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Microsatellite genetic linkage maps of myrobalan plum and an almond-peach hybrid—location of root-knot nematode resistance genes

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Abstract Inheritance and linkage studies were carried out with microsatellite [or simple sequence repeat (SSR)] markers in a F_1 progeny including 101 individuals of a cross between Myrobalan plum (*Prunus cerasifera* Ehrh) clone P.2175 and the almond (*Prunus dulcis* Mill.)-peach (*Prunus persica* L. Batsch) hybrid clone GN22 [‘Garfi’ (G) almond × ‘Nemared’ (N) peach]. This three-way interspecific *Prunus* progeny was produced in order to associate high root-knot nematode (RKN) resistances from Myrobalan and peach with other favorable traits for *Prunus* rootstocks from plum, peach and almond. The RKN resistance genes, *Ma* from the Myrobalan plum clone P.2175 and R_{MiaNem} from the ‘N’ peach, are each heterozygous in the parents P.2175 and GN22, respectively. Two hundred and seventy seven *Prunus* SSRs were tested for their polymorphism. One genetic map was constructed for each parent according to the ‘double pseudo-testcross’ analysis model. The *Ma* gene and 93 markers [two sequence characterized amplified regions (SCARs), 91 SSRs] were placed on the P.2175 Myrobalan map covering 524.8 cM. The R_{MiaNem} gene, the *Gr* gene controlling the color of peach leaves, and 166 markers (one SCAR, 165 SSRs) were mapped to seven linkage groups instead of the expected eight in *Prunus*. Markers belonging to groups 6 and 8 in previous maps formed a single group in the GN22 map. A reciprocal translocation, already reported in a G × N F_2, was detected near the *Gr* gene. By separating markers from linkage groups 6 and 8 from the GN22 map, it was possible to compare the eight homologous linkage groups between the two maps using the 68 SSR markers heterozygous in both parents (anchor loci). All but one of these 68 anchor markers are in the same order in the Myrobalan plum map and in the almond-peach map, as expected from the high level of synteny within *Prunus*. The *Ma* and R_{MiaNem}genes confirmed their previous location in the Myrobalan linkage group 7 and in the GN22 linkage group 2, respectively. Using a GN22 F_2 progeny of 78 individuals, a microsatellite map of linkage group 2 was also constructed and provided additional evidence for the telomeric position of R_{MiaNem} in group 2 of the *Prunus* genome.

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Introduction

The *Prunus* genus, containing over 400 species (Rehder 1954), is characterized by species that produce drupes as fruits, commonly called ‘stone fruits’ and includes peach (*Prunus persica* L. Batsch), apricot (*Prunus armeniaca* Linn.), cherry (*Prunus avium* L. and *Prunus cerasus* L.), plum (*Prunus salicina* Lindl. and *Prunus domestica* L.), and almond (*Prunus dulcis* Mill.). Several other species such as Myrobalan plum (*Prunus cerasifera* Ehrh.) or Sainte Lucie cherry (*Prunus mahaleb* L.) are mainly used as *Prunus* rootstocks. Peach and almond belong to the *Amygdalus* subgenus; plum and apricot belong to the *Prunophora* subgenus which is divided into two sections (i.e., Euprunus and Armeniaca, respectively). All the *Prunus* species have an 8-based chromosome number with various ploidy levels, $2n=2\times=16$ for peach, almond, sweet cherry and Myrobalan plum, $2n=4\times=32$ for sour cherry, and $2n=6\times=48$ for European plum (*P. domestica* L.).

Several *Prunus* linkage maps have been developed based on interspecific crosses between peach and almond (Foolad et al. 1995; Joobeur et al. 1998; Jáuregui et al. 2001; Bliss et al. 2002) or intraspecific crosses of peach (Chaparro et al. 1994; Rajapakse et al. 1995; Dirlewanger et al. 1998; Lu et al. 1998; Dettori et al. 2001; Yamamoto et al. 2001), almond (Viruel et al. 1995; Joobeur et al. 2000), apricot (Hurtado et al. 2002; Lambert et al. 2004), sour cherry (Wang et al. 1998). The almond × peach map, Texas × Earlygold (T × E), is highly saturated and is considered as the reference *Prunus* linkage map (Joobeur et al. 1998; Aranzana et al. 2003).

Most of the cultivated *Prunus* species are mainly grown in regions with Mediterranean climates where root-knot nematode (RKN) species *Meloidogyne arenaria* (MA), *Meloidogyne incognita* (MI) and *Meloidogyne javanica* (MJ) are widely distributed. These nematodes are major pests in *Prunus* orchards and are an important component of the replanting problem (Layne 1987; Nyczepir 1991). Until now, RKN have been mainly controlled by pre-plantation fumigations with methyl bromide (that will be completely removed for *Prunus* species by the end of 2004) or other highly toxic fumigants. The search for RKN resistant *Prunus* rootstocks as an alternative control method has been a long and continuous task in many countries (Minz and Cohn 1962; Sharpe et al. 1969; Kochba and Spiegel-Roy 1975; Sherman et al. 1981; Ramming and Tanner 1983; Kester and Grassely 1987; Lu et al. 1999; Yamamoto et al. 2001; Lecouls et al. 2004). The efficiency of RKN resistance in rootstocks depends on the source of resistance (Scotto La Massese et al. 1984; Esmenjaud et al. 1997). In France, interspecific hybrids termed P.2175 × GN22 between the Myrobalan plum clone P.2175 and the almond–peach (‘Garfi’ × ‘Nemared’) 22 (‘Felinem’; Gómez Aparisi et al. 2001), were produced to generate high-performance rootstocks for Mediterranean environments (Salesses et al. 1998) by combining their complementary traits: high resistance to RKN (by pyramiding RKN resistance genes from P.2175 and Nemared), adaptation to chlorosis and drought (from almond), tolerance to waterlogging (from plum) together with graft compatibility with peach (from peach) and good rooting ability (from plum).

The Myrobalan plum parental clone P.2175 was shown to be resistant to MA, MI, MJ and *Meloidogyne* sp. Florida (FL) (Lecouls et al. 1997; Rubio-Cabetas et al. 1999) and to the minor species *Melpidogyne mayaguensis* (Fargette et al. 1996; Rubio-Cabetas et al. 1999). This resistance is conferred by the *Ma* dominant resistance gene in the heterozygous state in P.2175 (Esmenjaud et al. 1996b; Rubio-Cabetas et al. 1998). The *Ma* resistance was not overcome by any of the 30 RKN species and isolates tested (Esmenjaud et al. 1994, 1997; Fernandez et al. 1994) and was not modified under conditions known to affect plant defenses to RKN such as high temperature and high inoculum pressure (Esmenjaud et al. 1996a). Moreover, the clone P.2175 shows a satisfactory graft compatibility with peach, this not being the case for all the Myrobalan clones, and thus appears particularly useful for breeding of RKN-resistant rootstocks. The peach rootstock Nemared (Ramming and Tanner 1983) is derived from the peach Nemaguard, one of the first RKN resistance source detected in the subgenus *Amygdalus*. Nematode resistance in Nemared was first reported to be controlled by two genes (*Mi* controlling the resistance to MI, and *Mij* the resistance to MI and MJ) found by Lu et al. (1998) using a ‘Lovell’ × ‘Nemared’ F_2 population. QTLs controlling the RKN from Nemared were also detected by Jauregui (1998) with a ‘Garfi’ × ‘Nemared’ F_2-like population. More recently two genes (*Mia* and *Mja* for resistance to MI and MJ, respectively) were found in the ‘Akame’ × ‘Juseitou’ F_2 population (Yamamoto and Hayashi 2002), and one gene (*Mi*) controlling MI was mentioned by Bliss et al. (2002) in a peach × peach (PMP2) population. By using P.2175 × GN22 hybrids, we have detected a single dominant gene for resistance to both MI and MA in Nemared (designated R_{MiaNem} ‘resistance to *M. incognita* and *M. arenaria* from Nemared’) which has been shown to be closely linked to *Mia* from Juseitou and thus might be the same gene (Claverie et al. 2004).

In this paper, we present the first linkage map in the Myrobalan plum P.2175, and a saturated map of the almond-peach GN22. The comparison between the P.2175 and the GN22 maps was made possible by using markers heterozygous in both parents. The reciprocal translocation between ‘Garfi’ and ‘Nemared’ was detected and the breakpoint was placed near the *Gr* gene controlling the red leaf color in peach. Two independent root-knot nematode resistance genes were located on the *Prunus* maps: *Ma* from Myrobalan was located in the P.2175 map, and R_{MiaNem} from Nemared peach was located in both GN22-F_1 and GN22-F_2 maps.

Materials and methods

Two progenies were analyzed for nematode resistance and for molecular marker linkage analyses: (1) an F_1 progeny of 101 hybrids issued from a three-way cross between the Myrobalan plum P.2175 and the almond–peach hybrid GN22; and (2) an F_2 progeny of 78 hybrids issued from the self-fertilization of GN22 (GN22⊗). The P.2175 × GN22 progeny segregates for the two RKN resistance genes, *Ma* from P.2175 and R_{MiaNem} from Nemared, and also for leaf color, controlled by a single gene (*Gr*), red being dominant and green recessive (Blake 1937). P.2175 has green leaves and is heterozygous for *Ma* (*Ma*/*ma*, r_{MiaNem}/r_{MiaNem}, gr/gr); GN22 is heterozygous for R_{MiaNem} and for the color of the leaves: (*ma*/*ma*, R_{MiaNem}/r_{MiaNem}, *Gr*/*gr*). The GN22⊗ progeny segregates for the R_{MiaNem}resistance gene. These two progenies were planted in the orchard of INRA-Domaine ‘Les Jarres’ (Unité Expérimentale Arboricole), near Bordeaux (France) and maintained in the field under standard culture conditions.

Nematode isolates and RKN resistance evaluation

One isolate of each of the RKN species MI and *Meloidogyne* sp. Florida (FL), was used. RKN isolates were maintained on tomato (*Lycopersicon esculentum* Mill.) cv. St Pierre and their identity at the species level was verified via their isoesterase phenotype before inoculation (Janati et al. 1982).

Homogenous softwood cuttings (25 cm long, 5 mm diameter) sampled from adult trees were harvested in June in the 'Unité de Recherches sur les Espèces Fruitières et la Vigne' at INRA (Villenave d'Ornon, France), rooted individually in alveolated plates in the nursery up to the next late autumn to allow for the development of rooted plants. Rooted plants were supplied to the 'Nematology Team' at INRA (Antibes, France) in December for resistance evaluation. RKN resistance evaluations were then performed according to the procedure described by Esmenjaud et al. (1992).

The *Ma* gene has a complete spectrum [comprising in particular MI and *Meloidogyne* sp. Florida (FL)]. The R_{MiaNem} gene does not control the FL isolate and thus this RKN can be used to separate the resistant individuals within the Myrobalan×almond-peach progeny carrying the *Ma* resistance allele from P.2175 from the susceptible individuals lacking it (Lecouls et al. 1997). Only susceptible individuals (homozygous recessive *ma/ma*) were evaluated separately for resistance to the MI isolate in order to identify homozygous and heterozygous hybrids for R_{MiaNem}. All 101 individuals of the P.2175 × GN22 cross were evaluated with FL. Twenty-seven of the susceptible individuals from this cross and 23 of the GN22⊗ individuals were then evaluated with MI.

DNA isolation

Fifteen milligram of young expanded terminal leaves were collected in a plate of 96 collection microtubes, each of 1.2 ml containing a tungsten carbide bead (3 mm diameter). They were ground in the presence of liquid nitrogen by using a Mixer Mill MM 300 (Retsch) for 1 min 30 s. Genomic DNA was then directly extracted following the method described by Viruel et al. (1995). DNA concentrations were measured using a spectrophotometer (μQuant BIO-TEK Instruments) and diluted to a final concentration of 50 ng/μl.

SCAR or sequence tagged site analysis

Two reliable SCAR markers, $SCAL19_{690}$ and $SCAFLP2_{202}$, already reported to be linked in coupling to the dominant resistant allele *Ma* (Bergougnoux et al. 2002; Lecouls et al. 1999) were PCR-amplified as described by Lecouls et al. (1999). The five STS markers obtained by Yamamoto and Hayashi (2002), STS-OPAP4, STS-OPS14a, STS-834b, STS-OPA11, STS-OPAC9, linked to the resistance loci of the peach 'Juseitou' were tested as described by these authors.

SSR analysis

Several sources of *Prunus* SSRs were tested for their polymorphism in the P.2175 × GN22 progeny (Table 1). As all genes described so far to determine disease resistance to RKN coming from peach have been placed on linkage group 2 of *Prunus* (Lu et al. 1998; Jáuregui 1998; Yamamoto et al. 2001; Bliss et al. 2002, Claverie et al. 2004), only the microsatellites located on linkage group 2 in *Prunus* linkage maps were tested on the GN22⊗ population.

The SSRs were PCR-amplified as follows: PCR buffer (20 mM Tris-HCl, 50 mM KCl), 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, 0.7 U of *Taq* DNA polymerase (Gibco BRL) and 20 ng of peach genomic DNA in a 15 μl final volume. PCR reactions were performed on a GeneAmp 9600 thermal cycler (Perkin-Elmer Cetus) with an initial denaturation for 1 min at 94°C followed by 35 cycles of 45 s at 94°C, 45 s at 57°C, 2 min at 72°C, then a final extension of 4 min at 72°C. Five microliters of the PCR products were separated on a 2% agarose gel and stained with ethidium bromide to check the PCR amplification and determine the approximate size of the amplified fragments. The PCR products were then denatured by the addition of 1 vol of 95% formamide/dye solution (loading dye: 95% deionized formamide, 10 mM NaOH, 0.05% xylene cyanol, 0.05% bromophenol blue), heated for 5 min at 94°C, chilled on ice and then 1.5 μl of the denatured preparations were loaded on 6% polyacrylamide sequencing gels containing 7.5 M urea in 0.5× TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA). Gels were run for 2 h at 80 W. Following electrophoresis, the gel was silver-stained according to Cho et al. (1996). Fragment sizes were estimated with 10 bp ladder DNA sizing markers (GibcoBRL Life Technologies).

Segregation analysis and map construction

Each polymorphic marker was tested by χ^2 analysis for goodness of fit (P<0.01) to the segregation ratios expected for a F_1 or F_2 population. For the P.2175 × GN22-F_1 progeny, separate genetic linkage maps were constructed for each parent following the "double pseudo-testcross" model (Grattapaglia and Sederoff 1994).

Each linkage map was constructed using the MAPMAKER/EXP V3.0 software (Lander et al. 1987). Markers were first divided into linkage groups using a critical LOD score threshold of 5.0 and a maximum recombination fraction of 0.3. The Kosambi function was used to convert recombination units into genetic distances. Loci heterozygous in both parents were used as anchor loci for the alignment of the two maps. The data for each marker were scored independently by two people.

Table 1 Prunus microsatellites tested for their polymorphism in the P.2175 × GN22 progeny. The SSR GDR (genome database for Rosaceae) tested were SSR 96D14-B4, SSR 96D14-C4, SSR 70 O11-B6, SSR 81P4-B7, SSR 13 C23-B11, SSR 65 P19-B8

SSR names	*Prunus* species	Repeats	Origins	References
UDP	*P. persica*	CT, GT	Two enriched genomic libraries from 'Redhaven'	Cipriani et al. (1999) and Testolin et al. (2000)
CPPCT	*P. persica*	CT	Enriched genomic libraries from 'O'Henry'	Aranzana et al. (2002)
BPPCT	*P. persica*	CT	Enriched genomic libraries from 'O'Henry'	Dirlewanger et al. (2002)
Pchgms	*P. persica*	CT, CA	Genomic library from 'Bicentennial'	Sosinski et al. (2000)
Pchgms	*P. persica*		cDNA library from 'Suncrest'	
MA	*P. persica*	GA	Genomic DNA from 'Akatsuki'	Yamamoto et al. (2002)
M	*P. persica*	CT	cDNA library from 'Akatsuki'	Yamamoto et al. (2002)
Pms	*P. avium*	CT, CA	Genomic library from 'Valerij Tschakhalov'	Cantini et al. (2001)
PS	*P. avium*	GA, GT, GTT	Enriched genomic library from 'Napoleon'	Joobeur et al. (2000), Cantini et al. (2001)
PceGA	*P. cerasus*	GA	Genomic library from 'Erdi Botermo'	Cantini et al. (2001), Downey and Iezzoni (2000)
SsrPaCITA	*P. armeniaca*	CT	Genomic library from 'Ungarische Beste'	Lopes et al. (2002)
AMPA	*P. armeniaca*	CT, CA, GA, GT, AT, CTT	cDNA library from 'Bergeron' and genomic library from 'Polonais'	INRA, Avingon, L. Hagen (unpublished)
PacD	*P. armeniaca*	CT, GA	cDNA library from 'Stark Early Orange'	INRA, Bordeaux, V. Decrocq (unpublished)
CPSCT	*P. salicina*	CT, GA	Enriched genomic library from 'Suite Rosa'	Mnejja et al. (2004)
CPDCT	*P. dulcis*	CT, GA	Enriched genomic library from 'Texas'	IRTA, Cabrils, P. Arús (unpublished)
SSR GDR	*P. persica*		BAC library from 'Nemared'	GDR database (http://www.genome.clemson.edu)

Conflicting results were re-examined. After mapping, the 'error detection' option of Mapmaker was used, and possible errors were examined again. Map figures were obtained using the FITMAPS software (Graziano and Arús 2002).

Detection of the translocation

One GN22 group, obtained by using a critical LOD score threshold of 5.0 and a maximum recombination fraction of 0.3, included markers located on linkage groups 6 and 8 in other *Prunus* maps (T × E map, Aranzana et al. 2003; P × E map, Dettori et al. 2001) and markers not already mapped. Markers included in this group were first mapped on a single linkage group as described below.

In order to construct the two separate linkage groups 6 and 8, markers already mapped in other *Prunus* maps were separated into two groups and linkage analyses were then performed. To determine the group that included the non-previously mapped markers, the "two point" and "try" commands of Mapmaker were used. These markers were located either in G6 or G8.

Results

SCAR analysis

$SCAL19_{690}$ and $SCAFLP2_{202}$, the two SCARs linked to the *Ma* gene (Lecouls et al. 2004), were analyzed in the progeny. $SCAL19_{690}$ could be read directly from agarose gels: all susceptible and resistant plants contained two fragments of 750 bp and 720 bp respectively, and a single smaller fragment of 690 bp. To determine thesegregation of marker $SCAFLP2_{202}$, acrylamide gels were needed to separate the fragments. All the resistant plants had profiles with the lower fragment (202 bp).

The five STS markers obtained by Yamamoto and Hayashi (2002), STS-OPAP4, STS-OPS14a, STS-834b, STS-OPA11, STS-OPAC9, linked to the resistant loci of the peach 'Juseitou', were tested. STS-834b gave no amplification product under our PCR conditions. STS-OPAP4 revealed no polymorphism in the progeny. STS-OPS14a and STS-OPAC9 gave complex profiles which were not easily readable. Only STS-OPA11 had a readable profile, with a fragment of 481 bp segregating in the P.2175 × GN22-F_1 progeny. In the GN22⊗ progeny, the amplification profile obtained with this marker was not clear enough to be used for the mapping analyses.

SSR analysis

The 277 SSRs tested for their polymorphisms originated from several *Prunus* species: 141 were from peach, 9 from sweet cherry, 4 from sour cherry, 58 from apricot, 28 from Japanese plum, 31 from almond and 6 from Myrobalan plum (Table 2). Only 13 (4.7%) gave no amplification

under our amplification conditions and the highest percentage of non-amplification was obtained with apricot SSRs (6.9%). Among the SSRs producing an amplification product, 46 (16.6%) had complex profiles on acrylamide gels, 14 (5%) revealed no polymorphism, 104 (37.5%) revealed polymorphism in P.2175, 184 (66.4%) revealed polymorphism in GN22 and 84 (30.3%) were polymorphic in both parents.

Among the SSRs revealing polymorphisms, those having easily readable profiles were selected, especially those revealing two alleles in each parent. As the polymorphism rate was much higher in GN22 than in P.2175, SSRs revealing polymorphism in P.2175 were selected.

Inheritance and map construction

Among the 169 SSRs selected for the mapping analysis, only four revealed several loci: CPPCT003 revealed four dominant loci in the GN22 map, CPPCT010 revealed three dominant loci in the GN22 map, CPSCT007 revealed three loci (one co-dominant and two dominant loci) in the P.2175 map, while CPSCT039 revealed two dominant loci on GN22.

Segregation of the Ma, R_{MiaNem} and Gr genes

The 101 individuals from 2175 × GN22 tested with *Meloidogyne* sp. Florida to determine the status of their the *Ma* gene were shown to segregate at a ratio of 40 resistant:61 susceptible. This segregation deviated from the expected 1:1 ratio (P=0.036). The 61 susceptible individuals, together with the GN22⊗ hybrids, were then evaluated for their resistance to *M. incognita*, in order to determine the status of their R_{MiaNem} gene. Nevertheless, only a subset of both mapping populations could be evaluated, due to unsuccessful rooting of the cuttings. Within the 27 P.2175 × GN22 hybrids evaluated with MI, 13 were resistant and 14 were susceptible, thus fitting the expected 1:1 ratio. Among the 23 GN22⊗ hybrids evaluated with MI, 17 were resistant and 6 were susceptible; also fitting the 3:1 ratio expected in an F_2 population. Among the P.2175 × GN22 hybrids, a high distorted segregation ratio (P=0.00059) was observed for leaf color, with 32 red-leaf and 66 green-leaf individuals.

GN22 linkage map

The map of the interspecific almond-peach GN22 parent from the P.2175 × GN22 progeny was constructed by analyzing the segregation of the R_{MiaNem} gene, the *Gr* gene, and 166 markers (165 SSRs and one STS). The

Table 2 Number of Prunus microsatellites tested and polymorphic in the P.2175 × GN22 progeny

SSR names	*Prunus* species	No. of SSR tested	No. of SSR amplified	Complex profiles	No. of polymorphic SSR	Polymorphic		In both
						In P.2175	In GN22	
UDP	*P. persica*	26	25	0	23	13	22	12
CPPCT		34	33	2	26	9	25	8
BPPCT		40	39	4	34	20	34	20
Pchgms		6	6	0	6	4	6	4
Pchcms		4	4	0	3	1	3	1
MA		24	24	7	17	9	14	6
M		7	7	1	6	3	5	2
Total		141	138	14	115	59	109	53
Pms	*P. avium*	6	6	0	5	3	5	3
PS		3	2	0	2	1	2	1
Total		9	8	0	7	4	7	4
PceGA	*P. cerasus*	4	4	0	3	1	3	1
SsrPaCITA		21	20	7	13	9	10	6
AMPA	*P. arme-*	34	31	12	18	6	17	5
PacD	*niaca*	3	3	0	3	2	3	2
Total		58	54	19	34	17	30	13
CPSCT	*P. salicina*	28	25	6	19	11	17	9
CPDCT	*P. dulcis*	31	29	7	22	9	17	4
SSR GDR	*P. persica*	6	6	0	4	3	1	0
	Total	**277**	**264** (95.3%)	**46** (16.6%)	**204** (73.6%)	**104** (37.5%)	**184** (66.4%)	**84** (30.3%)

segregation of 69 SSRs (41.5%) deviated significantly from the expected ratio ($P<0.01$). They were located on linkage groups 1 (2), 2 (5), 3 (16, corresponding to all markers from the group), 4 (8), 5 (18 among the 19 of the group), 6 (18) and 8 (2).

With a LOD >5.0, all markers were grouped into seven linkage groups instead of the eight expected. The 27 plants for which we had information on their resistance to MI allowed the R_{MiaNem} gene to be mapped to linkage group 2 (G2) with a LOD >4.7. Six of the linkage groups, G1, G2,

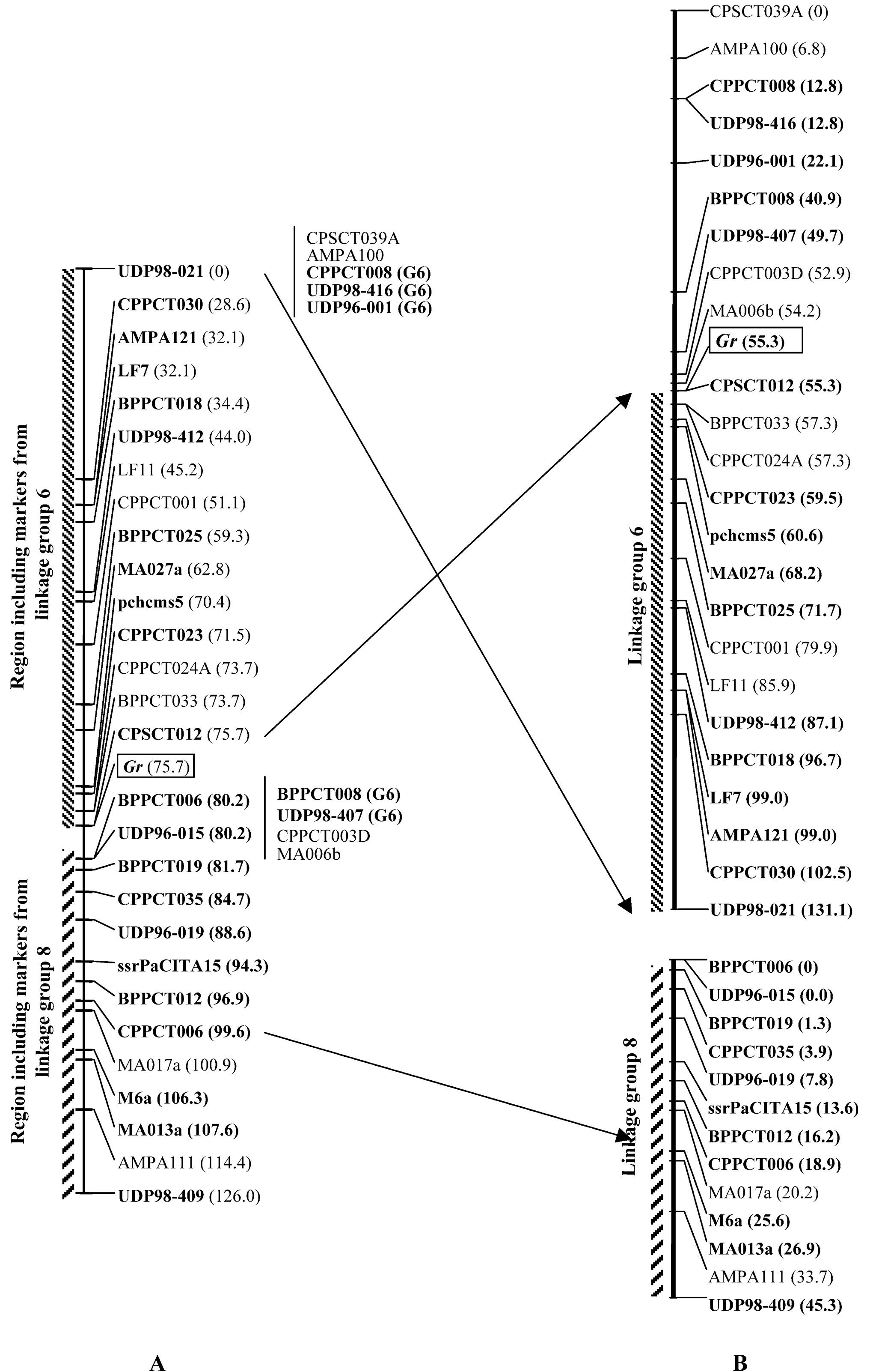

Fig. 1 **a** GN22 linkage group, obtained with a LOD >5.0, including markers already mapped within linkage groups 6 and 8 of other *Prunus* maps (indicated in *bold*). The upper part is homologous to linkage group 6 in other maps (indicated by a *segment*), upside down. The lower part is homologous to the linkage group 8 (indicated by a *segment*). **b** Position of imprecisely mapped markers is indicated on the *right* side of the group GN22 linkage groups 6 and 8 constructed using separate markers previously assigned to each group

G3, G4, G5, G7, were homologous to those found in T × E with identical locus order and similar distances (data not shown). Thirty-eight markers formed a single group in the GN22 map using the 'group' command of MAPMAKER. Among them, 16 were already mapped on linkage groups 6 and 11 on linkage group 8 in other maps [T × E (Aranzana et al. 2003), P × F (Dettori et al. 2001), J × F (F. Dirlewanger, unpublished)]; 11 markers were not previously mapped. Ordering of this group (G6–G8) was difficult and a linear order containing all loci could not be established. The map with the most markers included only 29 loci; 11 were already mapped in other maps on G6 and 11 on G8 (Fig. 1). The top of G6–G8 contained markers already mapped in G6 but in the inverse order in comparsion with the T × E map, the bottom of G6–G8 contained markers already mapped in G8 in exactly the same order as in the T × E map. This pseudo-linkage between G6 and G8 is a consequence of a reciprocal translocation between the chromosomes corresponding to G6 and G8 in the two parents of this cross. This has already been reported in a 'Garfi' × 'Nemared' F_2 population (Jáuregui et al. 2001). Nine markers could not be placed precisely: five were located on the top of G6–G8, and four near the *Gr* gene.

In order to determine the two separate groups G6 and G8, the 16 markers already mapped within G6 and the 11 markers already mapped within G8 were mapped independently. The 10 markers not previously assigned were then added to either group 6 or group 8 by using the "two point" and "try" commands. Each of them were located unambiguously in one of the two groups. Twenty-four markers and *Gr* were located on G6 (including eight markers not previously mapped) and 13 markers were located on G8 (with two markers not previously mapped).

With a LOD >5.0, the GN22 map covered 716.0 cM, including the *Gr* gene located on G6 and 166 markers (165 SSRs and one STS) (Fig. 2). With a LOD >4.7, the R_{MiaNem} gene controlling the nematode resistance from 'Nemared' was placed on G2 near the top of the group, between ssrPaCITA27 (13 cM) and the SCAR STSOPA11 that cosegregates with the SSR MAO24a (7.4 cM). The mean density of the map was 4.3 cM between markers. The biggest gap was 19.9 cM long and is located on G3.

P.2175 Myrobalan linkage map

The P.2175 Myrobalan linkage map was constructed by analyzing the segregation of the *Ma* gene and of 94 markers (92 SSRs, 2 SCARs) (Fig. 2). The segregation of ten SSRs (10.6%) deviated significantly from the expected 1:1 segregation ratio ($P<0.01$). They were located on G6 (3), G7 (6) and G8 (1).

With a LOD >5.0, chromosomes 1, 3, 5 and 7 were separated into two linkage groups. The two separated linkage groups belonging to the same chromosome were grouped in a single linkage group with a LOD >3.2 for chromosome 1 and 3, with a LOD >4.2 for chromosome 5, and with a LOD >4.1 for chromosome 7. Only one marker remained unlinked: BPPCT018. The P.2175 linkage map covered 524.8 cM with a LOD >5.0 and 653.8 cM with a LOD >3.2.

The *Ma* gene, already reported to cosegregate with the SCAR marker SCAFLP2 (Lecouls et al. 2004), co-segregated also with the SCAR $SCAL19_{690}$ and the SSR 96D14-B4. This SSR was identified within a BAC clone from the Nemared library (Georgi et al. 2002), containing $SCAFLP2_{202}$ (A.C. Lecouls, personal communication) The *Ma* gene and SSR 96D14-B4 segregated with the expected Mendelian 1:1 ratio; the two SCARs showed distorted segregation (P=0.037 each). They were located on P.2175 G7 at 12.4 cM from the top of the linkage group.

Comparison of the P.2175 and GN22 maps

Seventy-three SSRs were polymorphic in both parents. Five of them were not placed on the homologous linkage group in both maps: CPPCT024 (P.2175 G2 and GN22 G6), BPPCT006 (P.2175 G1 and GN22 G8), BPPCT019 (P.2175 G2 and GN22 G8), MA040a (P.2175 G6 and GN22 G3), UDP96–019 (P.2175 G5, GN22 G8). CPPCT024 has already been reported to reveal several loci and mapped onto linkage groups 1, 2, 4 and 7 in the T × E reference map (Aranzana et al. 2003). BPPCT006 and BPPCT019 were located in T × E G8. MA040a was located in G6 in the sweet cherry maps 'Regina' and 'Lapins' and in G7 in the peach map 'Jalousia' × 'Fantasia' (E. Dirlewanger, unpublished). Thus, among the 73 SSR markers polymophic in both parents, 68 were placed on both maps on homologous linkage groups. Four to fifteen anchor markers were detected according to the linkage group: 8 (G1), 15 (G2), 10 (G3), 10 (G4), 4 (G5), 4 (G6), 10 (G7), 7 (G8). The order of all the 68 anchor markers, except two which were inverted (UDP96–013 and BPPCT013 in G2), was exactly

Table 3 Genetic distance comparison between homologous regions of linkage groups in the P.2175 and GN22 maps

Linkage group	Interval	Genetic distance (cM)	
		P.2175	GN22
G1	PacD51-UDP96-005	48.3	56.5
	CPPCT026-BPPCT028	36.7	33.9
G2	ssrPaCITA27-CPSCT034	94.0	99.3
G3	MA034a-BPPCT039	35.1	29.0
	ssrPaCITA10-AMPA119	20.3	17.8
G4	BPPCT010-PS12a2	78.8	86.6
G5	AMPA105-BPPCT026	13.4	0.0
	BPPCT038-BPPCT032	4.1	17.6
G6	BPPCT008-BPPCT025	45.9	30.8
G7	AMPA107-pchgms6	16.4	22.8
	CPSCT042-CPPCT007	32.4	19.0
G8	CPPCT035-UDP98-409	37.4	45.6
Total		462.8	458.9

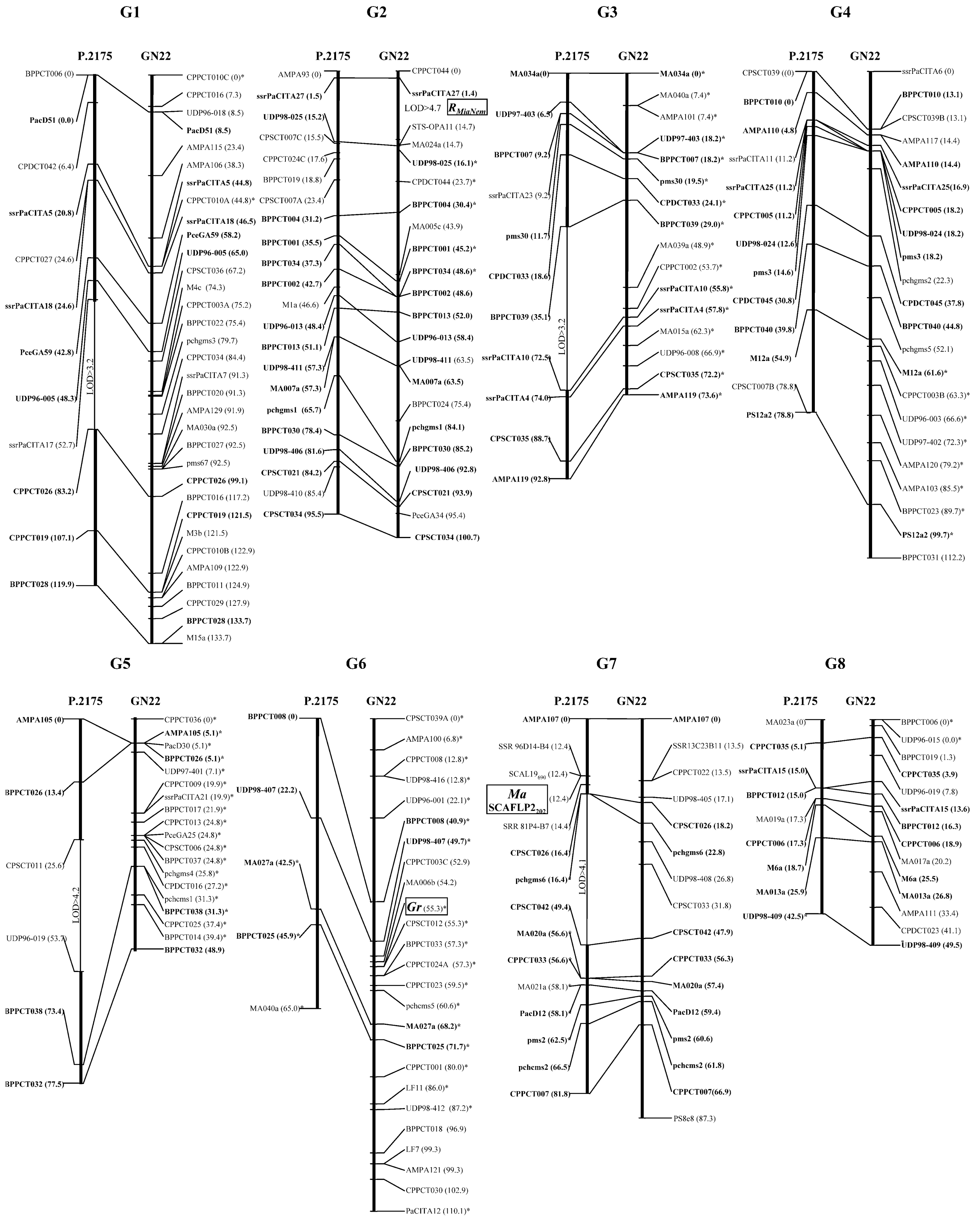

Fig. 2 Genetic maps obtained with the interspecific Myrobalan plum (P.2175) × almond-peach (GN22) F_1 progeny. Anchor loci between the P.2175 and the GN22 maps (in *bold*) are connected by *lines*. Distorted loci (P<0.01) are indicated by an *asterisk* after the name

the same in the two maps, and the distances between the markers were very similar in both maps (Table 3).

GN22⊗ map of linkage group 2

Using 19 of the SSRs mapped in G2 of the GN22 map, we elaborated a map of this group in the GN22⊗ progeny. The map covered a total distance of 47 cM and the position of the markers compared with the GN22 map was colinear, with two exceptions (BPPCT024 and CPSCT021) that were placed in a slightly different order relative to other tightly linked loci (Fig. 3). For the analysis of the R_{MiaNem} locus, data from 23 individuals (17 resistant and 6 susceptible hybrids) allowed the placement of this gene with a LOD ≥4.3 within the region of 13 cM delimited by markers ssrPaCITA27 and CPDCT044, in agreement with its position in the GN22 parent map of the three-way F_1 progeny.

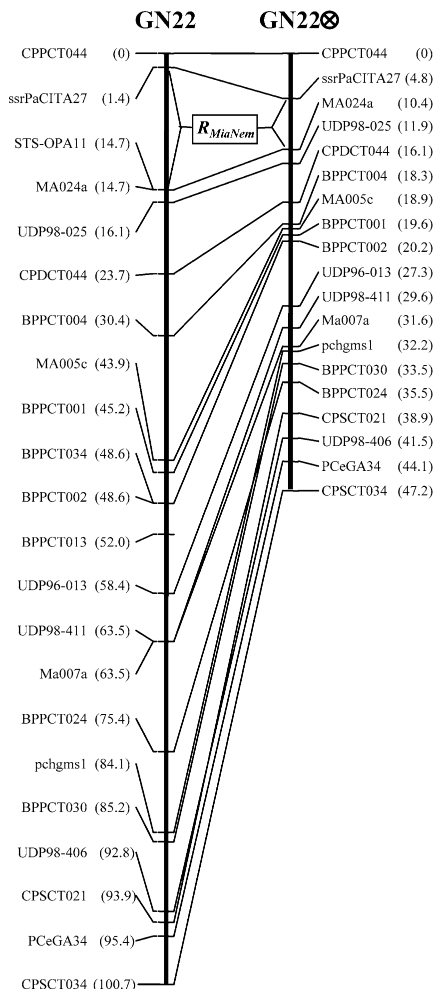

Fig. 3 Comparison between the maps of linkage group 2 of GN22 and GN22⊗, with indication of the most probable position of the R_{MiaNem} gene. Distorted loci are indicated by an *asterisk* after the name.

Discussion

Cross-species portability among *Prunus* species and segregation of the SSR markers

Among the 277 *Prunus* SSRs analyzed, most of them were obtained from peach (141) and others from apricot (58), almond (31), Japanese plum (28), sweet cherry (9), sour cherry (4) and Myrobalan plum (6). Only 4.7% gave no amplification confirming the high degree of microsatellite portability among *Prunus*. This is in agreement with previous data (Cipriani et al. 1999; Dirlewanger et al. 2002).

The number of polymorphisms detected in GN22 (66.4%) was much higher than that detected in P.2175 (37.5%). The high degree of heterozygosity in GN22 results from the fact that it is an interspecific almond-peach hybrid. As P.2175 is a Myrobalan plum, which is a self-incompatible species, a high degree of heterozygosity was also expected. The fact that few Myrobalan SSRs were used may explain the low degree of polymorphism detected in P.2175. The highest percentage was obtained with Myrobalan SSRs in P.2175 (50%) and with peach SSRs in GN22 (77.3%). Most heterozygous SSRs in P.2175 were also heterozygous in GN22 (80.8%). For the construction of the maps, simple locus SSRs revealing two alleles in P.2175 were preferred; 176 SSRs were selected and analyzed in the progeny.

More SSRs deviated significantly from the expected ratio in GN22 (41.5%) than in P.2175 (10.6%); the interspecific status of GN22 may explain these results. They were not located in the same region in the P.2175 and the GN22 maps. Among the 166 SSRs heterozygous in GN22, all those located in G3 and nearly all those located in G5 and G6 showed distorted segregation. In most cases, distorted segregations are more frequent in interspecific crosses than in intraspecific ones (Guo et al. 1991; Kianian and Quiros 1992). Mistakes between the coupling of homologous chromosomes during metaphase 1 may occur in interspecific crosses. In P.2175, these markers were located mainly within G6 and G7. Only the central part of G6 contained markers showing distorted segregation in both maps.

Many SSR markers (92) were already located on the T × E map (Aranzana et al. 2003), others were mapped on the peach P × F map (Dettori et al. 2001), on the apricot 'Stark Early Orange' and 'Polonais' maps (Lambert et al. 2004) or in the almond 'Ferragnès' and 'Tuono' maps (Joobeur et al. 2000). Here, 75 SSR markers were mapped for the first time.

GN22 almond-peach map

All markers were grouped into seven linkage groups; loci from the linkage groups G6 and G8, according to markers already mapped in other maps, appeared as a single group. The fusion of G6 and G8 observed in GN22 is attributed to the presence of a reciprocal translocation between the

'Garfi' almond and the 'Nemared' peach. This translocation has already been detected in the F_2 from the same parents (Jáuregui et al. 2001). Reciprocal translocations are one of the most common structural chromosome rearrangements and have been detected in many species through the study of pollen viability and chromosome pairing during meiosis (Garber 1972).

When linkage analysis was performed with loci of either G6 or G8 only, the locus order was again identical to that in the corresponding homologous T × E groups (Aranzana et al. 2003) and the P.2175 groups.

The GN22 map includes the R_{MiaNem} and *Gr* genes together with 166 markers (165 SSRs, one STS). Taking into account co-dominant markers, it can be considered as the most saturated *Prunus* map, after the T × E map. The number of markers per linkage group ranges from 33 (G1) to 14 (G8), and the density of markers ranges from 2.6 cM between markers (G5) to 5.4 cM (G7) with an average of 4.3 cM (Table 4).

The positions of the 19 G2 markers common to GN22 and the selfed progeny GN22⊗ were almost identical. The slight differences in the order of two loci between both maps are attributable to sampling errors produced by the small number of individuals used for mapping and the proximity between these markers. The R_{MiaNem} gene mapped to the same region of the map in both progenies, confirming the location previously established by Claverie et al. (2004) and thus providing additional evidence for its telomeric position in G2 of the *Prunus* genome.

P.2175 Myrobalan map

This is the first map of *P. cerasifera*. It includes the *Ma* gene and 93 markers (91 SSRs, 2 SCARs). The number of markers per linkage group ranges from 22 (G2) to 5 (G6), and the density of the markers ranges from 3.2 cM between markers (G7) and 13.0 cM (G6) with an average of 5.6 cM (Table 3). Some gaps in G1 (30.5 cM), G3 (37.4 cM), G5 (28.1 cM) and G7 (33 cM) will have to be filled. However, among all the 277 SSRs tested, all those that were polymorphic in P.2175 and had already been located in those regions have been analyzed in the progeny. Thus, additional SSRs will be necessary to complete this map.

Comparison of the Myrobalan P.2175 and almond-peach GN22 maps

All the 68 anchor loci were in the same order on both maps, except for UDP96-013 and BPPCT013 in G2, which were inverted; these two markers are very close (2.7 cM in P.2175 and 6.4 cM in GN22). The genetic distances of the homologous regions in the two maps were very similar (Table 4). The total genetic distance covered by the regions between the anchor markers was 462.8 cM in P.2175 and 458.9 cM in GN22. This suggests a high level of colinearity between Myrobalan plum and the peach and almond genomes. This has already been observed between apricot 'Stark Early Orange', 'Polonais' and T × E (Lambert et al. 2004). These results reveal a strong homology between the genomes of *Prunophora* and the *Amygdalus* subgenera.

Among the 176 SSRs analyzed within the P.2175 × GN22 progeny, 101 had already been mapped in other *Prunus* maps [T × E, Aranzana et al. 2003; 'Jalousia' × 'Fantasia' F_2 peach map (J × F) and 'Regina' × 'Lapins' F_1 sweet cherry maps (R) and (L) (E. Dirlewanger, unpublished)]. Only five SSRs were not located on homologous linkage groups on these maps: MA040a was located in G6 on P.2175, R and L;, in G3 on GN22; in G7 on J × F; while MA027a was located in G6 on P.2175 and GN22 but in G7 on J × F; BPPCT006 was

Table 4 Number of markers and total genetic distance covered in each linkage group in the P.2175 and GN22 linkage maps

Linkage group	Agronomical characteristics		Number of markers			Total genetic distance (cM)			
	P.2175	GN22	P.2175	Anchor markers	GN22	P.2175 LOD>5	GN22	P.2175 LOD>3.2	GN22
G1			12	8	33	89.4 (52.7+36.7)	133.7	119.9 LOD >3.2	133.7
G2		R_{MiaNem}	22	15	22	95.5	100.7	95.5	100.7
G3			11	10	16	55.4 (35.1+20.3)	73.6	92.8 LOD >3.2	73.6
G4			13	10	22	78.8	112.2	78.8	112.2
G5			6	4	19	49.4 (25.6+23.8)	48.9	77.5 LOD >4.2	48.9
G6		Gr	5	4	24	65.0	110.1	65.0	110.1
G7	Ma		15	10	16	48.8 (16.4+32.4)	87.3	81.8 LOD >4.1	87.3
G8			9	7	14	42.5	49.5	42.5	49.5
Total			93	68	166	524.8	716.0	653.8	716.0
Unlinked markers			1		0				

located in G8 on GN22 and T × E but in G1 on P.2175; BPPCT019 was located in G8 on T × E and GN22 and in G2 on P.2175. These SSRs may reveal several loci but only a few are polymorphic in a specific mapping population. By comparing all the *Prunus* maps sharing common markers it is now possible to identify a set of single SSR loci covering all the genomes as proposed by Aranzana et al. (2003). Translocation events are now easily detected by using already mapped markers.

Marker-assisted selection for RKN resistance in *Prunus* rootstocks

Markers cosegregating with the *Ma* gene, two SCARs ($SCAL19_{690}$, $SCAFLP2_{202}$) (Lecouls et al. 2004) and four SSRs(SSR 96D14-B4, SSR 81P4-B7, SSR6, SSR12) located on P.2175 G7, can already be used for MAS for RKN resistance from Myrobalan. For MAS of the R_{MiaNem} gene, located on the top of G2, more tightly linked markers need to be obtained. Nevertheless, the joint use of two SSRs flanking this gene (ssrPACITA27 and MA024a) will make it possible to select this gene with a high probability (0.996) considering that it falls between both markers, which are separated by 13.3 cM in GN22.

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