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# Hessian fly resistance gene *H13* is mapped to a distal cluster of resistance genes in chromosome 6DS of wheat

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**Abstract** *H13* is inherited as a major dominant resistance gene in wheat. It was previously mapped to chromosome 6DL and expresses a high level of antibiosis against Hessian fly (Hf) [*Mayetiola destructor* (Say)] larvae. The objective of this study was to identify tightly linked molecular markers for marker-assisted selection in wheat breeding and as a starting point toward the map-based cloning of *H13*. Fifty-two chromosome 6D-specific microsatellite (simple sequence repeat) markers were tested for linkage to *H13* using near-isogenic lines Molly (PI 562619) and Newton-207, and a segregating population consisting of 192 $F_{2:3}$ families derived from the cross PI 372129 (*Dn4*) × Molly (*H13*). Marker *Xcfd132* co-segregated with *H13*, and several other markers were tightly linked to *H13* in the distal region of wheat chromosome 6DS. Deletion analysis assigned *H13* to a small region closely proximal to the breakpoint of del6DS-6 (FL 0.99). Further evaluation and comparison of the *H13*-linked markers revealed that the same chromosome region may also contain *H23* in KS89WGRC03, an unnamed *H* gene ($H_{WGRC4}$) in KS89WGRC04, the wheat curl mite resistance gene *Cmc4*, and a defense response gene *Ppo* for polyphenol oxidase. Thus, these genes comprise a cluster of arthropod resistance genes. Marker analysis also revealed that a very small intercalary chromosomal segment carrying *H13* was transferred from the *H13* donor parent to the wheat line Molly.

**Keywords** Wheat · Hessian fly · Resistance gene · *H13* · Gene mapping · Marker

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## Introduction

Historically, the use of resistance genes in wheat has been the most effective, cost-efficient, and environmental-friendly approach to control infestations of Hessian fly (Hf), *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), one of the most destructive pests of wheat (*Triticum aestivum* L.) worldwide (Cox and Hatchett 1994; Ratcliffe and Hatchett 1997). So far, over 30 major Hf resistance genes have been identified (Ratcliffe and Hatchett 1997; McIntosh et al. 2003; Williams et al. 2003; Liu et al. 2002). Among these genes, *H13*, *H22*, *H23*, *H24*, and *H26* originated from *Aegilops tauschii* Coss. [syn. *Ae. squarrosa* L.; *T. tauschii* (Coss.) Schmal.], the diploid progenitor, and donor of the D genome of common wheat, and an important source for pest resistance (Hatchett and Gill 1981; Gill and Raupp 1987; Ratcliffe and Hatchett 1997). *H13* is a dominant resistance gene expressing a very high and stable level of antibiosis against a wide range of Hf biotypes and geographic populations (Hatchett et al. 1981; Gill et al. 1987; Ratcliffe and Hatchett 1997; El Bouhssini et al. 1999). The *H13* resistance was traced to KU2076 (TA2452) of a synthetic hexaploid ($2n = 42$), KU221-19, formed from an interspecific cross between KU138, an accession of *T. persicum* L. var. *stramineum* Zhuk. (tetraploid, $2n = 28$) and KU2076, an accession of *Ae. tauschii* (diploid, $2n = 14$) (Tanaka 1961; Hatchett et al. 1981). Previously, *H13* was mapped to wheat chromosome 6D using monosomic analysis, and further mapped to 6DL, $35.0 \pm 8.0$ recombination units from the centromere by telocentric analysis (Gill et al. 1987). *H22*,

*H23*, *H24*, and *H26* are located on chromosomes 1D, 6D, 3D, and 4D, respectively (Raupp et al. 1993; Cox and Hatchett 1994).

Significant advances in molecular genetics and genomics recently have produced many molecular markers that are being employed increasingly to facilitate identification and mapping of resistance gene (Yencho et al. 2000; Sourdille et al. 2004), marker-assisted selection (MAS) in molecular breeding (Melchinger 1990; Gupta and Varshney 2000), and map-based cloning of resistance genes (Huang et al. 2003; Feuillet et al. 2003; Yahiaoui et al. 2004).

Marker-based mapping and MAS are much more accurate and effective than conventional cytological mapping and phenotype-based breeding. A variety of molecular markers has been identified to map Hf resistance genes in wheat. Ma et al. (1993) identified restriction fragment length polymorphism (RFLP) markers linked to the *H23* gene on chromosome 6DS and *H24* on 3DL. Dweikat et al. (1994, 1997, 2002) developed random amplified polymorphic DNA and sequence-tagged site (STS) markers associated with *H3*, *H5*, *H6*, *H9-H14*, *H16*, and *H17*. Williams et al. (2003) mapped *H31* on chromosome 5BS with AFLP and STS markers. Most recently, we identified several microsatellite [or simple sequence repeat (SSR)] markers that were tightly linked to a Hf resistance gene (temporarily named *Hdic*) on wheat chromosome 1AS (X.M. Liu et al., unpublished data; http://maswheat.ucdavis.edu/protocols/HF/index.htm). In general, SSR markers are very promising for genetic mapping as well as for MAS, because they are abundant, highly polymorphic, reliable, and chromosome locus specific (Rafalski and Tingey 1993; Röder et al. 1998; Pestsova et al. 2000).

The objectives of this study were to identify SSR markers linked to *H13* and determine its precise chromosome map position and the linkage relationships with other resistance genes in order to facilitate the efficient use in breeding programs and the positional cloning of *H13*.

## Materials and methods

### Hessian fly populations

The Hf biotype GP originated from a laboratory colony collected from Ellis County, Kansas (Gagne and Hatchett 1989). The insects were maintained on wheat seedlings of the Hf-susceptible cultivars (Karl 92 or Newton). A biotype L culture was supplied by S.E. Cambron, USDA–ARS, West Lafayette, Ind., USA. The insects were maintained on seedlings of cultivars Ike (*H3*), Magnum (*H5*), Caldwell (*H6*), and Seneca (*H7H8*) sequentially. The Hf pupae together with infested wheat plants were stored at 4°C until Hf adults were needed.

### Plant materials and DNA isolation

Molly (PI 562619) is an *H13*-containing wheat germplasm with the pedigree of Newton-207*7/3/KU221-19/Eagle//KS806 (Patterson et al. 1994) and is a near-isogenic line of Newton-207. KS89WGRC03 (PI535766) is an *H23*-containing wheat germplasm derived from the cross TA1642 × 2*Wichita (Gill et al. 1991a). KS89WGRC04 (PI535767) is a wheat germplasm containing an unnamed *H* gene ($H_{WGRC4}$) developed from the cross TA1695 × 3*Wichita (Gill et al. 1991b). To map the *H13* gene in Molly, a mapping population consisting of 192 $F_{2:3}$ families was developed from the cross PI 372129 × Molly. PI 372129 is an Hf-susceptible wheat germplasm containing *Dn4*, a gene conferring resistance to biotype A of Russian wheat aphid, *Diuraphis noxia* (Mordvilko).

Seeds of Molly, Newton-207, and PI 372129 were provided by Dr. H. Ohm, Department of Agronomy, Purdue University, Ind., USA, and the USDA–ARS National Small Grains Research Facility in Aberdeen, Idaho. Wichita, KS89WGRC03, KS89WGRC04, and *Ae. tauschii*-resistant sources TA2452 (KU2076, *H13*), TA1642 (*H23*), and TA1695 ($H_{WGRC4}$) were provided by the Wheat Genetics Resource Center (WGRC) at Manhattan, Kansas. Wheat genetic stocks used for marker localization including cultivar Chinese Spring (CS), CS nullisomic-tetrasomic (N6DT6B, Sears 1966), ditelosomic (Dt6DS, Dt6DL, Sears 1954), and deletion lines (del6DS-2, del6DS-4, and del6DS-6, Endo and Gill 1996), were also kindly provided by the WGRC. Wheat genomic DNA was extracted from leaf tissue of each $F_2$ plant according to the modified CTAB procedure as described by Gill et al. (1991c). The DNA concentration was quantified spectrophotometrically.

### Evaluation of Hf resistance

Parents, $F_1$ plants, some $F_2$ plants, and the $F_{2:3}$ population were evaluated for phenotypic reaction to Hf infestation as described previously (Hatchett et al. 1981; Maas et al. 1987) with modification. Briefly, approximately 15–20 seeds of each wheat line or family were planted in uniformly spaced rows (12 rows or 24 half-rows per flat) in flats (52 × 36 × 10 cm) containing a mixture (1:1) of soil and vermiculite in growth chambers at 18 ± 1°C with 14:10 h (light/dark) photoperiod. Seedlings at the one-leaf stage were infested by confining ~200 newly mated Hf females in each flat within a cheesecloth tent. Three weeks after infestation, the seedlings were examined to identify susceptible and resistant phenotypes. Susceptible plants were stunted with dark green leaves and harbored live larvae (or prepupae). Resistant plants grew normally (unstunted), with light green leaves, and contained dead larvae between the leaf sheaths. Chi-square ($\chi^2$) tests were conducted to determine the goodness of fit of plant segregation ratios to theoretical mendelian segregation ratios.

### Microsatellite (SSR) analysis

Molecular markers selected from wheat chromosome 6D were screened for linkage to *H13* by bulked segregant analysis (Michelmore et al. 1991) as well as by evaluation of the near-isogenic lines Molly and Newton-207. Two DNA bulks were assembled, using equal amounts of DNA from five homozygous resistant and five susceptible progeny lines, respectively. The DNA samples of resistant and susceptible near-isogenic lines, parents, and bulks were evaluated for polymorphisms with SSR primers. Once a primer pair amplified polymorphic fragments between two bulks and between near-isogenic lines, they were further used to determine the genetic linkage between the Hf resistance gene and the markers with the entire mapping population.

The sequences of SSR primers were obtained from the GrainGenes Database at http://wheat.pw.usda.gov/ggpages/ggtabledefs.html. Specific information on primers and PCR protocols with the prefixes listed herein can be found in the respective references—WMS (or GWM): Röder et al. 1998; PSP: Stephenson et al. 1998, Devos et al. 1995; GDM: Pestsova et al. 2000; BARC: R. Ward et al. (http://www.scabusa.org/pdfs/BARC_SSRs_011101.html); WMC: Gupta et al. 2002; and CFA and CFD: P. Sourdille (http://wheat.pw.usda.gov/ggpages/SSRclub/Sourdille/), Guyomarc'h et al. 2002; Sourdille et al. 2004. Because *H13* was known to be located on wheat chromosome 6D, a total of 52 SSR primer pairs specific to wheat chromosome 6D were evaluated for genetic linkage to *H13*.

The PCR amplification was performed in a volume of 25 µl, as described by Röder et al. (1998), with minor modifications. The reaction mixture contained 0.2 m *M* of each dNTPs, 1.8–2.0 m *M* $MgCl_2$, 1 U *Taq* DNA polymerase (Promega, Madison, Wis., USA), 1X Thermophilic DNA polymerase buffer (10 m *M* Tris-HCl, 50 m *M* KCl, and 0.1% Triton X-100), 0.4 µ*M* each of the forward and reverse primers, and 100 ng of template DNA. The PCR amplifications were performed in an MJ Research PTC-200 Thermal Cycler (Watertown, Mass., USA) programmed at: 94°C for 3 min, followed by 40 cycles of 94°C for 1 min, 50–60°C (based on primer's annealing temperature) for 1 min, and 72°C for 2 min, and then a final extension step at 72°C for 10 min before cooling to 4°C. Particularly, PCR annealing temperature for primers CFD132 and GDM136 is 55°C. The primer sequences (from 5′ to 3′) are CFD132F: CAAATGCTAATCCCCGCC, CFD132R: TGTAAACAAGGTCGCAGGTG; GDM36F: ATGCAAAGGAATGGATTCAA, and GDM36R: CAAATCCGCATCCAGAAAAT. PCR-amplified fragments were separated on 3% agarose gels (Sigma, St. Louis, Mo., USA) under electrophoresis at 5 V/cm in 1X TAE buffer. The DNA banding patterns were visualized under UV light with ethidium bromide staining.

### Genetic and physical mapping

A genetic linkage map was constructed by converting recombination frequencies to genetic map distance (centiMorgans) with the Kosambi mapping function (Kosambi 1944) using MAPMAKER software, version 3.0 (Lander et al. 1987), at LOD > 3.0.

To determine the physical location of the SSR markers, genomic DNA from euploids, aneuploids, and deletion lines of CS wheat were amplified using SSR primers of the *H13*-linked markers. The presence or absence of a specific fragment amplified from a deletion stock indicates that the corresponding marker is located proximal or distal to the breakpoint of the tested deletion stock. In this manner, the markers and linked genes were physically localized into chromosome interval regions (bins) within the chromosome arm.

## Results and discussion

### Inheritance and phenotypic expression of *H13*

Segregation ratio of $F_{2:3}$ families confirmed that Hf resistance in Molly is controlled by a single dominant gene (*H13*), which was consistent with the previous report of Hatchett et al. (1981). All the tested seedlings of the resistant parent Molly and $F_1$ plants derived from the cross PI 372129 × Molly exhibited complete and consistent resistance to both Hf biotypes GP and L. The observed segregation of 47 homozygous resistant, 87 segregating (heterozygous), and 58 homozygous susceptible $F_{2:3}$ families fit a one gene segregation ratio 1:2:1 ($\chi^2 = 2.948$, $df = 2$, $P = 0.235 > 0.05$).

### SSR markers linked to *H13*

Of the 52 pairs of 6D SSR primers tested, no markers with loci on the long arm of the chromosome detected polymorphisms between Molly and the susceptible near-isogenic line Newton, or between the resistant and susceptible bulks. On the other hand, the 6DS primers CFD132, GDM36, CFD213, CFD42, and GDM141 amplified DNA fragments (with the expected sizes similar to those from CS wheat) polymorphic between Molly and Newton-207 (Table 1). The primer pairs CFD132, GDM36, CFD42, GDM141, GDM132, CFD75, and BARC173 amplified DNA fragments polymorphic between Molly and PI 372129 as well as between the resistant and susceptible bulks. Evaluation of the entire $F_{2:3}$ mapping population indicated that these SSR markers from the short arm of chromosome 6D are linked to *H13*.

Marker *Xcfd132* co-segregated with *H13* in the population consisting of 192 $F_{2:3}$ families and is completely linked to Hf resistance. The CFD132 primer pair amplified a 160-bp DNA fragment from the resistant parent Molly and all the homogenous resistant

**Table 1** The DNA fragments (base pair size) amplified from wheat parents and related sources with microsatellite primers of *H13*-linked markers

| Primers | KU2076 (*H13*) | Newton-207 | Molly (*H13*) | PI372129 (*Dn4*) | WGRC3 (*H23*) | TA1642 (*H23*) | Wichita | TA1695 (*H*) | WGRC4 (*H*) |
|---|---|---|---|---|---|---|---|---|---|
| BARC173 | 235 | 235 | 235 | 250 | 210 | 210 | 235 | 220 | 235 |
| CFD75 | Null | 310 | 310 | Null | 310 | 310 | 305 | 300 | 300 |
| GDM132 | Null | 155 | 155 | Null | 160 | 160 | 150 | 140 | 140 |
| GDM141 | Null | Null | 135 | 130 | 130 | 130 | Null | 140 | 140 |
| | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 | 120 |
| CFD42 | Null | 190 | 180 | 170 | 190 | 190 | 180 | 190 | 190 |
| GDM36 | 170 | 130 | 170 | 130 | 130 | 130 | 130 | 170 | 170 |
| | 120 | 120 | 120 | Null | 120 | 120 | 120 | 120 | 120 |
| CFD132 | 160 | 120 | 160 | 120 | 170 | 170 | 110 | 160 | 160 |
| CFD213 | 230 | 230/190 | 230 | 230 | 230 | 230 | 230/190 | 190 | 230/190 |
| BARC54 | 170 | 170 | 170 | 170 | 160 | 170 | 160 | 190 | 190 |
| | 140 | Null | Null | Null | Null | 140 | Null | 160 | 160 |

$F_{2:3}$ families, and a 120-bp fragment DNA from the susceptible parent PI 372129 and susceptible progeny. Both the 160- and 120-bp fragments are present in the heterozygous (segregating) progeny (Fig. 1).

In addition to *Xcfd132*, four other co-dominant markers are linked to *H13* at various distances. Marker *Xgdm36* is located 2.7 cM distal to *H13* (Fig. 2). The GDM36 primer pair amplified a 170-bp DNA fragment associated with *H13* resistance (a 120-bp fragment was not related), and a 130-bp DNA fragment from the susceptible parent PI 372129 and susceptible progeny. Both the 170- and 130-bp fragments are present in the heterozygous progeny (Table 1). *Xcfd42* is linked to *H13* at a distance of 5.8 cM. The CFD42 primer pair amplified a 180-bp DNA fragment associated with the resistance in Molly (*H13*), and a 170-bp DNA fragment from the susceptible parents PI 372129 and susceptible progeny. Both the 180- and 170-bp fragments are present in the heterozygous progeny (Table 1). Markers *Xgdm141* and *Xbarc173* are located 6.0 cM and 15.3 cM, respectively, distal to *H13* in Molly. The GDM141 and BARC173 primer pairs detected polymorphism between Molly and PI 372129 as shown in Table 1.

In addition to the co-dominant markers described as above, *Xgdm132* and *Xcfd75* are dominant markers linked to *H13* in the coupling phase at the same distance of 14.4 cM (Table 1; Fig. 2). These two markers were completely linked in the mapping population of 192 $F_{2:3}$ families. A genetic linkage map (Fig. 2) of *H13* and the linked SSR markers was constructed using MAPMAKER (Lander et al. 1987).

To determine the physical locations of the *H13*-linked markers, DNA samples of CS, N6DT6B, Dt6DS, Dt6DL, del6DS-2 [fraction length (FL)0.45], del6DS-4 (FL0.79) and del6DS-6 (FL0.99) were amplified using primer pairs of each SSR markers. The deletion mapping results demonstrate that *Xbarc173*, *Xcfd75*, *Xgdm132*, *Xgdm141*, and *Xcfd42* are physically located distal to the breakpoint of 6DS-6 in the terminal 1% of the 6dS chromosome arm. The completely linked marker *Xcfd132* and the very tightly linked marker *Xgdm36* together with *Xcfd213* and *Xbarc54* are physically located in bin 6DS4-0.79-0.99. The physical intervals of *Xbarc173* and *Xgdm141* are consistent with those of Sourdille et al. (2004) and R. Ward et al. (http://www.graingenes.org/dbs_images/graingenes/ BARC_1A.jpg). The exact physical bins for the other linked markers were newly established in this study. Considering the very small genetic distance from *H13* to *Xcfd42*, *H13* is located proximal and very close to the breakpoint of 6DS-6 (FL0.99).

Although *Xcfd213* could not be mapped using our mapping population, it can be placed proximal to *Xcfd132* based on its physical location described as above and the fact that *Xcfd213* detected polymorphisms between Molly and Newton, WGRC3 and Wichita, and WGRC4 and TA1695 (Table 1). The *Xcfd213* is located proximal to *Xcfd132* at 0.55 cM (http://www.grs.nig.ac.jp/wheat/komugi/maps/markerMap.jsp?, Chromosome = 6) or 1.1 cM (http://wheat.pw.usda.gov/ggpages/SSRclub/Sourdille/18). Another SSR marker, *Xbarc54*, is located proximal to *Xcfd132* at a distance of 12 cM (Somers et al. 2004).

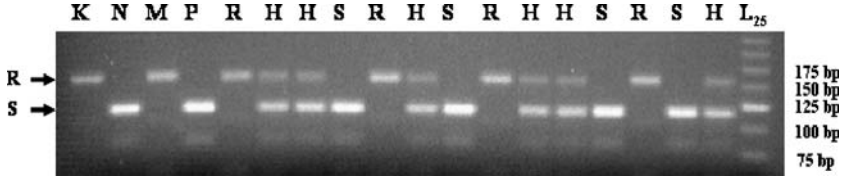


**Fig. 1** DNA fragments amplified with simple sequence repeat primer CFD132. The DNA samples were prepared from KU2076 (*K*), Newton-207 (*N*), Molly (*M*), PI372129 (*P*), and $F_2$ plants from the cross PI372129 × Molly. *R* Hessian fly (Hf)-resistant (homozygous), *S* Hf-susceptible, and *H* heterozygous progeny (segregating in $F_{2:3}$ families). $L_{25}$ 25-bp DNA ladder. *H13* is 100% co-segregating with *Xcfd132*

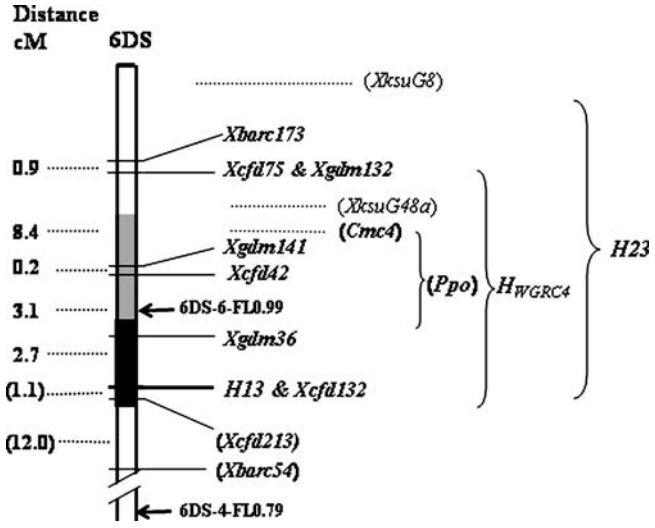


**Fig. 2** Linkage map of *H13* and an arthropod-resistant gene cluster on wheat chromosome 6DS. The *dark region* of the chromosome represents the *Aegilops tauschii* KU2076 (TA2452) segment containing *H13* in common wheat Molly (PI562619). The *light region* represents the Newton background. The *gray region* may be derived from cultivars either Eagle or KS806. Markers or genes in *parentheses* were cited for comparison. The breakpoints of the deletions are indicated with *arrows*

The present molecular mapping of *H13* on 6DS is consistent with the previous monosomic mapping result that *H13* is on chromosome 6D, but contrary to the previous ditelosomic arm mapping results that placed *H13* on 6DL arm (Gill et al. 1987). The original ditelosomic stocks of Dt6DS and Dt6DL must be correct, as they have been used in numerous mapping studies, and no anomalous results have been reported. Most probably, the previous crosses may have been mislabeled or there was an error in crossing as the identities of the diteosomics Dt6DL and Dt6DS was not verified independently.

## Size of the *Ae. tauschii* KU2076-derived chromosomal segment in common wheat Molly

The origin of the chromosomal segments carrying *H13* in Molly can be tracked by comparing the marker polymorphisms among the donor line KU2076 (or KU-221-19), the recipient line Newton-207, and Molly. According to the marker polymorphisms (Table 1) and marker order (Fig. 2), a very small intercalary chromosomal segment (proximal to the breakpoint of del6DS-6-FL0.99), carrying *H13* along with *Xgdm36*, *Xcfd132*, and *Xcfd213*, was introgressed from the original *H13* source KU2076 (dark region, Fig. 2). A small intercalary chromosomal fragment (around the breakpoint of del6DS-6-FL0.99) carrying *Xgdm41* and *Xcfd42* loci might derive from either cultivars Eagle or KS806 (gray region, Fig. 2). The chromosomal region distal to and including *Xgdm132* and the region proximal to and including *Xbarc54* derived from the recipient (recurrent) parent Newton (light region, Fig. 2).

## A cluster of arthropod resistance genes in the distal region of 6DS

The linked markers identified in this study facilitate the exact mapping of *H13* gene on the distal gene-rich region of wheat chromosome arm 6DS instead of 6DL, and provide new information about the genetic relationship between *H13* and other resistance genes on wheat chromosome 6DS that will facilitate their rational use.

Previous genetic analysis indicated *H23* and *H13* were linked at a distance of $25 \pm 5.0$ map units (Raupp et al. 1993). Ma et al. (1993) used RFLP markers to map *H23* 15.6 cM distal to *XksuG48a* on chromosome 6DS. Pestsova et al. (2000) located *XksuG48a* between markers *Xgdm132* and *Xgdm141*. The unnamed gene in KS89WGRC04 (here as referred to as $H_{WGRC4}$) is either tightly linked or allelic to *H23*, because no recombination or segregation was observed between these two genes (S. Singh, personal communication). In addition, Li et al. (1999) mapped a defense response gene *Ppo* for polyphenol oxidase, with close linkage and proximal to *XksuG48a*. Malik et al. (2003) mapped the wheat curl mite, *Aceria tosichella* Keifer, resistance gene *Cmc4* between *XksuG48a* and *Xgdm141*.

To confirm the potential relationships among *H13*, *H23*, and $H_{WGRC4}$, *H13*-linked markers were used to detect *H23* in KS89WGRC03, which derived from the cross TA1642 × 2*Wichita, and $H_{WGRC4}$ in KS89WGRC04 with pedigree TA1695/3*Wichita. Microsatellite polymorphism detected among KS89WGRC03, TA1642 (*Ae. tauschii*, *H23* donor) and Wichita (Table 1) indicated that a small terminal chromosomal segment distal to *Xbarc54* was derived from TA1642 and may carry *H23* in KS89WGRC03 (Fig. 2). Polymorphism among KS89WGRC04, TA1695 (*Ae. tauschii*, $H_{WGRC4}$ donor), and Wichita (Table 1) also revealed that a small intercalary chromosomal segment between *Xcfd75* and *Xbarc54* was derived from TA1695 and may carry $H_{WGRC4}$ in KS89WGRC04 (Fig. 2). Further investigation is needed to clarify whether *H13*, *H23*, and $H_{WGRC4}$ are allelic.

Our present results and those of others indicated that *H13*, *H23*, $H_{WGRC4}$, *Cmc4*, and a *Ppo* defense response gene are clustered in a small distal region on the short arm of chromosome 6D. This gene cluster in wheat may provide useful clues that demonstrate common characteristics of their evolution to those of disease resistance genes. Race-specific disease resistance genes in plants are commonly clustered in a linked array, and most likely evolved from the same progenitor resistance gene through duplication and diversification (Richter and Ronald 2000). Polyphenol oxidases play a defensive role in plant–pest interactions, and the *Ppo* genes possess both constitutive and inducible defensive modes. The

*Ppo* gene is currently the only defense response gene that has been mapped to the distal region of wheat chromosome arm 6DS. The close proximity of *H13*, and several other insect-resistance genes, to *Ppo*, suggests the possible involvement of polyphenol oxidase in insect resistance or the possibility of allelic relationship between the *H* and *Ppo* genes.

## The potential use of *H13*-linked markers

Rapid breeding and deployment of Hf-resistant wheat cultivars are critically needed to control Hf for the sustaining wheat production. Conventional plant breeding depends upon phenotypic selection for Hf resistance through bioassays or selection based on morphological or agronomic traits. All of these practices are labor-intensive, time-consuming, and sometimes inconclusive. In contrast, molecular breeding employs molecular markers linked to resistance genes for MAS, through which the accurate detection of specific resistance genes and efficient selection of desirable resistant genotypes can be achieved (Melchinger 1990; Yencho et al. 2000). As MAS is unaffected by environmental conditions or plant developmental stages, it can facilitate the selection of target genes or favorable genotypes at the seedling stage in early segregating generations. It is also practicable to use molecular markers to select against unwanted chromosomal segments, thus reducing linkage drag and accelerating cultivar development.

*H13* expresses a very high level of antibiosis against a wide range of Hf biotypes, including biotype L, the most virulent population known (Ratcliffe and Hatchett 1997; Ratcliffe et al. 2000). Linked markers identified in this study will be very useful in wheat breeding programs for MAS and gene pyramiding. The completely linked marker *Xcfd132* and several tightly linked markers can be used to tag and track *H13*, and to select accurately for *H13*. We used the *H13*-linked markers and the previously identified *Dn4*-linked markers (Liu et al. 2002), selected 15 $F_{2:3}$ families homozygous for both Hf resistance and Russian wheat aphid resistance among 192 families of a mapping population. Work is currently in progress to pyramid these and other resistance genes using MAS.

Results in this study revealed that *H13* is located in a distal gene-rich region (which has a potentially high rate of recombination) on wheat chromosome arm 6DS. The absence of recombination between *H13* and the co-segregating marker *Xcfd132* in a highly recombinagenic telomeric region (Sourdille et al. 2004) suggests that their physical distance should be small enough within a feasible range to allow a map-based cloning approach to isolate *H13*.

In summary, *H13* is completely or tightly linked to several SSR markers and is located in a cluster of arthropod resistance and defense response genes in the distal region of wheat chromosome 6DS. These linked markers are not only very useful for molecular breeding (MAS) and molecular mapping, but may also provide a good opportunity for the map-based cloning of *H13*.

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## References

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

Devos KM, Bryan GJ, Collins AJ, Stephenson P, Gale MD (1995) Application of two microsatellite sequences in wheat storage proteins as molecular markers. Theor Appl Genet 90:247–252

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

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

El Bouhssini M, Hatchett JH, Wilde GE (1999) Hessian fly (Diptera: Cecidomyiidae) larval survival as affected by wheat resistance alleles, temperature and larval density. J Agric Urban Entomol 16:245–254

Endo TR, Gill BS (1996) The deletion stocks of common wheat. J Hered 87:295–307

Feuillet C, Travella S, Stein N, Albar L, Nublat A, Keller B (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. Proc Natl Acad Sci USA 100:15253–15258

Gagne RJ, Hatchett JH (1989) Instars of the Hessian fly (Diptera: Cecidomyiidae). Ann Entomol Soc Am 82:73–79

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

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

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

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TS (1991c) A genetic linkage map of *Triticum tauschii* (DD) and its relation to the D genome of bread wheat (AABBDD). Genome 34:362–374

Gupta PK, Balyan HS, Edwards KJ, Isaac P, Korzun V, Röder 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

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113:163–185

Guyomarc'h H, Sourdille P, Charmet G, Edwards KJ, Bernard M (2002) Characterization of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D-genome of bread wheat. Theor Appl Genet 104:1164–1172

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 hexaploid wheats derived from *Triticum tauschii* (Coss.) Schmal. Crop Sci 21:731–734

Huang L, Brooks SA, Li W, Fellers JP, Trick HN, Gill BS (2003) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics 164:655–664

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

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Li WL, Faris JD, Chittoor JM, Leach JE, Hulbert SH, Liu DJ, Chen PD, Gill BS (1999) Genomic mapping of defense response genes in wheat. Theor Appl Genet 98:226–233

Liu XM, Smith CM, Gill BS (2002) Identification of microsatellite markers linked to Russian wheat aphid resistance genes *Dn4* and *Dn6*. Theor Appl Genet 104:1042–1048

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

Maas FB III, Patterson FL, Foster JE, Hatchett JH (1987) Expression and inheritance of resistance of 'Marquillo' wheat to Hessian fly biotype D. Crop Sci 27:49–52

Malik R, Brown-Guedira GL, Smith CM, Harvey TL, Gill BS (2003) Genetic mapping of wheat curl mite resistance genes *Cmc3* and *Cmc4* in common wheat. Crop Sci 43:644–650

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)

Melchinger AE (1990) Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed 104:1–19

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Patterson FL, Mass III FB, Foster JE, Ratcliffe RH, Cambron S, Safranski G, Taylor PL, Ohm HW (1994) Registration of eight Hessian fly resistant common winter wheat germplasm lines (Carol, Erin, Flynn, Iris, Joy, Karen, Lola, and Molly). Crop Sci. 34:315–316

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

Rafalski JA, Tingey SV (1993) Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. Trends Genet 9:275–280

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

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

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

Richter TE, Ronald PC (2000) The evolution of disease resistance genes. Plant Mol Biol 42:195–204

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

Sears ER (1954) The aneuploids of common wheat. Univ Mo Agric Exp Stn Bull 572:1–58

Sears ER (1966) Nullisomic–tetrasomic combinations in hexaploid wheat. In: Rilly R, Lewis KR (eds) Chromosome manipulations and plant genetics. Oliver and Boyd, Edinburgh, pp 29–45

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

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

Stephenson P, Bryan G, Kirby J, Collins A, Devos K, Busso C, Gale M (1998) Fifty new microsatellite loci for the wheat genetic map. Theor Appl Genet 97:946–949

Tanaka M (1961) New amphidiploids, synthesized 6X-wheats, derived from Emmer wheat × *Aegilops squarrosa*. Wheat Info Serv 12:11

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

Yahiaoui N, Srichumpa P, Dubert R, Keller B (2004) Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. Plant J 37:528–538

Yencho GC, Cohen MB, Byrne PF (2000) Applications of tagging and mapping insect resistance loci in plants. Annu Rev Entomol 45:393–422