Theor Appl Genet (2002) 105:766–770
DOI 10.1007/s00122-002-0946-9

I. Dweikat · W. Zhang · H. Ohm

Development of STS markers linked to Hessian fly resistance gene *H6* in wheat

Received: 20 April 2001 / Accepted: 10 January 2002 / Published online: 19 June 2002

Abstract Hessian fly is one of the world's most destructive insect pests of wheat *Triticum aestivum* L. We have used the combination of near-isogenic lines (NIL) and random amplified polymorphic DNA (RAPD) analysis to screen up to 2,000 primers to identify DNA markers that are linked to gene *H6* that confers resistance to biotype B of the insect. This screen produced six primers that show polymorphic fragments associated with resistance by *H6*. We have screened 440 F_2 individuals from a cross of the susceptible cultivar Newton and a NIL that contains *H6* to verify the linkage between these markers and the resistance gene. A high-resolution genetic map was constructed based on recombination frequency. Two of the markers were tightly linked to the gene with no recombination observed, three were within 2.0 cM, and one was 11 cM from the gene. Three of the six markers were successfully converted to sequence tagged site (STS) markers. Both RAPD and STS primers were used to screen for the presence or absence of the resistance gene in wheat varieties. The identification of markers and construction of the genetic high resolution map provide the first steps toward localization of this resistance gene.

Keywords RAPD · *Triticum aestivum* L. · Hessian fly · STS

Communicated by A.L. Kahler

I. Dweikat (✉)
Department of Agronomy and Horticulture,
University of Nebraska, Lincoln, NE 68583 USA
e-mail: idweikat2@unl.edu
Fax: +1-402-472-7904

W. Zhang · H. Ohm
Department of Agronomy, Purdue University, West Lafayette,
IN 47907 USA

Introduction

Hessian fly (*Mayetiola destructor* Say) is a serious pest of wheat, spreading to almost all major wheat-growing areas. Losses due to Hessian fly infestation have been reduced significantly by the use of wheat cultivars that contain resistance genes to the fly and by planting late enough to avoid infestation. A gene-for-gene relationship has been demonstrated for host resistance and avirulence in the insect (Hatchett and Gallun 1970). To-date, 27 resistance genes designated *H1* to *H27* (Mclntosh 1988; Cox and Hatchet 1994; Ohm et al. 1997) have been effective against the 16 reported biotypes of the insect (designated GP and A to O) that have been identified (Gallun 1977). The resistance phenotype of gene *H6* is dominant (Gallun and Patterson 1977).

Singly deployed Hessian fly (H. fly) resistance genes have been effective for a period of 6–8 years until virulence becomes prevalent. One strategy for lengthening the life of each gene is the recycling of these genes. Foster et al. (1991) reported on the effectiveness of the redeployment strategy for several H. fly resistance genes. Gene *H6* was derived from durum wheat (Allen 1959) and deployed in several wheat cultivars. A reduction in infestation level of H. fly occurred for up to 8 years. Redeployment of *H6*, after a 10-year absence from commercial use, was highly effective (Foster et al. 1991).

Screening for resistance to the H. fly requires maintenance of the H. fly biotypes, a 3-week period post-infestation for symptom screening, prior incubation conditions, and controlled environment and containment facilities. In some cases, it is difficult to determine the identity of a resistance gene using the standard screening method, because many genes confer resistance to the same biotype of the fly. Consequently, a more rapid, cost-efficient and reliable screening procedure would greatly facilitate the selection process. In addition, a DNA-based screen for H. fly resistance would allow the pyramiding of multiple resistance genes, one possible means of enhancing the durability of resistance.

The application of random amplified polymorphic DNA (RAPD) analysis (Williams et al. 1990) has proven useful in the development of DNA markers that are linked to disease and insect resistance in several crops. The combination of near-isogenic lines and RAPD analysis was used effectively in tomato (Martin et al. 1991), lettuce (Paran et al. 1991), oat (Penner et al. 1993), common bean (Haley et al. 1993) and wheat (Dweikat et al. 1994) for the purpose of identifying DNA markers for disease and insect resistance genes. However, marker-assisted selection with RAPDs is not always feasible because RAPD markers identified in one population are not always useful for another, and some markers lack reliability. To address these problems, it is useful to convert a RAPD polymorphic fragment to a sequence tagged site (STS, Olson et al. 1989), in which the DNA sequence of the polymorphic fragment is determined and used to design DNA primers that will consistently discriminate between lines and serve as a marker for a unique region of the genome. Such STSs are relatively easy to develop from DNA fragments identified by arbitrarily primed PCR, though they do not always result in a detectable polymorphism. STSs, as specific PCR products, allow for reproducibility of the technique across a relatively wide range of reaction conditions in different laboratories. As allele-specific markers they are useful for marker-assisted selection within segregating populations. The objective of this study was to identify several RAPD markers that are linked to the gene *H6*, construct a high-resolution genetic map encompassing *H6*, convert the RAPD markers to STS markers, and test the STS markers for their usefulness in marker-assisted selection.

Materials and methods

Plant materials

The plant materials used for this study consisted of the *Triticum aestivum* L. cultivar Newton, susceptible to all known H. fly biotypes, the near-isogenic line (NIL) Newton*8/*H6*, six released cultivars containing the gene *H6* (Adder, Benhur, Caldwell, Clark, Filmore and Knox 62) and two cultivars containing the gene *H3* but not *H6* (Knox and Monon). An F2 population of 440 individuals was derived from the cross Newton × Newton*8/*H6.*

DNA isolation, PCR reactions, gel electrophoresis

DNA was isolated from about 100 mg of fresh tissue from 2-week-old seedlings of Newton, Newton*8/*H6* and all the cultivars mentioned above, and from the F_2 individuals. DNA was extracted using the Leaf Squeezer (Ravenel Specialties, Inc., Seneca, S.C.). Leaves were placed between the two rollers, and the extraction buffer was immediately added to the roller to allow mixing with the extracted 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 et al. 1994).

Oligonucleotide primers (10-mers) were purchased from Operon Technologies (Alameda, Calif.), and the University of British Colombia (Vancouver, Canada). Polymerase chain reaction (PCR) conditions were as described by Williams et al. (1990). The amplified products were fractionated on 1.2% agarose gels in 0.5 × TBE buffer. Gels were stained in ethidium bromide, photographed over a UV light-source and scored for presence or absence of a particular polymorphism.

Screening for resistance

The F_2 plants from which DNA was isolated were allowed to self-pollinate to produce F_3 seeds. Resistance in F_3 families to Hessian fly biotype B was evaluated as described by Ohm et al. (1995). F_3 seedlings (20 per $F_{2:3}$ family) and all cultivars used for this study were germinated in wooden flats. One week after germination, seedlings were infested with biotype B of H. fly. About 3 weeks after infestation, progeny rows were classified as resistant, segregating, or susceptible.

STS design and analysis

The amplification products of the six selected RAPD primers were fractionated in 1.2% agarose and the polymorphic bands linked in coupling to the resistance phenotype were excised, eluted, re-amplified under the same conditions, and cloned using the TA Cloning Kit (Invitrogen Corp., San Diego, Calif.). The identity of the cloned DNA fragment was checked by hybridization to DNA gel blots of RAPD amplification products from F_2 individuals that segregated for the corresponding RAPD. Double-stranded sequencing of the cloned fragments was performed by the dideoxy termination method (SequiTherm Long–Read Kit, Epicentre Technologies, Madison, Wis.) with M13 forward and reverse primers. A pair of 20–25-bp oligonucleotides, defining a STS for each of the cloned fragments, was then designed using OLIGO 5.0 software (Rychlik et al. 1990). Primers were used to amplify the specific marker under the following conditions: the 25-µl reaction volume contained 10 mM of Tris–HCL (pH 8.9), 1.8 mM of $MgCl_2$, 50 mM of KCl, 300 µm of each dNTP, 0.2 µM of each primer, 1 u of *Taq* DNA polymerase (Promega), and 25 ng of genomic DNA. Reactions were preformed in a MJR PTC-100 thermocycler (MJ Research, San Francisco, Calif.) programmed for 2 min at 94 °C, then 30 cycles of 35 s at 94 °C, 1 min at 55 °C and 1 min at 72 °C, followed by a final elongation for 7 min at 72 °C.

Linkage analysis

The linkage map was constructed with MAPMAKER V2.0 (Hunt 1997) with LOD = 3.0 and recombination fraction (0) = 0.40. All markers were first identified using the "Group" command, and then verified by scanning two-point linkage data. An approximate order for the linkage group was obtained by using the "Multipoint, First order" command. The order was checked using the "Multipoint, Ripple" command. The maximum-likelihood map for each specified order of the markers was computed by Mapmaker using the "Multipoint, Compare" command. "Multipoint, Try" command was used to determine the exact position of the gene. Map units (cM) were derived using the Kosambi function (Kosambi 1944).

Results

A total of 2,000 random 10-mers were PCR-screened with genomic DNA isolated from the susceptible cultivar Newton and its NIL Newton*8/*H6*. Of the 2,000 primers, six (Op B01, Op AF 08, Op S15, UBC 511, UBC 691 and E1H6) produced polymorphic fragments present in the NIL that contains the resistance gene *H6* and absent from Newton as shown in Fig. 1. All the markers were scored as dominant loci. The resultant PCR products were fractionated with standard agarose-gel electro-

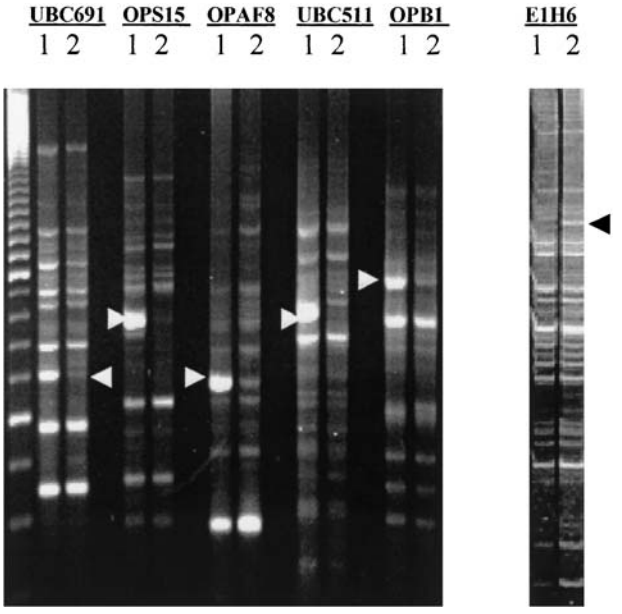

Fig. 1 PCR amplification products using RAPD primers to identify markers linked to *H6*. The polymorphisms linked to the *H6* gene are marked by *arrows*. *First lane* 100-bp molecular-weight ladder. The *first lane* for each pair used Newton DNA as a template and the *second lane* used Newton*8/*H6*. Primers used were, from left to right, OP B01, UBC 511, OP AF08, OP S15 and UBC 691. Reaction products using primer E1H6 were fractionated on a denaturing gradient gel (Dweikat et al. 1993)

phoresis except for those derived from the primer E1H6, where denaturing gradient gel-electrophoresis (Dweikat et al. 1993) was used to resolve the polymorphism (Fig. 1).

To verify the genetic linkage between the polymorphism and the *H6* resistance gene, each individual primer was then used to screen a total of 440 F_2 individuals produced from the cross Newton × Newton*8/*H6*. The F_2 plant classifications for resistance were verified by testing 20 F_3 progenies from each individual F_2 plant against Hessian fly biotype B. The six identified markers co-segregated with the *H6* resistance gene, demonstrating linkage in coupling phase. Out of 440 F_2 individuals,

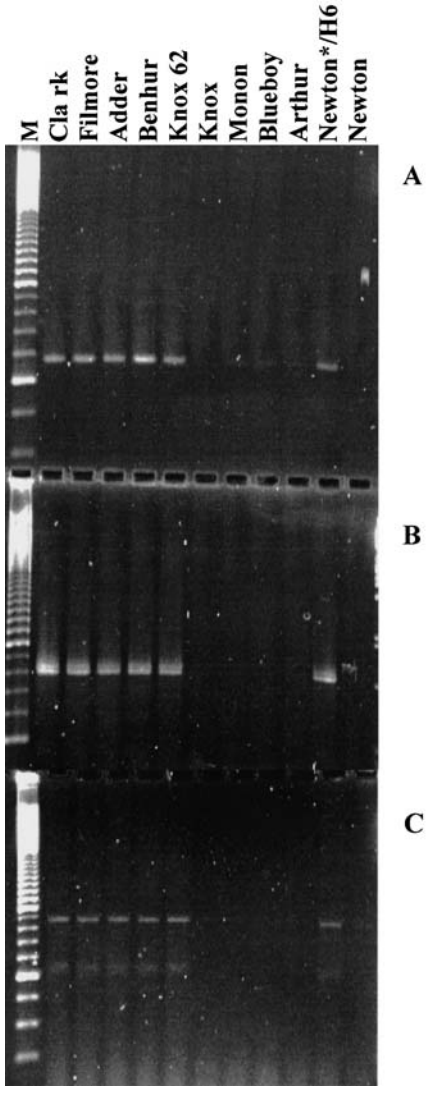

Fig. 2A–C STS markers associated with resistance gene *H6* on different genetic backgrounds. **A** Newton, Arthur, Blueboy, Monon and Knox do not contain the *H6* gene, while Newton*8/*H6*, Knox 62, Benhur, Adder, Filmore and Clark contain the *H6* gene. Note the presence of the *H6*-associated polymorphic fragment in all lines containing *H6* using STS AF08. **B, C** Same lines indicated in panel **A** screened with STS 511 and STS E1H6, respectively. *M* designates a 100-bp molecular-weight ladder

Table 1 Sequence of oligonucleotide primers for each STS locus derived from RAPD markers linked to Hessian fly resistance gene *H6*

Locus	Primer	STS sequence (5′–3′)[a]	Size (bp)	Phenotype
H6 F8	OP AF08	CTCTGCCTGATTGAAGACATCG CTCTGCCTGACCTTATTACTTT	545	Polymorphic
H6 S15	OPS 15	CAGTTCACGGGGAGCAATGGT CAGTTCACGGGCCTCTTGTGAAGC	782	Monomorphic
H6 511	UBC 511	GA ATGGTGAGACGGAGGGAAT GAATGGTGAGATGGTGAGGCAG	807	Polymorphic
H6 E01	E1H6	GCGAATTCCGGTAATAGCTTGT GCGAATTCCGAACAAGATGAA	864	Polymorphic
H6 B01	OP B01	GTTTCGCTCC TGTGTGGTCCG GTTTCGCTCCCCCTCTATAAATA	968	Monomorphic
H6 691	UBC 691	AAACCAGGCGAGGTGACGAC AAACCAGGCGCCAGCATGGAAAA	610	Monomorphic

[a] The underlined sequences indicate the progenitor RAPD primers

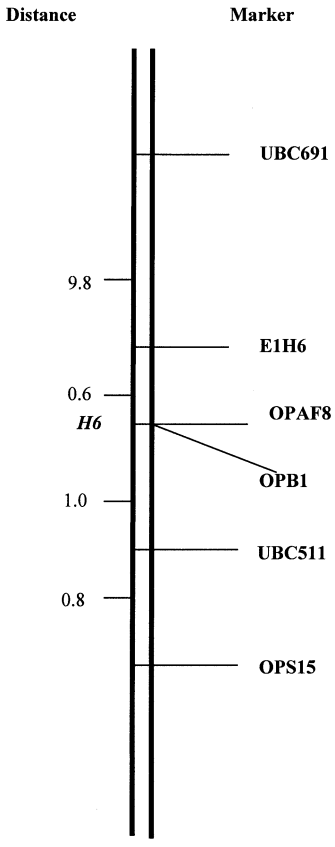

Fig. 3 Linkage group showing the *H6* Locus (resistance to Hessian fly) and linked markers. Gene distance and markers order were based on the maximum-likelihood estimation as determined with MAPMAKER software

no recombinants were observed between OP B01 and *H6* or between OP AF08 and *H6,* which indicates tight linkage of these two markers to the gene *H6*. Two recombinants were detected between UBC 511 and gene *H6*, two between OP S15 and *H6*, three recombinants between E1H6 and *H6*, and 27 recombinants between UBC 691 and the gene *H6* as shown in Fig. 2. The amplified products of six RAPDs linked to gene *H6* were cloned, sequenced and converted to STS markers by extending the original decamer primer sequence at both ends of the polymormphic fragment (Table 1). Sizes of the cloned polymorphic fragments ranged between 500 and 943 bp (Table 1). The identities of the cloned products were verified by hybridization of the cloned fragments to Southern blots of individuals that segregated for the particular RAPD.

Genomic DNAs from the parents of the mapping population, Newton and Newton*8/*H6*, were used as a template for PCR amplification with each pair of STS primers. Of the six STS markers, three (OPAF08, UBC 511 and E1H6) were polymorphic and co-segregated with the gene *H6*, while the three STS markers produced monomorphic bands. The alternative monomorphic fragments amplified from the susceptible parent resulting from UBC 691, OP B01 and OP S15 were cloned and sequenced to identify any sequence variations that exist between the sequence that derived from the resistant and the susceptible parent. Based on the sequence analysis, one difference was observed between the two fragments. The three monomorphic STS primers are still informative and mapped as RAPD markers.

Applicability of the RAPD markers and three corresponding STS markers was tested on nine wheat cultivars with different genetic backgrounds. Of these wheat genotypes, five have been reported to contain the gene *H6* (Adder, Benhur, Caldwell, Clark and Knox 62). All of the RAPD primers except UBC 691 were useful in detecting the presence of gene *H6* in the wheat cultivars possessing the resistance gene (data not shown). Likewise, three STS markers, STS 511, STS AF08 and STS E1H6, revealed a polymorphic fragment associated with resistance as shown in Fig. 3.

Discussion

In this study, we used a combination of NIL and RAPD analysis to identify markers linked to Hessian fly resistance in wheat. This approach has been used successfully in a number of studies to identify markers linked to important agronomic traits (Martin et al. 1991; Paran et al. 1991; Haley et al. 1993; Penner et al. 1993; Dweikat et al. 1997; Hu et al. 1997; Laroche et al. 2000). Because of the ability to quickly assay a large number of potential markers with RAPD primers, we were able to screen up to 2,000 random primers. In combination with DNA isolated from susceptible Newton and a resistance NIL containing gene *H6*, six primers produced polymorphic fragments that were only present in the resistant genotypes. Five of these markers were closely linked to the gene at less than 2.0 cM distance. Only one primer, UBC 691, was over 10 cM distant from *H6*. The first five markers are, therefore, ideal for marker-assisted selection since generally markers with a distance of less than 10 cM from the target locus are suitable for this purpose.

The tightly linked markers will provide a means of detecting *H6* presence in developed lines. This resolution is essential for gene pyramiding. Gene pyramiding is a practical goal in the case of Hessian fly in wheat because any particular gene is only effective for a period of 8–10 years when deployed singly. The efficacy of pyramiding for enhancing the durability of resistance has not been shown empirically for H. fly resistance yet, but a marker-based strategy should facilitate such testing.

Gene *H6* resides about 2.0 cM from *H9* on chromosome 5A. Interestingly, a recent study (Schulte et al. 1999) reported that the avirulence loci in the Hessian fly that correspond to resistance genes *H6* and *H9* are linked within discrete physical limits on Hessian fly chromosome XI. This apparent corresponding linkage in host and parasite represents an interesting avenue for investigating the co-evolution of this host-pest relationship and its changes in the field over time. Such corresponding linkage may be indicative of local gene duplication pro-

cesses in both host and parasite in response to selection pressures. We are beginning the process of physically localizing the *H6* gene on chromosome 5A using a set of deletion lines (Endo and Gill 1996).

One disadvantage of the RAPD technique is its sensitivity to PCR conditions. This shortcoming can be offset by converting polymorphic RAPD markers to stable and reproducible STS markers for mapping purposes (Paran and Michelmore 1993). When RAPD markers OP AF08, UBC 511 and E1 H6 were converted to STS markers they each produced fragments present in only the resistant F_2 individuals and cultivars that contained gene *H6*. Consequently, although conversion from RAPD to STS markers is not 100% efficient, the benefits of STS markers in minimizing laboratory to laboratory variation render them an appropriate alternative even in the large genome of wheat.

The identification of at least two markers encompassing the *H6* gene with a linkage of less than 1 m.u. should provide an effective strategy for *H6* selection. The availability of additional markers allows reduction of linkage drag and facilitates effective gene intogression

References

Allen RE, Heynen EG, Jones ET, Johnston CO (1959) Genetic analysis of ten sources of Hessian fly resistance, their interrelationships and association with leaf rust reaction in wheat. Kansas Agric Exp Station and USDA-ARS Tech Bull 104:51–108

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

Dweikat I, Mackenzie S, Levy M, Ohm H (1993) Pedigree assessment using PAPD-DGGE in cereal species. Theor Appl Genet 85:497–505

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

Endo T, Gill B (1996) The deletion stocks of wheat. J Hered 87:295–307

Foster JE, Ohm H, Patterson F, Taylor P (1991) Effectiveness of deploying single gene resistance in wheat for controlling damage by the Hessian fly (Diptera: *Cecidomyiidae*). Environ Entomol 20:964–969

Gallun RL (1977) Genetic basis of Hessian fly epidemics. Ann N Y Acad Sci 287:223–229

Gallun RL, Patterson FL (1977) Monosomic analysis of wheat for resistance to Hessian fly. J Hered 68:223–236

Haley SD, Miklas PN, Stavely JR, Byrum J, Kelly JD (1993) Identification of RAPD markers linked to a major rust resistance gene block in common bean. Theor Appl Genet 86:505–512

Hatchett JH, Gallun RL (1970) Genetics of the ability of the Hessian fly, *Mayetiola destructor*, to survive on wheat having different genes for resistance. Ann Ent Soc Amer 63:1400–1407

Hu XY, Ohm HW, Dweikat I (1997) Identification of RAPD markers linked to the gene *Pm*1 for resistance to powdery mildew in wheat. Theor Appl Genet 94:832–840

Hunt GJ (1997) Construction of linkage maps with RAPD markers. In: Micheli MR, Bora R (eds) Fingerprinting method based on arbitrarily primed PCR. Springer, New York, pp 187

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

Laroche A, Demeke T, Gaudet DA, Puchalski B, Frick M, Makenzie R (2000) Development of a PCR marker for rapid identification of the *Bt*-10 gene for common bunt resistance in wheat. Genome 43:217–223

Martin GB, Williams JGK, Tanksley SD (1991) Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA 88:2336–2340

Mclntosh RA (1988) Catalogue of gene symbols for wheat. In: Miller TE, Koebner RMD (eds) Proc 7th Intl Wheat Genet Symp, Bath Press, Bath, UK, pp 1225–1323

Ohm HW, Sharma HC, Patterson FL, Ratcliffe RH, Obanni M (1995) Linkage relationships among genes on wheat chromosome 5A that condition resistance to Hessian fly. Crop Sci 35:1603–1607

Ohm HW, Ratcliffe RH, Patterson FL, Cambron S (1997) Resistance to Hessian fly conditioned by gene *H19* and proposed gene *H27* of durum wheat line PI422297. Crop Sci 37:113–115

Olson M, Hood L, Cantor C, Dotstein D (1988) A common language for physical mapping of the human genome. Science 254:1434–1435

Paran I, Kesseli R, Michelmore RW (1991) Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near-isogenic lines. Genome 34:1021–1027

Paran MG, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew gene in lettuce. Theor Appl Genet 85:985–993

Penner GA, Chong J, Levesque-Lemay M, Molner SJ, Fedak G (1993) Identification of a RAPD marker linked to the oat stem rust gene *Pg*3. Theor Appl Genet 85:702–703

Rychlick MR, Spencer WJ, Rhoads RE (1990) Optimization of the annealing temperature for DNA amplification in vitro. Nucleic Acids Res 18:6409–6412

Schulte SJ, Rider SD, Hatchett JH, Stuart JJ (1999) Molecular genetic mapping of three X-linked avirulance genes vH6, vH9, vH13, in the Hessian fly. Genome 42:821–828

Williams J, Kubelik A, Livak K, Rafalski J, Tingey S (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535