) as modified by Osborn et al. (1987). Between 0.5 and 2 g of frozen leaves from single 2-week-old seedlings were ground to a fine powder in the presence of liquid nitrogen and 1 g of 20–30 mesh Ottawa sand (Fisher, Pittsburgh, PA). DNA was dissolved in TE (10 mM Tris-HCl pH 7.5 and 0.1 mM EDTA) and quantified by fluorometry.

AFLP analysis

The Gibco BRL AFLP Analysis System I (Gibco BRL Life Technologies, Gaithersburg, MD) was used with Promega *Taq* Polymerase and 10 x PCR buffer (Promega, Madison, WI) with $MgCl_2$ concentrations between 1.5 and 3 mM and [^{33}P]dCTP. AFLP samples (2–3 μl) were then loaded on a 6% denaturing gel [acrylamide/bisacrylamide 19:1, 8 M urea with 1 × TBE (100 mM Tris, 100 mM boric acid, 2 mM EDTA)]. Electrophoresis was performed at 60 W constant power for approximately 2.5 h. AFLP gels were exposed to Kodak Biomax MR film (Kodak Corporation, Rochester, NY).

AFLP markers linked to *H31* were identified by bulked-segregant analysis (BSA; Michelmore et al. 1991). Resistant and susceptible bulks each contained DNA pooled from ten F_2 plants from the segregating P921696/Ning 7840 population. All bands that were considered candidates for linkage to *H31* were polymorphic between the bulks as well as between the two parental lines. Linkage between markers and the *H31* resistance locus was estimated in the F_2 population by MAPMAKER V2.0 for Macintosh (Lander et al. 1987) using a LOD of 3.00 and the Haldane function (Haldane 1919).

Sequence-specific PCR primers for two *H31*-linked markers

AFLP bands were excised and the DNA isolated by crushing the gel in 30 μl of TE and heating for 10 min at 70°C followed by a 5 s centrifugation. A 5 μl aliquot of this mixture was reamplified by the pre-selective AFLP primers. The amplification products were then cloned into the pGEM-T Vector System I (Promega). The clones were sequenced using the Thermo Sequenase Primer Cycle Sequencing Kit 7-deaza-dGTP (Amersham Pharmacia Biotech, Piscataway, NJ) and a fluorescent-labeled primer (Integrated DNA Technologies, Coralville, Iowa). From the sequence of the cloned AFLP fragments sequence-tagged site (STS) primer pairs were designed with the program Primer3 (Rozen and Skaletsky 2000). STS primer names were derived from the AFLP primer combinations that generated the original markers, according to the primer designations at: http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html

STS-E35M47-Forward *5'- CCT TGA CAG CAT CTG TGT GC -3'*
STS-E35M47-Reverse *5'- GTT CTC GTT GGC AGG TCT CT -3'*
STS-E41M48-Forward *5'- TCC TAC CTC CAT TCC CCT TT -3'*
STS-E41M48-Reverse *5'- TCA AAA TGA ATC GGA AGG GT -3'*.

To confirm that the two STS markers map to the same locus as the original AFLP markers, the STS markers were amplified from the individual DNA samples that had been pooled to construct the resistant and susceptible DNA bulks used for BSA (DNA from ten individual plants constituted each bulk sample). PCR samples (50 μl) consisted of 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 150 μM of each nucleotide, 20 pmols of either the STS-E35M47 or the STS-E41M48 forward and reverse primers, 100 ng of template DNA and 1.25 units of *Taq* DNA polymerase (Promega). DNA was amplified as follows in a PTC-100 thermal cycler (MJ Research, Waltham, MA): 4 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C; ending with 7 min at 72°C.

Mapping the marker locus linked to *H31*

The chromosomal location designated as *Xupw4148* corresponded to STS marker E41M48. *Xupw4148*, which is linked to the resistance locus *H31*, was mapped using DNA from 114 of the recombinant inbred lines of the International Triticeae Mapping Initiative (ITMI) mapping population, Synthetic (W7984) × Opata 85 (Van Deynze et al. 1995). In order to construct the map, the E41M48 marker segregation data were combined with the segre-

gation data for the set of 1,409 markers that were downloaded from the GrainGenes website: http://www.graingenes.org/.

The map position of *Xupw4148* was determined by the program MAPMAKER 3.0 for PC (Lander et al. 1987) using a LOD of 5.00 and the Haldane mapping function (Haldane 1919).

Cloning the genomic region adjacent to the *Xupw4148* marker locus

In order to generate a fragment suitable for probing blots, the Universal GenomeWalker Kit (Clontech, Palo Alto, CA) was employed to amplify a genomic region adjacent to the locus corresponding to the AFLP marker E41M48. Genomic DNA of the P921696 line was restricted with *Pvu*II and the digested DNA was ligated to the GenomeWalker Adaptor. The adaptor-ligated DNA was used as a template for a primary PCR with an adaptor primer (AP1) and a marker-specific primer (GP1) designed from within the sequence of the AFLP marker. The amplified product was diluted and used as a template for a secondary PCR with a nested adaptor primer (AP2) and a nested marker-specific primer (GP2). The following marker-specific primers were designed to carry out the primary and secondary PCRs:

GP1: *5' GAGTCACGAGTTGGATCCTAATATCGTGG 3'*, and
GP2: *5' AGCCTCCCTCCACCCTTCCGATTCATTTT 3'*.

The major product of the secondary PCR was a 745-bp fragment that was cloned into the pCR4-TOPO vector using the TOPO-TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, CA). This clone is named UPW4148. The cloning reaction was used to transform Electromax DH10B cells (Invitrogen) by electroporation (Cell Porator Electroporation System I, Gibco BRL Life Technologies Inc). White colonies of transformed cells were selected for PCR analysis using the marker-specific primer GP2 and AP2 to verify the size of the plasmid insert. Plasmids were isolated from the transformed cells and the insert was sequenced using the ABI PRISM DYEnamic ET Terminator Cycle Sequence kit (Amersham Biosciences) on an ABI PRISM 3700 sequencer (Applied Biosystems, Foster City, CA) following the manufacturer's protocol.

An internal primer (IP) was designed from the distal end of the newly cloned region for use in PCR amplification along with the STS-E41M48-Forward primer. The sequence of the internal primer (IP) is as follows:

IP: *5' TTACACTTGAAGAGGTGTCCCA 3'*

The 710-bp PCR product was named E41M48-FLANK and used as a probe in Southern analysis in physical mapping experiments.

Physical mapping of *H31*

We used seven lines of wheat ('Chinese Spring' derivatives) with terminal chromosomal deletions in the short and long arms of group-5 chromosomes (Endo and Gill 1996) for physical mapping of *Xupw4148*, the marker locus linked to *H31*. The following terminal deletion lines were used: 5BS-4, -5, -6, -8, 5AS-3, -10 and 5BL-16.

Ten micrograms of DNA from each of the chromosome deletion lines as well as from Chinese Spring and the P921696 line were restricted with *Hin*dIII (New England Biolabs, Beverly, MA). The digested DNA samples were separated by electrophoresis on an 0.8% agarose gel in 0.5 × TAE (20 mM Tris-acetate, 0.5 mM EDTA). DNA was transferred from the gel to a Hybond-XL membrane (Amersham Pharmacia Biotech) according to the manufacturer's instructions. After the transfer was complete the membrane was neutralized in 2 × SSC (0.3 M NaCl, 30 mM sodium citrate) and UV-crosslinked (70,000 μJ/cm^2, UVC500, Hoefer, Amersham Pharmacia Biotech).

The membrane was pre-hybridized at 65°C in PerfectHyb Plus Hybridization Buffer (Sigma, St. Louis, MO) for 6 h. Twenty-five nanograms of the PCR product E41M48-FLANK DNA served as a template for random priming (Prime-It II Random Primer Labeling Kit, Stratagene, La Jolla, CA) using [^{32}P]dCTP. The probe was column-purified (QIAquick Nucleotide Removal Kit, Qiagen, Valencia, CA), denatured at 100°C for 10 min and allowed to hybridize to the membrane at 65°C for 22 h. The membrane was washed at 65°C for 20 min with 2 × SSC and 0.1% (w/v) SDS, followed by 10 min with 0.5 × SSC and 0.1% (w/v) SDS, and rinsed with 2 × SSC at room temperature. The membrane was exposed to an X-ray film (Kodak Biomax MR, Kodak Corp) for 3 days.

Results

Phenotype and number of Hessian fly-resistance loci in the P921696 line

All 90 of the P921696 parental plants exhibited resistance to biotype L, showed no stunting and harbored dead first-instar red larvae. A plant line is considered resistant, and of potential use in our breeding program, if at least 75% of the homozygous plants exhibit the resistant phenotype.

To determine the number of loci conferring resistance in the parental P921696 line, the segregation ratio of resistant to susceptible BC_1 test plants was calculated from phenotypic data of the Cardinal*2/P921696 BC_1 F_2 progeny. The expected ratio of resistant to susceptible BC_1 individuals, assuming one resistance locus, is one to one. Because BC_1 F_2 plants were scored to determine BC_1 genotypes, we expected a ratio of one susceptible BC_1 F_2 family for each BC_1 F_2 family that was segregating with both resistant and susceptible individuals. Data for P921696 were consistent with the single-locus hypothesis; 52.2% of the BC_1 F_2 families segregated for resistant and susceptible progeny ($\chi^2_{1:1}$=0.178, 1 *d*f, p=0.673).

Backcrosses to assess the degree of dominance of *H31*

Data from the subset of Cardinal*2/P921696 BC_1 F_2 families that were segregating for resistance provided an estimate of the degree of dominance of the *H31* resistance locus when heterozygous. A line containing a completely dominant, single resistance gene would yield segregating BC_1 F_2 families in which approximately 75% of the plants are resistant and 25% are homozygous susceptible. In the P921696 population, 47 segregating families (1,410 plants) were tested in a Hessian fly biotype L bioassay along with approximately 90 homozygous P921696 plants as experimental controls. The percentage of resistant individuals in the Cardinal*2/P921696 BC_1 F_2 segregating families was lower than predicted for a fully dominant gene, with 59% of the segregants being resistant rather than the expected 75% ($\chi^2_{3:1}$=275.95, 1 *d*f, p<0.001).

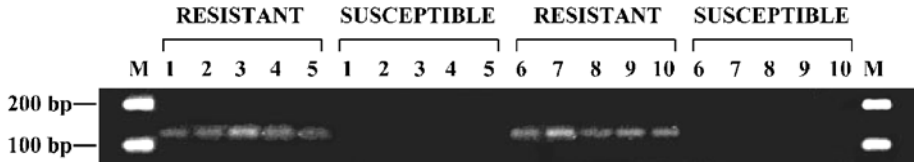

Fig. 1 Amplification of the *H31*-linked STS-E41M48 marker. The ethidium bromide-stained gel contains samples from ten resistant and ten susceptible individual F_2 plants (P921696 and Ning 7840 parental lines) that were used in BSA to identify the linked AFLP marker from which the STS was derived. *Lane M* is a 100 bp DNA molecular-weight ladder. *Figures* on the left represent the molecular weight in bp

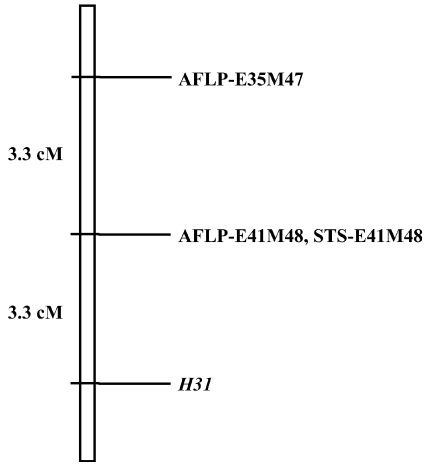

Fig. 2 Map of *H31* with linked marker loci. Loci were mapped in the F_2 population from the cross of P921696 to Ning 7840. E35M47 is an AFLP marker that maps in repulsion with the *H31* resistance locus. The AFLP-E41M48 marker and its derived STS-E41M48, map in coupling with the resistance locus. Genetic distances in cM are shown on the left, with the map being a total of 6.6 cM. Orientation of these loci with respect to the centromere is unknown

Identification of AFLP markers linked to the *H31* resistance locus in P921696

Sixty-four AFLP primer combinations produced approximately 5,700 selectively amplified DNA fragments. Although 54 polymorphisms were identified in the resistant and susceptible F_2 bulks as well as in the resistant P921696 and susceptible Ning 7840 parental lines, most appeared to be false positives, with only two of these fragments being linked to the resistance gene. Selective primer combination E41M48 (*Eco*RI+AGG/*Mse*I+CAC) amplified a 172-bp marker fragment that was linked in coupling to within 3.3 cM of the *H31* allele for Hessian fly resistance in the P921696/Ning 7840 F_2 population. The STS marker, STS-E41M48-Forward and -Reverse, that was derived from this AFLP sequence, was a dominant 128-bp fragment that amplified in samples from resistant plants only (Fig. 1). It mapped to the same location as its progenitor, the original E41M48 AFLP marker (Fig. 2). Primer combination E35M47 (*Eco*RI+ACA/*Mse*I+CAA) generated a 197-bp marker that was linked in repulsion to within 6.6 cM of the resistance locus (Fig. 2). The fragment generated by the STS-E35M47 primers was present in both resistant and susceptible samples, and as a result could not be mapped with respect to the *H31* resistance locus.

Mapping of the STS linked to *H31* using the ITMI population

The location of *H31* in the wheat genome was inferred by using the STS -E41M48 primers to map the *H31*-linked locus, *Xupw4148*, with respect to segregation data for 1,409 markers in the ITMI population. The amplification product from the ITMI population was a single band of the expected size and was present in samples of DNA from the Opata 85 parent but absent in samples from the Synthetic parent. Linkage analysis placed *Xupw4148* as the distal-most locus on the short arm of chromosome 5B at a distance of 9.8 cM from the nearest RFLP locus, *Xfbb277* (Fig. 3). The orientation of the segment from *Xupw4148* to the resistance gene *H31,* with respect to the rest of the chromosome, is not known. Although the exact position of the resistance gene could not be mapped with respect to the previously mapped markers, because *H31* is linked to the *Xupw4148* marker at a distance of only 3.3 cM, it too most likely lies near the end of chromosome 5BS.

Physical mapping of the marker linked to *H31* using deletion lines

Southern hybridization of the labeled amplicon, E41M48-FLANK, with DNA from the P921696 line, Chinese Spring and the chromosome 5AS and 5BL terminal-deletion lines, revealed a single band of approximately 0.8 kb corresponding to the presence of the *Xupw4148* locus associated with *H31* (Fig. 4). However, no hybridization was seen with DNA from the four 5BS terminal deletion lines, confirming that *Xupw4148* and, by association, the linked resistance locus *H31* were located near the end of the chromosome (Fig. 5).

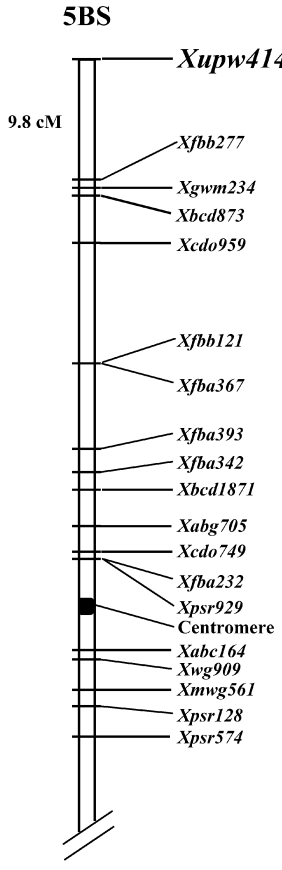

Fig. 3 Location of *Xupw4148* on chromosome 5BS. The linkage map shows RFLP loci (data from GrainGenes web site), *Xgwm*234 the closest microsatellite locus (Röder et al. 1998) and the *Xupw4148* marker locus that is associated with the *H31* resistance gene. All markers were mapped in the ITMI recombinant inbred line population derived from the cross Synthetic (W7984) × Opata 85. The *Xupw4148* locus maps 9.8 cM distal to the previous terminal marker locus, *Xfbb277*. The centromere is shown as a *black band*

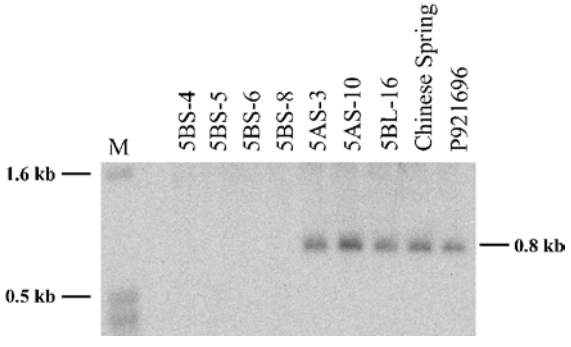

Fig. 4 Deletion mapping of the *H31*-linked marker to the terminus of chromosome 5BS. Genomic DNAs from P921696, Chinese Spring and the terminal deletion lines were digested with *Hin*dIII (does not cut within the probe sequence) and hybridized with the ^{32}P-labeled E41M48-FLANK probe. *Lane M* contains a 1-kb DNA molecular-weight ladder. *Figures* on the left and right denote molecular weight

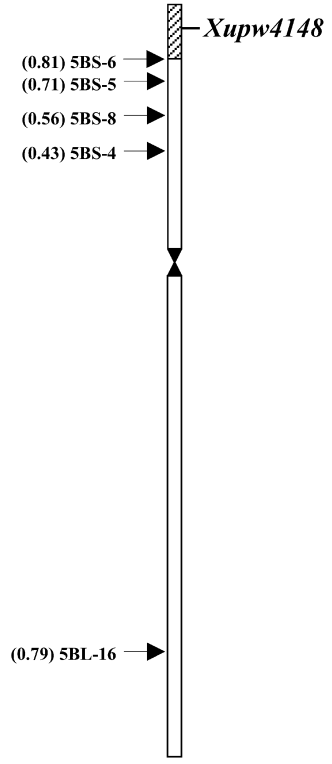

Fig. 5 Physical map of the *H31*-linked marker with respect to deletion break points. The fraction-lengths for break points of the Chinese Spring 5BS terminal deletion lines are shown on the left. The *terminal shaded area* corresponds to the region of chromosome 5BS containing the *H31*-linked *Xupw4148* marker locus. The centromere is shown as a constriction

Discussion

Level of resistance

The percentage of homozygous *H31* plants that were resistant (100%, 90 P921696 plants tested) was comparable to the resistance of wheat lines containing other recent Hessian fly-resistance genes when tested with the same laboratory stock of biotype L: *H9* (100.0% of the plants were resistant), *H10* (92.8%), *H13* (92.2%), *H14* (98.7%), *H16* (100.0%), *H17* (100.0%), *H18* (100.0%), *H19* (91.2%) (Ratcliffe et al. 1996) and *H20* (100.0%) (R.H. Ratcliffe, personal communication, and C.E.W., unpublished data). But unlike the temperature-sensitive resistance of *H10*, *H18* and *H20* (C.E.W. and H.W.O., unpublished data), *H31* resistance was stable under the standard test conditions (18°C with 14 h of light) and is expected to be robust under field conditions.

Other Hessian fly-resistance genes are characterized as dominant or partially dominant (El Bouhssini et al. 1999). Heterozygous resistant plants were challenged with the avirulent Great Plains biotype to determine the proportion that was phenotypically resistant (unstunted). In that study, 100% of the plants that were heterozygous for resistance genes *H7H8, H11*, *H13* and *H22* were unstunted. Between 90 and 99% of the plants that were

heterozygous for *H3*, *H5*, *H9* and *H10* were unstunted, and 64% of the *H6* heterozygotes were unstunted. But at least 50% of the unstunted heterozygotes with genes *H3*, *H5*, *H6*, *H7H8*, *H9*, *H10* and *H11* harbored living larvae.

The resistance of *H31* in the P921696 line of wheat is moderately dominant, with 59% of the plants in segregating families exhibiting the resistant, unstunted phenotype (75% expected if fully dominant, 25% expected if recessive). Although fully dominant resistance genes that allow no stunting or survival of larvae have been highly sought after for use in cultivars, *H13* is the only deployed gene that falls into this category (El Bouhssini et al. 1999). Moderately dominant resistance genes have proven useful as single-gene-releases since the 1955 release of *H3,* and are expected to also be effective in cultivars with pyramided resistance genes.

Hessian fly populations

Biotype L of the Hessian fly is now predominant in populations found in Indiana, Illinois, Missouri, Northern Arkansas and Alabama, Tennessee, Maryland, Virginia, North Carolina (Ratcliffe et al. 1996, 1997, 2000) and more recently in Delaware (R.H. Ratcliffe, personal communication). However, because the biotype L designation gives no information about virulence to genes that are newer than *H7H8*, new resistance genes must be tested against regional Hessian fly populations if they are to be effectively deployed. In addition to being resistant to the laboratory stock of biotype L, preliminary results indicate that the P921696 line containing *H31* is also resistant to wild Hessian fly populations collected in Spalding County, GA, Barnwell County, SC, Sussex County, DE and Wicomico County, MD (tested in 2000, personal communication by R.H. Ratcliffe). In contrast, some not yet deployed biotype L-resistant wheat lines containing genes *H12* and *H17* are susceptible to regional populations such as the Wicomico County MD population of Hessian fly.

Chromosomal location

The genomic location of the resistance gene could not be determined in the P921696 mapping population because previously mapped markers that resided outside the introgressed region originating from the durum donor were not polymorphic. However, the location of the resistance gene was inferred by determining the genomic location of the linked marker locus, *Xupw4148*, with the highly polymorphic ITMI mapping population. Because seed is freely distributed for these recombinant inbred lines, and the mapping data for 1,409 markers are available on the GrainGenes web site, the position of *Xupw4148* could be determined by adding our mapping data to the web-based data set and constructing a new map.

Both deletion-mapping and mapping with respect to the 1,409 markers in the ITMI population demonstrated that the marker *Xupw4148* and probably the associated resistance gene, *H31,* reside near the terminus of chromosome 5BS. *Xupw4148* is now the terminal-most marker on this chromosome arm. Due to the terminal position and small size of the introgressed polymorphic region containing *H31,* flanking markers were not identified in either our mapping efforts or by comparison to markers used with the ITMI mapping population. *H31* is the first gene, conferring resistance to the Hessian fly, to be mapped to chromosome 5BS. Six other Hessian fly-resistance genes originating from *T. turgidum* reside on chromosome 5A, including *H9, H10, H12, H15, H16* and *H17* (Ohm et al. 1995). In addition, *H3* and *H6* are also on chromosome 5A. In order for the process of constructing a gene pyramid to be successful, candidate genes cannot be allelic or tightly linked. Currently, the *H31* resistance gene from line P921696 is being combined in a pyramid with resistance genes *H9* and *H13*, which reside on chromosomes 5AL (Patterson and Gallun 1977; Stebbins et al. 1982) and 6DL respectively (Gill et al. 1987).

Molecular markers are already available for *H9* and *H13* (Dweikat et al. 1997). These markers plus the marker for the *H31* resistance gene are being used in the construction of breeding lines containing all three resistance loci. The marker-assisted selection will prevent inadvertent unstacking of the three resistance loci during cultivar development.

Acknowledgements Valuable discussions with K.M. Devos, R.H. Ratcliffe and G.J. Hunt are gratefully acknowledged. We thank F.B. Maas III for assistance in construction of mapping populations and scoring of resistance phenotypes. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA. This is a joint contribution by the USDA Agricultural Research Service and Purdue University. The work was supported by funding from USDA CRIS number 3602-2200-010-00D. Purdue University Agricultural Experiment Station Journal Article No. 16993.

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