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I. Dweikat · H. Ohm · F. Patterson · S. Cambron

Identification of RAPD markers for 11 Hessian fly resistance genes in wheat

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Abstract The pyramiding of genes that confer race- or biotype-specific resistance has become increasingly attractive as a breeding strategy now that DNA-based marker-assisted selection is feasible. Our objective here was to identify DNA markers closely linked to genes in wheat (*Triticum aestivum* L.) that condition resistance to Hessian fly [*Mayetiola destructor* (Say)]. We used a set of near-isogenic wheat lines, each carrying a resistance gene at 1 of 11 loci (*H3*, *H5*, *H6*, *H9*, *H10*, *H11*, *H12*, *H13*, *H14*, *H16* or *H17*) and developed by backcrossing to the Hessian fly-susceptible wheat cultivar 'Newton'. Using genomic DNA of these 11 lines and 'Newton', we have identified 18 randomly amplified polymorphic DNA (RAPD) markers linked to the 11 resistance genes. Seven of these markers were identified by denaturing gradient gel electrophoresis and the others by agarose gel electrophoresis. We confirmed linkage to the Hessian fly resistance loci by cosegregation analysis in F_2 populations of 50–120 plants for each different gene. Several of the DNA markers were used to determine the presence/absence of specific Hessian fly resistance genes in resistant wheat lines that have 1 or possibly multiple genes for resistance. The use of RAPD markers presents a valuable strategy for selection of single and combined Hessian fly resistance genes in wheat improvement.

Key words Wheat · RAPD · Marker-assisted selection · Hessian fly

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I. Dweikat (✉) · H. Ohm · F. Patterson
Agronomy Department, 1150 Lilly Hall, Purdue University, West Lafayette, IN 47907, USA

S. Cambron
Entomology Department and USDA-ARS, Purdue University, West Lafayette, IN 47907, USA

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Introduction

Genes in wheat that confer resistance to the Hessian fly (*Mayetiola destructor*) provide the most efficient and economical means of crop protection against this damaging insect. To date, 26 resistance genes (*H1–H26*) in wheat have been reported (McIntosh 1988; Cox and Hatchett 1994). Among these, 8 loci (*H3*, *H6*, *H9*, *H10*, *H12*, *H15*, *H16* and *H17*) have been located on wheat chromosome 5A by means of monosomic and segregation analysis (Ohm et al. 1995), while genes *H5* and *H11* have been located on chromosome 1A and are separated by 4 m.u. (Roberts and Gallun 1984). A gene-for-gene interaction was demonstrated for host resistance and virulance loci in the insect (Hatchett and Gallun 1970). Subsequently, a number of cultivars, usually containing single genes for resistance, were deployed sequentially and widely grown, and the result has been a diminished effectiveness of resistance within 8–10 years (Foster et al. 1991). The loss in effectiveness of resistance genes apparently occurs with the evolution of virulent biotypes by mutation and/or selection pressure (Cox and Hatchett 1986; Foster et al. 1991; Gould 1986). Therefore, wise management of these resistance genes is important to maximize their longevity and effectiveness.

Random amplified polymorphic DNA (RAPD) analysis using near-isogenic lines has been one effective approach to identify DNA markers associated with important traits in several agronomic crops such as tomato (Martin et al. 1991), lettuce (Paran et al. 1991), oat (Penner et al. 1993) and common bean (Johnson et al. 1995). We have identified a RAPD marker tightly linked to gene *H9* that could be detected by means of denaturing gradient gel electrophoresis (DGGE) (Dweikat et al. 1994). Our objectives for this study were to identify RAPD markers associated with 11 Hessian fly resistance genes that are located in two linkage blocks on chromosomes 5A and 1A and to use these

markers to confirm, within a single line, the presence of 2 or more resistance genes that confer resistance against the same biotype of the insect. This genotypic confirmation has not previously been practical in the wheat-Hessian fly interaction because of the difficulties in detecting multiple genes by phenotypic testing.

Materials and methods

Plant materials

The plant materials used for this study consisted of a series of near-isogenic lines (NILs), each containing a different gene for Hessian fly resistance. All NILs were backcrossed four to eight cycles to 'Newton', a hard winter wheat susceptible to all known biotypes of Hessian fly. Among these NIL lines are those with genes *H3*, *H5*, *H6*, *H9*, *H10*, *H11*, *H12* or *H13* (Patterson et al. 1994), *H14*, *H16* or *H17* (Ohm et al. 1995). F_2 populations of 50–125 individuals each were developed from crosses between the susceptible parent ('Newton') and the various resistant lines, each containing an individual gene for resistance. Resistant lines, developed at Purdue University, include IN86981RC1-10-3 [parentage: 'Clark' (*H5H5*)// 'Auburn' (*H6H6*)*3/'Ella' (*H9H9*)] and IN85809A5-6 [parentage: 'Clark'//'Caldwell' (*H6H6*)*2//'Auburn' (*H6H6*)/KS10-9 (*H13H13*)]. KS10-9 was obtained from the Wheat Genetics Resource Center, Kansas State University, Manhattan, Kansas (Hatchett et al. 1981). Also used were the Purdue lines IN8268G1-19-49 [parentage: 'Auburn' (*H6H6*)/3/CI17647 (*H14H14*)/'Arthur'//IN72468C21-186-1 (*H5H5*)], IN9373 [IN861A1-38-50 (*H13H13*)//IN86982C2-11 (*H9H9*)/IN871327B1-25-2-4 (*H6H6*)] and line IN72468C21-186-1 ['Beau' (*H5H5*)/'Caldwell' (*H6H6*)].

DNA isolation

Wheat genomic DNA was isolated from 'Newton', the resistant NILs and the individuals in each F_2 population segregating for resistance. DNA was isolated from 100 mg of fresh leaf tissue using a sap extraction method. Leaves of 2-week-old seedlings were placed between the two rollers, and the extraction buffer was slowly added to the rollers, immediately mixing with the sap for collection in 1.5-ml microfuge tubes. The tubes were then placed in a 65°C water bath for 1 h, and the DNA was extracted as previously described (Dweikat 1994).

PCR and gel electrophoresis

Oligonucleotide primers (10-mers) were purchased from Operon Technologies (Alameda, Calif.) and the University of British Columbia (Vancouver, Canada). Polymerase chain reaction (PCR) conditions were as described by Williams et al. (1990). The amplified PCR products were fractionated either on 1.2% agarose gels in 0.5 × TBE buffer or by DGGE in 12% polyacrylamide with a denaturant gradient of 10–50% (Dweikat et al. 1994). Gels were stained in ethidium bromide (1 μg/ml 0.5 × TBE buffer) for 30 min, destained in water for 1 h and photographed over a UV light source.

Screening for resistance

The F_2 plants from which the DNA was extracted were self-pollinated to produce F_3 seeds. Resistance in F_3 families to Hessian fly biotypes C (*H3*), B (*H5*, *H6*, *H10*, *H11* and *H12*), or biotype *L* (*H9*, *H13*, *H14*, *H16* and *H17*) was evaluated as described by Ohm et al. (1995). F_3 seedlings (14–15 per $F_{2:3}$ family) were germinated in wooden flats. Nine progeny rows of 15 seeds per row and two half-rows of 10 seeds of parental lines (controls) were planted per flat. One week after germination, seedlings were infested with the appropriate fly biotype. Three weeks after infestations, progeny rows were classified as resistant, susceptible, or segregating.

Results

Efficiency of marker identification

We used genomic DNA extracted from ten individual near-isogenic lines, each containing a different gene for Hessian fly resistance, and the susceptible line 'Newton' to screen 1600 random 10-mer primers. At least 1 RAPD marker was identified in association with each gene, with

Table 1 RAPD primer sequences producing polymorphisms that demonstrate genetic linkage to Hessian fly resistance genes

Gene symbol	Primer I.D.	Sequence	Fragment approximate size (bp) resolved by	Number of F_2s	Number of recombinants
H3	3-1	TTCATACGCG	520, DGGE	60	0
H5	5-1	CGCATTTGCA	520, Agarose	110	0
	5-2	CGAATTCGGC	800, DGGE	110	0
H6	6-1	GTTTCGCTCC	900, Agarose	125	0
	6-2	GAATGGTGAG	800, Agarose	125	0
	6-3	GCGAATTCCG	660, DGGE	125	0
H9	9-1	GGGTAACGCC + GACCGCTTGF	220, DGGE	124	0
	9-2	CCCAGTCACT	1000, Agarose	124	0
H10	10-1	GCTGGACATC	510, DGGE	80	0
	10-2	TGGTCGCAGA	490, Agarose	80	1
H11	11-1	GTAGACGAGC	1090, Agarose	120	1
	11-2	TTCAGGGTGG	980, Agarose	120	1
H12	12-1	CTACCCGTGC	1110, Agarose	50	0
	12-2	ACGCCAGTTC	610, DGGE	50	1
H13	13-1	TTGCTGGGCG	920, Agarose	63	0
H14	14-1	TTCCCCGTCG	1000, Agarose	80	0
H16	16-1	TGCCCAGCCT	850, Agarose	72	0
H17	17-1	CGCTGCAGAC	400, DGGE	80	0

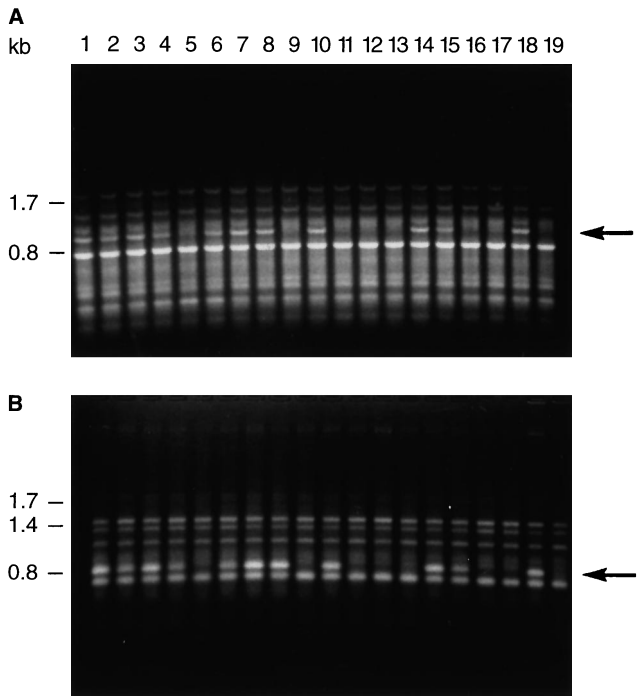

Fig. 1 Genomic DNA extracted from a F_2 population (*lanes 1–17*) segregating for the presence of resistance gene *H6*. The population was screened with 2 different RAPD primers – 6-1 **(A)** and 6-2 **(B)** – that produce polymorphisms linked to the *H6* allele. The F_2 population was derived from 'Newton' × 'Newton'-*H6* NIL. *Lanes 18* and *19* contain the resistant and susceptible parental lines, respectively. *Arrows* indicate the cosegregating DNA amplification product

2 or 3 markers for some genes, providing 18 markers for the 11 genes.The same 1600 primers were used each time to screen for each individual gene separately. It was necessary to screen, on average, approximately 900 primers for each marker identified. The markers were visualized on agarose gels or by DGGE (Table 1), and all identified markers demonstrated coupling phase linkage to the targeted resistance loci. Genetic linkage between the marker(s) and the respective gene was confirmed by screening F_2 populations of 50–125 plants segregating for each individual gene. Figure 1 shows an example of linkage between the gene *H6* and 2 markers in a segregating F_2 population. The majority of the markers demonstrated linkage to the target locus with few or no recombinants (Table 1). Of the 1600 primers screened, only 2 "false-positive" polymorphisms arose; these were polymorphisms that subsequently demonstrated independent segregation relative to the target resistance locus.

Usefulness of markers for breeding applications

We have evaluated the usefulness of some of these markers for marker-assisted selection. DNA markers linked to Hessian fly resistance gene *H6* were tested on eight cultivars, six of which contain the resistance gene. All the resistant cultivars demonstrated the presence of both *H6* markers, 6-1 and 6-2, and the susceptible cultivars lacked the markers (Fig. 2).

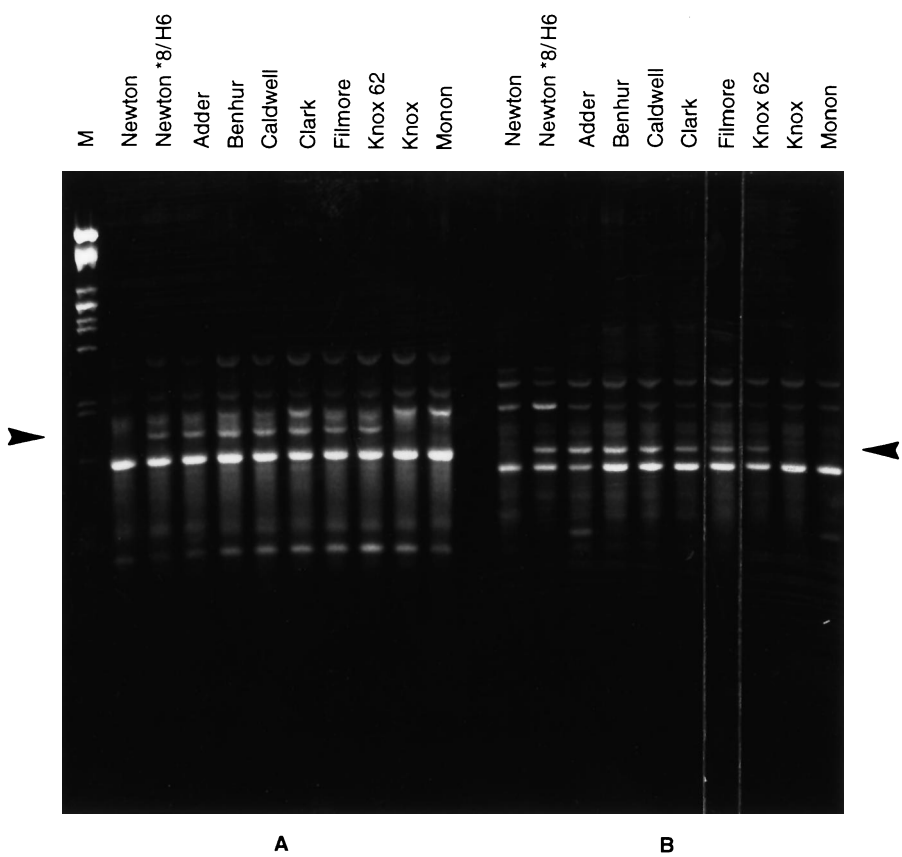

Fig. 2A, B Test for effect of different genetic backgrounds on the detection of DNA markers associated with resistance gene *H6*. Products were separated on agarose gels. **A** 'Newton', 'Knox' and 'Monon' do not contain the *H6* allele, while 'Newton'-*H6* NIL, 'Adder', 'Benhur', 'Caldwell', 'Clark', 'Fillmore' and 'Knox 62' contain the *H6* gene. Note the presence of the *H6*-associated polymorphic fragment in all lines containing *H6* as a result of using primer 6-1 **B** Same plant lines indicated in **panel A** screened with primer 6-2 that produces an *H6*-linked DNA polymorphism. Again, note the presence of the *H6*-associated polymorphism in all lines that contain the *H6* gene

To test the feasibility of using the identified markers to facilitate gene pyramiding, we intercrossed the NILs (*H5* × *H11*) and (*H9* × *H16*). Genomic DNAs were extracted from the F_1 plants presumed to contain 2 resistance genes, and the primers that produce associated polymorphisms were used singly or in combination. As shown in Fig. 3, the F_1 plants that contain genes *H5* and *H11* in combination produced corresponding PCR-amplified markers for both genes. Also shown are similar results obtained when RAPD markers for *H9* and *H16* were used to test for the presence of both genes in the F_1 plants. In most cases, it was necessary to assay for the presence of each gene singly because the combination of primers in one reaction produced an altogether distinct profile (see Fig. 3). Two released cultivars and four advanced wheat lines were used to further test the efficiency of marker-based gene detection. The presence or absence of resistance loci, estimated on the basis of breeding pedigree, was in most cases confirmed by correspondencing DNA marker data (Table 2).

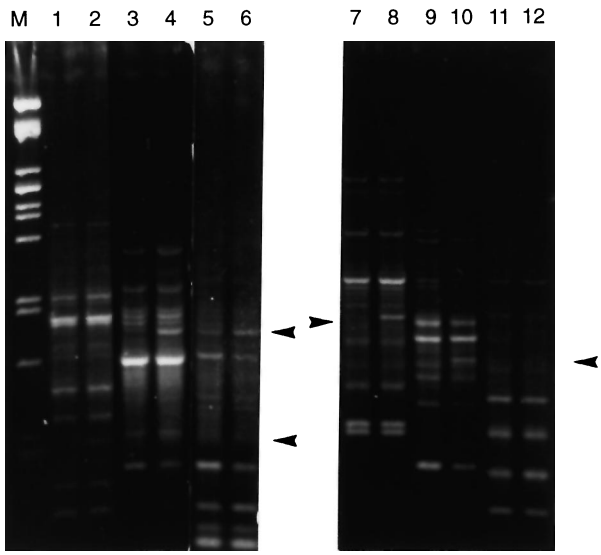

Fig. 3 Amplification of genomic DNA isolated from F_1 plants containing two resistance genes in combination. *Lanes 1*, *3*, *7*, *9* and *11* contain samples amplified from 'Newton', *lanes 2*, *4* and *6* contain samples amplified from an F_1 plant containing genes *H5* and *H11* in combination. Primer 5-1 was used in *lanes 1* and *2*, primer 11-1 in *lanes 3* and *4* and the combination of both primers was used in *lanes 5* and *6*. *Lanes 8*, *10* and *12* contain samples amplified from an F_1 plant that contains genes *H9* and *H16*. Primer 9-2 was used in *lanes 7* and *8*, primer 16-1 for *lanes 9* and *10*, and the combination of both primers was used in *lanes 11* and *12*. *Arrows* indicate the relevant polymorphic bands. *Lane M* contains *Pst*I-digested λ DNA for molecular-weight estimation

Discussion

The development of near-isogenic lines greatly facilitated this study by enhancing the efficiency of RAPD primer screening. Markers linked to 11 different Hessian fly resistance loci were identified with concurrent screening of all the NILs for polymorphism. The rapidity with which multiple markers, tightly linked to these loci, were obtained greatly encourages our current efforts toward high-resolution mapping of several of these loci.

Our emphasis in this study was to identify RAPD markers that would facilitate breeding efforts in the development of lines displaying more durable resistance to the Hessian fly. Toward this aim we have demonstrated that the markers identified in this study not only show reasonably tight linkage with resistance loci but also allow discrimination between breeding lines with and without the corresponding resistance gene. An important, and somewhat surprising, feature of the markers is that they all proved to be allele-specific. Consequently, the markers appeared only in lines containing the corresponding resistance gene but never appeared in lines devoid of the locus of interest. This high degree of marker : locus correspondence allowed for unambiguous genotypic classification. Furthermore, this correspondence permitted the detection of more than 1 resistance gene in combination, which is especially useful for those that confer resistance against the same biotype(e.g. *H5* and *H11* confer resistance to biotype B). This has not been possible using a standard biotype infestation procedure. This observation implies that gene pyramiding will now be feasible with the assistance of DNA markers. The pyramiding of

Table 2 Hessian fly resistance genes assumed to be contained within tested wheat lines and cultivars based on pedigree (*S* susceptible, *R* resistant)

Cultivar/line	Putative genes by parentage	Genes based on RAPD analysis	Reaction to biotype		
			L	D	B
Grant	H5, H6	*H5*[a], *H6*	S	R	R
Patterson	H5, H6	*H5*, *H6*	S	R	R
86981RC1-10-3	H9, H5, H6	*H9*, *H5*, *H6*	R	R	R
85809A5-6	H13, H5, H6	*H13*, *H5*	R	R	R
8268G1-19-49	H14, H5, H6	*H14*, *H5*	R	R	R
9373G1-1	H13, H9, H6	*H13*, *H9*, *H6*	R	R	R

[a] Genes *H5* and *H6* do not provide resistance to biotype L. *H5* confers resistance to biotypes B and D; *H6* confers resistance to biotype B. Genes *H9*, *H13* and *H14* confer resistance to biotypes B, D and L

multiple Hessian fly resistance loci may provide one strategy for enhancing the durability of resistance in wheat lines.

By virtue of the efficiency with which we were able to identify multiple markers per locus, the strategy outlined here will assist our efforts toward the ordering of resistance loci within a linkage group (several of the described resistance loci reside on chromosome 5A Ohm et al. 1995) and the identification of rare recombinations in proximity to target loci required for their physical localization.

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