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Genetic characterization and molecular mapping of a Hessian fly-resistance gene transferred from *T. turgidum* ssp. *dicoccum* to common wheat

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Abstract A gene (temporarily designated *Hdic*) conferring resistance to the Hessian fly (Hf) [*Mayetiola destructor* (Say)] was previously identified from an accession of German cultivated emmer wheat [*Triticum turgidum* ssp. *dicoccum* (Schrank ex Schübler) Thell] PI 94641, and was transferred to the Hf-resistant wheat germplasm KS99WGRC42. The inheritance of *Hdic* resistance exhibited incomplete penetrance because phenotypes of some heterozygous progenies are fully resistant and the others are fully susceptible. Five simple sequence repeat (SSR) markers (*Xgwm136*,*Xcfa2153*, *Xpsp2999*,*Xgwm33*, and *Xbarc263*) were linked to the *Hdic* gene on the short arm of wheat chromosome 1A in the same region as the *H9*,*H10*, and *H11* loci. Flanking markers *Xgwm33* and *Xcfa2153* were mapped at distances 0.6 cM proximal and 1.4 cM distal, respectively. Marker analysis revealed that a very small intercalary chromosomal segment containing *Hdic* was transferred from emmer wheat to KS99WGRC42. This is the first emmer-derived Hf-resistance gene that has been mapped and characterized. The *Hdic* gene confers a high level of antibiosis to biotypes GP and L, as well as to strains v*H9* and v*H13* of the Hf, which is different from the biotype reaction patterns of the known Hf-resistance genes on chromosome 1A (*H5* and *H11* susceptible to biotype L, *H9* and *H10* susceptible to strain v*H9*). These results suggested that *Hdic* is either a new gene or a novel allele of a known H gene on chromosome 1A. The broad spectrum of resistance conferred by the *Hdic* gene makes it valuable for developing Hf resistant wheat cultivars.

Keywords Wheat · Hessian fly · Resistance gene · Gene mapping · Marker · KS99WGRC42

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Introduction

The Hessian fly (Hf), *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is one of the most destructive pests of wheat (*Triticum aestivum* L.) worldwide, and causes annual losses ranging from 5 to 10% of wheat production to US farmers (Hatchett et al. 1987; Buntin 1999). Historically, the use of wheat resistance genes has been the most effective and cost-efficient approach in protecting wheat from Hf damage and minimizing the use of insecticides (Ratcliffe and Hatchett 1997). However, the success of the plant resistance strategy has been challenged by the ability of the Hessian fly to develop different virulent populations or biotypes that overcome the specific resistance genes in deployed cultivars (Ratcliffe et al. 2000). To counter the development of new biotypes, it is necessary to identify and utilize different resistance genes derived from diverse sources (Ratcliffe and Hatchett 1997).

Todate, 31 major Hf-resistance genes (named *H1* through *H31)* have been identified from wheat and its relatives (Ratcliffe and Hatchett 1997; McIntosh et al. 2003; Harris et al. 2003; Williams et al. 2003). These genes were identified from different sources, including

common wheat (*H1*,*H2*, *H3*,*H4*, *H5*,*H7H8*, *H12*), *Aegilops tauschii* (*H13*,*H22*, *H23*,*H24*, *H26*), *T. turgidum* ssp. *durum* (*H6*,*H9*, *H10*,*H11*, *H14*,*H15*, *H16*,*H17*, *H18*,*H19*, *H20*,*H28*, *H29*,*H31*), *Ae. ventricosa H27*), *Ae. triuncialis H30*), and rye (*H21*,*H25*). A Hf-resistance gene, temporarily designated *Hdic*, was recently identified from an accession of German cultivated emmer wheat [AABB genomes, *T. turgidum* ssp. *dicoccum* (Schrank ex Schübler) Thell] PI 94641, and was transferred to the Hf-resistant hard red winter wheat germplasm KS99WGRC42 (PI 635054) (Brown-Guedira et al. 2004).

As a result of changes in virulence of Hessian fly populations in the field in response to the deployment of resistant cultivars, resistance genes such as *H3*,*H5*, and *H6* no longer provide effective resistance (Patterson et al. 1990; Ratcliffe and Hatchett 1997; Ratcliffe et al. 2000). To ensure the continued success of the plant resistance strategy, different breeding and gene deployment strategies have been suggested (Cox and Hatchett 1986; Smith et al. 1999). Use and rotation (or sequential release) of wheat cultivars containing different resistance genes may reduce new biotype outbreaks. Combining several genes in a single cultivar may also increase the level, duration, and breadth of resistance. However, conventional breeding based on phenotypic selection is time-consuming and sometimes inconclusive, particularly for combining multiple genes. More efficient and accurate techniques are needed to incorporate diverse genes into different cultivars for rotation and to pyramid multiple genes into single cultivars to increase the durability of resistance.

Molecular markers can greatly facilitate the identification of new resistance genes. This is particularly true for Hessian fly resistance because more than 30 resistance genes have been identified, and distinguishing new genes is difficult by traditional phenotypic differentiation with biotypes. Molecular markers can also greatly accelerate breeding for resistance through marker-assisted selection (MAS) (Yencho et al. 2000). Compared with traditional phenotypic selection, MAS can detect desired resistant genotypes in early generations, allowing breeders to avoid carrying numerous unwanted lines. More importantly, MAS makes pyramiding of multiple effective resistance genes a reality.

A variety of molecular markers linked to several Hf-resistance genes have been identified. Ma et al. (1993) identified restriction fragment length polymorphism (RFLP) markers that are linked to the *H23* gene on chromosome 6D and *H24* on chromosome 3DL. Delaney et al. (1995) tagged the *H25* gene on 6BL with RFLP markers. Dweikat et al. (1994, 1997, 2002) developed a series of random amplified polymorphic DNAs (RAPD) and sequence tagged site (STS) markers that were associated with the *H3*,*H5*, *H6*,*H9*- *H14*,*H16*, and *H17* genes. Seo et al. (1997, 2001) identified several amplified fragment length polymorphism (AFLP), RAPD, and STS markers linked to the *H21* gene. Martin-Sanchez et al. (2003) identified an isozyme marker that was linked with *H30*, and Williams et al. (2003) mapped a new gene (*H31*) on chromosome 5BS with AFLP and STS markers. Identification of microsatellite or simple sequence repeat (SSR) markers linked to Hf resistance genes should be useful for MAS because SSRs are abundant, reliable, highly polymorphic, chromosome-specific, and can be easily handled in high-throughput systems (Weber and May 1989; Röder et al. 1998; Pestsova et al. 2000). A SSR marker (*Xgwm397*) linked to the rye-derived Hf-resistance gene *H25* on chromosome 4A was reported (E. D. Souza, http://maswheat.ucdavis.edu/protocols/HF/index.htm). More recently, Liu et al. (2005) mapped *H9*,*H10*, and *H11* to the distal region of wheat chromosome 1AS with tightly linked SSR markers.

In this study, we characterized the emmer wheat-derived Hf-resistance gene (tentatively named *Hdic*), and determined its chromosomal location on the short arm of wheat chromosome 1A using SSR markers.

Materials and methods

Hessian fly populations

Biotype GP was from a laboratory colony collected from Ellis County, Kansas (Gagne and Hatchett 1989). The insects were maintained on susceptible wheat seedlings (Karl 92 or Newton). Biotype L culture was supplied by S. E. Cambron, USDA-ARS, West Lafayette, Indiana. The insects were maintained on seedlings of Ike (*H3*), Magnum (*H5*), Caldwell (*H6*) and Seneca (*H7H8*) sequentially. The *H9*-virulent strain (v *H9*) and the *H13*-virulent strain (v*H13*) were provided by J. J. Stuart, Purdue Univ., West Lafayette, Indiana. The v*H9* and v*H13* flies were maintained on seedlings of Iris (*H9*) and Molly (*H13*), respectively. Hf pupae together with the infested wheat plants were stored at 4°C until ready for use.

Plant materials and DNA isolation

The original source of the Hf-resistance (PI 94641) was an accession of a German cultivated emmer wheat (*T. turgidum* ssp. *dicoccum*) obtained from the USDA/ARS National Small Grains Collection. The resistance gene was transferred to common wheat through the cross Karl 92/PI 94641//Jagger*2/3/Karl 92. A resulting F_6–derived line 01-337-1-20 homozygous for resistance to Hessian fly biotypes GP and L was selected for release as Hf-resistant hard red winter wheat germplasm KS99WGRC42 (Brown-Guedira et al. 2004). A mapping population consisting of 121 $F_{5:6}$lines (equivalent to $F_{2:3}$families) was derived from eight heterozygous F_4plants from the cross Karl 92/PI 94641//Jagger*2 /3/Karl 92. Data from each family were recorded separately and were pooled for analyses. Two additional populations of 134 and 118 F_2plants, respectively, were derived

from crosses between Hf-susceptible cultivar Tugela-*Dn1* and two homozygous resistant $F_{4:5}$lines, 01-20-12 and 01-186-11. In addition, F_2 populations consisting of 188 and 134 plants were developed from reciprocal crosses between PI 94641 and Hf-susceptible emmer accession CI 7685. Wheat genomic DNA was extracted from leaf tissue according to the modified CTAB procedure as described by Gill et al. (1991). DNA concentration was quantified spectrophotometrically.

Seeds of PI 94641 and CI 7685 were provided by the USDA/ARS National Small Grains Research Facility in Aberdeen, Idaho. Seeds of susceptible cultivars Karl 92 and Jagger, were provided by the developer at Kansas State Universtiy. Tugela- *Dn1* was provided by the Small Grain Institute, Bethlehem, South Africa. Wheat lines 01-20-12, 01-186-11, and KS99WGRC42 were maintained at USDA/ARS and the Wheat Genetics Resource Center (WGRC) at Manhattan, Kansas. Seeds of wheat genetic stocks used for marker localization including Chinese Spring (CS), CS nullisomic-tetrasomic (NT: N1A-T1D) (Sears 1966), ditelosomic (Dt1AL, Dt1AS) (Sears 1954), 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 kindly provided by WGRC.

Evaluation of Hf-resistance

Parents (used as checks), F_1 plants, and segregating populations were evaluated for phenotypic reaction to Hf infestation in growth chambers at 18 ± 1°C with 14:10 h (L:D) photoperiod or in the greenhouse (20–32°C), 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. Seedlings in each flat at the one-leaf stage were infested by confining ~200 newly mated Hf females with 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 pre-pupae). Resistant plants grew normally (unstunted), with light green leaves, and contained dead larvae between the leaf sheaths. Chi-square (χ^2) tests were conducted to determine the goodness-of-fit to theoretically expected Mendelian segregation ratios.

Bulked segregant analysis (BSA)

Molecular markers putatively linked to the resistance gene were identified by bulked segregant analysis (Michelmore et al. 1991) of the $F_{5:6}$population. Resistant and susceptible DNA bulks were assembled, using equal amounts of DNA from five homozygous resistant and five susceptible families, respectively. The DNA samples of resistant and susceptible parents and bulks were amplified and screened for polymorphisms with SSR primers. Once polymorphisms between resistant and susceptible bulks were identified by BSA, genetic linkage between the Hf-resistance gene and the markers was determined in the segregating population.

SSR analyses

The sequences of SSR primers and PCR protocols were obtained from GrainGenes at http://wheat.pw.usda.gov/ggpages/DEM/ggtabledefs.html/. Information for specific primers 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 (Ward et al.: http://www.graingenes.org/dbs_images/graingenes/BARC_1A.jpg), WMC (Gupta et al. 2002), and CFA (Sourdille: http://wheat.pw.usda.gov/ggpages/SSRclub/Sourdille/; Sourdille et al. 2004). Because the majority of Hf-resistance genes have been localized on chromosomes 1A and 5A (Ratcliffe and Hatchett 1997), we initially chose markers from these two chromosomes to screen for linkage to resistance. A total of 43 SSR primer pairs specific for wheat chromosome 1A and 32 primer pairs specific for 5A were used for screening.

PCR amplification was essentially performed as described by Röder et al. (1998), with minor modification (Liu et al. 2002). The reaction mixture contained 0.2 mM each of dNTPs, 1.8–2.0 mM $MgCl_2$, 1 U *Taq* DNA polymerase (Promega, Madison, WI, USA), 1× Thermophilic DNA polymerase buffer (10 mM Tris–HCl, 50 mM KCl and 0.1% Triton X-100), 0.4 μM of each of the forward and reverse primers, and 100 ng of template DNA. PCR amplifications were performed in an MJ Research PTC-200 Thermal Cycler (Watertown, MA, 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, then a final extension step at 72°C for 10 min before cooling to 4°C. PCR amplified fragments were separated on 3% agarose gels (Sigma, St. Louis, MO) through electrophoresis at 5 V/cm in 1× TAE buffer. DNA banding patterns were visualized under UV light with ethidium bromide staining.

Aneuploid and deletion line analyses

To determine the physical location of the SSR markers, DNA from euploids, aneuploids, and deletion lines of CS wheat was amplified by using SSR primers corresponding to loci located on chromosome 1AS. The NT lines for group 1 chromosomes and the Dt lines for the short and long arms of chromosome 1A were used to assign the chromosome and chromosomal arm location of markers. Amplification (or no amplification) of a specific fragment from a deletion stock indicates that the

corresponding marker is located proximally (or distally) 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.

Linkage analysis and genetic mapping

The linkage map of SSR markers and the resistance gene was constructed by converting Recombination frequencies (RF) to genetic map distance (cM) with the Kosambi mapping function (Kosambi 1944) using MapMaker software version 3.0 (Lander et al. 1987) at LOD > 3.0.

Results

Inheritance of resistance

The mechanism of the resistance conferred by the *Hdic* gene is antibiosis, which results in the death of the first-instar larvae 3–4 days after hatching. All plants of homozygous resistant lines, including the resistant donor PI 94641, the derived homozygous resistant $F_{4:5}$ lines 01-20-12 and 01-186-11, and the wheat germplasm KS99WGRC42, exhibited resistance (no stunting) to Hf biotypes GP and L as well as strains v*H9* and v*H13*. In all cases, presence of alive larvae within the leaf sheaths and stunting phenotype were observed on all infested plants of Karl 92 and Jagger.

When tested with Hf biotype L, the F_2 populations derived from reciprocal crosses between PI 94641 and the Hf-susceptible emmer wheat CI 7685 phenotypically segregated 139-R: 49-S, and 97-R: 37-S. The observed segregation ratios fit a model for a single dominant gene ($\chi^2_{3:1} = 0.114$, df = 1, $P = 0.743 > 0.05$, and $\chi^2_{3:1} = 0.667$, df = 1, $P = 0.438 > 0.05$, respectively). In addition, we also analyzed a population of 121 $F_{5:6}$lines derived from eight heterozygous F_4individual plants (from a cross of Karl 92/PI 94641//Jagger*2/3/Karl 92), which were equivalent to F_2-derived F_3 families at the resistance gene locus. Among the 121 lines, 32 were homozygous resistant, 61 were segregating, and 28 were homozygous susceptible (1RR:2Rr:1rr; $\chi^2 = 0.273$, df = 2, $P = 0.875 > 0.05$). This result also indicated the Hf resistance of PI 94641 is controlled by a single dominant gene.

However, analysis of the 793 individuals within the 61 segregating $F_{5:6}$ lines indicated that the *Hdic* gene did not behave in a typical dominant manner. The 793 progeny segregated 374-R: 419-S plants, which is obviously significantly different from 3R:1S ratio. Since the expected number of homozygous resistant and susceptible $F_{4:5}$ lines was observed, the deviation among F_6individuals within the segregating lines must be due to phenotypic variation of the heterozygous plants.

To investigate if the emmer-derived resistance has variable penetrance, F_1 progeny from crosses of Tugela-*Dn1* and two homozygous resistant lines, 1-20-12 and 01-186-11, were evaluated for reaction to the Hf biotype L. Lines 1-20-12 and 01-186-11 were evaluated for reaction to Hf-biotype L multiple times and no susceptible plants were observed, indicating that the lines were indeed homozygous for the resistance gene. In addition, subsequent marker analysis of progeny from the plants used to make the crosses with Tugela- *Dn1* indicated that these plants were homozygous for PI 94641 alleles in the region flanking the resistance gene. However, 22 of the 37 F_1 individual plants evaluated (59.4%) were susceptible to Hf. Observed segregation of 72R: 62S and 65R: 53S in the F_2 populations derived from resistant F_1 plants of these crosses deviated significantly from the 3R:1S ratio expected for a single dominant resistance gene ($\chi^2 = 32.328$, df = 1, $P < 0.001$, and $\chi^2 = 24.960$, df = 1, $P < 0.001$). The genotypes of individual F_2 plants derived from the two crosses were determined with markers flanking the resistance gene (*Xbarc263* and *Xcfa2153*, or *Xgwm33* and *Xcfa2153*) identified in our marker analysis. These markers were expected to provide selection accuracy greater than 99.97% for the resistance gene (see the Discussion section). In both populations, all plants homozygous for the *T. dicoccum*-derived alleles for the flanking markers were resistant to Hf. All plants homozygous for the susceptible parent alleles at the marker loci were susceptible. However, 60 of a total of 116 F_2plants (51.7%) that were heterozygous at the flanking marker loci were resistant, while 56 heterozygous F_2plants (48.3%) were susceptible to Hf. The phenotypes of *Hdic* heterozygotes were inconsistent (some were R and others were S), whereas homozygotes were consistently resistant. Both the F_1 and F_2 results demonstrated that the *Hdic* gene is incompletely penetrant, instead of partially dominant (which implies that all heterozygotes are intermediate to the parents in phenotype).

SSR markers linked to the *Hdic* gene

Of the 75 SSR primer pairs tested, five (GWM136, PSP2999, CFA2153, GWM33, and BARC263) from chromosome 1AS gave amplification products of expected sizes that were polymorphic between the resistant and susceptible parents as well as two bulks. Analysis of the $F_{5:6}$lines indicated that these SSR markers were linked to *Hdic*. A genetic linkage map was constructed as Fig. 1a based on the $F_{5:6}$ population evaluation.

The most closely linked proximal marker, *Xgwm33*, is dominant and 0.6 cM proximal to the *Hdic* gene (Fig. 1a) in the coupling phase. The GWM33 primer pair amplified a 130-bp DNA fragment from Jagger, PI 94641, homozygous resistant lines 01-20-12, 01-186-11, and KS99WGRC42 (Fig. 2a). No corresponding band was amplified from DNA of susceptible lines Karl 92 or Tugela- *Dn1*. A co-dominant marker, *Xbarc263*, 2.0 cM proximal to the resistance gene was also identified. Amplification with the BARC263 primer pair generated

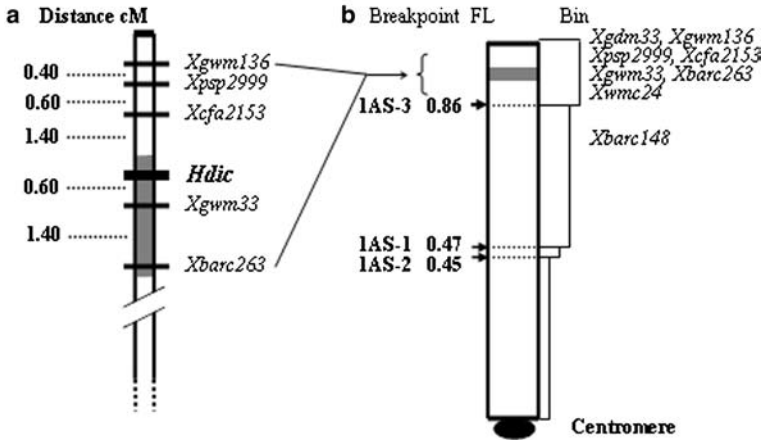

Fig. 1 Genetic map (**a**) and physical map (**b**) of the *Hdic* gene and linked SSR markers on wheat chromosome arm 1AS. The *grey region* of the chromosome represents the emmer-derived segment containing the *Hdic* gene in common wheat germplasm KS99WGRC42, and the white regions represent the genetic background of recipient parents (Karl 92 or Jagger)

a specific 220-bp DNA fragment in samples from the resistant donor parent PI 94641, KS99WGRC42 and 01-20-12. A 200-bp fragment was amplified from the susceptible parents Karl 92, Jagger (Fig. 2b), as well as Tugela- *Dn1* and 01-186-11. Both bands were amplified in most heterozygous progeny.

The most closely linked distal marker was *Xcfa2153*, located 1.4 cM from the *Hdic* gene (Fig. 1a). The CFA2153 primer pair amplified a 230-bp DNA fragment from the resistant parents PI 94641, 01-20-12, and 01-186-11, as well as from most homozygous resistant $F_{5:6}$ lines, and amplified a 190-bp DNA fragment from the susceptible parents Karl 92, Jagger and Tugela-*Dn1* (Fig. 2c), as well as from KS99WGRC42. Both the 230- and 190-bp fragments were amplified from heterozygous progeny.

Distal markers *Xgwm136* and *Xpsp2999* are dominant and linked to *Hdic* in the repulsion phase. The GWM136 primer pair amplified a 320-bp DNA fragment from susceptible parents, Karl 92 and Jagger, and a 280-bp fragment from the susceptible parent, Tugela-*Dn1*. The 320-bp fragment was also present in KS99WGRC42 amplifications. In contrast, no DNA amplification was achieved from the resistant parents PI 94641, 01-20-12, and 01-186-11, or from most homozygous resistant progeny (data not shown). The PSP2999 primer pair amplified three DNA fragments (with sizes 130, 140 and 145 bp, respectively) from Karl 92 and Jagger, and the susceptible and segregating progeny, as well as from KS99WGRC42. Only the 140-bp fragment was amplified from the resistant parents PI 94641, 01-20-12, and 01-186-11, as well as from most homozygous

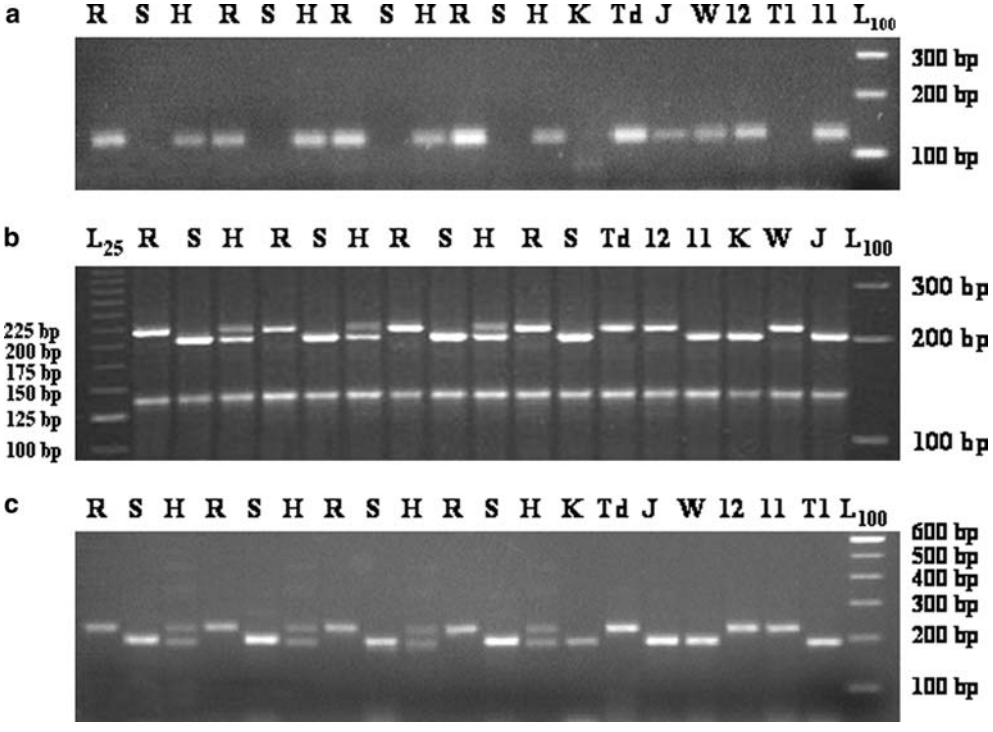

Fig. 2 DNA fragments amplified with SSR primers GWM33 (**a**), BARC263 (**b**), and CFA2153 (**c**). DNA samples were prepared from $F_{5:6}$ lines of "Karl 92/PI 94641// Jagger*2 /3/ Karl 92", PI 94641(Td), Karl 92(K), Jagger (J), and Tugela- *Dn1* (T1), 01-20-12 (12), 01-186-11 (11), and KS99WGRC42 (W). *R* Hf-resistant progeny, *S* Hf-susceptible progeny, *H* heterozygous progeny. L_{25} = 25-bp DNA ladder, L_{100} = 100-bp ladder

resistant progeny. Linkage analysis revealed that *Xgwm136* and *Xpsp2999* are distal to *Hdic* at distances of 2.4 and 2.0 cM, respectively.

To determine the physical locations of the linked SSR markers, DNA samples of CS, NT, Dt, and deletion lines of chromosome 1A were amplified by using SSR primer pairs GWM136, CFA2153, PSP2999, GWM33, and BARC263. Each primer pair amplified DNA fragments of the expected size(s) from CS and 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 markers are located distal to the breakpoint of 1AS-3 in the terminal 14% of the chromosome arm (Fig. 1b).

Size of the *T. dicoccum* derived chromosomal segments

To determine the size of the introduced chromosome segment in the derived wheat lines and to determine recombination breakpoints of the transfers, allele sizes for the five SSR markers linked to Hf-resistance were determined in resistant lines 01-20-12, 01-186-11, and KS99WGRC42, as well as in the donor PI 94641 and the recipients Karl 92 and Jagger. Two SSR markers (*Xwmc24* and *Xbarc148*) previously mapped proximal to *Xbarc263* (http://www.grs.nig.ac.jp/wheat/komugi/maps/markerMap.jsp?chromosome=1) and one SSR marker (*Xgdm33*) located distal to *Xgwm136* (Somers et al. 2004) were also included to determine the chromosomal breakpoints more accurately. In our physical mapping, marker *Xwmc24* was located in the same interval (1AS3-0.86-1.00) as the *Hdic* gene, whereas marker *Xbarc148* was located in the more proximal deletion interval 1AS1-0.47-0.86 (Fig. 1b).

Differences detected by these markers among the three homozygous resistant lines indicated that chromosomal segments in different sizes were transferred from PI 94641 in each case. Two recombination events took place during the transfer of *Hdic* from the donor to germplasm KS99WGRC42 (Fig. 1a). Only two markers, *Xgwm33* and *Xbarc263*, detected polymorphism between KS99WGRC42 and the susceptible recipients (Table 1). Alleles of common wheat parents Jagger and Karl92 were present for the markers distal to *Xgwm33* and the markers proximal to *Xbarc263*. A double crossover, or two separate recombination events, occurred at breakpoints between *Xcfa2153* and *Hdic*, as well as between *Xwmc24* and *Xbarc263*. These crossover events resulted in the transfer of a small intercalary fragment carrying the *Hdic* gene along with alleles of *Xgwm33* and *Xbarc263* from the donor parents to KS99WGRC42. In the line 01-20-12, a terminal fragment (distal to *Xwmc24*) containing *Hdic* was transferred from the donor into the recipient. Similarly, the terminal segment distal to *Xbarc263* in the wheat line 01-186-11 was derived from the donor parent (Table 1)

Discussion

The interaction between wheat and Hessian fly operates in a typical gene-for-gene model (Hatchett and Gallun 1970). Most of the 31 characterized resistance genes are dominant or incompletely (partially) dominant, the exceptions are the *H4* gene, which is recessive (Hatchett and Gallun 1970), while *H7* and *H8* exhibit complementary action (Amri et al. 1990; Ratcliffe and Hatchett 1997). The resistance conditioned by *H3*,*H5*, *H6*,*H9*, *H10*,*H11*, and *H31* was reported as incompletely dominant (Cox and Hatchett 1994; El Bouhssini et al. 1999; Williams et al. 2003). In the present study, we determined that inheritance of Hf-resistance in wheat derived from PI 94641 is due to a single major gene (*Hdic*). The observed inheritance of resistance was dominant in the F_2 population from a cross between resistant and susceptible emmer wheat lines. However, in a hexaploid wheat background, the *Hdic* gene did not exhibit a typical dominant or recessive action. Phenotypic variation (either completely resistant or fully susceptible) was observed for plants that were heterozygous at the resistance locus in both common wheat populations in the study. In each case, about 50% of heterozygotes were

Table 1 Polymorphisms of PCR amplified fragments (sizes in base pairs) among donor parent, recipient parents, and derived wheats

Primer Pair	PI94641	Karl92	Jagger	01-20-12	01-186-11	KS99WGRC42
GWM136	N[1]	320	320	N*[2]	N*	320
PSP2999	N	130	130	N*	N*	130
	140	140	140	140	140	140
	N	145	145	N*	N*	145
CFA2153	230	190	190	230*	230*	190
GWM33	130	N	130	130*	130*	130*
BARC263	220	200	200	220*	200	220*
WMC24	150	170	160	170	170	170
BARC148	215	205	205	205	205	205

"N" means no amplification or null allele

Asterisks (*) represent the PCR amplifications from the derived wheat lines are the same as those from the donor, but different from those amplified from the recipients. Polymorphic patterns in the PCR products between the recipient parents 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

resistant and 50% were susceptible to the Hf. This was not a typical incompletely dominant gene action in which heterozygotes have an intermediate phenotype. The variable phenotypes (either R or S) were only observed in heterozygous plants, and not in any homozygous individuals or lines. This indicated that the *Hdic* gene does not have full penetrance in heterozygotes, and that phenotype variation was not likely caused by the impurity in the tested Hf stocks.

The *Hdic* gene is the first Hf-resistance gene identified from emmer wheat. *Hdic* has a different biotype reaction than that reported for Hf-resistance genes *H5* (derived from common wheat), *H9*,*H10*, and *H11* (from durum wheat) which are also located on chromosome 1A (Stebbins et al. 1983; Roberts and Gallun 1984, Liu et al. 2005). The *H5* and *H11* genes confer resistance to biotype GP, but not to biotype L (Sosa 1978, 1981; Ratcliffe et al. 1996; Ratcliffe and Hatchett 1997). *H9* and *H10* confer resistance to biotypes GP and L (Ratcliffe and Hatchett 1997), but susceptibility to Hf strain v*H9* (Liu et al. unpubl. data). PI 94641 and the derived hexaploid lines in this study are resistant to biotypes GP and L, as well as to strains v*H9* and v*H13*. Accordingly, the *Hdic* gene may either represent a new locus that is different from all previously named genes or may be a novel allele of an existing H gene. Biotype L is the most virulent population identified so far and has been the predominant biotype in the eastern wheat growing regions in USA (Ratcliffe et al. 2000) The v*H13* strain of Hf is virulent to the most stable Hf-resistance gene *H13*. The broad spectrum of resistance conferred by *Hdic* makes it a promising gene for use in wheat breeding programs. In addition to biotypes GP and L and strains v*H9* and v*H13* that were tested, *Hdic* was also effective to field wild populations collected in 2002 from Idaho, Texas, Oregon, and Washington states (data not shown). In an attempt to isolate a biotype virulent to *Hdic*, KS99WGRC42 seedlings were infested with every Hessian fly populations we possessed, but no virulent insects were identified in several experiments. This indicated the frequency of virulent insects to *Hdic*, if any, was very low in the tested Hf stocks.

The markers identified in this study will facilitate the use of the *Hdic* gene in breeding programs. Since variation in reaction to the Hessian fly was observed in heterozygous progeny, accurate phenotypic selection for this gene needs to be delayed until lines are inbred. However, MAS for the resistance gene could be done on hybrid individuals and/or at early selfing generations. The flanking co-dominant SSR markers can determine the genotype at the resistance locus with a high degree of accuracy. For example, the two co-dominant markers *Xcfa2153* and *Xbarc263* are linked to the *Hdic* gene at 1.40 and 2.00 cM, respectively (Fig. 1a). The recombination frequency (RF) between *Xcfa2153* and *Hdic* is 1.38% (with a Kosambi map distance of 1.40 cM) (Kosambi 1944). The RF between *Xbarc263* and *Hdic* is 1.96% (with a map distance of 2.00 cM). These RFs for *Xcfa2153* and *Xbarc263* translate into selection accuracies of 98.62 and 98.04%, respectively, if they are used separately. The selection accuracy will increase to 99.97% when these two flanking markers are used together based on the product rule of probability. In addition to markers for MAS, the presence of only a small segment transferred from the emmer parent increases the usefulness of the germplasm for breeding programs, since this segment would be less likely to be detrimental to wheat quality and yield. The very small terminal and intercalary transfers and the high degree of polymorphisms between the emmer wheat and common wheat parents also provide effective tools to construct a fine map for the purpose of map-based cloning of the *Hdic* gene.

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