Theor Appl Genet (1998) 97: 888–895

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Genetic linkage map of peach [*Prunus persica* (L.) Batsch] using morphological and molecular markers

Received: 1 November 1997 / Accepted: 31 March 1998

Abstract A genetic linkage map of peach [*Prunus persica* (L.) Batch] was constructed in order to identify molecular markers linked to economically important agronomic traits that would be particularly useful for long-lived perennial species. An intraspecific F_2 population was generated from self-pollinating a single F_1 plant from a cross between a flat non-acid peach, 'Ferjalou Jalousia®' and an acid round nectarine 'Fantasia'. Mendelian segregations were observed for 270 markers including four agronomic characters (peach/nectarine, flat/round fruit, acid/non-acid fruit, and pollen sterility) and 1 isoenzyme, 50 RFLP, 92 RAPD, 8 inter-microsatellite amplification (IMA), and 115 amplified fragment length polymorphism (AFLP) markers. Two hundred and forty-nine markers were mapped to 11 linkage groups covering 712 centiMorgans (cM). The average density between pairs of markers is 4.5 cM. For the four agronomic characters studied, molecular markers were identified. This map will be used for the detection of QTL controlling fruit quality in peach and, particularly, the acid and sugar content.

Key words *Prunus persica* · Linkage map · RFLP · RAPD · AFLP

Introduction

Peach [*Prunus persica* (L.) Batch] is the second most important fruit crop in Europe, apple being the most important, and ranks first in the genus *Prunus*. Moreover, peach is the best genetically characterised species of the genus. Peach breeding is time-consuming, especially for fruit-specific characters as the trees must be grown for at least 3 or 4 years before they bear fruit. Therefore, molecular markers linked to these traits are of great value for the identification and selection of plant genotypes with the desired characters long before the traits are expressed.

Molecular marker linkage maps are useful for localising important genes controlling both qualitative and quantitative traits in numerous plant species (Tanksley et al. 1989). Recent mapping studies using molecular markers have provided several linkage maps of peach (Belthoff et al. 1993; Chaparro et al. 1994; Dirlewanger and Bodo 1994; Rajapakse et al. 1995; Warburton et al. 1996) or those based on interspecific crosses between peach and other *Prunus* (Foolad et al. 1995; Dirlewanger et al. 1996; Joobeur et al. 1998). However, to date, only a few peach agronomic characters have been mapped: weeping (Dirlewanger and Bodo 1994) or pillar (Chaparro et al. 1994; Rajapakse et al. 1995) shape of the tree, red leaves (Chaparro et al. 1994), double flower (Chaparro et al. 1994; Rajapakse et al. 1995), and fruit characters, i.e., peach/nectarine (Chaparro et al. 1994), flesh colour (Rajapakse et al. 1995; Warburton et al. 1996), stony hard flesh and freestone (Warburton et al. 1996). The peach/nectarine character shows only a very loose linkage (23.4 cM) with a random amplified polymorphic DNA (RAPD) marker (Chaparro et al. 1994). Very few results have been reported for quantitative characters, and those that have been reported are only concerned with disease resistance (Dirlewanger et al. 1996; Viruel et al. 1998).

In this paper we report our investigation of a number of molecular markers [isoenzyme, restriction fragment length polymorphism (RFLP), RAPD, inter-microsatellite amplification (IMA) and amplified fragment length polymorphism (AFLP)] that segregate in an

Communicated by M. A. Saghai Maroof

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intraspecific peach offspring. Some of the RFLPs of this map were used for the development of a densely populated *Prunus* map in a cooperative effort with other European research groups (Arùs et al. 1994; Joobeur et al. 1998). Consequently, it was possible to determine homology between linkage groups of the two maps.

For the four agronomic characters segregating in the peach offspring studied here i.e., peach or nectarine, flat or round fruit, acid or non-acid fruit, and pollen sterility, molecular markers were identified. The nectarine character is controlled by a recessive gene, *g*, which determines the glabrous character of the fruit skin (Blake 1933). The flat fruit character is controlled by a dominant gene, *S* (Lesley 1940). Breeders would like to associate this character to a specific taste of the fruit, i.e., the non-acid fruit character. As the consumer cannot perceive visually the non-acid character, the flat shape of the fruit would be a useful morphological marker even though no genetic linkage exists between the two characters. The non-acid character, originating from the south of China (Reimer 1904), is reported to be controlled by a single dominant gene, *D* (Yoshida 1970, Monet 1979). The acid content is a quantitative measure of a continuous nature. However, fruits can be classified into two groups, acid and non-acid, depending upon their pH value: fruits below pH 3.9 are considered acid and those above pH 4.0, non-acid (Monet 1979). The non-acid character is very interesting one for breeders, especially with respect to its introduction in early-maturing varieties whose fruits are often too acidic. It was first introduced into 'Robin' and 'Redwing', two peaches widely cultivated in France (Monet 1979). Most cases of pollen sterility originate from the 'J. H. Hale' variety, which has a very high agronomic value. Therefore, as this variety is frequently used for breeding, this character can be a problem for growers. Male sterility was first reported to be controlled by a single recessive gene, designated as *ps* (Scott and Weinberger 1944). Chaparo et al. (1994) identified the existence of another gene, designated as *ps2*, non-allelic to *ps*.

Our long-term objectives are to develop a saturated linkage map of peach and to identify molecular markers tightly linked to fruit quality components. Quantitative trait loci (QTLs) associated with soluble sugars and organic acid contents have already been identified, and candidate genes for these component are being searched for. Molecular markers linked to fruit quality components should be used through marker-assisted selection (MAS) in peach but also in other *Prunus*, such as apricot, where similar quality characters are being searched for (Bassi and Selli 1990).

Table 1 Agronomic characters of 'Ferjalou Jalousia®', 'Fantasia' and of the F_1 individual which was self-pollinated to give the F_2 population

Ferjalou Jalousia®	Fantasia	F_1
Peach (G/g)	Nectarine (g/g)	Peach (G/g)
Flat fruit (Ss)	Round fruit (s/s)	Flat fruit (S/s)
Non-acid fruit (D/D)	Acid fruit (d/d)	Non-acid fruit (D/d)
Male sterility (ps/Ps)	Male sterility (ps/Ps)	Male sterility (ps/Ps)

Materials and methods

Mapping population

An intraspecific F_2 population was generated by self-pollination of a single F_1 plant from a cross between peach variety 'Ferjalou Jalousia®', a non-acid flat peach, and 'Fantasia', an acid round nectarine. The fruit characteristics of the parents and of the F_1 individual which was self-pollinated to give the F_2 population, are summarised in Table 1. The F_1 parent of the F_2 offspring is heterozygous for all of the studied agronomic characters.

The F_2 population consists of 63 plants, all of which were maintained in the field under standard cultural conditions for phenotypic evaluation. For each F_2 tree, the monogenic characters segregating in the progeny were noted.

Genotyping

Five types of molecular markers were used to elaborate the linkage maps : isoenzymes, RFLPs, RAPDs, IMAs and AFLPs.

Sixteen isoenzyme systems were tested: malate dehydrogenase (MDH), acid phosphatase (ACP), alcohol dehydrogenase (ADH), menadione reductase (MNR), glutamate oxaloacetate transaminase (GOT), esterase (EST), isocitrate dehydrogenase (IDH), hexokinase (HEX), glutamate dehydrogenase (GDH), 6-phosphogluconate dehydrogenase (6PGD), leucine amino peptidase (LAP), phosphoglucose isomerase (PGI), alanine amino peptidase (AAP), phosphoglucomutase (PGM), NADH dehydrogenase (NADHDH), shikimate dehydrogenase (SKDH). Proteins were extracted from lyophilised young leaves using the procedure described by Monet and Gibault (1991). The extracts were fractionated in 7.5% acrylamide gels (16 V/cm) and examined by staining the gels for specific enzyme activity.

Genomic DNA extraction and hybridisation for the RFLP analysis were performed according to the method of Viruel et al. (1995). Polymorphisms were tested using four restriction enzymes (*Hin*dIII, *Eco*RI, *Hpa*II, *Bgl*II) for all probes and with *Eco*RV and *Bam*HI for a few of them. Three sets of probes were used. The first set originated from genomic or cDNA isolated from the libraries of several *Prunus* (*P. persica*, *P. amygdalus*, *P. avium*, *P. ferganensis*) developed in the European mapping project AIR3-CT93-1585 (Arùs et al. 1994; Joobeur et al. 1998), i.e., peach cDNA (PC), almond cDNA (AC), almond genomic (AG), cherry cDNA (CC), and *P. ferganensis* genomic (FG). The second set was composed of probes corresponding to known genes of almond, extensin (*Ext1*), oleosin (*Ole1*), phosphoglyceromutase (*Pgl1*), lipid transfer protein (*Ltp2*), jasmonic-induced protein (*Pij1*), tonoplast intrinsic protein (*Tip1*) and two genes homologous to tagged sequences of *Arabidopsis thaliana* (*TSA2* and *TSA3*). The third set was composed of cDNAs isolated from a peach fruit cDNA library and expressed during early fruit development (Rothan et al. 1998). They correspond to genes putatively involved in the control of peach fruit development and composition in sugars and acids, i.e., the phosphoenolpyruvate carboxylase (*PEPc*), a S28 ribosomal protein (S28), and a gene homologous to a tagged sequence of rice (EST-rice). In total, 171 RFLP probes were tested. The RFLP markers are named by the probe origin followed by a code number. When more than one marker was

generated from a single clone, different letters were used at the end of clone identification (e.g., AG25c: almond genomic probe, number 25, fragment c).

RAPD was performed as described by Dirlewanger et al. (1996). Three hundred and eighty primers from Operon Technologies (Alameda, Calif.) were tested. The RAPD markers obtained were designed by the letter and the number of the Operon code and the size of the amplified fragment in kilobases (e.g., A01–0.4). Eleven additional primers corresponding to microsatellite sequences were also used. They were named IMA:IMA1:GCG(CA)^{8}C, IMA2:CCGG(AC)^8, IMA3:GCG(AC)^{8}A, IMA4:(CA)^{8}CG, IMA5:(CA)^{8}GT, IMA6:CGC(GA)^{8}G, IMA7:CGCT(GA)^7, IMA8:(GA)^{8}GT, IMA9:(GA)^{8}CG, IMA11:(GA)^{8}CG, and IMA12:(CA)^{8}TG. The amplification was performed under the same conditions as those used for the Operon primers except for the annealing temperature which was 50°C instead of 37°C.

AFLP analysis was performed according to Vos et al. (1995) with some modifications. The same purified DNA as used for the RFLP analysis, was digested with *Eco*RI and *Mse*I restriction enzymes. Digestion was carried out in a final volume of 35 µl in 10 m*M* Tris-HAc, 10 m*M* MgAc, 50 m*M* KAc, 5 m*M* DTT, 12.5 U *Eco*RI (Pharmacia, Orsay, France), 8 U *Mse*I (New England Biolabs, Hitchin, UK), and 500 ng of genomic DNA for 4 h at 37°C. Two different adaptors, one for the *Eco*RI sticky end and one for the *Mse*I sticky end (Table 2), were ligated to the DNA by adding 5 µl of a mixture containing 5 pmol *Eco*RI adaptator, 50 pmol *Mse*I adaptator, 8 m*M* ATP, 10 m*M* Tris-HAc, 10 m*M* MgAc, 50 m*M* KAc, 5 m*M* DTT and 1.7 U T4 DNA ligase (Pharmacia, Orsay, France) to the digestion. The ligation was incubated for 3 h at 37°C and then overnight at 4°C. The 40-µl aliquot of the ligation reaction was then diluted with 160 µl of sterile water.

A first preselective polymerase chain reaction (PCR) amplification was performed using *Eco*RI + A and *Mse*I + C primers (Table 2) in a 50-µl volume of 20 m*M* Tris-HCl (pH 8.4), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.2 m*M* of each dNTP, 30 ng of each primer, and 1 U *Taq* DNA polymerase (GibcoBRL, Cergy Pontoise, France). The reactions were carried out in a Perkin Elmer 9600, and the samples were subjected to 28 cycles of melting at 94°C for 30 s, annealing at 56°C for 60 s, and extension at 72°C for 60 s. The preamplification products were diluted to be used as the starting material for the selective radioactive amplification: 35 µl of preamplified material was diluted by adding 300 µl H_2O.

Table 2 Adapter, +1 primer, +2 primer, and +3 primer sequences used for AFLP analysis

Adapter *Eco*RI	5′-CTCGTAGACTGCGTACC-3′ 3′-CATCTGACGCATGGTTAA-5′
Adapter *Mse*I	5′-GACGATGAGTCCTGAG-3′ 3′-TACTCAGGACTCAT-5′
*Eco*RI + A	5′-GACTGCGTACCAATTCA-3′
*Mse*I + C	5′-GATGAGTCCTGAGTAAC-3′
*Eco*RI + AA	5′-GACTGCGTACCAATTCAA-3′
*Eco*RI + AC	5′-GACTGCGTACCAATTCAC-3′
*Mse*I + CA	5′-GATGAGTCCTGAGTAACA-3′
*Mse*I + CG	5′-GATGAGTCCTGAGTAACG-3′
*Eco*RI + ACA	5′-GACTGCGTACCAATTCACA-3′
*Eco*RI + AGA	5′-GACTGCGTACCAATTCAGA-3′
*Eco*RI + AGG	5′-GACTGCGTACCAATTCAGG-3′
*Mse*I + CAA	5′-GATGAGTCCTGAGTAACAA-3′
*Mse*I + CAC	5′-GATGAGTCCTGAGTAACAC-3′
*Mse*I + CAG	5′-GATGAGTCCTGAGTAACAG-3′
*Mse*I + CAT	5′-GATGAGTCCTGAGTAACAT-3′
*Mse*I + CTG	5′-GATGAGTCCTGAGTAACTG-3′

For selective amplification *Eco*RI and *Mse*I primers with two or three selective nucleotides were used (Table 2), one of them being [^{33}P]-labelled using T4 polynucleotide kinase. The PCR reaction was performed in a 20-µl volume of 20 m*M* Tris-HCl (pH 8.4), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 5 ng *Eco*RI primer, 30 ng *Mse*I primer, 1 U *Taq* DNA polymerase (GibcoBRL, Cergy Pontoise, France), and 5 µl of diluted preamplified DNA. The selective amplification was carried out using the following cycling parameters: 1 cycle of 30 s at 94°C, 30 s at 65°C, 1 min at 72°C followed by 11 cycles in which the annealing temperature was lowered by 0.7°C per cycle, followed by 24 cycles of 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C.

At the end of the selective radioactive PCR, the samples were denatured by adding an equal volume (20 µl) of formamide buffer containing 98% formamide, 10 m*M* EDTA, 0.05% bromo-phenol blue, and 0.05% xylene cyanol. The samples were heated for 3 min at 90°C and immediately placed in ice. Two µl of each sample was loaded on a 4.5% acrylamide/bisacrylamide (20:1), 7.5 *M* urea and 0.5 × TBE gel (40 × 50 cm). The samples were then electrophoresed at a constant power of 95 W for 90 min. Gels were dried on a standard gel drier for 2 h and exposed for 3 days to an X-ray film. In this study, 18 primer combinations were tested. The AFLP markers were designated by the primer combination used and by the code of the band for this combination (e.g., AC-CAG11: *Eco*RI + AC/*Mse*I + CAG, band 11).

Statistical analysis

Segregation data were tested for deviation from the expected Mendelian ratio using a Chi-square test. The genetic linkage map was constructed using the MAPMAKER/EXP V3.0 software (Lander et al. 1987). Linkage groups were established with a LOD threshold of 4.0 and a recombination fraction of 0.3. The Kosambi function was used to convert a recombination units into genetic distances. Data were scored independently by two people. Conflicting results were re-examined. After mapping, the 'error detection' option of MAPMAKER was used, and possible errors were examined again by rereading the molecular marker patterns.

Results and discussion

Polymorphism

From the 16 isoenzyme systems studied, only MDH was polymorphic; among the 171 RFLP probes tested, only 39 (22.8%) were polymorphic (Table 3). The percentage of polymorphic probes varied according to the origin of the probes, but the differences are not significant according to a likelihood ratio Chi-square test, G^2 test (or 2î test) (Arbonnier 1966). Among the 11 known genes, 4 (36%) were polymorphic: PEPc, ESTrice, *Pgl*1, S28. For only 6 probes, the polymorphism was detected with two enzymes while for all the others it was detected by only one enzyme, suggesting that most of the polymorphisms were due to point mutations rather than deletion or insertion events. Among the six enzymes tested, *Hpa*II, a four-base restriction enzyme, revealed more polymorphisms than the other five, which are six-base restriction enzymes.

Among the 380 primers tested for RAPD only 65 revealed polymorphism (17%). With these 65 primers, a total of 92 RAPD markers were detected (1.4 markers

Table 3 Number of polymorphic and non-polymorphic probes based on probe origin and the enzyme tested

Probe origin	Number of probes tested	Number of polymorphic probes	Percentage of polymorphic probes	Number of polymorphic probes according to the enzyme tested					
				*Hpa*II	*Hin*dIII	*Eco*RI	*Bgl*II	*Bam*HI	*Eco*RV
Peach cDNA (PC)	33	6	18.2	3	1	1	1	0	0
Almond genomic (AG)	38	15	39.5	10	3	2	3	0	0
Almond cDNA (AC)	16	4	25.0	2	1	1	0	1	0
P. ferganensis genomic (FG)	40	8	20.0	6	2	1	0	0	0
Cherry cDNA (CC)	33	2	6.1	2	1	0	0	0	0
Known genes	11	4	36.4	0	2	1	0	0	1
Total	171	39	22.8	23	10	6	4	1	1

Table 4 Number of AFLP markers detected according to the primer combination used

*Eco*RI + **AA**-*Mse*I + **CA**	10	*Eco*RI + **AA**-*Mse*I + **CAA**	13	*Eco*RI + **ACA**-*Mse*I + **CAC**	5
*Eco*RI + **AA**-*Mse*I + **CG**	11	*Eco*RI + **AA**-*Mse*I + **CAC**	5	*Eco*RI + **ACA**-*Mse*I + **CAT**	4
*Eco*RI + **AC**-*Mse*I + **CA**	2	*Eco*RI + **AA**-*Mse*I + **CAG**	4	*Eco*RI + **AGA**-*Mse*I + **CAA**	5
*Eco*RI + **AC**-*Mse*I + **CG**	3	*Eco*RI + **AA**-*Mse*I + **CAT**	15	*Eco*RI + **AGA**-*Mse*I + **CAT**	2
		*Eco*RI + **AC**-*Mse*I + **CAA**	9	*Eco*RI + **AGG**-*Mse*I + **CAT**	4
		*Eco*RI + **AC**-*Mse*I + **CAC**	3	*Eco*RI + **AGG**-*Mse*I + **CTG**	5
		*Eco*RI + **AC**-*Mse*I + **CAG**	11		
		*Eco*RI + **AC**-*Mse*I + **CAT**	4		

per primer on average): 43 primers revealed only 1 marker, 18 revealed 2 markers, 3 revealed 3 markers and 1 revealed 4 markers.

The 18 primer combinations used for AFLP detected polymorphism, and 115 AFLP markers were identified (6.1 markers per primer combinations on average) (Table 4). The number of markers identified varied according to the primer combination used from 2 (*Eco*RI + AC-*Mse*I + CA, *Eco*RI + AGA − *Mse*I + CAT) to 15 (*Eco*RI + AA − *Mse*I + CAT). The lowest and highest number of markers were obtained, respectively, with primer combinations including three selective nucleotides for both primers (4.2 markers per combination of primers on average), and with primer combinations including three selective nucleotides for 1 primer and two selective nucleotides for the other primer (8 markers per combination of primers on average). With 2 primers including three selective nucleotides each, the number of amplified fragments was too low. On the other hand, with primers including two selective nucleotides each, the number of amplified fragments was too high to allow a reliable reading of the gel. These results suggest that the best alternative is to use combination primers with two selective nucleotides for one primer and three for the other.

Genetic linkage map

The map is organized into 11 linkage groups covering 712 cM and is composed of 249 markers: four agronomic characters [non-acid fruit (*D*), peach or nectarine (*G*), flat fruit (*S*), and male sterility (*ps*)] and 1 isoenzyme (MDH), 47 RFLPs, 82 RAPDs, 6 IMAs, and 109 AFLPs (Fig. 1). Twenty-one additional markers remain independent (3 RFLPs, 10 RAPDs, 2 IMAs, and 6 AFLPs). Among the molecular markers analysed in the progeny, only 5, all included in different linkage groups, presented significant deviation from the expected Mendelian ratio.

Linkage group identification numbers 1–7 were assigned based on the linkage map constructed with the interspecific F_2 cross *P. amygdalus* cv 'Texas' × *P. persica* cv 'Earlygold' using the same RFLP markers in the two maps (Joobeur et al. 1998). The two groups 4a and 4b were also determined according to the 'Texas' × 'Earlygold' map. AC41 located in the group 4a and CC133, AG12 in the group 4b were all in the same group 4 in the 'Texas' × 'Earlygold' map . Two RFLP markers, FG215 and FG42c, located on linkage group 8 of the present map were located, respectively, on linkage group 6 and 7 of the 'Texas' × 'Earlygold' map (Joobeur et al. 1998). As the FG42 probe enabled the identification of 3 dominant RFLP markers in different linkage groups (FG42a on group 6, FG42b on group 7, FG42c on group 8), linkage group 8 of the present map may correspond to part of the chromosome that includes linkage group 6 of the 'Texas' × 'Earlygold' map. For groups 9 and 10, which do not include any previously mapped markers, the group numbers were assigned arbitrarily. Twenty-six RFLP markers are common to the present map and to the 'Texas' × 'Earlygold' map: 1–6 per linkage group. Except for the tightly linked RFLP markers for which some

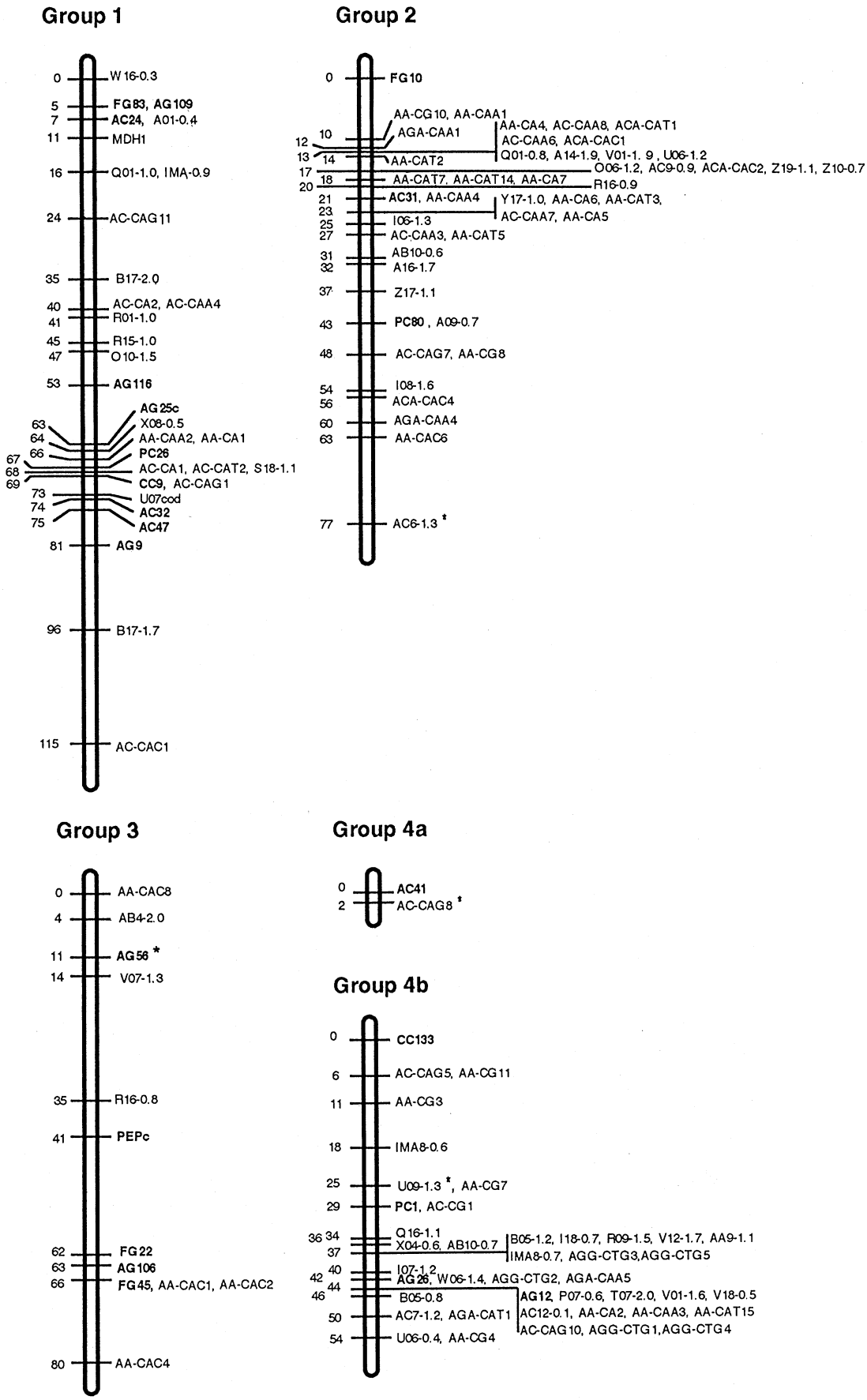

Fig. 1 See page 893 for legend

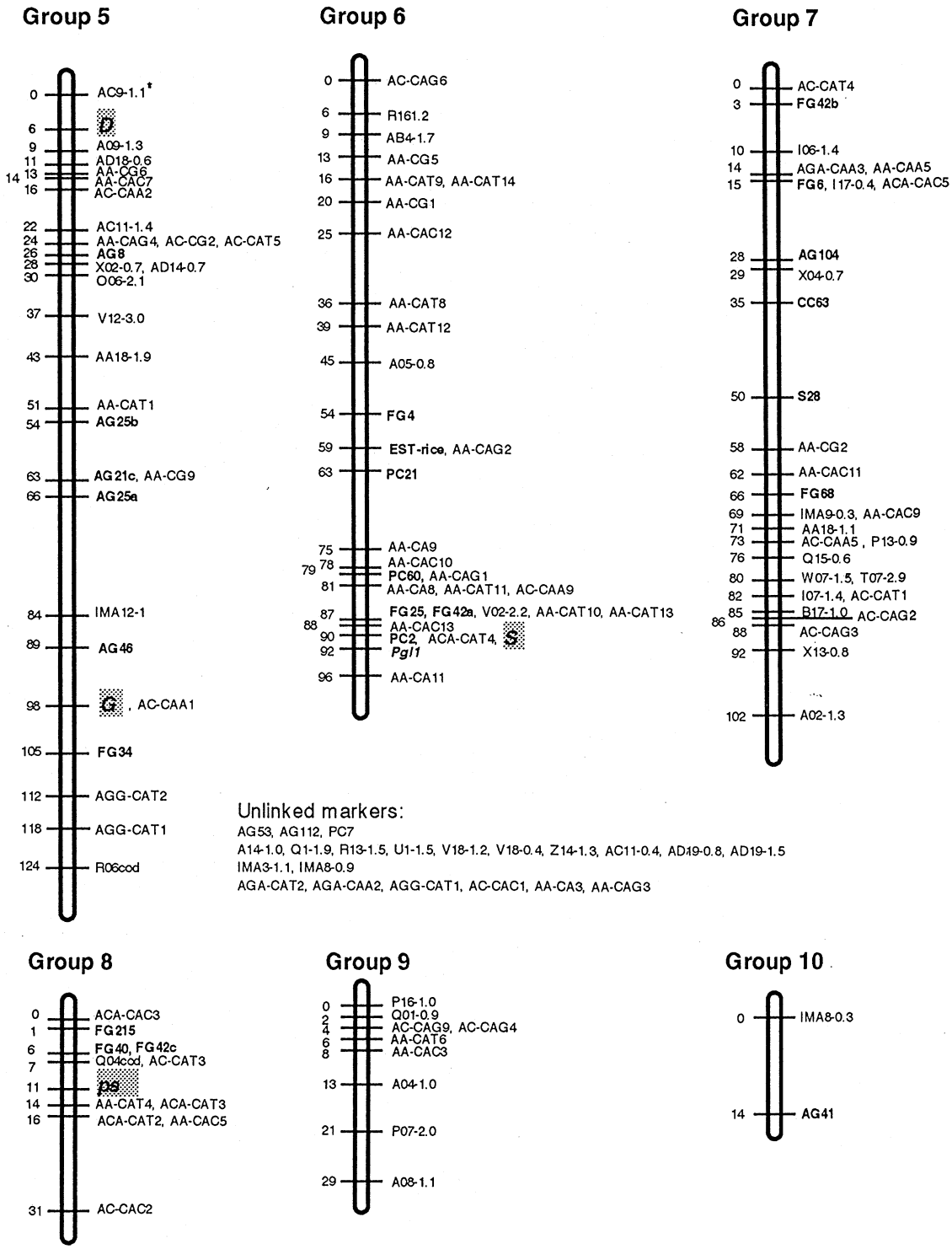

Fig. 1 Molecular linkage map of peach generated from the F_2 population resulting from the intraspecific peach cross 'Ferjalou Jalousia®' × 'Fantasia'. Linkage groups were established with a LOD threshold of 4.0 and a recombination fraction of 0.3. The Kosambi function was used to convert recombination units into genetic distances. The names of the loci are shown on the right: morphological markers are in *italics*; RFLP markers are in *bold* (*PC* peach cDNA, *AC* almond cDNA, *AG* almond genomic, *CC* cherry cDNA, *FG P. ferganensis* genomic). RAPDs are indicated by operon code-fragment size; AFLP, selective *Eco*RI nucleotides – selective *Mse*I nucleotides. The centiMorgan distances are shown on the left. * Indicates significant ($p < 0.05$) deviation from the expected segregation

inversions are observed between the two maps, the order is well preserved within each linkage group. However, as the present population is composed of only 63 trees, the order for very tightly linked markers, i.e., less than 1 cM, cannot be defined with precision, and the genetic distance reported only corresponds to that with highest probability.

In some regions, numerous markers cosegregate. Some primer combinations used for AFLP may produce the same markers; for example, on linkage group 1, the AFLP markers AC-CA2 and AC-CAA4; AA-CA1 and AA-CAA2; AC-CA1 and AC-CAT2 cosegregate. As the two first selective nucleotides in each combination couple are common, these markers may be identical. Consequently, the average density between pairs of markers is 4.5 cM.

For the four agronomic characters, male sterility, peach/nectarine, non-acid/acid, and flat/round fruit, the segregations fit the expected 1 : 3 ratio for a single gene. For all of them, linked molecular markers were obtained. The *D* gene, determining the non-acid fruit, and the *G* gene, controlling the peach or nectarine character, are located on linkage group 5. The *D* gene is located between the RAPD markers AC9-1.1 (6 cM) and A09-1.3 (3 cM), and the *G* gene cosegregates with the AFLP marker (AC-CAA1) and is between 2 RFLP markers AG46 (9 cM) and FG34 (7 cM). The *S* gene, controlling fruit shape (flat or round) and located on linkage group 6, cosegregates with the RFLP marker PC2 and with AFLP marker ACA-CAT4. The *ps* gene, controlling male sterility, is located on linkage group 8, between a codominant RAPD marker Q04 cod cosegregating with AC-CAT3 (4 cM) and 2 cosegregating AFLP markers AA-CAT4 and ACA-CAT3 (3 cM).

Conclusion

Linkage maps in the *Prunus* genus have been constructed for peach (Belthoff et al. 1993; Chaparro et al. 1994; Dirlewanger and Bodo 1994; Rajapakse et al. 1995; Warburton et al. 1996). In the current study, we present a map generated from an intraspecific F_2 population. This map, combining morphological characters, isoenzymes, RFLPs, RAPDs, IMAs, and AFLPs, consists of 11 linkage groups. The level of polymorphism observed with isoenzymes, RFLPs, and RAPDs was low compared to that obtained with other *Prunus* species, especially almond in which polymorphism is very high (Viruel et al. 1995). These results are in agreement with those previously obtained with isoenzymes (Mowrey et al. 1990; Monet and Gibault 1991) and with RAPDs in genetic mapping studies (Chaparro et al. 1994; Dirlewanger and Bodo 1994; Dirlewanger et al. 1996). With AFLP the polymorphism detected was much higher, especially with some primer combinations. The low level of polymorphism is due to the fact that peach is predominantly self-pollinating and that relatively few parents have been used for breeding European or American cultivars (Reynders and Monet 1987; Scorza et al. 1995; Arulsekar et al. 1986; Dirlewanger et al. 1998). The existence of gaps in the map (20 cM) without markers and the identification of more linkage groups (11) than the haploid chromosome number ($x = 8$) indicate that additional markers must be mapped in order to achieve a complete and uniform coverage of the entire genome.

Prunus linkage maps can be used to locate genes of economic and biological value. For the four agronomic characters, male sterility, peach/nectarine, non-acid/acid, and flat/round fruit tightly linked molecular markers were obtained. Peach breeders generate large populations to recombine traits from different varieties, and plant breeding is mostly based upon phenotypic selection. Markers that would allow seedling-stage selection of traits expressed late in development would be particularly useful in trees because of their long generation cycle. Moreover, markers linked to recessive alleles such as nectarine or round fruit would allow the identification and selection of heterozygous individuals not expressing the trait.

The F_2 population used in the present study is particularly interesting because it segregates for the qualitative characters analysed here and also for several quantitative characters involved in fruit quality. Consequently, the map developed here will be used for determining QTL for the major components of fruit quality in peach by measuring several tree characters possibly interfering with fruit quality, such as bloom date, fruit maturity date and the primary sugar, and organic acid contents of fruit.

Acknowledgements The authors thank P. Arus (IRTA, Cabrils, Spain), R. Quarta (ISF, Rome, Italy), K.R. Tobutt (HRI, East Malling, UK) and their groups for providing AG and AC, FG, CC probes, respectively. We thank the "Les Jarres" INRA Experimental Station team in Toulenne for growing the plant material in their orchard. This work was supported by a grant from the European Community in the context of the *Prunus* mapping project (AIR3-CT93-1585), INRA (AIP Génome des Plantes Cultivées and AIP 'AGRAF') and the Aquitaine Region program 'Détection de gènes d'intérêt écologique et économique chez les arbres forestiers et fruitiers'.

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