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M. Claverie · N. Bosselut · A. C. Lecouls · R. Voisin ·
B. Lafargue · C. Poizat · M. Kleinhentz · F. Laigret ·
E. Dirlewanger · D. Esmenjaud

Location of independent root-knot nematode resistance genes in plum and peach

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Abstract *Prunus* species express different ranges and levels of resistance to the root-knot nematodes (RKN) *Meloidogyne* spp. In Myrobalan plum (*Prunus cerasifera*), the dominant *Ma* gene confers a high-level and wide-spectrum resistance to the predominant RKN, *Meloidogyne arenaria*, *Meloidogyne incognita*, *Meloidogyne javanica* and the isolate *Meloidogyne* sp. Florida which overcomes the resistance of the Amygdalus sources. In Japanese plum (*Prunus salicina)*, a similar wide-spectrum dominant resistance gene, termed R_{jap}, has been hypothesized from an intraspecific segregating cross. In peach, two crosses segregating for resistance to both *M. incognita* and *M. arenaria* were used to identify single genes that each control both RKN species in the Shalil (R_{Mia557}) and Nemared (R_{MiaNem}) sources. Localisation of these genes was made possible using the RFLP and SSR- saturated reference *Prunus* map T×E, combined with a BSA approach applied to some of the genes. The *Ma1* allele carried by the Myrobalan plum accession P.2175 was localised on the linkage group 7 at an approximate distance of 2 cM from the SSR marker pchgms6. In the Japanese plum accession J.222, the gene R_{jap} was mapped at the same position in co-segregation with the SSR markers pchgms6 and CPPCT022. The peach genes R_{Mia557} and R_{MiaNem}, carried by two *a priori* unrelated resistance sources, were co-localized in a subtelomeric position on linkage group 2. This location was different from the more centromeric position previously proposed by Lu et al. (1999) for the resistance gene *Mij* to *M. incognita* and *M. javanica* in Nemared, near the SSR pchgms1 and the STS EAA/MCAT10. By contrast, R_{Mia557} and R_{MiaNem} were flanked by STS markers obtained by Yamamoto and Hayashi (2002) for the resistance gene *Mia* to *M. incognita* in the Japanese peach source Juseitou. Concordant results for the three independent sources, Shalil, Nemared and Juseitou, suggest that these peach RKN sources share at least one major gene resistance to *M. incognita* located in this subtelomeric position. We showed that plum and peach genes are independent and, thus, can be pyramided into interspecific hybrid rootstocks based on the plum and peach species.

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M. Claverie · N. Bosselut · A. C. Lecouls · R. Voisin ·
D. Esmenjaud (✉)
Unité “Interactions Plantes-Microorganismes et Santé Végétale” (IPMSV), Equipe de Nématologie,
Institut National de la Recherche Agronomique (INRA),
B.P. 2078, 06606 Antibes Cedex, France
e-mail: esmenjau@salis.antibes.inra.fr

B. Lafargue · C. Poizat · M. Kleinhentz · F. Laigret · E. Dirlewanger
Unité de Recherche sur les Espèces Fruitières
et la Vigne (UREFV), INRA,
B.P. 81, 33883 Villenave d’Ornon Cedex, France

Introduction

The *Prunus* genus comprises over 400 species, most of them being well adapted to Northern temperate areas and widely distributed in Europe (Rehder 1954). They include fruit-producing species (e.g. peach, almond, plum, apricot and cherry), and several rootstock and ornamental species. Plums and apricots belong to the Prunophora subgenus which is divided into two sections (i.e. Euprunus and Armeniaca, respectively). Peaches and almonds belong to the Amygdalus subgenus. All the *Prunus* species have an 8-basis chromosome number with various ploidy levels: ($2n=2x=16$) for peach, almond, Myrobalan and Japanese plums, apricot and sweet cherry, ($2n=4x=32$) for sour cherry and ($2n=6x=48$) for European plums (Salesses et al. 1994).

Root-knot nematodes (RKN) (*Meloidogyne* spp.) are major crop pests all over the world (Sasser 1977; Lamberti 1979). The most economically damaging are the Mediterranean and tropical species, *Meloidogyne arenaria*, *Meloidogyne incognita* and *Meloidogyne javanica*, that are highly polyphagous and reproduce through parthenogenesis (Triantaphyllou 1985) on hundreds of cultivated and wild plant species (de Guiran and Netscher 1970). Genetic resistance of plants has been used to control main RKN species (Minz and Cohn 1962; Kochba and Spiegel-Roy 1975 Kester and Grasselly

1987; Layne 1987; Nyczepir 1991). However, the efficiency of RKN resistance in rootstocks depends on the source of resistance (Scotto La Massese et al. 1984; Esmenjaud et al. 1997). In the subgenus Amygdalus, three types of plant response have been identified. Most of the rootstock material is susceptible to RKN. The peach Shalil and its peach-almond hybrid GF.557 are resistant to *M. arenaria* and *M. incognita* but susceptible to *M. javanica* (Esmenjaud et al. 1994) and to a RKN population from Florida [considered as belonging to a new species and designated as *Meloidogyne* sp. Florida (Esmenjaud et al. 1997)]. The peach Nemaguard and related material, such as the peach Nemared and the hybrids Garfi almond × Nemared (termed G×N), are also resistant to most *M. javanica* populations (Ramming and Tanner 1983) but not to *Meloidogyne* sp. Florida.

Within the Prunophora subgenus, plums are the most taxonomically diverse and are adapted to a broad range of climatic and edaphic conditions (Ramming and Cociu 1991; Salesses et al. 1993). Some are used for their fruits (e.g. the Japanese plum, *Prunus salicina*, or the domestic plums, *Prunus domestica* and *Prunus insititia*), the large majority being used as rootstocks for other *Prunus* species. Among them, the Myrobalan plum (*Prunus cerasifera*), an outbreeding diploid species, has been recently introduced into selection schemes since some of its clonal selections exhibit beneficial agronomic features (Salesses et al. 1993, 1994) or express resistance to root-knot nematodes (RKN). Clones P.2175, P.1079 and P.2980 of the Myrobalan plum proved to be resistant to the population *Meloidogyne* sp. Florida (Esmenjaud et al. 1997; Lecouls et al. 1997; Rubio-Cabetas et al. 1999). All three clones carry one dominant allele of a single resistance gene, designated *Ma1, Ma2* and *Ma3,* respectively (Esmenjaud et al. 1996b; Rubio-Cabetas et al. 1998). Each of these *Ma* resistance alleles confers a high and wide-spectrum resistance to *M. arenaria, M. incognita*, *M. javanica* and *M*. sp. Florida (Lecouls et al. 1997; Rubio-Cabetas et al. 1999) and to the minor species *Meloidogyne mayaguensis* (Fargette et al. 1996; Rubio-Cabetas et al. 1999). This resistance was not overcome by any of the over-30 RKN species and isolates tested (Esmenjaud et al. 1994, 1997; Fernandez et al. 1994), and was not modified under conditions known as affecting plant defences to RKN such as high temperature and high inoculum pressure (Esmenjaud et al. 1996a). Thus Myrobalan plum appears particularly useful for RKN-resistant rootstock breeding because of the high-level and wide-spectrum RKN resistance of certain accessions. Within perennials, where the genetics of RKN resistance is poorly documented, the *Ma* gene from Myrobalan plum represent the first genetic system fully characterized. This is also the only system extensively investigated in the *Prunus* genus for resistance to a plant pest (Lecouls et al. 1997; Lecouls 2000).

Molecular studies have been conducted in order to develop marker-assisted selection (MAS) for *Ma*. Two reliable SCAR (Sequence Characterized Amplified Region) markers, $SCAL19_{690}$ and $SCAFLP2_{202}$, were shown to be linked in coupling to the dominant resistance alleles *Ma1* and *Ma3* (Lecouls et al. 1999; Bergougnoux et al. 2002). They have been identified by bulked segregant analysis (BSA) (Michelmore et al. 1991) using intraspecific progenies involving P.2175 (*Ma1 ma*) and several susceptible parents (*ma ma*). SCAL19 is located less than 1 cM from *Ma* and SCAFLP2 is co-segregating with *Ma*, as shown by the analysis of 340 individuals belonging to diverse intra- and inter-specific progenies (M. Claverie, unpublished).

Peach RKN resistance has been first studied in the Nemared rootstock. Markers have been obtained using different F2 progenies such as the intraspecific cross between Lovell and Nemared (Lu et al. 2000) or the almond-peach cross Garfi × Nemared (Jauregui 1998). Recently additional data have been obtained from the Japanese RKN resistant peach Juseitou (Yamamoto and Hayashi 2002).

Here we report results on the precise location of the Myrobalan plum *Ma* gene in comparison with the putative location of another RKN gene from the Japanese plum on the reference *Prunus* map. We also give the location of genes for RKN resistance in the two peach sources Nemared and Shalil, in comparison to other available information on peach RKN genes. These data are discussed in the perspective of pyramiding strategies based on marker-assisted selection (MAS) for RKN resistance in *Prunus* rootstocks.

Materials and methods

Characteristics of plant material and progenies for resistance to RKN species

Various Prunophora and Amygdalus parents (Table 1) showing different RKN spectra for resistance (Esmenjaud et al. 1994, 1997; Lecouls et al. 1997) were used to produce intra- and inter-specific progenies (Table 2). These progenies segregate for several RKN genes carried either by Prunophora or by Amygdalus parents.

Material segregating for Prunophora RKN genes

This material includes intraspecific progenies from Myrobalan or Japanese plums. Myrobalan progenies are crosses of the same resistant clone P.2175 (carrying the heterozygous dominant *Ma* gene) with each of the three susceptible parents P.2646, P.16.5 and P.2032 (homozygous recessive for *Ma*) (Esmenjaud et al. 1996b; Lecouls et al. 1997). The segregating progeny of the Japanese plum is a cross between the resistant accession J.222 (resistant to *M. arenaria, M. incognita, M. javanica* and *Meloidogyne* sp. Florida) and the susceptible accession J.13. The dominant gene evidenced in this cross has been named R_{jap} and the corresponding resistant and susceptible parental genotypes proposed are (R_{jap} r_{jap}) and (r_{jap} r_{jap}), respectively (Lecouls 2000).

Material segregating for Amygdalus RKN genes

The segregating progenies are interspecific crosses between a Myrobalan plum accession and almond-peach hybrids. Two peach resistance sources, Shalil and Nemared, were considered. The Shalil peach was used through its almond-peach hybrid GF.557 that expresses the same RKN resistance to *M. arenaria* and *M.*

Table 1 Spectrum of resistance of parental *Prunus* material used in this study

Accession	Resistance status to				RKN resistance gene and genotype
	M. arenaria (MA)	*M. incognita* (MI)	*M. javanica* (MJ)	*Meloidogyne* sp. Florida (FL)	
Prunophora					
Myrobalan plum (*P. cerasifera*)					*Ma* gene controlling MA, MI, MJ and FL
P.2175	R[a]	R	R	R	(*Ma1 ma*)
P.2032	S[a]	S	S	S	(*ma ma*)
P.16.5	S	S	S	S	Idem
P.2646	S	S	S	S	Idem
Japanese plum (*P. salicina*)					R_{jap} gene controlling MA, MI, MJ and FL
J.222	R	R	R	R	(R_{jap} r_{jap})
J.13	S	S	S	S	(r_{jap} r_{jap})
Amygdalus					
Almond (*P. dulcis*)					
Garfi (G)	S	S	S	S	Susceptible to all RKN species
Peach (*P. persica*)					
Shalil					
GF.557 = Almond × Shalil peach					R_{Mia557} gene controlling MA and MI
GF.557	R	R	S	S	(R_{Mia557} r_{Mia557})
Nemared (N)					R_{MiaNem} gene controlling MA and MI
Nemared	R	R	R/S[b]	S	(R_{MiaNem} R_{MiaNem})
$(G{\times}N)_{15}$	R	R	R/S	S	(R_{MiaNem} r_{MiaNem})
$(G{\times}N)_{22}$	R	R	R/S	S	Idem

[a] R = resistant; S = susceptible
[b] R/S: variable behaviour in function of *M. javanica* isolates

Table 2 *Prunus* material segregating for resistance to root-knot nematodes (RKN) *Meloidogyne* spp. and numbers of individuals from the different progenies used for localisation of RKN resistance genes in this study

Segregating progenies	RKN species	Total numbers	Genes involved
Myrobalan plum (*P. cerasifera*)			
P.2175×P.2646 P.2175×P.16.5 P.2175×P.2032	*Meloidogyne* sp. Florida (or other RKN species[a])	288	*Ma*
Japanese plum (*P. salicina*)			
J.13×J.222	*Meloidogyne* sp. Florida (or other RKN species[a])	26	R_{jap}
Myrobalan plum × [almond (*P. dulcis*) × peach (*P. persica*)]			
Myrobalan plum x (almond × Shalil peach)			
P.2032×GF.557	*M. incognita* and *M. arenaria*	36	R_{Mia557}
Myrobalan plum x almond-peach			
Myrobalan plum × [Garfi (G) almond × Nemared (N) peach]			
$P.2175{\times}(G{\times}N)_{22}$			
All individuals	*Meloidogyne* sp. Florida[b]	101	*Ma* (+R_{MiaNem})
Susceptible individuals (*ma ma*)	*M. incognita* and *M. arenaria*	61	R_{MiaNem}

[a] *M. arenaria*, *M. incognita* and *M. javanica*
[b] This isolate was used to discriminate the individuals lacking the *Ma* gene that were then evaluated for resistance to *M. arenaria* and *M. incognita* (see Table 1)

incognita as Shalil. GF.557 is heterozygous for resistance, and segregation was obtained by crossing it (as a male parent) with the susceptible Myrobalan plum P.2032. The dominant gene for resistance to both *M. incognita* and *M. arenaria* in GF.557 is designated R_{Mia557} (= 'resistance to *M. incognita* and *M. arenaria* from GF.557'). The peach Nemared (N) was used through its almond-peach hybrids with the RKN susceptible almond Garfi (G). Accession $(G{\times}N)_{22}$ (= 'Felinem'), heterozygous resistant to *M. incognita* and *M. arenaria*, was crossed as a male parent with the Myrobalan plum accession P.2175. This Myrobalan × almond-peach progeny was firstly evaluated for its resistance to *Meloidogyne* sp. Florida, which is not controlled by the Amygdalus resistance sources and thus allows the separation of the resistant individuals carrying the *Ma1* resistance allele from P.2175 and the susceptible individuals lacking it. These susceptible individuals (homozygous recessive for *Ma*) were then evaluated separately for their resistance to each of the *M. incognita* and *M. arenaria* species. The same segregation was observed whatever the RKN species, and

Table 3 SSR markers from *Prunus* LG7, species and the laboratory of origin, and polymorphism between resistant and susceptible bulks from Myrobalan plum crosses (P.2175×P.2646 and P.2175×P.16.5) and in the resistant Japanese plum J.222 (cross J.13×J.222). P = polymorphic; NP = non-polymorphic

SSR name	Species of origin	Reference or laboratory	Polymorphism between Myrobalan bulks	Polymorphism in the Japanese plum J.222
CPPCT022	*P. persica*	Aranzana et al. 2002a	NP	P
pchgms6	*P. persica*	Clemson University	P	P
UDP98–405	*P. persica*	Cipriani et al. 1999	P	NP
UDP98–408	*P. persica*	Cipriani et al. 1999	NP	NP
CPPCT033	*P. persica*	Aranzana et al. 2002a	P	P

Table 4 SSR markers from *Prunus* LG2, species and the laboratory of origin, and polymorphism between resistant and susceptible bulks from G×N [cross P.2175×(G×N)] and from GF.557 (cross P.2032×GF.557). P = polymorphic; NP = non-polymorphic

SSR name	Species of origin	Reference or laboratory	Polymorphism between bulks	
			In [P.2175×(G×N)]	In (P.2032×GF.557)
CPPCT024	*P. persica*	Aranzana et al. 2002a	NP	NP
UDP98–025	*P. persica*	Testolin et al. 2000	P	P
BPPCT004	*P. persica*	Dirlewanger et al. 2002	P	P
BPPCT001	*P. persica*	Dirlewanger et al. 2002	P	P
BPPCT002	*P. persica*	Dirlewanger et al. 2002	P	P
BPPCT013	*P. persica*	Dirlewanger et al. 2002	P	P
UDP96–013	*P. persica*	Cipriani et al. 1999	NP	NP
pchgms1	*P. persica*	Sosinski et al. 2000	P	P
BPPCT030	*P. persica*	Dirlewanger et al. 2002	P	P
PceGA34	*P. cerasus*	Downey and Lezzoni 2000	P	P

thus a single dominant gene for resistance to both nematodes, designated R_{MiaNem} (= 'resistance to *M. incognita* and *M. arenaria* from Nemared'), was proposed.

Nematode isolates and RKN resistance evaluation

One isolate representative of each predominant species *M. arenaria, M. incognita* and *M. javanica* completed with the isolate *Meloidogyne* sp. Florida was used (Lecouls et al. 1997). RKN resistance evaluations were performed according to the procedure described by Esmenjaud et al. (1992). All the RKN isolates were maintained on tomato (*Lycopersicon esculentum* Mill.) cv St Pierre and their identity, at the species level, was verified before inoculation via their isoesterase phenotype (Janati et al. 1982).

DNA extraction and PCR experiments

Genomic DNA of *Prunus* material was extracted from frozen leaves according to the procedure of Saghai-Maroof et al. (1984) with some modifications. DNA concentrations and quality were evaluated by electrophoresis. For SSR markers, amplifications were performed in a 15-µl final volume containing 40–60 ng of genomic DNA, 0.7 U of *Taq* polymerase (Life Technologies), 0.2 µM of each primer, 200 µM of each dNTP (Promega Corp., Madison, Wis.), 1.5 mM of $MgCl_2$ and 1× reaction buffer provided with the enzyme. For each SSR, 0.3 pmol of the forward primer was γ^{33}P-ATP end-labeled with polynucleotide kinase (Invitrogen, Cergy-Pontoise, France). PCR conditions were as follows: 94°C for 4 min, then 35 cycles of [94°C for 45 s, annealing temperature provided by the authors (see Tables 3 and 4) for 45 s, 72°C for 45 s], and finally 72°C for 4 min. The labeled PCR products were separated on a 5% denaturing polyacrylamide gel containing 7.5 M urea, in 0.5× TBE running buffer then dried and autoradiographed on X-ray films. For the SCAR or STS (sequence tagged site) markers, PCR amplifications were performed as described by Lecouls et al. (1999) for SCAL19 and SCAN12, Lu et al. (1999) for EAA/MCAT10 STS, and Yamamoto and Hayashi (2002) for OPAP4, OPS14a and OPA11. To recover polymorphism for the SCAL19 marker in the resistant Japanese plum J.222, the multiplex amplification procedure reported by Lecouls (2000) using two forward and one reverse primer was performed. Sequences of those primers are: SCAL19JF1 (5′-TTAGGTGCAGGAATACCA-3′), SCAL19JF2 (5′-CAAATTGATCACCAATGATAC-3′) and SCAL19–2 (5′-CATTGGAGAAGATTGGCCC-3′), respectively.

Elaboration of the resistant and susceptible bulks and evaluation of polymorphism for RFLP and SSR markers in segregating progenies

Segregating crosses, RKN species considered, and the number of individuals used in the different progenies are reported in Table 2. Resistant (R) and susceptible (S) bulks were constituted by 12–15 individuals. Two couples of R and S bulks were constructed for *Ma* with the two intraspecific progenies P.2175×P.2646 and P.2175×P.16.5. One R and one S bulk was constructed from each of the peach segregating crosses (genes R_{Mia557} and R_{MiaNem}). In Japanese plum, no bulks were elaborated from the small-sized cross J.13×J.222 (26 individuals).

Localisation of the *Ma* gene was initiated using restriction fragment length polymorphism (RFLP) markers from the reference almond-peach map T×E (Joobeur et al. 1998). A set of 46 probes covering the entire genome and separated approximately by a mean distance of 20 cM were chosen. DNAs were digested with *Eco*RI, *Hind*III and *Hpa*II, and hybridized with the RFLP probes. The putative location was then more precisely defined by the same BSA approach using SSR markers. Those SSR markers obtained from various teams (Table 3) have been recently placed on the T×E reference map by Aranzana et al. (2002b). The localisation of R_{Mia557} and R_{MiaNem} was carried out using an equivalent BSA strategy, only based on SSR markers. As preliminary results obtained in Nemared by Jauregui (1998) and Lu et al. (1999) suggest a location of resistance factors in Nemared on linkage group (LG) 2, all ten available SSRs from LG2 (Table 4; Aranzana et al. 2002b) were tested in both segregating progenies for polymorphism or differences in amplification signal intensity between alleles in resistant and susceptible bulks. All parents and grandparents were also deposited in the gels to confirm the origin of the alleles linked to the R genes.

Linkage analysis

The MAPMAKER software version 3.0 (Lander et al. 1987) was used with a minimum LOD score of 3.0 to construct the local maps around *Ma* and R_{MiaNem}. Linkage analyses were performed using the Kosambi mapping function (Kosambi 1944) to convert recombination units into genetic distances. The Myrobalan plum local map around the *Ma* gene was established from SSR and SCAR markers using progenies of the three intraspecific crosses totalizing 288 individuals (Table 2). Concerning the gene R_{MiaNem}, as the BSA strategy confirmed its location on LG2, all the individuals were genotyped to construct the map of this linkage group for the parent G×N. These individuals were also evaluated for resistance to *Meloidogyne* sp. Florida and, among them, the susceptible individuals were tested for segregation of R_{MiaNem} to both *M. arenaria* and *M. incognita*. These latter segregation data were analysed to localise the gene R_{MiaNem} on the aforementioned map of LG2 in G×N. The STS markers OPAP4, OPS14a and OPA11, linked to the *Mia* gene and obtained by Yamamoto and Hayashi (2002), and the STS EAA/MCAT linked to the *Mij* gene and obtained by Lu et al. (1999), were also mapped. For the genes R_{Mia557} and R_{jap}, the number of individuals available in the progenies was limited (Table 2). Nevertheless, an indicative map of the linkage group carrying the gene R_{Mia557} was constructed from recombination frequencies. In the same way, the local map around R_{jap} was compared with the corresponding T×E reference map (Joobeur et al. 1998; Aranzana et al. 2002b) and with the local map of the *Ma* gene.

Results

Location of RKN genes in the Prunophora subgenus (Myrobalan and Japanese plums)

In the Myrobalan plum, only three RFLPs among the 46 probes distributed all over the *Prunus* genome, revealed polymorphic fragments between the resistant and the susceptible bulks, digested with *Eco*RI, *Hind*III and *Hpa*II. These were AG104 (with both *Eco*RI and *Hpa*II) (Fig. 1), AG63 (with *Eco*RI) and TSAIII (with *Hpa*II). All three RFLP markers lie on the LG7 of the reference map (Joobeur et al. 1998) and cover 32 cM. This preliminary position of *Ma* on LG7 was confirmed by the detection of an SSR polymorphism, or the difference in amplification signal intensity between bulks for three SSR markers located on this group, pchgms6, UDP98–405 and CPPCT033 (Table 3). Figure 2 shows an example of the polymorphim observed for pchgms6. Genotyping the individuals of the couples of bulks completed by all other individuals previously characterized for *Ma* (Table 2), allowed us to locate these markers on the same side of the gene at 2.3, 9.5 and 21.3 cM, respectively. These SSR markers are located on the other side of the gene relative to the SCAR markers SCAL19 and SCAN12 (Bergougnoux et al. 2002) (Fig. 3).

In the Japanese plum, the SCAR markers linked to *Ma* and all the SSRs available for this LG7 region were evaluated for their polymorphism in the J.222 and J.13 parents. Polymorphic markers (the SSRs pchgms6, CPPCT033 and CPPCT022), and in particular the new multiplex marker derived from SCAL19, were then directly evaluated on the 26 individuals of the segregating progeny J.13×J.222. On this small-sized cross, the markers pchgms6, CPPCT022 and SCAL19 co-segregat-

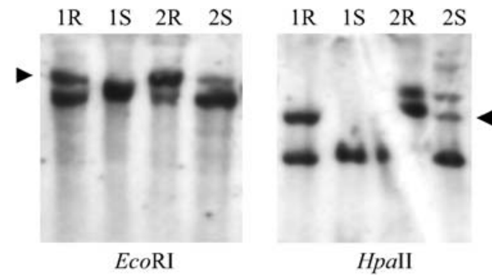

Fig. 1 RFLP patterns obtained for the bulks of *Ma1*-resistant and -susceptible individuals hybridized with the probe AG104. 1R, 1S, 2R and 2S, correspond to resistant (*R*) and susceptible (*S*) bulks for the segregating crosses P.2175×P.2646 (1) and P.2175×P.16.5 (2). The *arrows* indicate the location of the polymorphic bands

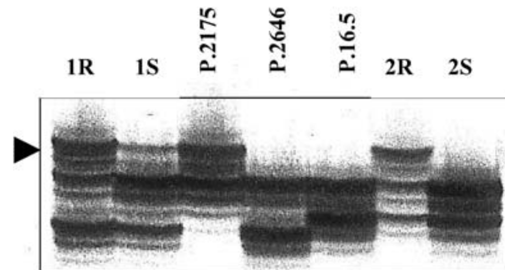

Fig. 2 Amplification pattern of the SSR pchgms6 in intraspecific Myrobalan progenies segregating for the *Ma* gene. P.2175 is the resistant (*R*) parent; P.2646 and P.16.5 are the susceptible (*S*) parent. *1R*, *1S*, *2R* and *2S* correspond to resistant (*R*) and susceptible (*S*) bulks for the segregating crosses P.2175×P.2646 (*1*) and P.2175×P.16.5 (*2*). The *arrow* indicates the location of the resistant allele in P.2175. For this allele, the less intense band recovered in *lane 1S* is due to one recombinant individual in this bulk

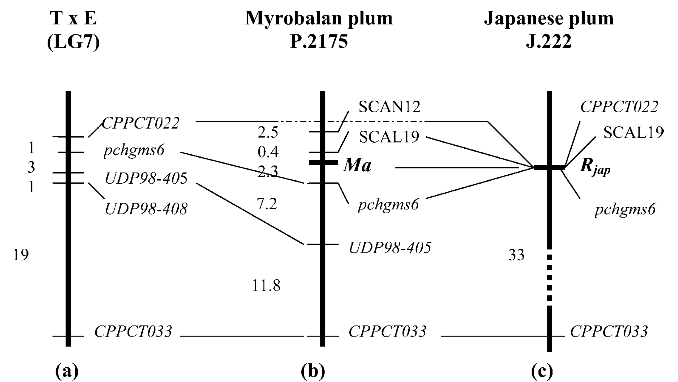

Fig. 3a–c Local maps pf SSR (*in italics*) and SCAR (*normal letters*) markers linked to the *Ma* gene in the Myrobalan plum P.2175 (**b**) and to the R_{jap} gene in the Japanese plum J.222 (**c**) in comparison with SSR markers located on the LG7 of the almond × peach reference *Prunus* map Texas × Earlygold (T×E) (**a**) (Aranzana et al. 2002b). For the *Ma* gene, distance are expressed in cM using the Kosambi distance given by the MAPMARKER software version 3.0 (Lander et al. 1987) with a minimum LOD score of 3.0. For the R_{jap} gene, distance are expressed in recombination percentages

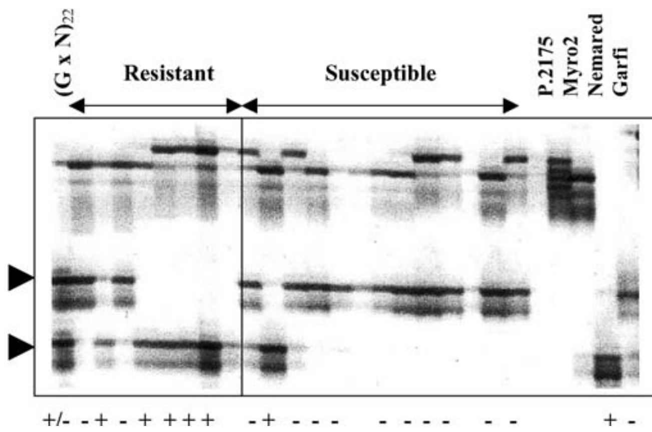

Fig. 4 Amplification pattern of the SSR UDP98-025 in the interspecific progeny P.2175×(G×N) segregating for the R_{MiaNem} gene. $(G{\times}N)_{22}$ and Nemared (*N*) are the resistant (*R*) parent and grandparent, respectively; Garfi (*G*) is the susceptible (*S*) grandparent; P.2175 and Myro2 are Myrobalan controls. The *arrows* indicate the location of the resistant (low) and susceptible (high) marker alleles in $(G{\times}N)_{22}$. (+) and (−) indicate the presence of these alleles in coupling with resistance (+) or susceptibility (−) in the individuals of the progeny

ed with the R_{jap} gene (Fig. 3), which shows that this gene lies on the LG7 probably in the same position as *Ma*.

Location of genes in the Amygdalus subgenus (Shalil and Nemared peaches)

Eight SSRs from LG2 in the reference T×E *Prunus* map (Aranzana et al. 2002b) expressed a clear polymorphism simultaneously in both couples of bulks. In order to localise more precisely R_{MiaNem}, each of the individuals of the two couples of bulks was genotyped together while the other RKN characterized individuals of the progeny. Figure 4 shows an example of the polymorphism of the alleles for the SSR UDP98-025 in segregating individuals from the cross P.2175×(G×N). Data from this cross were integrated in the map of LG2, and the respective positions of the markers were compared in that map and in the T×E map. R_{MiaNem} and R_{Mia557} are placed on the LG2 in an *a priori* equivalent subtelomeric position (Fig. 5). In the Japanese peach source Juseitou, Yamamoto and Hayashi (2002) have obtained five STS markers for resistance to *M. incognita* (gene *Mia*) and *M. javanica* (gene *Mja*), both genes being approximately 3.5-cM apart. Three of these STSs were polymorphic in at least one of our segregating progenies (Fig. 5). The STSs OPA11 and OPS14a, located on one side of *Mia*, were also located on the same side and in the same order as for R_{Mia557} and R_{MiaNem}. The STS OPAP4 located on the other side of *Mia* in Juseitou was also located on the other side of R_{Mia557}.

Discussion

Our data illustrate the respective positions of two plum and two peach loci involved in RKN resistance in *Prunus* species. Thanks to a RFLP approach (for plum) completed with a SSR approach (for plum and peach), plum genes were localised on LG7 and peach genes were shown to reside on LG2. *Ma* and R_{jap} mapped very close to the SSR marker pchgms6. The location of these genes in a cluster of SSR markers on LG7 makes the localisation of

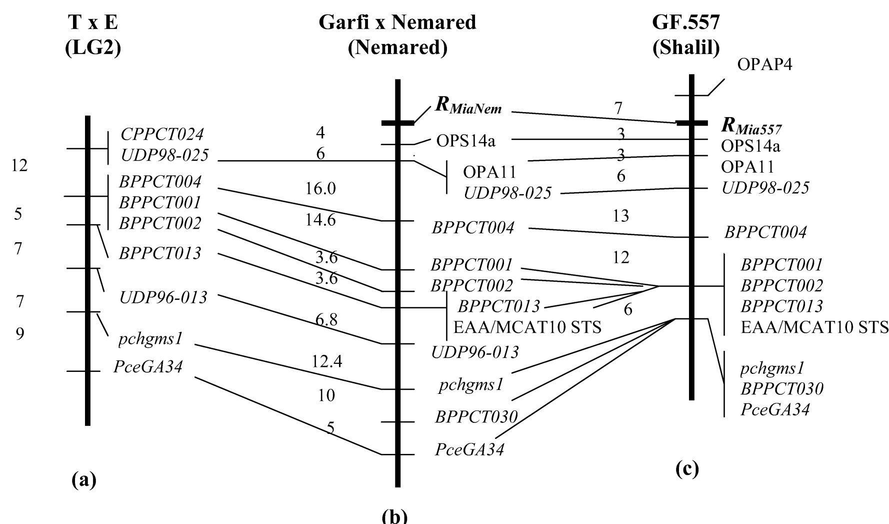

Fig. 5a–c Maps of SSR markers (*in italics*) linked to the genes R_{MiaNem} and R_{Mia557} for resistance to *M. incognita* in Nemared (**b**) and Shalil (**c**) peaches, respectively, in comparison with markers located on the LG2 of the almond × peach reference *Prunus* map Texas × Earlygold (T×E) (**a**) (Aranzana et al. 2002b). For the R_{MiaNem} gene, distance are expressed in cM using the Kosambi distance given by the MAPMARKER software version 3.0 (Lander et al. 1987) with a minimum LOD score of 3.0. For the R_{Mia557} gene, distances are expressed in recombination percentages. The SCAR markers OPAO4, OPS14a and OPA11, linked to the *Mia* gene in Juseitou (Yamamoto and Hayashi 2002), and the marker EAA/MCAT10 STS, linked to the *Mij* gene in Nemared (Lu et al. 1999), are indicated in normal letters

homeologous regions in other *Prunus* crosses easier, and illustrates the good cross-species transportability of these markers within *Prunus* species. Nevertheless the amplification of the Myrobalan alleles of pchgms6 in the three-way hybrid crosses [P.2175×(G×N)] was difficult to obtain, presumably because of competition between Prunophora and Amygdalus alleles. A new primer combination, more specific to the Myrobalan alleles, could be defined to solve this problem (M. Claverie, unpublished).

Ma is the first evidence of the precise localisation of a resistance gene in the *Prunus* genus. The location of R_{jap}, based on a 26-individual cross, is less fine but seems to be the same as *Ma*: R_{jap} co-segregates with the two markers flanking *Ma* in a 2.7-cM interval and with the tightly linked SSR marker (in the T×E map) CPPCT022. Additional segregating individuals should provide recombination events that would precisely define its location. It is likely that the location of *Ma* and R_{jap} is conserved in cultivated and wild plum species, including diploid to hexaploid species. Locations of R_{MiaNem} and R_{Mia557} suggest that both genes might be the same: the order of the SSR markers is conserved and, whatever the SSR, the coupling-phase alleles for resistance from Nemared are identical to the coupling-phase alleles for resistance from the Shalil parent (Fig. 5b and c). This could be explained by the limited genetic variability of peach and the almond-peach nature of both segregating parents. Nevertheless, the precise location of peach genes on LG2 in a subtelomeric position appears different from that obtained by Jauregui (1998) who placed one gene or a major QTL in a more centromeric position, in the vicinity of the SSR marker *pchgms1*. Moreover, our location of R_{MiaNem} was also different from that previously obtained by Lu et al. (1999) in the intraspecific peach cross Lovell × Nemared. These authors have found by a BSA approach that the *Mij* locus for resistance to *M. incognita* and *M. javanica* was located at about 3 cM from the EAA/MCAT10 STS marker (derived from an AFLP marker). We could clearly map this STS marker in a central position on LG2 (Fig. 3b) at more than 45 cM from R_{MiaNem}. Since an equivalent location is observed for R_{Mia557}, our data based on two independent crosses appear quite reliable and one can be confident for the map position of both peach genes. The differences between Nemared gene locations in different studies could be explained by the presence of a major QTL for *M. javanica* and *M. incognita* resistance, acting as a complete-resistance gene depending on either the nematode isolate or the inoculation procedure, or resistant versus susceptible definition or the genetic background. Taken together, these results completed by those of Lu et al. (2000) support the hypothesis of two genes lying on the same linkage group but at an approximate distance of 45 cM.

Since the origin of Nemaguard, the resistant ancestor of Nemared, remains unclear, co-location of R_{MiaNem} and R_{Mia557} could be explained by a relatively close parentage strongly suggested by the identity of SSRs alleles in coupling with resistance in both Nemared and GF557. It is highly probable that both genes also co-localize with the gene *Mia* in the peach Juseitou (Yamamoto and Hayashi 2002) since STS markers flanking *Mia* also flank R_{MiaNem} and R_{Mia557} in the same order. Concordant results from the three different sources, Shalil, Nemared and Juseitou, suggest that peach RKN sources share at least one major gene (or gene cluster) of resistance to *M. incognita* located in this subtelomeric position.

In Prunophora, differences in allelism and polymorphism of the genetic markers linked to resistance, associated with co-location of the *Ma* and R_{jap} genes in Myrobalan and Japanese plums, suggest the conservation of a resistance locus acquired before species separation. This last result, the usual transportability of SSR markers between *Prunus* species, together with the conservation of locus order and genetic distances around *Ma*, suggest an even higher level of synteny between *Prunus* species than previously observed (Joobeur et al. 1998).

Our most beneficial and applied result is that *Ma*, on the one hand, and the gene(s) specifically controlling *M. incognita* and *M. arenaria* in both Nemared and GF.557, on the other hand, are independent, and can be pyramided into new interspecific hybrid rootstock material. Introgression of *Ma* and peach genes into the genome of new *Prunus* rootstocks by interspecific hybridisation (e.g. Myrobalan plum × Amygdalus) has been undertaken. These hybrids can cumulate favorable agronomic traits from both origins, together with the complete-spectrum resistance controlled by the Myrobalan *Ma* gene and the more restricted-spectrum of Amygdalus genes. Indeed, the pyramiding of several genes in the same genotype may limit the risk of resistance breaking (Johnson 1983; Cook and Evans 1987; Roberts 1995), and thus extend the useful life of new rootstocks. For that purpose MAS for *Ma* is now greatly improved by the availability of the two flanking markers SCAL19 and pchgms6 in a 2.7 cM genetic interval.

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