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RAPD and SCAR markers linked to the *Ma1* root-knot nematode resistance gene in Myrobalan plum (*Prunus cerasifera* Ehr.)

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Abstract The Myrobalan plum (*Prunus cerasifera*) is a self-incompatible species in which the clones P.2175, P.1079 and P.2980 are highly resistant to all root-knot nematodes (RKN), *Meloidogyne* spp. Each clone bears a single major dominant gene, designated *Ma1*, *Ma2* and *Ma3* respectively, that controls a high and wide-spectrum resistance. Bulked segregant analysis (BSA) and random amplified polymorphic DNA (RAPD) analysis were both performed to detect markers linked to the *Ma1* gene using three segregating progenies from P.2175 (*Ma1 ma1*) crossed by three host parents (*ma1 ma1*). Four dominant coupling-phase markers were identified from a total of 660 10-base primers tested. The resulting linkage map spans 14.7 cM and comprises three markers located on the same side of *Ma1* and one marker located on the other side. The nearest markers ($OPAL19_{720}$ and $OPA16_{1400}$) are located at 3.7 and 6.7 cM, respectively, on each side of the gene. Among the three markers that could be successfully converted into sequence characterized amplified region (SCAR) markers, two of them ($SCAL19_{690}$ and $SCAN12_{620}$) were scored as dominant markers whereas the third ($SCAO19_{770}$) failed to produce any polymorphism. SCAL19, and to a lesser extent SCAN12, can be used reliably in the marker-assisted selection of *Prunus* rootstocks. These markers are adequate to identify the *Ma1* RKN resistance gene in intraspecific segregating progenies and will be suitable for the creation of interspecific rootstocks involving Myrobalan plum. Some of the RAPD and SCAR markers for *Ma1* were also recovered in clones P.1079 and P.2980, but not in additional host clones, suggesting that *Ma1*, *Ma2* and *Ma3* are either allelic or at least closely linked.

Key words Bulked segregant analysis · Marker-assisted selection · *Meloidogyne* spp · *Prunus cerasifera* · SCAR marker

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Introduction

Root-knot nematodes (RKN) of the genus *Meloidogyne* are obligate plant endoparasites with extensive host ranges including many species of economic importance in temperate and Mediterranean regions (Sasser 1977; Lamberti 1979), and particularly the Rosaceae family (e.g. stone and pome fruits, strawberry and ornamental species). In Mediterranean regions, the RKN *M. arenaria*, *M. incognita* and *M. javanica* represent one of the major problems of the *Prunus* crops (peach, almond, apricot, plum and cherry) (Rom and Carlson 1987; Nyczepir and Halbrendt 1993) because of the substantial losses of vigour and yield that they can cause.

Limitation of these pests on perennial crops has generally been based on costly pre-plant fumigations with highly toxic active ingredients, now widely restricted in agriculture due to increasing concern over the environment (Scotto La Massese et al. 1991). Thus, the use of resistant rootstocks appears as the best alternative control. Several sources of RKN resistance are already available in the subgenus *Amygdalus* (peach and almond), but their resistance is not complete as illustrated by the multiplication of a RKN population from Florida on these sources (Esmenjaud et al. 1997). Moreover, the rootstocks selected from these sources are poorly adapted to the edapho-climatic conditions

of most Mediterranean areas (Rom and Carlson 1987). In Myrobalan plum (*Prunus cerasifera*), subgenus *Prunophora* (plum and apricot), a few clones have recently been selected because they offer diverse favourable agronomic features (graft compatibility with peach and almond, wide adaptation to different soils; (Layne 1987) together with a broad and durable resistance (Esmenjaud et al. 1994; 1996 a; 1997) to *Meloidogyne* spp. The host suitability to RKN in Myrobalan plum material ranges from susceptible clones (P.2646, P.16.5 and P.2032) to highly resistant clones (P.2175, P.1079 and P.2980). Resistance to *M. arenaria* is monogenic and completely dominant in P.2175 (gene *Ma1*, heterozygous) and in P.1079 (gene *Ma2*, homozygous) (Esmenjaud et al. 1996 b). Data on the respective spectra of these *Ma* genes showed that they also control resistance to the other predominant RKN species, *M. javanica* and *M. incognita*, and to the population *M.* sp. Florida (Lecouls et al. 1997).

Since *P. cerasifera* resistance depends on major genes, marker-assisted selection (MAS) can be developed, thereby limiting the difficult, and very time and space-consuming, screening procedures in woody plants (Bernatsky and Mulcahy 1992; Williams and Neale 1992). Because of the self-incompatibility of Myrobalan plum, genetic studies are based on a diallel-cross design completed with backcrosses and test crosses. Consequently, the genetics of this species has been poorly investigated and up to now is limited to RKN resistance. Nevertheless, Myrobalan plum provides favourable characteristics such as its diploid status ($2n = 2x = 16$) and its small genome. Its estimated size is equivalent to the DNA content of the diploid peach genome (0.58×10^9 bp or 0.60 pg) (Baird et al. 1994), i.e. approximately twice the size of *Arabidopsis thaliana* (Arumuganathan and Earle 1991), and should facilitate the detection of molecular markers linked to traits of interest.

Because of their low cost, and the simplicity and rapidity of their detection, RAPD (random amplified polymorphic DNA) markers are commonly chosen for this kind of study. Nevertheless, a poor sensitivity and a lack of reproducibility are also associated with this technique. In order to address these problems, the informative RAPD fragments can be converted into SCAR markers (sequence characterized amplified regions) (Paran and Michelmore 1993) that generate a simple, reliable, and sometimes co-dominant, banding pattern.

We report here the detection of RAPD markers (Williams et al. 1990) linked to the *Ma1* resistance gene in P.2175 in combination with bulked segregant analysis (BSA) (Michelmore et al. 1991) and the elaboration of reliable SCAR markers. The procedure of RAPD/SCAR/BSA has already been used with success for detecting markers closely linked to economically important resistance traits concerning diverse diseases and crops, such as downy mildew in lettuce (Michelmore et al. 1991), rust, anthracnose and bacterial blight in common bean (Haley et al. 1993; Adam-Blondon et al. 1994; Bai et al. 1997), black leaf spot in Chinese elm (Benet et al. 1995) or verticillium wilt in tomato (Kawchuk et al. 1998). In addition, we provide some information about the relationship between the *Ma1* and *Ma2* genes.

Materials and methods

Plant material

Clones and G1 hybrids used in this study have been selected or created by INRA, Bordeaux, and propagated by softwood cuttings as previously described (Esmenjaud et al. 1996 a). A diallel cross involving eight selected Myrobalan plum parental clones was established. The phenotypic and genotypic characteristics of these clones are listed in Table 1 (Esmenjaud et al. 1997; Lecouls et al. 1997). The basic numbers of hybrids and their resistance response to RKN are reported in Table 2. All the hybrids were characterized, at least twice, for their host response to RKN. Among the available segregating material, the crosses P.2175 × P.2646 and P.2175 × P.16.5 were used in the bulked segregant analysis. Segregation of the identified RAPD or SCAR markers was then achieved on the whole G1 progenies of the previous crosses completed with the cross P.2175 × P.2032 (Table 2).

DNA extraction and bulk composition

Genomic DNA was extracted from frozen leaves using a MTAB protocol according to the procedure of Saghai-Maroof et al. (1984) with some modifications. RNA was removed from the DNA preparation by adding 10 µl of RNAse (10 mg/ml) and then incubating for 30 min at 37°C. Sample DNA concentration was estimated by DNA fluorometry (Hoefer TK100, Hoefer Scientific Instrument, San Francisco, Calif.).

Four different bulks (Table 2) were created for both phenotypic classes of plants as follows: bulks 1R (resistant) and 1H (host) corresponded to the cross P.2175 × P.2646 and contained respectively a mix of an equal amount of DNA from 15 resistant and 15 host individuals chosen at random. Similarly, bulks 2R and 2H corresponded to the cross P.2175 × P.16.5 and contained respectively a mix of DNA from 15 resistant and 15 host individuals. Each of the RAPD primers was screened simultaneously on these four

Table 1 Phenotypic and genotypic characteristics of Myrobalan plum parental clones for resistance to *Meloidogyne* spp.

Clone	Phenotype	Putative genotype[a]
P.2175	R[b]	*Ma1*[c] *ma1*; *ma2 ma2*; *ma3 ma3*
P.1079	R	*ma1 ma1*; *Ma2 Ma2*; *ma3 ma3*
P.2980	R	*ma1 ma1*; *ma2 ma2*; *Ma3 ma3*
P.2646	H	*ma1 ma1*; *ma2 ma2*; *ma3 ma3*
P.16.5	id.	id.
P.2032	id.	id.
P.18	id.	id.
P.2794	id.	id.

[a] All genes expressed in a dominant fashion
[b] R = resistant; H = host
[c] *Ma1*, *Ma2* and *Ma3* are independent, linked or allelic

Table 2 Nature and number of the G1 hybrids used in this study. The numbers between brackets correspond to clones that have been employed to construct the DNA bulks

Parent type		Host parent (*ma1 ma1*)			Total
		P.2646	P.16.5	P.2032	
Resistant parent	R[a] (*Ma1 ma1*)	23 (15)	18 (15)	36	77
P.2175 (*Ma1 ma1*)	H (*ma1 ma1*)	24 (15)	15 (15)	24	63

[a] R = resistant; H = host

DNA pools and on the parental clones P.2175, P.2646 and P.16.5. Amplifications of DNA from the other whole individuals of the previous crosses completed by the cross P.2175 × P.2032 were conducted after markers were found.

RAPD analysis

Six hundred and sixty 10-base primers (kits A–D, G–I, K, L. O–Z, AA–AD an AI–AO from Operon Technologies, Alameda, Calif.) were screened. Amplification patterns of the four DNA pools and the three parents were viewed for the detection of bands present in the resistant pools and the resistant parent, and absent in the host bulks and in both host parents.

Each amplification was performed in a 18-µl vol containing 30 ng of genomic DNA, 0.7 U of *Taq* polymerase (Gibco Brl), 20 ng of 10-base primers, 100 µM of each dNTP (Promega), 1.5 mM $MgCl_2$ and reaction buffer provided with the enzyme. Amplifications were carried out in a MJ Research PTC-100 thermal cycler with amplification conditions adapted from Williams et al. (1990): DNA denaturation at 95°C for 1 min and 40 cycles of melting at 95°C for 10 s, annealing at 37°C for 15 s and extending at 72°C for 75 s. RAPD fragments were size-fractionated in a 1.5% agarose gel in TAE 1 × buffer, with a 1-kb ladder molecular-weight marker (Gibco Brl). Gels were stained in an ethidium bromide solution and then photographed.

Cloning and sequencing of RAPD fragments

The polymorphic RAPD fragments were rescued from the agarose gel by excising the corresponding DNA band and purified using the Quiagen PCR gel-extraction kit. The purified fragment was cloned into the pGEM-T^R plasmid vector (Promega) following the manufacturer's instructions and the resulting ligation mixture was used to transform the electrocompetent *Escherichia coli* strain DH5α or DH12S. The identity of the cloned RAPD product was confirmed by PCR using the universal T7 and SP6 primers and by Southern-blot hybridization. The RAPD amplifications were transferred onto positively charged nylon membranes (Boehringer Mannheim) using a vacuum blotter. The cloned polymorphic RAPD products were labelled with digoxygenin-11-dUTP by insert-amplification and then used as probes. Southern blotting, probes labelling and hybridization were all performed as described by Viruel et al. (1995). The first 300 bases from each end of the RAPD insert were then sequenced.

SCAR design and analysis

For each cloned amplification product, oligonucleotide primer pairs were synthesized. These new primers were designed according to the OLIGO 4.0 program (Rychlik et al. 1990) and either included or did not include the original 10 bases of the RAPD primer plus the next 7–13 adjacent internal bases. Amplification of genomic DNA with SCAR primers was done under the same conditions as the RAPD reaction except for the annealing temperature (55–65°C). The SCAR amplification products were resolved on 1.5% agarose gels in TAE 1 × running buffer.

Sequence analysis

The sequences of the polymorphic RAPD fragments were submitted to existing databases like GeneBank, EMBL or the *A. thaliana* database. Nucleotide homologies were searched using Blastx and Blastn (Altschul et al. 1990).

Linkage analysis

Linkage between the polymorphic markers and the *Ma1* gene was confirmed and quantified by using the segregating progenies from which the bulks were generated completed, by the P.2175 × P.2032 progeny (Table 2). The JOINMAP 1.4 program (Stam 1993) was used to estimate recombination frequencies and corresponding LOD values. A LOD score of 3.0 was chosen to establish the degree of linkage. Recombination fractions were converted to approximative map distances using the Kosambi mapping function (Kosambi 1944)

The nomenclature for the markers follows the convention of primer designation together with the fragment size in bp.

Results

Identification of RAPD markers linked to the *Ma1* gene

Of the 660 10-base RAPD primers screened, 582 (88.2%) provided 2924 fragments (an average of five bands per primer) while the remaining 78 did not amplify *P. cerasifera* DNA at all. Among the amplified fragments, 41 were specific for the clone P.2646 and 64 for the clone P.16.5. Seventy one bands permitted us to characterize the resistant parent P.2175, but only four clear and repeatable polymorphic fragments (0.8%) appeared to be linked to the resistance trait. These four markers, designated $OPA16_{1400}$, $OPAL19_{720}$, $OPAN12_{650}$ and $OPAO19_{820}$, were present in P.2175, and also in both resistant bulks 1R and 2R but were absent in the susceptible bulks 1H and 2H (Fig. 1). As expected from the use of heterozygous-resistant and homozygous host bulks, only markers in coupling phase

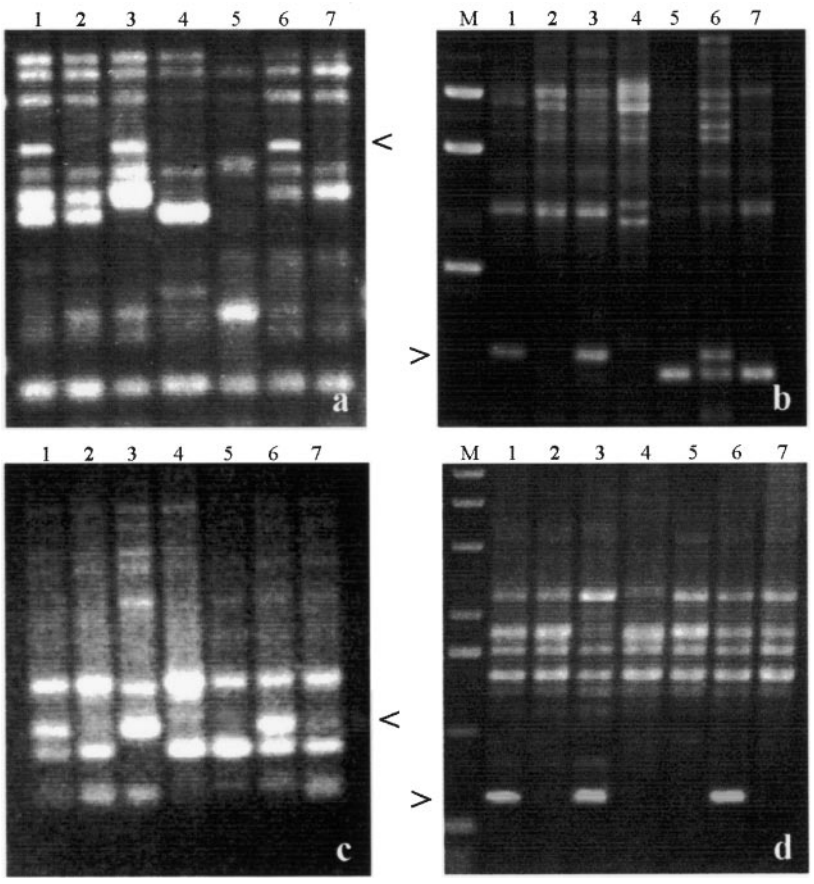

Fig. 1 RAPD amplification patterns using the primers OPA16 (**a**), OPAL19 (**b**), OPAO19 (**c**) and OPAN12 (**d**). Markers are indicated by *arrows*. *Lanes 1–2* 1R and 1S (resistant and host bulks from P.2175 × P.2646), *lanes 3–4–5* P.2175 (R), P.2646 (H) and P.165 (H), *lanes 6–7* 2R and 2S (resistant and host bulks from P.2175 × P.16.5), *M* molecular-weight marker

Table 3 Loci, phenotypic frequencies, relative LOD scores and distances between loci[a] in the identification of molecular markers for *Ma1*

Locus		Phenotype[b]				Missing data	Distance (cM)	LOD score
A	B	AB	Ab	aB	ab			
Ma1	OPAL19	74	3	6	55	11	3.70	27.09
Ma1	OPAO19	75	2	9	50	3	5.69	24.34
Ma1	OPA16	61	7	6	54	11	6.67	20.27
Ma1	OPAN12	71	6	9	51	2	7.97	20.68
OPA16	OPAL19	64	6	6	52	11	10.37	21.98
OPA16	OPAO19	65	3	9	50	12	12.36	20.72
OPA16	OPAN12	63	5	10	50	11	12.36	20.72
OPAL19	OPAO19	79	0	4	53	3	1.99	33.10
OPAL19	OPAN12	77	3	3	54	2	4.27	30.54
OPAO19	OPAN12	80	4	0	51	4	2.28	30.10

[a] Data from the three hybrid progenies were pooled for JOINMAP analysis
[b] Number of hybrids: A or B correspond to resistant hybrids or to the presence of the RAPD marker; a or b correspond to host hybrids or to the absence of the RAPD marker

were detected. In our case, the rate of informative markers is theoretically twice lower than in the classical case where both coupling- and repulsion-phase markers can be identified.

The co-segregation of the four markers and the *Ma1* locus was verified using the 139 individuals of the three hybrid progenies P.2175 × P.2646, P.2175 × P.16.5 and P.2175 × P.2032 (Table 2). A total of 9, 11, 13 and 15 recombinant plants were identified between the resistance gene and the markers $OPAL19_{720}$, $OPAO19_{820}$, $OPA16_{1400}$ and $OPAN12_{650}$, respectively. The pooled recombination frequencies were then estimated (Table 3) and converted into genetic distances using the JOINMAP 1.4 program (Stam 1993). The resulting map based on a LOD score of 3 and the Kosambi mapping function is presented in Fig. 2. Three RAPD markers are located on one side of *Ma1* and one marker is located on the other side. In total the markers cover a distance of 14.7 cM. The nearest marker ($OPAL19_{720}$) is located 3.7 cM from *Ma1*.

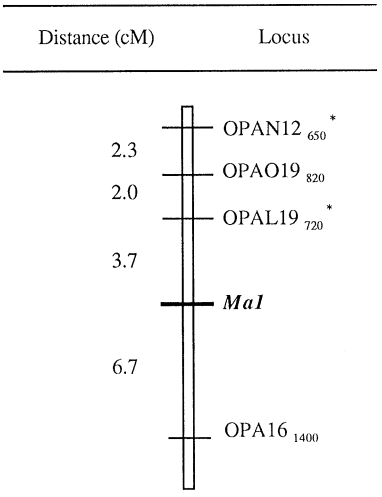

Fig. 2 Genetic map of the chromosome region containing the *Ma1* resistance gene. RAPD markers supplemented with * were successfully converted into SCAR markers

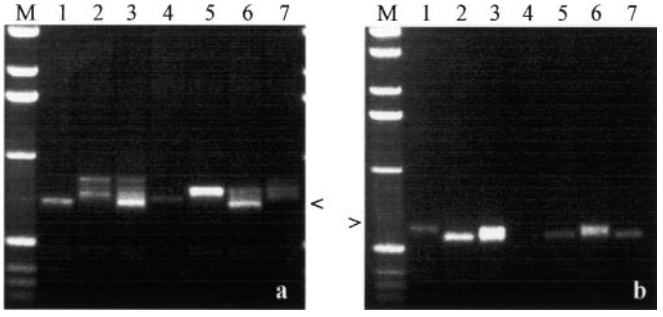

Fig. 3 Amplification patterns of the SCAR markers $SCAL19_{690}$ (**a**) and $SCAN12_{620}$ (**b**) on bulks and on parental clones. Markers are indicated by *arrows*. *Lanes 1–2* 1R and 1S (resistant and host bulks from P.2175 × P.2646), *lanes 3–4–5* P.2175 (R), P.2646 (H) and P.16.5 (H), *lanes 6–7* 2R and 2S (resistant and host bulks from P.2175 × P.16.5), *M* molecular-weight marker

Conversion of RAPD markers into SCAR markers

The resistant discriminant bands $OPAL19_{720}$, $OAPO19_{820}$ and $OPAN12_{650}$ were first cloned. The similarity of the cloned products with the selected RAPD fragments was verified by Southern-blot hybridization. For each marker, both the RAPD profile and the hybridization pattern were identical indicating that the SCAR fragment was derived from the amplified product. At least 300 bp from each end of the RAPD cloned products were sequenced. A pair of specific primers, designated SCAR-1 and SCAR-2, were deduced from each sequence, either including or not including the original RAPD primer and varying from 17 to 23 bp (Table 4).

The same four bulks and the same three parents as in the RAPD assay were used for PCR amplification with each pair of SCAR primers. The annealing temperature was optimized for all pairs in order to obtain the less-complex banding pattern in each case.

With two of the pairs of primers deduced from the sequences of OPAL19 and OPAN12, the same polymorphism as in RAPDs was observed: bands of the expected size appeared in the resistant bulks and in the parent P.2175 but were absent in the host bulks and in both host parents. These new markers, designated $SCAL19_{690}$ and $SCAN12_{620}$ (Fig. 3), were then tested on the hybrids of the plant mapping progenies and led to the same conclusions: the 690- and 620-bp SCAR markers co-segregated with the original RAPD fragment in the resistant plants as well as in the recombinant hybrids (Fig. 4). This result confirmed that the same locus was involved in the polymorphism observed with the two techniques. In contrast with most of the studies reported in the literature (Ohmori et al. 1996; Deng et al. 1997; Jiang and Sink 1997; Kawchuk et al. 1998), amplifications with the SCAL19-1 and SCAL19-2, and with the SCAN12-1 and SCAN12-2, primers did not generate a single fragment present only in resistant plants. The polymorphism observed here consisted in a variation of fragment length. A similar result was also obtained with oak species where variant length polymorphism had been justified by the presence of subrepetitions within the RAPD sequences (Bodenes et al. 1996). In our case, for $SCAN12_{620}$, the presence of a second band, smaller than the resistance marker, could be explained by the same hypothesis.

Table 4 Primer sequences and PCR conditions for SCAR amplifications

RAPD marker	SCAR Primer	Sequences[a]	Annealing temperature	Polymorphism
$OPAL19_{720}$	SCAL19-1 SCAL19-2	TCTGCCAGTGAAATATAAT (20 bases) CATTGGAGAAGATTGGCCC (19 bases)	55–56°C	Dominant
$OPAO19_{820}$	SCAO19-1 SCAO19-2	CGCGCTGTTTACCACTATC (19 bases) GACTCTTGTATTTGTATAGGTCG (23 bases)	60°C	Non-polymorphic after digestion
$OPAN12_{650}$	SCAN12-1 SCAN12-2	CGGCGGTCATTTTAAAA (17 bases) CACACGAACAGACGTTT (17 bases)	58°C	Dominant

[a] The underlined sequences correspond to the original RAPD primer

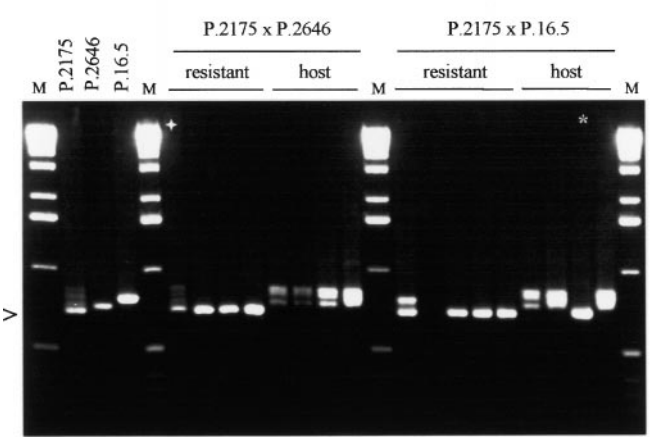

Fig. 4 Segregation of $SCAL19_{690}$ in P.2175 × P.2646 and P.2175 × P.16.5 progenies. The marker is indicated by an *arrow*. The lane supplemented with * corresponds to a recombinant hybrid, the lane supplemented with † corresponds to a hybrid expressing the P.2175 parental pattern. *M* molecular-weight marker

Concerning $SCAL19_{690}$, the amplification in P.2175 generates one major resistance-marker band and three longer additional bands that are less intense. In the segregating progenies, the first band is recovered in the resistant hybrids whereas the band triplets are observed in the host hybrids. The probe derived from the RAPD cloned product hybridizes with all four bands, which indicates that the amplified fragments are homologous. This result suggests that the major band and the triplets could be allelic. Nevertheless, one resistant individual expressing the phenotype of the resistant parent has been identified (see Fig. 4). Consequently, the two markers are non-allelic and cannot be considered as co-dominant.

When used as primers, SCAO19-1 and SCAO19-2 amplified a 770-bp fragment in both resistant and host individuals, suggesting that the RAPD polymorphism was caused by a mismatch in one of the targeted primers' sequence. Curiously, this mismatch did not appear in the sequence of the cloned product for which the sequences of the binding site were recovered. As suggested by Paran and Michelmore (1993), one can hypothesize that nucleotide differences between the primer and the genomic sequences could have been lost in the initial cycle of amplification. In the case of $SCAO19_{770}$, the loss of the initial polymorphism could have resulted from the use of longer primers and stronger PCR conditions. Digestion of the monomorphic band with 15 different endonucleases (4–6 base-cutter enzymes) was carried out but failed to produce any fragment-length polymorphism.

Sequence analysis

The sequenced RAPD fragments were compared for homologies with known sequences in the data banks (Genebank, EMBL or *A. thaliana* database) using Blastx or Blastn. Significant nucleotide similarities were found with *A. thaliana* mRNA for peroxidase ATP ($OPAL19_{720}$) or with mustard flower bud-expressed sequence tags of *Brassica campestris* ($OPAO19_{820}$).

Relationship between the *Ma* genes in Myrobalan plum

Three different single genes conferring resistance to *Meloidogyne*, spp. have been identified (Table 1) in the Myrobalan plum (Esmenjaud et al. 1996 b; Rubio et al. 1998). In order to establish the relationships between these *Ma* genes, the RAPD markers linked to *Ma1* were tested on all three resistant parental clones and on the other host parents (recessive for all *Ma* genes). As illustrated in Fig. 5A, the *Ma1* $OPA16_{1400}$ marker is present in P.2175, P.1079 and P.2980, but is absent in all the host parents. Markers $OPAL19_{720}$ and $OPAN12_{650}$ also segregate as expected in a backcross involving P.1079 (data not shown).

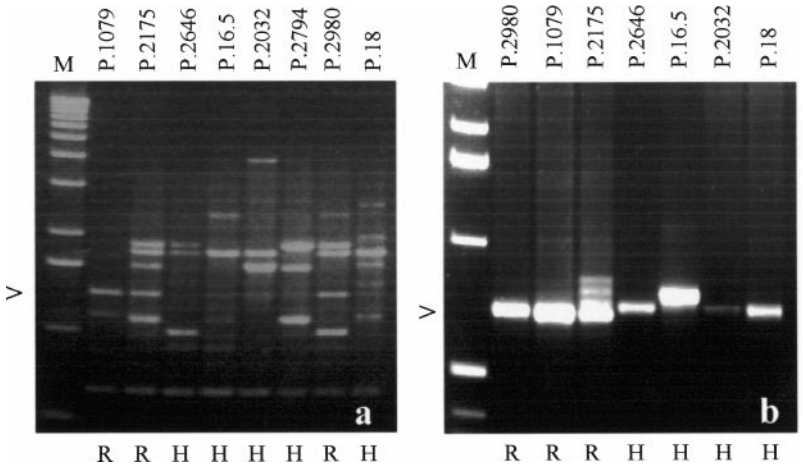

Fig. 5 RAPD and SCAR markers on the parental clones. **a** $OPA16_{1400}$, **b** $SCAL19_{690}$. Markers are indicated by *arrows*. *M* molecular-weight marker, *R* resistant, *H* host

Previous RAPD data were confirmed using both SCAR markers, as illustrated for SCAL19 in Fig. 5B. The 690-bp SCAR fragment generated by the primers pairs SCAL19-1 and SCAL-19-2 in P.2175, P.1079 and P.2980 was rescued from the gel and sequenced. The three resulting sequences were then aligned and compared for homologies using the Align program. A high percentage of homology was found between P.2175 and P.1079 (98%), P.2175 and P.2980 (96.5%), and P.1079 and P.2980 (96%) when compared for the first 350 bases of each marker.

These results suggest that *Ma1*, *Ma2* and *Ma3* could be closely linked or else be three allelic forms of the same gene. Linkage maps of the genomic regions flanking the *Ma2* and *Ma3* genes are currently under construction.

Discussion

The resistance to *Meloidogyne* spp. in Myrobalan plum depends on the major *Ma* dominant genes. Molecular tagging of *Ma1* led to the identification of four RAPD markers in coupling phase. Two of them, $OPAL19_{720}$ and $OPAN12_{650}$, were successfully converted into SCAR markers in order to increase their reliability and reproducibility. These two markers are located respectively at 3.7 and 5.7 cM from *Ma1* but on the same side of the gene. The RAPD marker $OPA16_{1400}$ is located on the other side but at a longer distance from *Ma1* (6.7 cM). According to Tanksley (1983), the application of MAS requires a tight linkage (less than 5 cM) between the marker and the gene of interest or else the use of two loosely linked markers flanking the targeted locus. $SCAL19_{690}$ and, to a lesser extent, $SCAN12_{620}$ conform to these conditions and so can be used in MAS. Nevetheless, additional markers have to be sought particularly on the side where no SCAR is available and a fine mapping of *Ma1* is in progress using more resolutive markers like AFLPs (Vos et al. 1995).

The *Ma* genes control resistance to the all predominant RKN species, i.e. *M. arenaria*, *M. incognita* and *M. javanica* (Lecouls et al. 1997), and also to a new population from Florida (*M.* sp Florida) which overcomes the resistance of the *Amygdalus* sources (Esmenjaud et al. 1997). Moreover, the resistance conferred by the *Ma* genes in Myrobalan plum is not affected by high temperatures, inoculum pressure or the phenological stage of the plant (Esmenjaud et al. 1996 a). These characteristics illustrate the importance of these genes for all *Prunus* rootstocks. Actually, in stone fruits, the variety can be grafted onto a rootstock that can be of a different species (edible, ornamental or wild) or of an interspecific hybrid. In this way, the introduction of the *Ma* genes into the genome of new *Prunus* rootstocks by interspecific hybridization has been undertaken. With this objective in mind, the use of SCAR markers as tools for MAS will considerably simplify screening procedures in *Prunus*. Both SCAR markers will be useful in the characterization of simple interspecific rootstocks involving Myrobalan and peach or Myrobalan and almond, or in three-way hybrids pyramiding the Myrobalan, peach and almond genes (Lu et al. 1998).

The molecular approach, as well as genetic results, make *P. cerasifera* a simple model for resistance studies. It is highly probable that a single gene with a broad spectrum and three allelic forms is involved. Molecular markers for *Ma1* are also closely linked to *Ma2* and are recovered in P.2980 whose resistance shares the same characteristics as P.2175 and P.1079. *P. cerasifera* seems all the most interesting in that it is a diploid species with a small genome (twice the size of *A. thaliana*) (Arumuganathan and Earle 1991). This model could be particularly convenient for the study of genetic and molecular relationships among the different resistance systems existing in the subgenera *Amygdalus* and *Prunophora* (Esmenjaud et al. 1997). Different maps have been constructed within *Prunus* species (Foolad et al. 1995; Viruel et al. 1995; Dirlewanger et al. 1998). A RFLP core map established by Joobeur et al. (1998) on an almond-peach hybrid should allow one to locate the genes from both subgenera on the eight linkage groups. This approach should be facilitated in Myrobalan plum because of the high synteny expected between these diploid species.

Plum species range from diploid ($2n = 2x = 16$ for *P. cerasifera*) to hexaploid ($2n = 6x = 48$ for *P. domestica*) species (Rehder 1954). The diverse polyploid species are often supposed to include the Myrobalan genome as at least one of their components. Consequently, stable SCAR markers may also provide evidence for the *Ma* genes, or allelic forms of the *Ma* genes, within these plum species. This information could be of interest both in the detection of new resistant rootstocks or resistance sources and in phylogenetic studies of the plum species (Salesses 1973; Reynders and Salesses 1991).

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