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H9, *H10*, and *H11* compose a cluster of Hessian fly-resistance genes in the distal gene-rich region of wheat chromosome 1AS

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Abstract *H9*, *H10*, and *H11* are major dominant resistance genes in wheat, expressing antibiosis against Hessian fly [(Hf) *Mayetiola destructor* (Say)] larvae. Previously, *H9* and *H10* were assigned to chromosome 5A and *H11* to 1A. The objectives of this study were to identify simple-sequence-repeat (SSR) markers for fine mapping of these genes and for marker-assisted selection in wheat breeding. Contrary to previous results, *H9* and *H10* did not show linkage with SSR markers on chromosome 5A. Instead, *H9*, *H10*, and *H11* are linked with SSR markers on the short arm of chromosome 1A. Both *H9* and *H10* are tightly linked to flanking markers *Xbarc263* and *Xcfa2153* within a genetic distance of 0.3–0.5 cM. *H11* is tightly linked to flanking markers *Xcfa2153* and *Xbarc263* at genetic distances of 0.3 cM and 1.7 cM. Deletion bin mapping assigned these markers and genes to the distal 14% of chromosome arm 1AS, where another Hf-resistance gene, *Hdic* (derived from emmer wheat), was also mapped previously. Marker polymorphism results indicated that a small terminal segment of chromosome 1AS containing *H9* or *H10* was transferred from the donor parent to the wheat lines Iris or Joy, and a small intercalary fragment carrying *H11* was transferred from the resistant donor to the wheat line Karen. Our results suggest that *H9*, *H10*, *H11*, *Hdic*, and the previously identified *H9*- or *H11*-linked genes (*H3*, *H5*, *H6*, *H12*, *H14*, *H15*, *H16*, *H17*, *H19*, *H28*, and *H29*) may compose a cluster (or family) of Hf-resistance genes in the distal gene-rich region of wheat chromosome 1AS; and *H10* most likely is the same gene as *H9*.

Keywords Wheat · Hessian fly · Resistance gene · *H9* · *H10* · *H11* · Gene mapping · Marker

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Introduction

Hessian fly (Hf), *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is an important pest of wheat (*Triticum aestivum* L.) worldwide (Cox and Hatchett 1994; Ratcliffe and Hatchett 1997; Harris et al. 2003). The use of resistance genes in wheat provides the most effective and efficient method of controlling the Hessian fly. 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. 2005a). Many of these genes, including *H6*, *H9-H11*, *H14-H20*, *H28*, *H29*, and *H31*, were identified from tetraploid durum wheat, *T. turgidum* ssp. *durum* Desf., and most of them have been introgressed into common wheat (McIntosh et al. 2003; Williams et al. 2003). The greatest number of Hf-resistance genes identified to date were assigned to wheat chromosome 5A, including *H3*, *H6*, *H9*, *H10*, *H12*, *H14*, *H15*, *H16*, *H17*, *H19*, *H28*, and *H29* (Stebbins et al. 1982; Ohm et al. 1995, 1997; Cebert et al. 1996; McIntosh et al. 2003).

Resistance genes *H9*, *H10*, and *H11* were transferred individually into the background of common wheat cultivar 'Newton' (CI 17715) by backcrossing (Patterson

et al. 1994), resulting in the near isogenic wheat lines Iris (Newton-207*7 × Ella, *H9*), Joy (Newton-207*3 × IN76529A5-3-3, *H10*), and Karen (Newton-207*4 × IN916-1-3-1-47-1, *H11*). However, these genes have not yet been deployed in commercial cultivars (Williams et al. 2003). *H9* and *H10* originated from the same durum wheat selection Elva (CI 17714) and were transferred to common wheat through backcrossing that resulted in wheat germplasm lines Ella and 76529, respectively (Carlson et al. 1978; Stebbins et al. 1982). Further analysis revealed that *H9* was linked to *H10* at 36 map units (Carlson et al. 1978). It was found that *H9* was linked to *H3* at 15.5 ± 4.8 map units and to *H6* at 2.02 ± 2.01 map units, respectively (Stebbins et al. 1980, 1982). Because *H6* was located on chromosome 5A by monosomic analysis (Gallun and Patterson 1977), based on linkage, *H9* and *H10* were assigned to chromosome 5A (Carlson et al. 1978; Stebbins et al. 1982). Later, the chromosome location of *H10* on 5A was confirmed by monosomic analysis (Ohm et al. 1995). *H11* was derived from durum wheat accession PI 94587 and was linked to *H5* at 4.40 ± 1.78 map units on chromosome 1AS (Stebbins et al. 1983; Roberts and Gallun 1984).

A variety of molecular markers has been identified for mapping Hf-resistance genes in wheat. Dweikat et al. (1994, 1997, 2002) developed random amplified polymorphic DNA (RAPD) 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. Recently, simple-sequence-repeat (SSR) markers were found tightly linked with an Hf-resistance gene (tentatively named *Hdic*) on wheat chromosome 1AS (Liu et al. 2005a) and with *H13* on chromosome 6DS (Liu et al. 2005b).

The objectives of this study were to identify SSR markers linked to *H9*, *H10*, and *H11* for marker-assisted selection (MAS) and to further map these genes for map-based cloning. We discovered that none of the SSR markers on chromosome 5A showed linkage with either *H9* or *H10*, a result contrary to their previous assignment to 5A. Instead, *H9* and *H10*, together with *H11*, are linked with SSR markers on chromosome arm 1AS.

Materials and methods

Hf 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 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 'Ike' (*H3*), 'Magnum' (*H5*), 'Caldwell' (*H6*), and 'Seneca' (*H7H8*) sequentially. Hf pupae together with infested wheat plants were stored at 4°C until Hf adults were needed.

Plant materials and DNA isolation

Seeds of Hf-resistant wheat germplasm lines Iris (*H9*, PI 562615), Joy (*H10*, PI 562616), and Karen (*H11*, PI 562617) were kindly provided by Dr. H. Ohm, Purdue University, Lafayette, Ind., USA. The corresponding susceptible near-isogenic line Newton (CI 17715) and the original Hf-resistance sources Elva (*H9* and *H10*, CI 17714) and Ella (*H9*, CI 17938) were provided by the USDA–ARS National Small Grains Research Facility in Aberdeen, Idaho, USA. To map the Iris-*H9*, Joy-*H10*, and Karen-*H11* genes, three mapping populations consisting of 122 $F_{2:3}$ families, 71 $F_{2:3}$ families, and 97 $F_{2:3}$ families, were developed from the crosses Tugela-*Dn1* × Iris-*H9*, Tugela-*Dn1* × Joy-*H10*, and Tugela-*Dn1* × Karen-*H11*, respectively. Tugela-*Dn1* is an Hf-susceptible wheat germplasm containing *Dn1*, a gene conferring resistance to biotype A of Russian wheat aphid, *Diuraphis noxia* (Mordvilko).

Wheat genetic stocks used for physical mapping including 'Chinese Spring' (CS), CS nullisomic-tetrasomic [(NT, N1A-T1D) Sears 1966], ditelosomic [(Dt1AL, Dt1AS) Sears 1954; Sears and Sears 1978], and deletion lines del1AS-1 [with fraction length (FL) 0.47], del1AS-2 (FL 0.45) and del1AS-3 [(FL 0.86) Endo and Gill 1996], were provided by the Wheat Genetics Resource Center (WGRC) at Manhattan, Kan., USA.

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. (1991). DNA concentration was quantified spectrophotometrically.

Evaluation of Hf resistance

Parents, F_1 plants, and $F_{2:3}$ populations were evaluated for phenotypic reaction to Hf infestation in growth chambers at 18 ± 1°C with a 14h:10 h (light:dark) photoperiod as described previously (Hatchett et al. 1981; Maas et al. 1987), with modifications (Liu et al. 2005a, b). The *H11* populations were infested with HF biotype GP. The *H9* and *H10* populations were infested with biotype L. Chi-square (χ^2) tests were conducted to determine the goodness-of-fit of plant segregation ratios to theoretical Mendelian segregation ratios.

Microsatellite (SSR) analysis

Because *H9*, *H10*, and *H11* were previously assigned to wheat chromosomes 5A or 1A, a total of 32 SSR markers mapped to wheat chromosome 5A, and 62 SSR markers to chromosome 1A were screened for linkage to *H9*, *H10*, and *H11* by bulked segregant analysis [(BSA) Michelmore et al. 1991)] as well as by evaluation of the near-isogenic lines Iris-*H9*, Joy-*H10*, Karen-*H11*, and the recurrent susceptible parent Newton. Two DNA bulks were assembled, using equal amounts of DNA from five homozygous resistant and five susceptible F_2

plants of each mapping population. DNA samples of resistant and susceptible near-isogenic lines, parents, and bulks were evaluated for polymorphisms with SSR primers. Polymorphic markers indicative of linkage with resistance genes based on BSA analysis were further used to determine the genetic linkage between the Hf resistance genes *H9*, *H10*, *H11*, and the candidate markers using the $F_{2:3}$ mapping populations.

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 (Devos et al. 1995; Stephenson et al. 1998), BARC (Ward et al.: http://www.scabusa.org/pdfs/BARC_SSRs_011101.html), WMC (Gupta et al. 2002), GPW (Nicot et al. 2004), and CFA (Sourdille: http://wheat.pw.usda.gov/ggpages/SSRclub/Sourdille/; Guyomarc'h et al. 2002; Sourdille et al. 2004).

PCR amplification was performed in a volume of 25 µl as described by Röder et al. (1998), with minor modifications (Liu et al. 2005a, b). PCR amplified fragments were separated on 3% agarose gels (Sigma, St. Louis, Mo., USA) under electrophoresis at 5 V/cm in 1× TAE buffer. 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) using the Kosambi mapping function (Kosambi 1944) and MapMaker software, version 3.0 (Lander et al. 1987; Lincoln et al. 1992), 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 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

Phenotypic segregation of the mapping populations

Accurate phenotypic data on the mapping populations are critical for the correct mapping of the target genes. The observed segregation of 30 homozygous resistant, 61 segregating (heterozygous), and 31 homozygous susceptible $F_{2:3}$families in the F_3 populations derived from the cross Tugela-*Dn1* × Iris-*H9* fit a one-gene segregation ratio 1:2:1 ($\chi^2 = 0.0164$, $df = 2$, $P = 0.992$). The observed segregation of 17R:38H:16S in the F_3 populations derived from the cross Tugela-*Dn1* × Joy-*H10* fit a one-gene segregation ratio 1:2:1 ($\chi^2 = 0.3803$, $df = 2$, $P = 0.831$). The observed segregation of 26R:48H:23S in the F_3 populations derived fromTugela-*Dn1* × Kareen-*H11* fit a one-gene segregation ratio 1:2:1 ($\chi^2 = 0.1959$, $df = 2$, $P = 0.91$). Segregation ratios confirmed that Hf resistance in each of Iris (*H9*), Joy (*H10*), and Karen (*H11*) is controlled by a single, dominant gene.

Genetic mapping of *H9*, *H10*, and *H11*

Because *H9* and *H10* were previously assigned to wheat chromosome 5A, 32 5A-specific SSR markers were screened for linkage. No primer pairs detected polymorphisms between the susceptible line Newton and either near-isogenic line Iris (*H9*) or Joy(*H10*), or between the resistant and susceptible bulks (data not shown). To determine the actual locations of these two genes, SSR markers from other chromosomes were screened for potential linkage. No SSR markers other than a few from chromosome 1A displayed linkage with *H9* and *H10*, indicating that these two genes are on chromosome 1A instead of 5A. For the markers from chromosome 1A, 8 out of 63 SSRs, including GWM136, PSP2999, GPW7072, CFA2153, BARC263, WMC329, WMC95b, and WMC24, produced polymorphic DNA fragments from PCR amplification (with the expected sizes similar to those from CS wheat) between Newton and Iris or Joy (Table 1; Fig. 1). The primer pairs for these markers also amplified DNA fragments polymorphic between Tugela-*Dn1* and Iris (*H9*) or Joy (*H10*), as well as between the resistant and susceptible bulks. Evaluation of the F_3 mapping populations indicated that these SSR markers are tightly linked to *H9* (Fig. 2a) and *H10* (Fig. 2b) on the short arm of chromosome 1A. Markers *Xbarc263*, *Xwmc329*, *Xwmc95b*, and *Xwmc24* are proximal to *H9* at 0.3, 1.6, 1.9, and 7.7 cM, respectively. The markers *Xcfa2153*, *Xpsp2999*, and *Xgwm136* are distal to *H9* at 0.5, 3.5, and 3.8 cM, respectively (Fig. 2a). Marker *Xgpw7072*, which co-segregates with *Xpsp2999*, is also linked to *H9* at 3.5 cM.

In the wheat line Iris, a small terminal chromosomal segment carrying *H9* (the gray region of Fig. 2a) was found to be transferred from the donor parent to the recipient (recurrent) parent Newton. This is based on the fact that all the *H9*-linked SSR markers in the distal region of chromosome 1AS detected no polymorphisms among the resistant wheat lines Iris (*H9*), Ella (*H9*), and the original durum donor parent Elva (*H9H10*) (Table 1), but detected polymorphisms between Iris (*H9*) and Newton (the recurrent recipient). Similar results (Table 1) also indicated that a small terminal segment carrying *H10* (the gray region of Fig. 2b) was transferred from the donor parent to the recurrent parent Newton and resulted in the wheat line Joy.

Table 1 Polymorphisms of DNA fragments (base pair-sized) amplified from wheat parents and related sources with simple-sequence-repeat (SSR) primers of *H* gene-linked markers on 1AS

Primer	Elva (*H9H10*)	Ella (*H9*)	Newton (Susceptible)	Iris (*H9*)	Joy (*H10*)	Karen (*H11*)	Tugela-*Dn1* (Susceptible)
GWM136	**270**	**270**	null[a]	**270**	**270**	null	260
PSP2999	**160**	**160**	150	**160**	**160**	150	150
GPW7072	**240**	**240**	230	**240**	**240**	230	260 and 230
CFA2153	**180**	**180**	210	**180**	**180**	**195**	190
BARC263	**220**	**220**	210	**220**	**220**	210	null
	190	**190**	180	**190**	**190**	180	190
WMC329	**120**	**120**	130	**120**	**120**	130	null
	105	**105**	105	**105**	**105**	105	95
WMC95b	**220**	**220**	null	**220**	**220**	null	200
	200	**200**	null	**200**	**200**	null	180 and 160
WMC24	**120**	**120**	140	**120**	**120**	140	160
BARC148	190	190	200	200	200	200	200

Sizes in *boldface* represent the PCR amplifications in the derived resistant wheat near isogenic lines are the same as those from the donor, but different from those amplified from the susceptible near isogenic line Newton (recipient). Polymorphic patterns in the PCR products between the recipient parent and the derived wheat lines, together with the non-polymorphic pattern between the donor and the derived wheat lines, indicated that the loci of the SSR markers were derived from the donor parent

[a]*null* No amplification or null allele

We also investigated linkage of *H9*- or *H10*-linked SSR markers with *H11*, which was previously mapped to chromosome 1AS (Roberts and Gallun 1984). Indeed, the *H9*- or *H10*-linked SSR markers were also linked with *H11* on wheat chromosome 1AS (Fig. 2c). *H11* is tightly linked to flanking markers *Xcfa2153* and *Xbarc263* at genetic distances of 0.3 cM and 1.7 cM, respectively. Because CFA2153 is the only marker that detected polymorphism between Karen (*H11*) and the susceptible near isogenic line Newton among the 1AS SSR primer pairs (Table 1), it is most likely that a very small intercalary segment containing *H11* together with the locus of *Xcfa2153* was transferred from the donor parent to the Newton backcross derived line, Karen (*H11*).

Previously, Dweikat et al. (1997) reported RAPD markers that were linked to *H9*, although the chromosome locations of RAPD markers were unknown. We also tested these RAPD markers using our F_3 mapping populations to determine the relationship between the RAPD markers and the SSR markers. Under our experimental conditions, repeatable results were ob-

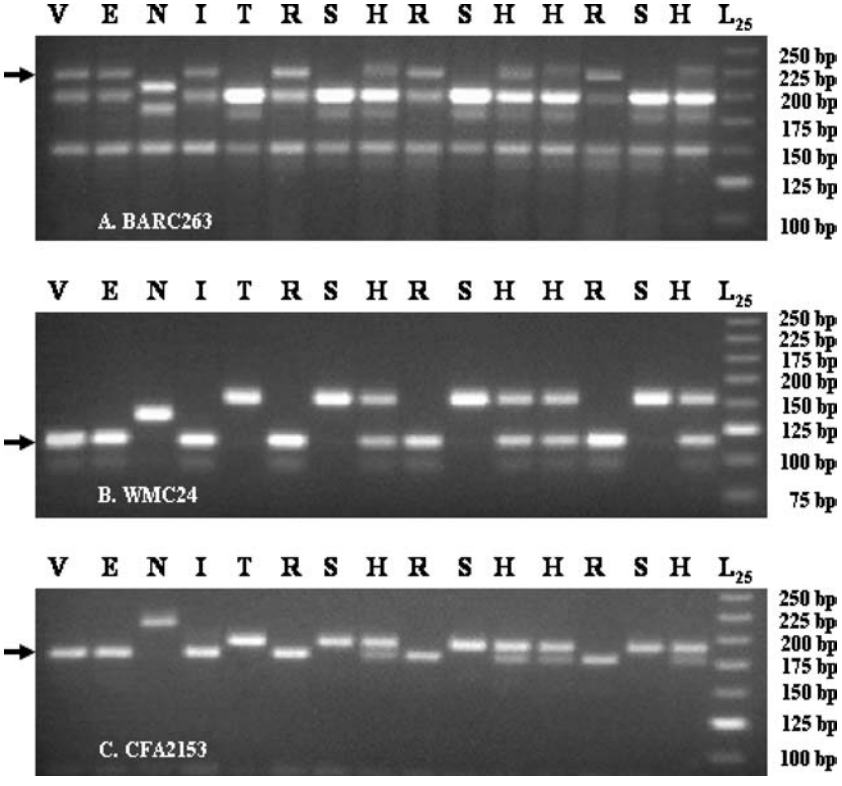

Fig. 1 DNA fragments amplified with simple-sequence-repeat primers BARC263 (**a**), WMC24 (**b**), and CFA2153 (**c**). DNA samples were prepared from Elva (*V*), Ella (*E*), Newton-207 (*N*), Iris (*I*), and Tugela-*Dn1* (*T1*), as well as F_2 plants from the cross Tugela-*Dn1* × Iris. *R* Hessian fly (Hf)-resistant progeny, *S* Hf-susceptible progeny, *H* heterozygous progeny. L_{25} Twenty-five-base pair DNA ladder. The *arrows* point to the PCR amplified fragments associated with the *H9* resistance

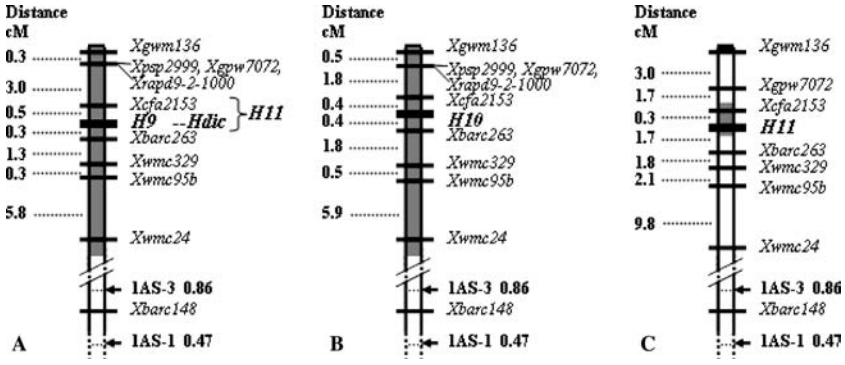

Fig. 2 Genetic maps of the *H9* (**a**), *H10* (**b**), and *H11* (**c**) genes and linked markers on wheat chromosome arm 1AS. The *gray regions* of the chromosome represent the donor-derived segment containing *H9*, *H10*, or *H11* in wheat germplasm Iris, Joy, and Karen, respectively. The *white regions* represent the genetic background of recipient parents Newton-207. The breakpoints of the deletions are indicated with *arrows*

tained using *Xrapd9-2-1000*. However, we did detect recombinations (6 recombinants out of 122) between *Xrapd9-2-1000* and *H9*, whereas no recombination was detected previously in a population of 124 F_2 plants (Dweikat et al. 1997). Furthermore, we found that marker *Xrapd9-2-1000* co-segregated with *Xpsp2999* and *Xgpw7072* and was linked to *H9* at 3.5 cM distally (Fig. 2a). Similar results were also obtained using the F_3 population of *H10*; *Xrapd9-2-1000* co-segregated with *Xpsp2999*, and *Xgpw7072*, and was linked to *H10* at 2.2 cM (Fig. 2b).

Physical mapping of the linked markers

To determine the physical locations of the linked SSR markers, DNA samples from CS, NT, Dt, and deletion lines of chromosome 1AS were amplified using SSR primer pairs, including GWM136, PSP2999, GPW7072, CFA2153, BARC263, WMC95b, and WMC24. Each primer pair amplified DNA fragments of the expected size(s) from CS and CS Dt1AS, but no corresponding fragments were amplified from Dt1AL, N1AT1D, del1AS-1 (FL 0.47), del1AS-2 (FL 0.45), and del1AS-3 (FL 0.86). The results demonstrate that all of these linked markers are located distal to the breakpoint of 1AS-3 in the terminal 14% of the chromosome short arm (distal to the arrow point of del1AS-3 as shown in Fig. 2). *Xbarc148* is proximal to the above-described SSR markers and was physically located in bin 1AS3-0.47-0.86.

Characteristics of the linked markers

All the *H9*- or *H10*-linked SSR markers used in the F_3 mapping populations of this study are standard co-dominant markers based on the sizes of the amplified fragments, except *Xbarc263*, which seems to be a dominant marker based on the sizes of the PCR fragment amplified from the resistant and susceptible parents. However, it showed a co-dominant inheritance pattern based on the sizes as well as the intensity of the amplified fragments. The BARC263 primer pair amplified two DNA fragments with sizes of 190 bp and 220 bp from DNA samples of the resistant donor Elva and the derived wheat lines Ella and Iris. However, the same primer pair amplified a 190-bp DNA fragment from the susceptible parent Tugela-*Dn1* (Table 1; Fig. 1a). Particularly, the 190-bp band amplified from Tugela-*Dn1* is about five times stronger than that from the resistant lines (Fig. 1a). The different intensities could be a result of different amplification efficiencies. The primer pair BARC263 may be perfectly complementary to the primer-binding sites of the template DNA from Tugela-*Dn1*, but imperfectly match with the primer-binding sites of the template DNA from Iris and the original resistance sources Elva and Ella, due to nucleotide-sequence alteration(s) or mutation(s). The same band pattern was observed in $F_{2:3}$ families, i.e., a 220-bp band and a weak 190-bp band were amplified from DNA of homozygous resistant families, and a strong 190-bp band was amplified from the susceptible families. Both the 220-bp and the stronger 190-bp DNA bands were amplified from the heterozygous families.

Discussion

Chromosome locations of *H9*, *H10*, *H11*, *Hdic*, and other *H9*-linked genes

The molecular marker analyses based on linkage and deletion mapping have provided conclusive evidence that Hf-resistance genes *H9* and *H10* are located in the distal gene-rich region on the short arm of wheat chromosome 1A. Previously, *H9* was assigned to 5A because of their linkage to *H6* (Stebbins et al. 1980, 1982), H6 was in turn mapped to 5A by monosomic analyses (Gallun and Patterson 1977). Later, *H10* was also mapped to chromosome 5A because of linkage to *H9* (Stebbins et al. 1982) as well as by monosomic analysis (Ohm et al. 1995). A reappraisal of the monosomic mapping papers (Gallun and Patterson 1977; Ohm et al.

1995) revealed that erroneous mapping of *H6* and *H10* on 5A probably occurred because of insufficient data or misinterpretation of the monosomic mapping data. In the *H6* monosomic analysis, the segregation test for critical or noncritical ratios was based on only 20 or less F_3 families for each monosomic type, which is usually not enough to correctly distinguish the critical segregation ratio from noncritical ratios. In the *H10* monosomic analysis, Ohm et al. (1995) only analyzed monosomic 5A crosses, and observed 5A monosomic F_2 segregation ratio of 35R : 58S, a clear departure from the 3:1 ratio but in the wrong direction (with excess of susceptible plants instead of excess of resistant plants). The reasons for the excess of susceptible plants are not known but may be due to the hemizygous plant weakness or heterozygous incomplete dominance as the authors suggested. However, it is obvious that this deviated segregation ratio (35R:58S) was improperly interpreted as an evidence that *H10* was located on chromosome 5A. If *H10* were critically located on 5A, the segregation ratio should be derived from 3R:1S ratio, with an excess of resistant 5A monosomic F_2 plants.

The present results also provide new information about the genetic relationship of *H9*, *H10*, *H11*, and the previously mapped emmer-derived Hf-resistance gene *Hdic* (Liu et al. 2005a), and will facilitate the rational use of these genes in breeding.

Although *H9* and *H10* were previously regarded as linked and different genes, the present study indicates it is most likely that *H10* in Joy is the same gene as *H9* in Iris, because: (1) all the primer pairs of the linked SSR markers, as well as the primer of a linked RAPD marker *Xrapd9-2-1000*, each amplified DNA fragments with exactly the same size(s) from DNA samples of resistant wheat lines Joy (*H10*), Iris (*H9*), Ella (*H9*), and the original resistant donor, the durum wheat Elva (*H9H10*) (Table 1); (2) both Joy (*H10*) and Iris (*H9*) carry the same terminal chromosome segment distal to and including the locus *Xwmc24* on 1AS (the gray region of Fig. 2a, b) transferred from the original resistant donor Elva; (3) the genetic linkage maps of *H9* (Fig. 2a) and *H10* (Fig. 2b) showed that both *H9* and *H10* are located in the same region between *Xcfa2153* and *Xbarc263*; and (4) a previous report indicated that Joy (*H10*) and Iris (*H9*) were linked and originally derived from the same resistant source Elva (Carlson et al. 1978). The reasons that an allelic pair of genes was regarded as different genes may be complicated. For any phenotype-based allelism test, it is critical that the parents used to test for allelic relationship must be from pure and homozygous seed stocks (Liu et al. 2002), and there should be no extraneous pollen contamination during the cross. The variation of virulence or impurity of Hf population and some undefined environmental factors may also affect the phenotypic evaluation of Hf resistance in wheat, and thus influence the results of allelism tests, especially when the phenotypic evaluation is based on the BC_1F_1 or F_2 plants.

Sunderman and Hatchett (1986) found that a powdery mildew resistance gene *Pm3* (located on 1AS) was tightly linked to *H3* in repulsion. Because of the previous report that *H9* was linked to *H3* and *H6* on chromosome 5A (Stebbins et al. 1980, 1982), the linkage was explained by a 1A-5A chromosome translocation in the powdery mildew-resistant wheat, because a ring of four chromosomes was observed in the F_1 microsporocytes. *Pm3* was reported to co-segregate with *Xpsp2999* on 1AS (Bougot et al. 2002). In the light of the present results, it is most likely that *H3* and *H6* are located in the same region as *H9*, which is linked to *Xpss2999* at 3.5 cM on chromosome 1AS.

The discovery of the actual chromosome location of *H9* and *H10* on 1AS also provided a new clue to reconsider the locations of *H3*, *H6*, *H12*, *H14*, *H15*, *H16*, *H17*, *H19*, *H28*, and *H29*, which were previously identified within the same linkage block of *H9* (and *H10*) on chromosome 5A (Stebbins et al. 1982; Ohm et al. 1995, 1997; Cebert et al. 1996). These genes most likely are located on wheat chromosome 1A.

In the present study, *H9*, *H10*, and *H11* were mapped to the small region between *Xcfa2153* and *Xbarc263* on 1AS, same as the previously mapped emmer-derived gene *Hdic* (Liu et al. 2005a). These results demonstrate that *H9* (*H10*), *H11*, and *Hdic* are either allelic or tightly linked genes. Results also indicate a cluster or family of Hf-resistance genes that are located in the distal gene-rich region on wheat chromosome arm 1AS. This cluster of H genes include *H9* (*H10*), *H11*, *Hdic*, and may also include *H3*, *H5*, *H6*, *H12*, *H14*, *H15*, *H16*, *H17*, *H19*, *H28*, and *H29*, although further research is needed to confirm this extended deduction.

The relationship of markers *Xpsp2999*, *Xgpw7072*, and *Xrapd9-2-1000*

Two SSR markers, *Xpsp2999* and *Xgpw7072*, and one RAPD marker, *Xrapd9-2-1000*, co-segregated and are distally linked to *H9* at 3.5 cM. Primer set PSP2999 was originally developed to amplify the microsatellite array in the low-molecular-weight glutenin gene *Glu-3* on wheat chromosome 1AS (Pitts et al. 1988; Devos et al. 1995; Stephenson et al. 1998). SSR marker *Xgpw7072* was developed from wheat expressed sequence tags (Nicot et al. 2004). RAPD marker *Xrapd9-2-1000* was previously reported to co-segregate with *H9* (Dweikat et al. 1997), but in the present study, we determined it was 3.5 cM distal to *H9*. It seems that all three markers are either within, or tightly linked to, the *Glu-3* gene.

The potential use of molecular markers

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 in the seedling stage in early generations (Rafalski and Tingey 1993; Somers et al. 2004).

The markers identified in this study will greatly facilitate the selection and use of the *H9* (*H10*) and *H11* genes in breeding programs. The flanking SSR markers can be used to determine the genotype at the resistance locus with a high degree of accuracy. For example, the two markers *Xcfa2153* and *Xbarc263* are linked to *H9* at 0.5 cM and 0.3 cM, respectively (Fig. 2a). The recombination frequency (RF) between *Xcfa2153* and *H9* is 0.5% (with a Kosambi map distance of 0.5 cM, Kosambi 1944). The RF between *Xbarc263* and *H9* is 0.3%. These RFs for *Xcfa2153* and *Xbarc263* translate into selection accuracies of 99.5% and 99.7%, respectively, if they are used separately. According to the product rule of the probability, the selection accuracy will increase to nearly 100% (i.e., $1-0.5\% \times 0.3\%$) when these two flanking markers are used together.

In summary, the tightly linked markers identified in this study will be useful not only to facilitate wheat molecular breeding (MAS) and molecular mapping, but may also provide a good opportunity for the map-based cloning of target genes. The discovery that the actual location of *H9* (*H10*) is in the same region as *H11* and *Hdic* on the short arm of chromosome 1A greatly helped to clarify the linkage relationship among many of the Hf-resistance genes, and is of critical importance for the rational use of these genes in wheat breeding and resistant cultivar deployment.

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