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ORIGINAL PAPER

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Molecular markers linked to papaya ring spot virus resistance and *Fusarium* race 2 resistance in melon

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Abstract In melon, the *Fom-1* gene confers monogenic resistance against the soil-borne fungus *Fusarium oxysporum* f. sp. *melonis*, races 0 and 2, while the closely linked *Prv* gene specifies resistance against the papaya ring spot virus. Markers linked to these resistance (R) genes were identified using two recombinant inbred line populations, derived from crosses between *Cucumis melo* Védrantais and *C. melo* PI 161375, and between *C. melo* Védrantais and *C. melo* PI 414723, respectively. Using bulked segregant analysis, as well as systematic scoring of the mapping populations, we developed two amplified fragment length polymorphism markers, two random amplified polymorphic DNA markers and five restriction fragment length polymorphism (RFLP) markers linked to this locus. Four of the RFLP sequences bear homology to nucleotide-binding site–leucine-rich repeat R genes, indicating the presence of a significant R-gene cluster in this locus. Our study provides the most closely linked markers published so far for these important traits. It also improves the resolution of the whole linkage group IX, which was difficult to order in our previous studies. Two of the markers were converted to cleaved amplified polymorphic sequence markers to facilitate their application in marker-assisted selection. Testing these two markers in several melon lines revealed different marker haplotypes in the melon germplasm and supported multiple, independent origin of the *Fusarium* races 0 and 2 resistance trait.

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Introduction

The soil-borne pathogen, *Fusarium oxysporum* f. sp. *melonis*, exclusively attacks melon (*Cucumis melo* L.), causing a severe wilt disease. Four races of the fungus, designated races 0, 1, 2, and 1.2, have been characterized (Risser et al. 1976). Melon genotypes that are resistant to the different races have been identified, and monogenic dominant inheritance was described against races 0, 1, and 2. The *Fom-1* gene, originally identified in cultivar Doublon, confers resistance to races 0 and 2 (Risser and Mas 1965). Another gene, *Fom-3*, controls resistance to races 0 and 2 in cultivar Perlita FR, but data about its possible allelism with *Fom*-1 are controversial (Zink and Gubler 1985; Risser 1987; Danin-Poleg et al. 1999). The *Fom-2* gene, originally described in accession CM 17187, is present in both PI 414723 and PI 161375. It confers resistance to races 0 and 1 (Risser 1973; Risser et al. 1976).

Potyviruses such as the zucchini yellow mosaic virus and the papaya ring spot virus (PRSV) form the largest and economically most important group of plant viruses (Riechmann et al. 1992). PRSV strains were divided into two biotypes whose virions were indistinguishable by serological tests, but differed in their ability to infect papaya (*Carica papaya*, Purcifull et al. 1984). PRSV-P naturally infects papaya and can be transmitted experimentally to cucurbits, but is seldom found in field-grown cucurbits (Gonsalves 1998). PRSV-W, formerly called watermelon mosaic virus 1, naturally infects *Cucurbitaceae* crops but is unable to infect papaya. It has been described as one of the five most important viruses in field-grown vegetables (Tomlinson 1987). There is evidence that PRSV-P

evolved by mutation from PRSV-W (Bateson et al. 1994). The two biotypes display 14% and 10% nucleotide and amino acids sequences divergence, respectively, in the sequences of coat protein from different isolates collected from different parts of the world (Bateson et al. 2002).

PRSV induces yellow mosaic symptoms on susceptible melon plants. Two resistant alleles were differentiated in the melon germplasm according to their reaction with different PRSV strains. Genotype PI 414723 possesses dominant monogenic resistance to this pathogen, encoded by the Prv^2 allele. It reacts by inducing systemic necrotic lesions following inoculation, while plants with the Prv^1 resistance allele are symptomless (Webb 1979; Pitrat and Lecoq 1983). *Fom-1* and *Prv* mapped at the extremity of linkage group IX in the melon genetic map of Périn et al. (2002); the two genes are tightly linked (~2 cM) to each other.

In a previous study, we isolated a set of melon sequences that shared homology with R genes of the nucleotide-binding site–leucine-rich repeat (NBS–LRR) gene family. These were mapped as restriction fragment length polymorphism (RFLP) markers on the melon map, and several such markers were linked to the *Fom-1*/*Prv*, *Fom-2*, and *Vat* (resistance to *Aphis gossypii* Glover) loci (Brotman et al. 2002). In the present study, we focused on the *Fom-1*/*Prv* locus in order to enrich the area with additional genetic markers. We identified two tightly linked random amplified polymorphic DNA (RAPD) markers and two amplified fragment length polymorphism (AFLP) markers, as well as several RFLPs, four of which represent NBS–LRR sequences. Two of the novel markers were converted into single-locus cleaved amplified polymorphic sequence (CAPS) markers. Such markers will be useful for the selection of *Fusarium* and PRSV-resistant alleles by breeders and may represent convenient starting points for positional cloning of the locus.

Materials and methods

Mapping populations

Two recombinant inbred line (RIL) populations developed at INRA, France, were used for this study (Périn et al. 2002). Population I was derived from a cross between a cantaloupe cultivar, *C. melo* Védrantais and a Korean accession, PI 161375; Védrantais is the donor of the *Fom-1* allele that confers resistance to *F. oxysporum* f. sp. *melonis* races 0 and 2. Population II originated from a cross between cv. Védrantais and an Indian accession, PI 414723, which is the source of PRSV resistance. Markers were tested also on a set of *Fusarium* race 2-resistant and-susceptible melon genotypes from different genetic backgrounds (see Table 2 for details).

RFLP analysis

Clones NBS 47-3, NBS 26-2, and NBS-1 represent genomic fragments homologous to NBS–LRR genes, obtained by applying degenerate PCR primers as described by Brotman et al. (2002). The MRGH-21 genomic fragment (Garcia-Mas et al. 2001) was amplified from *C. melo* Védrantais, PI 414723 and PI 161375, using a pair of specific primers (Table 1) and standard PCR conditions (94°C denaturation, 60°C annealing, 72°C elongation, 35 cycles). The CAG-1 cDNA clone was isolated from an early-stage floral bud cDNA library of cucumber (*Cucumis sativus* L., Perl-Treves et al. 1998). Accession numbers of RFLP clones are presented in Table 1. For Southern blots, 3–5-μg DNA samples were digested with restriction endonucleases according to the manufacturers' instructions (Roche Diagnostics, Basel, Switzerland, or MBI Fermentas, Vilnius, Lithuania), run on 0.8% agarose gels, and blotted to charged nylon

Table 1 Sequence and primers data on markers mapped to the *Fom-1*/*Prv* locus

Marker name	Marker type[a]	Accession number	Primers
NBS1-CAPS	CAPS	AF354504	5′ TATTGCTAAAGCTGTTTTCAAAAGCG 3′ 5′ AACAAAAACTTTTCGATTTCCTAAGTT3′
62-CAPS	CAPS	AY611532	RAPD: 5′ CCGCTGGAGC 3′ CAPS: 5′ GGAGAAGATGCTAGAGCCATTC 3′ 5′ AATCGGGCATCCTGTTTTGG 3′
53_{428}	RAPD	AY611533	RAPD: 5′ CCAGATGCAC 3′ Band-specific primers: 5′ CACACGAATGTATCAGATTTTG 3′ 5′ CCAGATGCACCATAACATACC 3′
MRGH21	RFLP	AJ251870	5′ CGCTAGAATCATTTACAAAAGTG 3′ 5′ GCATAATCTACAACTTGGCTAC 3′
ATC/CAT_{497}	AFLP	AY611531	5′ AACAATCATGGAAATGGCG 3′ 5′ AATGGACTTTTAGCTTCATC 3′
NBS47-3	RFLP	AY611534	
NBS26-2	RFLP	AF354511	
CAG1	RFLP	AF022377	
ACA/CAT_{90}	AFLP	AY611530	

[a] *CAPS* Cleaved amplified polymorphic sequence, *RAPD* random amplified polymorphic DNA, *RFLP* restriction fragment length polymorphism, *AFLP* amplified fragment length polymorphism

membranes (Genescreen Plus, Du Pont, Wilmington, Del., USA), using the manufacturer's alkaline blotting method. Samples of parental DNA of three genotypes, namely *C. melo* Védrantais, PI 414723, and PI 161375, were digested with four different enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I), blotted, and hybridized with [^{32}P]-labeled RFLP probes labeled by the random hexamer method (Roche protocol). About 60 individuals were scored from either one or both RIL populations, depending on availability of polymorphism between the mapping parents. Hybridization was performed at 65°C, in 6% (w/v) of polyethylene glycol, 5% (w/v) of SDS, 5× SSPE, and 50 μg/ml denatured salmon DNA.

Sequence analysis

Automated sequencing was carried out by Hylabs (Rehovot, Israel), using an ABI Prism 310 Genetic Analyzer and the BigDye Terminator Kit (PerkinElmer, Foster City, Calf., USA). Oligonucleotides were purchased from Hylabs. The BLAST method (Altschul et al. 1997) was used to reveal homology between our clones and sequences in the GenBank databases. For creating restriction maps and sequence alignment, we used the GCG-Wisconsin Package, version 10.3 (Accelrys, San Diego, Calif., USA). The marker sequences reported in this study were deposited in the GenBank database.

RAPD markers

RAPD-PCR reactions were performed according to Williams et al. (1993), using random decamer sets A, B, E, AC, and AH (Operon Technologies, Alameda, Calif., USA) and sets nos. 1, 2, 4, and 7 of the University of British Columbia Biotechnology Laboratory, Vancouver. Each reaction included 30 ng genomic DNA, 0.2 μmol/l primer, 0.5 U *Taq* DNA polymerase, 0.1 mmol/l of each dNTP, 1.5 mmol/l MgCl2, 10 mmol/l Tris-HCl (pH 9), 50 mmol/l KCl, 0.1% volume fraction of Triton X-100, and 0.2 mg/ml bovine serum albumin in a 25-μl volume. Amplification included 40 cycles of 1 min at 94°C, 90 s at 36°C, and 2 min at 72°C. To confirm RAPD genotyping of specific *Fom-1* linked markers, selected RAPD-PCR bands were gel-eluted and cloned in the pGEM-T easy plasmid vector (Promega). The RAPD products of the parental lines and segregating progeny were run on 1.8% agarose gel, blotted, and hybridized with the cloned probe. Marker names include a primer code number and the band size in base pairs. Primer and sequence data are summarized in Table 1.

AFLP markers

AFLP analysis was carried out according to Vos et al. (1995), with minor modifications, using population II. DNA was digested with *Eco*RI and *Mse*I and ligated to the appropriate adaptors, using the AFLP Core Reagent Kit; primers were taken from the AFLP Starter Primer Kit (Life Technologies, Carlsbad, Calif., USA). The first PCR reaction was preformed with primers having a single selective nucleotide, followed by a second, selective PCR reaction with primers having two or three selective nucleotides in order to produce multi-banded patterns of convenient complexity. *Eco*RI primers were labeled with γ–[^{33}P]dATP, using T4 polynucleotide kinase. Aliquots (3 μl) of AFLP reactions were separated on acrylamide–urea sequencing gels. Markers were named according to the three-base selective extension of the *Eco*RI adapters, followed by the *Mse*I site-selective nucleotides and the band size. The sequences of the 3′ selective nucleotides in the primers that we used for AFLP were *Eco*RI plus three selective nucleotides: AAC, AAG, ACA, ACT, ACC, ACG, AGA, AGC, AGG, ATC, GAG, TAG, AGT, CGA, CTC; *Mse*I plus two selective nucleotides: CA, GC, AG, CC; *Mse*I plus three selective nucleotides: CAA, CAC, CAG, CAT, CGA, CGC, CTA, CTC, CTG, CTT, GCA, TCG, ATC, AGC, TAG.

In order to clone *Fom-1*-linked AFLP markers, bands were cut from dried gels and eluted in H_2O at 55°C for 1 h, to be subjected to PCR re-amplification with the same primer combination used in the original AFLP reaction. Amplification products were separated on 1% TBE–agarose gels to check for the expected size, and cloned in the pGEM-T easy plasmid vector. Thirty-six cloning products were digested using *Eco*RI and *Not*I restriction enzymes to see whether all clones contained similar inserts. Following sequencing, a primer pair was designed to specifically amplify the genomic sequence present in AFLP band ATC/CAT$_{497}$ (Table 1).

CAPS markers

CAPS markers were developed based on RFLP marker NBS-1 and RAPD marker 62$_{1235}$. The CAPS primers are given in Table 1. For CAPS genotyping, we applied 35 PCR cycles to 30 ng genomic DNA in a total volume of 25 μl, using an annealing temperature of 56°C. A 10-μl aliquot of the PCR reaction was digested for 3 h at 37°C in a final volume of 30 μl by adding 3 μl of the appropriate buffer and 2 U of the appropriate restriction enzyme, and the whole sample was separated on a 2% TBE–agarose gel.

Inverse PCR for the isolation of genomic regions that flank the NBS 47-3 marker

Sequences from the NBS-47-3-flanking region were isolated by long-range inverse PCR, adapted from Meissner et al. (2000). Genomic DNA (4 μg) from the melon line Védrantais was digested overnight in a 100-μl final volume with 40 U of restriction enzyme (either *Hin*dIII, *Dra*I, *Eco*RI or *Xba*I). Following phenol–chloroform

extraction and ethanol precipitation, DNA was re-suspended in 90 µl H_2O, and 20 µl was run on a 1% agarose gel to verify full digestion. The remaining 70 µl underwent self-ligation overnight at 16°C in a 500-µl final volume with 36 U of T4 DNA-ligase (Fermentas) to obtain circularized genomic molecules. After ethanol precipitation, DNA was re-suspended in 20 µl, and 10 µl was subjected to PCR amplification using the forward primer 5′ GAT TTT AGA ATA TGG CAT GAG AAT C 3′ and the reverse primer 5′ AAA GCA TTT CCC GTT GTA ATG ACA C 3′. PCR reaction was performed in 50 µl, using the Expand Long Template PCR system with Buffer 3 (Roche), as recommended by the manufacturer. A 10-µl aliquot was separated on a 1% agarose gel. PCR fragments were isolated, sub-cloned in the pGEM-T easy plasmid vector, and sequenced.

Mapping procedure

The MAPMAKER software (Lander et al. 1987) was used to generate a linkage map. Markers were assigned to the same linkage group using the *group* command with a threshold of LOD = 3, theta = 0.3. A LOD threshold value of 3 was used to construct a framework of five markers for the linkage group, using the *two point:three point* command, and additional markers were progressively added to the group, using the commands *try* and *ripple*.

Results

Mapping populations and DNA bulks

Markers linked to the *Fom-1*/*Prv* locus were identified using 95 RILs of population I, derived from the cross *C. melo* Védrantais and PI 161375, and 62 RILs from population II, derived from the cross Védrantais × PI 414723 (Périn et al. 2002). Both populations segregated 1:1 between *Fusarium* race 2-resistant and -susceptible lines. In population II, PRSV resistance segregated at a 1:1 ratio (Périn et al. 2002). A residual 5% of the RIL lines segregated for these genes and were omitted from the analysis. In the present study, we searched for linked markers, using the bulked segregant approach (Michelmore et al. 1991). A pair of bulked DNA samples was prepared from each population. In population I, the first DNA mixture included 27 *Fusarium* race 2-resistant lines and 36 -susceptible lines. In population II, the first sample was composed of DNA from 25 plants that were PRV-resistant and *Fusarium* race 2-susceptible, while the opposite mix contained DNA from 25 *Fusarium*-resistant, PRSV-susceptible RILs.

RAPD analysis

We screened a total of 73 random decamer primers, previously shown to be polymorphic between the mapping parents of our two populations, to try and identify polymorphism between the two pairs of DNA mixtures. On average, each primer amplified six to seven fragments that ranged in size from 120 bp to 4 kbp. Six of these detected a polymorphic band between the two bulks and were analyzed in the whole population. Four of the bands either did not segregate properly or were unlinked to the target locus. The remaining two bands were linked to the *Fom-1*/*Prv* locus.

RAPD primer 62 generated a 1,235-bp fragment in PI 414723 and in the *Fom-1*-susceptible, PRSV-resistant mix, but not in Védrantais or in the opposite mix (Fig. 1a). The band was excised, cloned, and sequenced, and two locus-specific primers were designed (Table 1) that amplified a single DNA fragment of 420 bp from the three parents. A useful CAPS marker was obtained by cleaving the 420-bp amplification product with restriction endonuclease *Alw*26I. In Védrantais, the fragment is cleaved into 330-bp and 90-bp products; in PI 414723 and PI 161375 it remains uncut (Fig. 1b). The CAPS marker, due to robust PCR conditions and single-band amplification product, is easier to score reproducibly, compared to the original RAPD marker from which it was derived. The 62-CAPS marker mapped at a distance of 0.7 cM, one recombinant out of 95 RILs from the *Fom-1* gene, in population I. In population II we observed, out of 62 lines, three recombinants (6.3 cM) between this marker and *Fom-1*. After combining the data from both populations, the distance was 2.8 cM (Fig. 2).

A second RAPD primer, coded 53, generated a 428-bp fragment in PI 414723 (Fig. 1c), but not in Védrantais. We screened population II with this marker and found that it mapped at a distance of 2.6 cM from *Fom-1* (three recombinants, Fig. 2). The RAPD band was excised, cloned, and sequenced, and two locus-specific primers were designed (Table 1); these primers amplified a specific product from all three parents, but no single-base polymorphism was detected between them. (GenBank accession AY 611533).

AFLP analysis

For AFLP analysis we used population II and the above-mentioned DNA mixes derived from it. We applied 48 *Eco*RI/*Mse*I primer combinations to the parental lines and the respective pair of bulked samples. Two AFLP products were specific to the *Fusarium*-resistant, PRSV-susceptible bulk and were derived from the Védrantais parent; three bands were specific to the opposite DNA mix and were derived from the PI 414723 parent. These polymorphic primer combinations were further tested on individual DNA samples of the entire population, but all five bands turned out to be unlinked to the locus. We therefore decided to replace the bulked segregant analysis with individual testing of a small sample of the mapping population, consisting of four *Fom-1*/*prv* and four *fom-1*/*Prv* individuals, and test more RILs whenever apparent linkage (less than two rec-

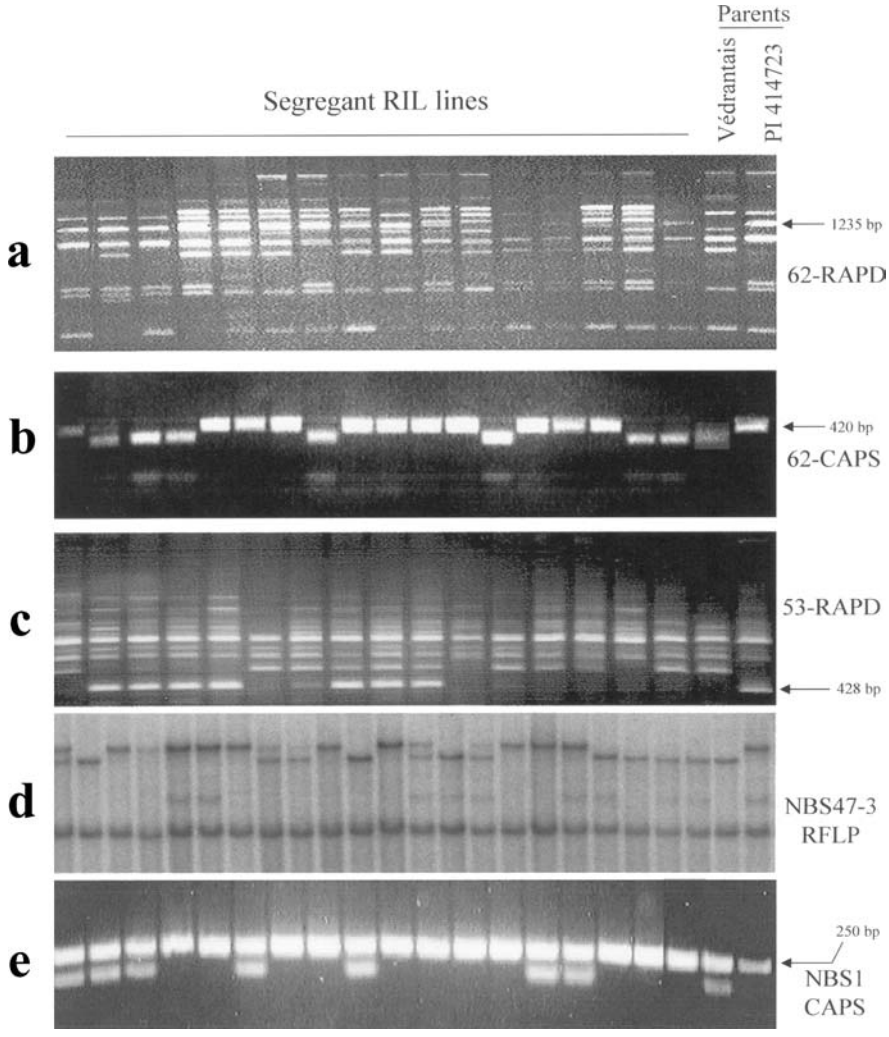

Fig. 1 Molecular markers linked to the *Fom-1*/*Prv* locus in melon. **a** Random amplified polymorphic DNA (*RAPD*) marker 62_{1235}. **b** The cleaved amplified polymorphic sequence (*CAPS*) marker derived from RAPD 62_{1235} by specific PCR amplification and cleavage with *Alw*26I. **c** RAPD marker 53_{428}. **d** Restriction fragment length polymorphism (*RFLP*) marker *NBS* 47-3. Genomic DNA (3 μg) was digested with *Xba*I, blotted to charged nylon membranes, and hybridized with radiolabeled, 250-bp NBS 47-3 fragment at high stringency. **e** CAPS marker derived by specific amplification of the NBS-1 sequence, followed by digestion with *Nco*I. Polymorphism among parental lines Védrantais and PI 414723 and a sample of the segregating recombinant inbred population (population II) is shown for each marker, except for panel e, where a sample of a back-cross population (parents: PI 414723 and PI 414723 x Védrantais) is shown. *Arrows* in **a** and **c** indicate the linked RAPD fragment

ombinants) was detected for a given band. We performed AFLP reactions, using 184 *Eco*RI/*Mse*I primer combinations. Three bands differentiated between the two sets of opposite genotypes and were further tested in the entire population. Two of them were linked to the locus of interest. Marker ATC/CAT_{497} consisted of a 497 bp-fragment specific to the PI 414723 parent, while marker ACA/CAT_{90} represents a 90-bp fragment (including the primers) amplified from the Védrantais genome (Table 1). The markers were located together at a distance of 1.7 cM (two recombinants of 62 lines) from the *Fom-1* gene (Fig. 2). The two bands were cloned from several independent PCR replicates and sequenced. Locus-specific primers were designed to amplify the ATC/CAT_{497} AFLP product as a single genomic band from the DNA of the two mapping parents. Amplification resulted in a mixture of two related products: the expected 497-bp band (Table 1) and a 400-bp band that shared 93% identity with the larger product over 387 bp of its sequence and differed in the remaining region. Database searches with both sequence variants revealed high homology to reverse transcriptase enzymes encoded by retrotransposon elements from plants and animals (e.g., GenBank accessions T02206, Tu and Orphanidis 2001; AAK12626, Hirochika et al. 1996). We could not convert these AFLP markers to single-locus markers, due to lack of polymorphism between the mapping parents.

Mapping RGH sequences and other known-sequence genes to the resistance locus

In a previous study we isolated a set of 15 melon sequences that were homologous to disease and pest resistance genes of the NBS–LRR family (Brotman et al. 2002). One of our clones, NBS 47-3, was mapped as an RFLP marker at a distance of 2.8 cM from *Fom-1* and 1.4 cM (a single recombinant) from *Prv* (Figs. 1d, 2). The 250-bp sequence of NBS 47-3 was identical in the two mapping parents. To search for molecular polymorphism, we cloned by inverse PCR larger fragments, 2,800 bp in total, from both mapping parents, but no polymorphism was detected. A database ‘‘conserved

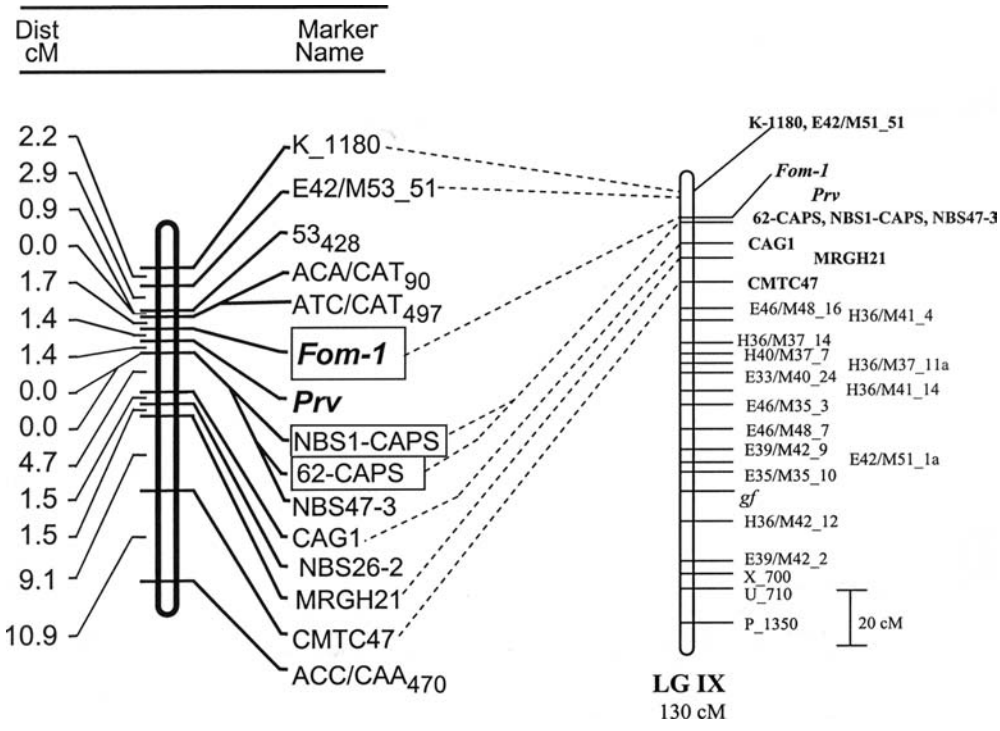

Fig. 2 Linkage map of the *Fom-1*/*Prv* locus. *Left* Merged map based on population I (Védrantais × PI 161375) and population II (Védrantais x PI 414723). *Boxed* markers/traits were scored in both populations. The *three underlined markers* were mapped by Périn et al. (2002) and served to orient the present map with other maps of the same linkage group. On the *right*, a complete scheme of linkage group IX was depicted, combining our present study (markers in *boldface*) with data from Périn et al. (2002). The maps were derived using the MAPMAKER software (Lander et al. 1987)

domain'' search with the translated NBS 47-3 sequence revealed homology to the TIR domain between nucleotides 1 and 335, an NB–ARC domain between nucleotides 820 and 1610, and an LRR domain at positions 2357–2807.

Two additional, 250-bp-long NBS-like sequences, NBS-1 and NBS 26-2, were mapped as RFLPs on population I, at distances of 2.8 cM (a single recombinant) and 9 cM, respectively, from the *Fom-1* gene (Fig. 2). NBS-26-2 was not polymorphic between the mapping parents of population II, while NBS-1 had a single-base polymorphism between these parents, allowing the development of a robust CAPS marker for both populations. The Védrantais allele was cleaved by restriction endonuclease *Nco*I, yielding 45-bp and 205-bp products, while the 250-bp allele of PI 414723 and PI 161375 remained uncut (Fig. 1e, the smaller product migrated out of the gel). We therefore used the NBS-1-CAPS, the 62-CAPS, as well as the *Fusarium* resistance trait, as anchor points to merge the two separate maps derived from the two populations (Fig. 2).

Garcia-Mas et al. (2001) found an NBS–LRR homologue, MRGH-21, which was likely to reside near the *Fom-1*/*Prv* locus. The assignment of MRGH-21 to the vicinity of *Fom-1* was based on anchor markers shared by two melon reference maps. The first map (Oliver et al. 2001) included MRGH-21 but did not include the *Fom-1* and *Prv* resistance genes, whereas the second map (Périn et al. 2002) included the two traits but not MRGH-21. In the present study, we directly mapped RFLP MRGH-21 with respect to *Fusarium* resistance on population I at a distance of 11.6 cM. MRGH-21-specific primers were designed (Table 1), and the amplification products consisted of a mixture of sequences that shared 85–93% nucleotide identity, but all variants were present in the three parental lines used in this study. CAG-1, a cDNA that encodes a MADS box-homologous protein (Perl-Treves et al. 1998), was also mapped as an RFLP in population I. It was linked to the *Fom-1*/*Prv* locus at a distance of 7.5 cM from the *Fom-1* gene (Fig. 2).

Orientation of the *Fom-1*/*Prv* locus within linkage group IX

The map shown in Fig. 2 included three additional markers that were developed by Périn et al. (2002), namely K_1180 (an inter-microsatellite amplification marker), E42/M53_51 (an AFLP marker), and CMTC47 (an SSR marker, Danin-Poleg et al. 2001). These markers enabled us to orient the *Fom-1*/*Prv* locus and anchor it to the entire linkage group (Fig. 2). In the melon map developed by Périn et al. (2002), linkage group IX was rather difficult to resolve, in particular at its distal part, where the *Fom-1*/*Prv* locus resides. Therefore, a final map of the region could not be depicted so far. Adding our newly developed markers strengthened the map order of the markers, and a new version of linkage group IX is displayed in Fig. 2. Moreover, SSR marker CM-CT47 is common also to the map by Oliver et al. (2001), where it mapped to linkage group 7, and to the map of Danin-Poleg et al. (2002) on linkage group II, allowing a better alignment of the various melon maps in this region.

Applications of CAPS markers across a set of melon genotypes

We applied the two CAPS markers, NBS1 and 62, to a set of melon genotypes from diverse horticultural types, that differ in *Fusarium* race 2 resistance (Table 2). These markers were developed on the basis of SNPs between Védrantais and the exotic accessions PI 414723 (var. *momordica*) and PI 161375 (var. *conomon*). We wished to determine whether these markers could be applied across different genetic backgrounds.

The selected genotypes belonged to three main horticultural types, Charentais, Ananas, and American cantaloupe, as well as several exotic accessions; each group included race 2-resistant and -susceptible genotypes, respectively. We see that the markers reveal polymorphism not only between the three parental lines, but also among other genotypes. However, they do not separate all the genotypes according to their resistance phenotype. Instead, each horticultural group appears to carry a characteristic haplotype regarding these two closely linked markers. For example, Charentais-type lines have an A/A haplotype (A allele at both marker loci), while American cantaloupe, Ananas, and *conomon* accessions have a B/B haplotype; some accessions have B/A or A/B haplotypes. The haplotype is shared by both resistant and susceptible genotypes within each group. Implications to the possible origin of resistant alleles and the potential use of these markers in breeding are discussed below.

Discussion

In this study we have enriched the genomic region around the *Fom-1*/*Prv* resistance locus with RFLP, AFLP, RAPD, and CAPS markers, using two mapping populations that shared a common parent, Védrantais. Three common points allowed us to merge the two maps into a composite one (Fig. 2). Périn et al. (2002) used the same two populations and the same merging approach to create a reference map for *C. melo*. Altogether, we assembled a linkage map that includes the two dominant resistance genes, *Prv* and *Fom-1*, and ten novel molecular markers that flank the genes on both sides. Two of the closest markers, RAPD-62_{1235} and RFLP marker NBS-1, were converted into robust CAPS markers, named 62-CAPS and NBS1-CAPS. They were found to co-segregate in both populations. However, we noted that the map distances between the two co-segregating markers and the *Fom-1* gene differed significantly between mapping populations I (0.7 cM, 0.7% recombination ± 0.008 SE) and II (6.3 cM, 6.1% recombination ± 0.03 SE, 2.8 cM in the merged map). Rates of recombination at specific genomic regions could vary among families; using relatively small mapping populations could also result in such variance.

In our hands, the bulk segregant analysis approach was more successful with RAPD as compared to AFLP. Out of 73 RAPD primers applied to the DNA mixes, two linked markers were recovered. Applying AFLP to the same samples resulted in artefactual polymorphism between the DNA mixtures. Due to its complex protocol, the AFLP technique could yield a higher proportion of sporadic bands that are only present in very few individuals, resulting in false DNA-mix-specific bands. We therefore decided to analyze eight individual samples for each AFLP primer combination instead of the DNA mixes, and this strategy yielded two closely linked markers out of 184 primer combinations that were tried.

Plant R genes often encode receptors that recognize ligands specified by pathogen avirulence (*Avr*) genes, either directly or, more likely, through the interaction of the Avr protein with target proteins within the plant cell. This specific recognition initiates a signal transduction cascade, leading to resistance responses. During the last decade, many R genes were cloned, and most of them belonged to the NBS–LRR gene family (Martin et al. 2003). Genes belonging to this family often appear in large clusters in the genomes of many plant species (Shen

Table 2 Testing of the 62- and NBS1-CAPS markers in selected melon germplasm

Horticultural type	Genotype name	Seed source	Fusarium races 0, 2 resistance	NBS1-CAPS[a]	62-CAPS[a]
Charentais	Védrantais	INRA, France	Resistant	A	A
	Doublon	INRA, France	Resistant	A	A
	CharentaisT	Bar-Ilan, Israel	Susceptible	A	A
American cantaloupe	Dulce	ARO, Israel	Resistant	B	B
	TopMark FR	USDA, USA	Resistant	B	B
	Perlita FR	USDA, USA	Resistant	B	B
	TopMark	Cornell, USA	Susceptible	B	B
Ananas	Line 33	Zeraim Gedera, Israel	Resistant	B	B
	Ein Dor	Hazera, Israel	Susceptible	B	B
Yellow canary	Noy amid	Hazera, Israel	Susceptible	A	B
Exotic accession	MR-1	USDA, USA	Resistant	B	A
Exotic accession (var. *momordica*)	PI 414723	INRA, France	Susceptible	B	B
Exotic accession (var. *conomon*)	PI 161375	INRA, France	Susceptible	B	B
	Freeman cucumber	ARO, Israel	Susceptible	B	B

[a]Marker genotype designation: *A* Védrantais-like allele, *B* PI 414723 and PI 161375 allele

et al. 1998; Ellis et al. 2000). In melon, Wang et al. (2002) described a cluster of nine R gene homologous sequences near the *Fom-2* resistance gene, which confers resistance against *F. oxysporum* f. sp. *melonis* races 0 and 1. In our previous study, eight NBS–LRR homologues mapped to the *Vat* locus that specifies resistance to the cotton–melon aphid (Klingler et al. 2001; Brotman at al. 2002; J. Klingler and R. Perl-Treves, unpublished data). The present study indicates the existence of an NBS–LRR cluster in the *Fom-1*/*Prv* resistance locus. This could suggest that the *Fom-1* and *Prv* genes themselves encode NBS–LRR proteins. The NBS 47-3 sequence that resides in this region contained typical TIR, NBS, and LRR motifs.

The distal end of linkage group IX was poorly resolved in the map by Périn et al. (2002). It is often harder to determine the marker order of distal portions of linkage groups, where markers could be sparse; moreover, the mapping algorithms tend to place markers that are problematic to map and involve scoring errors at the ends of linkage groups. By adding a set of ten markers to this region, including several RFLP and CAPS that were scored with high fidelity, the resolution of the whole linkage group was improved.

Testing our CAPS markers in a set of 14 genotypes (seven resistant to *Fusarium* race 2 and seven susceptible) revealed a complex situation. The markers do not divide the genotypes according to the *Fom-1* resistance genotype; instead, they seem to group the accessions according to the horticultural or botanical origin, each group including resistant and susceptible genotypes that share the group-specific marker haplotype. This probably indicates that the different groups have different alleles for *Fusarium* resistance that originated independently (and recently) in different lineages of the melon phylogeny. In fact, resistance in American cantaloupes was isolated independently and designated as *Fom-3* to distinguish it from the Charentais-derived resistance, *Fom-1*. Different authors disagreed whether *Fom-1* and *Fom-3* are alleles or closely linked loci (Zink and Gubler 1985; Risser 1987; Danin-Poleg et al. 1999). Also at the *Prv* locus, different resistance alleles were reported (Pitrat and Lecoq 1983). A different condition exists in tomato, where resistance genes were often introduced from a single source, usually a wild *Lycopersicon* species (e.g., Levesque et al. 1990); there, closely linked molecular markers are expected to work across many cultivars. As for the melon *Fom-2* gene, conferring resistance to *Fusarium* races 0 and 1, testing melon germplasm for markers linked to such locus gave different results from *Fom-1*; there, nearly all cultivars were separated according to resistance (Zheng et al. 1999; Burger et al. 2003), suggesting a single origin of resistance. In the case of our *Fom-1*-linked markers, linkage is tight, and polymorphism is common; however, a breeder could reliably use the markers only after testing his particular resistant source and recurrent parent for their CAPS haplotype, since these vary across the germplasm.

In conclusion, our study provides a rather detailed map of the *Fom-1*/*Prv* locus. The two closely linked CAPS markers that we report are the first ''user-friendly'' markers published for this locus and could be applied by breeders for marker-assisted selection in specific parental combinations. Moreover, markers that flank the resistance locus on both sides could provide good starting points toward positional cloning of this important locus.

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