Theor Appl Genet (2003) 107:239–247
DOI 10.1007/s00122-003-1243-y

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An apricot (*Prunus armeniaca L.*) F2 progeny linkage map based on SSR and AFLP markers, mapping plum pox virus resistance and self-incompatibility traits

Received: 13 September 2002 / Accepted: 4 November 2002 / Published online: 14 March 2003
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Abstract A genetic linkage map of apricot (*Prunus armeniaca* L.) was constructed using AFLP and SSR markers. The map is based on an F_2 population (76 individuals) derived from self-pollination of an F_1 individual ('Lito') originated from a cross between 'Stark Early Orange' and 'Tyrinthos'. This family, designated as 'Lito' × 'Lito', segregated for two important agronomical traits: plum pox virus resistance (PPV) and self-incompatibility. A total of 211 markers (180 AFLPs, 29 SSRs and two agronomic traits) were assigned to 11 linkage groups covering 602 cM of the apricot genome. The average distance (cM/marker) between adjacent markers is 3.84 cM. The PPV resistance trait was mapped on linkage group G1 and the self-incompatibility trait was mapped on linkage group G6. Twenty two loci held in common with other *Prunus* maps allowed us to compare and establish homologies among the respective linkage groups.

Keywords Genetic linkage map · *Prunus armeniaca* · Sharka resistance trait · Self-incompatibility · SSR · AFLP

Introduction

Apricot (*Prunus armeniaca* L.) ranks as the third most agronomically important species of the stone fruit crops. The main growing areas are China, the Mediterranean European countries, Turkey and USA (Faostat 2001). The worldwide apricot industry produces 2.3 millions of tons of fruit per year; however, the spread of plum pox virus (PPV) or sharka has seriously reduced this production level. PPV is the most important virus affecting stone fruit crops in Europe and causes significant losses of fruit, mainly in apricot and European plum. In the last few years, PPV has become the most limiting factor for the apricot industry and a severe problem for other *Prunus* species. The eradication of infected source plants has been not able to stop the disease in European countries (Llácer and Cambra 1998) suggesting that effective control of the disease will require PPV-resistant cultivars.

In order to solve this problem several breeding programs aimed at introducing resistance to PPV were initiated in France (Audergon 1995), Greece (Karayiannis and Mainou 1999), Italy (Bassi et al. 1995) and Spain (Egea et al. 1999; Badenes et al. 2002). All of them introduce resistance to sharka by conventional breeding methods from resistant sources, most of which are North American apricots. Those cultivars resistant to PPV have high chilling requirements for budbreak and are self-incompatible, which dictates that the European apricot breeding programs currently in progress will introduce these traits into their native germplasms. Both these characters are not desirable for the apricot cultivation in the Mediterranean climate and thus need to be eliminated in breeding programs. Therefore a major breeding goal is to obtain new self-compatible cultivars resistant to PPV with good agronomical and commercial quality for utilization in the Mediterranean region and, within this goal, the screening for detection of PPV resistance and self-compatibility is the 'bottle neck' of the selection process.

For instance, the methodology to determine sharka resistance, first described by Audergon and Morvan (1990) and later improved by Martínez-Gómez and Dicenta (2000) and Moustafa et al. (2001), is a very long procedure that uses 'GF-305' peach seedlings as woody indicators and involves several cycles of graft inoculation (Dosba et al. 1991; Audergon et al. 1995), followed by ELISA and PCR analysis (Olmos et al. 1997). On the other hand, screening for self-compatibility by traditional observation of pollen-tube growth (Burgos et al. 1997) is

Communicated by C. Möllers

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very time consuming and needs adult trees. Stylar ribonuclease assay to detect self-compatible seedlings is an alternative approach (Sassa et al. 1992; Boskovic and Tobutt 1996; Boskovic et al. 1997; Tao et al. 1997; Burgos et al. 1998; Boskovic and Tobutt 1999); however, it also requires flowering trees to collect pistils. More recently the molecular cloning and characterization of *S*-alleles from almond (Ushijima et al. 1998) and cherry (Tao et al. 1999) has provided a fast way of *S*-genotype identification utilizing PCR. With appropriate sequences, this approach could be used to solve the problem of breeding for self-compatibility in apricot breeding programs, introgressing characters such as Sharka resistance.

Molecular marker-assisted selection (MAS) for PPV resistance and self-compatibility would be very convenient in terms of increasing breeding efficiency, and genetic maps are a very useful tool to implement this strategy. In *Prunus* several linkage maps have been developed based on interspecific crosses of peach × almond (Foolad et al. 1995; Joobeur et al. 1998; Bliss et al. 2002) or intraspecific crosses of almond (Viruel et al. 1995; Joobeur et al. 2000), peach (Chaparro et al. 1994; Rajapakse et al. 1995; Dirlewanger et al. 1998; Lu et al. 1998; Dettori et al. 2001) and sour cherry (Wang et al. 1998). Within the *Prunus* genus, apricot is the most appropriate species for studies focused on marking PPV resistance since there are no known sources of resistance in peach, the genetically most highly characterized species of *Prunus*. In apricot, a genetic linkage map that included a sharka resistance locus was constructed by Hurtado et al. (2002), and a similar locus was also mapped by Salava et al. (2002) using bulked segregant analysis. However, to integrate the information on apricot resistance in different crosses and to merge this information with the general *Prunus* map of Joobeur et al. (2000), a saturated apricot map with co-dominant markers tightly linked to the trait is necessary.

The objective of this study was to construct an apricot linkage map including the most limiting traits for apricot breeding efficiency: resistance to sharka and self-incompatibility. This map also includes SSR markers mapped in other *Prunus* maps, allowing us to compare and establish genome homologies with other *Prunus* species maps.

Materials and methods

Plant material

An apricot intraspecific F_2 population (N = 76) was generated by self-pollination of an F_1 individual ('Lito'). 'Lito', a self-compatible (SC) cultivar resistant to sharka, derived from a cross between 'Stark Early Orange', a North American self-incompatible (SI) cultivar resistant to sharka, and 'Tyrinthos', a Greek SC cultivar susceptible to sharka. This progeny, designated as the 'Lito' × 'Lito' (L × L) family, is maintained at the Instituto Valenciano de Investigaciones Agrarias (IVIA).

DNA isolation

DNA was extracted from 50 mg of young leaves following the CTAB method described by Doyle and Doyle (1987). Concentration was measured using an spectrophotometer (UV-1601, Shimadzu Corp. Kyoto, Japan).

AFLP markers

DNA digestion, adaptor ligation and pre-selective and selective amplifications were carried out according to the manufacturer's instructions (GIBCO BRL, Gathersburg, Md., USA) and standard procedures (Vos et al. 1995). Selective amplification was carried out using the [^{33}P]-labeled *Eco*RI primer. Primer combinations including three selective bases for one primer and two for the other were selected. PCR reactions were mixed with 2.5 μl of formamide dye (98% formamide, 10 mM of EDTA, 0.05% bromophenol blue and xylene cyanol), heated at 95 °C for 5 min and immediately cooled on ice. Two microlitres of each sample were loaded on a 6% denaturing polyacrylamide gel (acrylamide-bisacrylamide 20:1) containing 7.5 M urea. The samples were then electrophoresed at 70 W for 2 h. Gels were dried on Whatmann 3 MM paper in a standard gel dryer for 1 h, and exposed to Kodak Biomax X-ray films for 2–4 days.

SSR markers

We screened 64 SSRs from peach (Cipriani et al. 1999; Sosinski et al. 2000; Testolin et al. 2000; Aranzana et al. 2002a) and five from cherry (Sosinski et al. 2000) on the mapping progeny. PCRs were performed in a GeneAmp PCR System 9700 thermal cycler (Perkin-Elmer Corp. Calif., USA) following Aranzana et al. (2002a). PCR products were separated by electrophoresis in 3% Metaphor-agarose or 6% non-denaturing polyacrylamide gels, stained with ethidium bromide (0.8 mg/ml) and visualized using UV light.

Screening for sharka resistance

Evaluation of sharka resistance in the L × L family was performed according to the biological test described by Moustafa et al. (2001). In this experiment we used the PPV Dideron strain 3.3 RB described by Asensio (1996) and 'GF-305' peach seedlings as woody indicators. The virus presence was analyzed by visual scoring of symptoms, ELISA-DASI, performed as described by Lommel et al. (1982), and RT-PCR, performed as described by Wetzel et al. (1991).

PCR detection of the self-incompatibility allele

The presence of the self-incompatibility allele was evaluated in the L × L family using a partial degenerated primer AS1 [5'-TTATTTCAATTTGTNCAGCAATGG-3'] and PRU-C4R [5'-GGATGTGGTACGATTGAAGCG-3'] primers (Tao et al. 1999). PCR was performed using a program of 35 cycles at 94 °C for 30 s, 54 °C for 45 s and 72 °C for 1 min and 15 s, with an initial denaturing of 94 °C for 3 min and a final extension of 72 °C for 10 min. The PCR reaction mixture contained 1 × PCR buffer (20 mM of Tris-HCl pH 8.4 and 50 mM of KCl), 2.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.25 μM of each primer, 30 ng of genomic DNA and 1 unit of *Taq* DNA polymerase (Invitrogen, San Diego, Calif., USA). PCR products were separated by electrophoresis in 1% agarose gels and DNA bands were visualized by ethidium bromide staining using UV light. Molecular sizes were determined by comparison with a 100-bp molecular-weight marker (Invitrogen, San Diego, Calif., USA).

Linkage analysis

The linkage analysis was carried out using Joinmap 3.0 software (Van Ooijen and Voorrips 2001) setting F_2 type data. In order to evaluate the segregation ratio, the chi-square (χ^2) goodness of fit test was performed using the '*locus genotype frequency*' command. Markers showing a distorted segregation ratio ($P < 0.05$) were added to the map at the end. Initially, the linkage groups were established using as a threshold a logarithm of odds (LOD) of minimum 5.0 with the 'LOD grouping' command. Once the groups were determined, the map was calculated using a maximum recombination fraction θ of 0.3 and a minimum LOD score of 1.0 with a threshold for removal of loci with respect to jumps in a goodness of fit of 5.0. The framework obtained was then fixed using the '*fixed order*' command. Markers not placed in the framework map were finally added using a minimum LOD score of 3.0 and a θ of 0.4. The Kosambi mapping funtion (Kosambi 1944) was used to convert recombination units into genetic distances.

Results

AFLPs

A total of 222 AFLP polymorphic bands were produ- ced using 25 *Eco*RI + 2/*Mse*I + 3 selective primer combinations (Table 1) and 180 of them were mapped. The number of markers identified varied according to the primer combination used from two (*Eco*RI-AA/*Mse*I-CTC, *Eco*RI-AC/*Mse*I-CAC and *Eco*RI-AC/*Mse*I-CTC) to 18 (*Eco*RI-AT/*Mse*I-CTT). On average, nine polymorphisms were scored per primer pair and seven out of them were mapped. Segregation data were tested for deviation from the expected Mendelian ratio (3:1) using the χ^2 test. Only 17 (9%) loci gave distorted segregation at $P < 0.05$, and five (3%) at $P < 0.01$.

SSRs

A total of 69 SSRs from different sources were screened (Table 2). Segregation was demonstrated for 29 (42%) of them, 35 (51%) did not show any polymorphism and five (7%) did not amplify any fragment. Eight primer pairs amplified two or three polymorphic loci each, thus bringing to 39 the number of segregating SSR loci. In total 29 SSR loci were mapped, 20 of them were codominant and nine were dominant (Table 3). Seven markers (24%) deviated significantly from the expected F_2 segregation ratio (3:1 and 1:2:1), two (7%) gave distorted segregation at $P < 0.05$ and five (17%) at $P < 0.01$. The distribution of SSR loci was uniform, ranging from one marker in G3, G7b, G8 and G11 to six markers in G1. Four markers departed from the expected size more than 50 bp (Table 3).

The SSR markers mapped allowed us to establish homologies with other *Prunus* maps: 15 SSRs were held in common with the 'Texas' × 'Earlygold' map obtained by Aranzana et al. (2002b), one with the 'Ferragnès' × 'Tuono' map (Joobeur et al. 2000), six with the 'Prunus persicae' × '(*Prunus ferganensis* × *P. persicae*)' map (Dettori et al. 2001) and five with the 'Goldrich' × 'Valenciano' map (Hurtado et al. 2002) (see Fig. 2). We

Table 1 Summary of the AFLP markers segregating in the L × L population

Primer combination	Marker code	Total no. of polymorphic bands	No. of polymorphic bands mapped
*Eco*RI-AA/*Mse*I-CAA	EAA-MCAA(x)[a]	4	4
*Eco*RI-AA/*Mse*I-CAC	EAA-MCAC(x)	12	9
*Eco*RI-AA/*Mse*I-CAG	EAA-MCAG(x)	11	9
*Eco*RI-AA/*Mse*I-CAT	EAA-MCAT(x)	12	9
*Eco*RI-AA/*Mse*I-CTA	EAA-MCTA(x)	17	14
*Eco*RI-AA/*Mse*I-CTC	EAA-MCTC(x)	2	2
*Eco*RI-AA/*Mse*I-CTT	EAA-MCTT(x)	8	6
*Eco*RI-AC/*Mse*I-CAA	EAC-MCAA(x)	14	10
*Eco*RI-AC/*Mse*I-CAC	EAC-MCAC(x)	2	1
*Eco*RI-AC/*Mse*I-CAG	EAC-MCAG(x)	4	3
*Eco*RI-AC/*Mse*I-CAT	EAC-MCAT(x)	16	12
*Eco*RI-AC/*Mse*I-CTC	EAC-MCTC(x)	2	1
*Eco*RI-AC/*Mse*I-CTG	EAC-MCTG(x)	5	5
*Eco*RI-AC/*Mse*I-CTT	EAC-MCTT(x)	7	5
*Eco*RI-AG/*Mse*I-CAA	EAG-MCAA(x)	10	9
*Eco*RI-AG/*Mse*I-CAC	EAG-MCAC(x)	9	9
*Eco*RI-AG/*Mse*I-CAG	EAG-MCAG(x)	6	5
*Eco*RI-AG/*Mse*I-CTG	EAG-MCTG(x)	5	5
*Eco*RI-AG/*Mse*I-CTT	EAG-MCTT(x)	4	1
*Eco*RI-AT/*Mse*I-CAA	EAT-MCAA(x)	4	4
*Eco*RI-AT/*Mse*I-CAC	EAT-MCAC(x)	15	14
*Eco*RI-AT/*Mse*I-CAG	EAT-MCAG(x)	17	16
*Eco*RI-AT/*Mse*I-CTC	EAT-MCTC(x)	15	11
*Eco*RI-AT/*Mse*I-CTG	EAT-MCTG(x)	3	3
*Eco*RI-AT/*Mse*I-CTT	EAT-MCTT(x)	18	13
Total		222	180

[a] (x) identifies the polymorphic bands numbered according to their sizes (the bigger the size the smaller the number)

Table 2 Origin and sources of SSR markers used to construct the linkage map of the L × L progeny

Acronym	Source	SSR origin	No. of SSR primer pairs used	No. of SSR primer pairs detecting > 1 locus	No. of SSR loci mapped
UDP	University of Udine (Italy) (Cipriani et al. 1999)	Peach	25	2	11
CPPCT	IRTA (Spain) (Aranzana et al. 2002a)	Peach	29	1	11
pch/ps	Clemson University (USA) (Sosinski et al. 2000)	Peach and Cherry	15	1	7
Total			69	4	29

Table 3 Sumary of SSRs mapped in the L × L population

SSR marker	Length[a]	Fragment size[b]	Linkage group	χ^{2} [c]
UDP96-005 (1)[d]	155	130	G1	0.2
UDP96-005 (2)[d]	–	260	G5	2.4
UDP96-010	131	110/90	G6	27.6**
UDP96-015	174	200/190	G5	6.6*
UDP97-401	130	130/125	G5	2.9
UDP98-405	104	110/105	G7a	3.6
UDP98-406	101	105/95	G2	1.0
UDP98-409	129	190/185	G8	3.7
UDP98-410 (1)	146	130/120	G2	1.0
UDP98-410 (2)[d]	–	280	G5	1.1
UDP98-412	129	110/90	G6	17.9**
CPPCT-2 (1)[d]	100	150	G3	0.1
CPPCT-2 (2)[d]	–	120	G6	0.3
CPPCT-3	160	525/500	G1	9.8**
CPPCT-5[d]	160	140	G4	1.0
CPPCT-8	161	185/180	G6	2.2
CPPCT-13	150	190/180	G5	5.9*
CPPCT-16	191	200/190	G1	0.7
CPPCT-19	184	285/280	G1	2.9
CPPCT-26	180	170/165	G1	3.1
CPPCT-27	114	80/70	G1	12.9**
CPPCT-33	151	165/155	G7a	0.6
pchcms 2[d]	180	190	G7a	28.5**
pchcms 3	220	300/290	G7a	3.0
pchcms 5 (1)	246	285/275	G6	0.8
pchcms 5 (2)[d]	–	200	G4	1.9
pchcms 5 (3)	–	175/170	G10	2.5
ps12a02	164	165/160	G4	2.1
ps05c03[d]	–	200	G7b	1.6

[a] Length of the fragment (bp) cloned by authors (Table 2)
[b] Fragment size (bp) obtained in the mapped progeny
[c] Chi-square of the goodness of fit to a 3:1 segregation in dominant markers or to a 1:2:1 segregation in co-dominant markers (*P < 0.05; **P < 0.01)
[d] SSR dominant markers

also mapped seven new SSR loci: pchcms5(2) in G4, UDP96-005(2), UDP98-410(2) and UDP96-015 in G5, CPPCT-2(2) in G6, pchcms3 in G7a and pchcms5(3) in G10.

Self-incompatibility trait

The self-incompatibility trait was coded as a dominant marker based on the amplified fragments obtained by PCR using PD2-F and PRU-C4 primers. 'Stark Early Orange', the SI parent, and 'Lito' showed one band around 1.5 kb, 'Tyrinthos', the SC parent, did not present any band and the L × L population showed segregation (39 individuals had the band, 35 did not and two were undetermined), therefore the amplified band would correspond to a self-incompatibility allele (Fig. 1). This trait deviated significantly (χ^2 = 19.6 at $P < 0.01$) from the expected segregation ratio for a single dominant locus (3:1), but is in agreement with a 1:1 segregation (χ^2 = 0.26) according to the semi-compatible reaction produced by selfing of a heterozygous F_1 at the *S*-locus. The trait was mapped at a LOD score of 4.0 at the end of linkage group G6 as was previously reported in almond by Ballester et al. (1998) and in almond × peach by Bliss et al. (2002).

Sharka resistance trait

The sharka resistance was coded as a single trait based on the resistance/susceptible phenotypes observed. From the analysis, 46 individuals of the L × L population were resistant and 30 susceptible. This trait deviated significantly (χ^2 = 8.5 at $P < 0.01$) from the expected segregation ratio for a single dominant locus (3:1) and was mapped at a LOD score of 4.0 in the group G1, as was previously reported by Hurtado et al. (2002) using a segregating F_1 population derived from the apricot intraspecific cross 'Goldrich' × 'Valenciano'.

Genetic linkage map

The map is organized into 11 linkage groups covering 602 cM and is composed of 211 loci: 180 AFLPs, 29 SSRs and two agronomic traits (Fig. 2). Fifty two additional markers remain unlinked (42 AFLPs and ten SSRs).

Linkage groups were numbered according to the nomenclature adopted by Joobeur et al. (2000), Dettori et al. (2001) and Aranzana et al. (2002b), because of the SSR loci held in common. G7b, a small linkage group composed of three loci, shares an SSR locus (Ps05c03) with the 'Texas' × 'Earlygold' map (Aranzana et al. 2002b) and therefore it was assigned to group G7. Groups G9 and G10 were named arbitrarily because they do not include any previously mapped markers. Loci order was

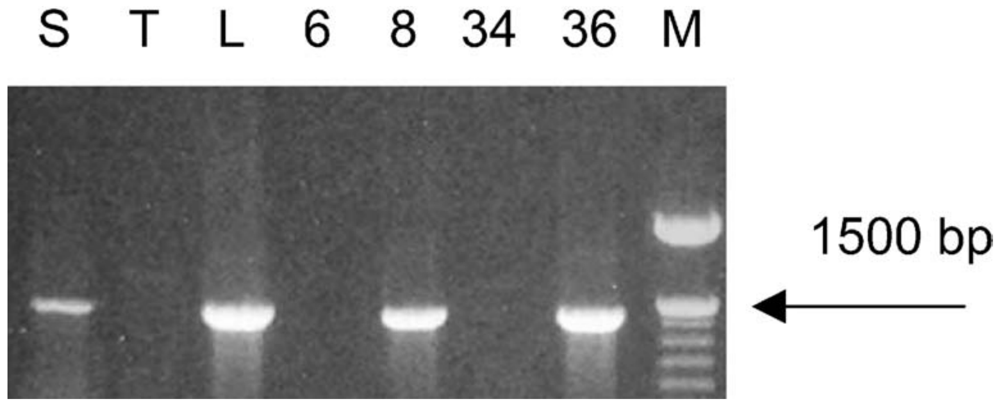

Fig. 1 PCR analysis of the self-incompatibility allele in the L × L population. Amplified fragments were obtained by PCR from genomic DNA using AS1 and Pru-C4R (Tao et al. 1999) primers. The picture below shows the PCR result in *L* ('Lito'), their parentals *S* ('Stark Early Orange') and *T* ('Tyrinthos'), and in a sample of four individuals of the L × L progeny (6, 8, 34 and 36). Band size was determined by comparison with a 100-bp molecular-weight marker (*M*) on the right

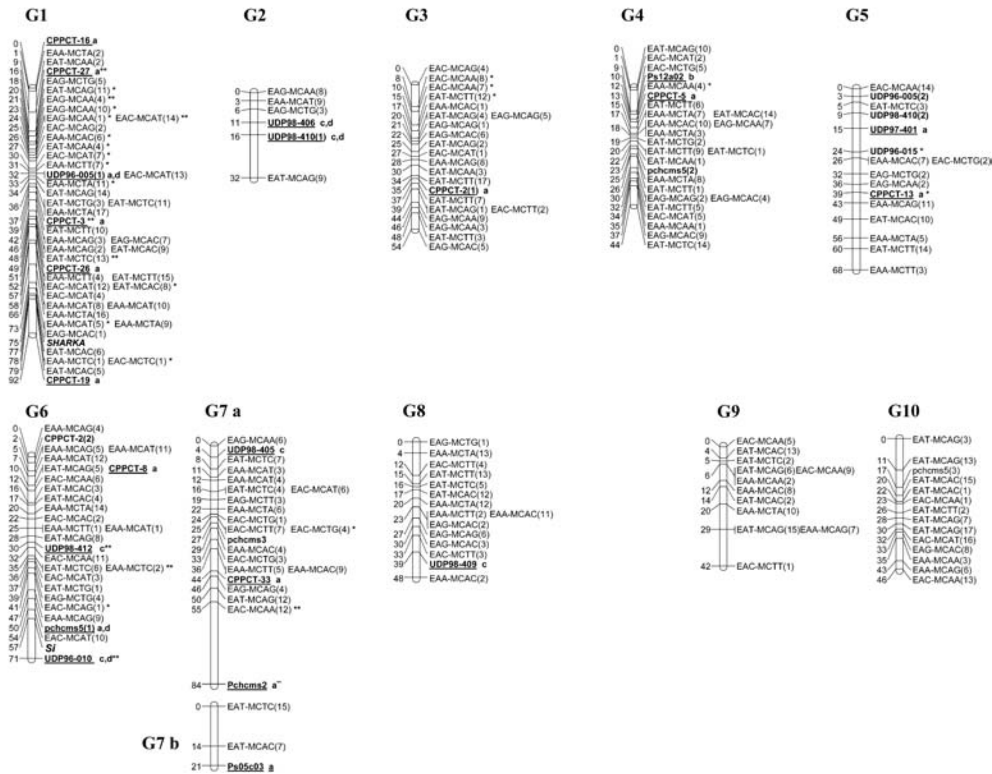

Fig. 2 Molecular linkage map obtained from the L × L F_2 progeny. Group numbers are according to Joobeur et al. (2000). Dettori et al. (2001) and Aranzana et al. (2002b) except groups number 9 and 10. SSR loci are *in bold* and those of them *underlined* are anchor points with the Aranzana et al. (2002b) map (*a*), Joobeur et al. (2000) map (*b*), Dettori et al. (2001) map (*c*) and Hurtado et al. (2002) map (*d*). SSR loci that can not be found in these maps are followed by (2) or (3). The rest of the markers are AFLPs developed for this map. Loci labelled with *asterisks* showed distorted segregations (*$P < 0.05$; **$P < 0.01$). Self-incompatibility (*Si*) and Sharka resistance traits are in *italics*. The cM distances are shown on the left

compared with the previously constructed maps and was maintained in all groups.

The average distance (cM/marker) between markers is 3.84 cM, considering only one marker in regions with co-segregating markers, and 3.28 cM taking all markers into account. The distance ranges from 1.7 cM in G4 to 5.3 cM in G2. The largest gap (>20 cM) is located in G7a, and six more gaps (≥10 cM) are located on G1 (1), G2 (1), G6 (1), G7b(1), G9(1) and G10(1). Twenty nine loci (14%) showed distorted segregations (19, $P < 0.05$ and ten, $P < 0.01$). Groups G1 and G6 have clusters of loci with distorted segregations. We also found loci with skewed

segregation in G3(3), G4(1) and G7a(2). Markers of groups G2, G5, G7b, G8, G9 and G10 do not depart from the expected Mendelian ratio.

Discussion

Molecular-marker analysis

We used AFLP markers to build the basic map backbone. In order to obtain the highest number of scorable bands, we selected primer combinations including three selective bases for one primer and two for the other (*Eco*RI + 2/ *Mse*I + 3). This combination had been previously reported as the best alternative in the peach genome (Dirlewanger et al. 1998). We found a total of 222 AFLP polymorphic fragments and nine markers per primer combination on average. This ratio is lower than that obtained by Hurtado et al. (2002) evaluating an F_1 apricot population, probably because the progeny analyzed in this case is an F_2 generated by self-pollination. In addition, a lower rate of polymorphism was found in peach (Dirlewanger et al. 1998) pointing out the narrower range of genetic variability in the peach material. AFLP distribution in this map is rather uniform and no clusters are evident, in contrast to the results obtained by Haanstra et al. (1999) in tomato, where AFLP markers developed with *Eco*RI/*Mse*I clustered in the centromeric regions of chromosomes. Most of the AFLP markers (83%) showed the expected normal segregation in the progeny which is a similar percentage to that found in apricot by Hurtado et al. (2002) and in peach by Lu et al. (1998) (88% and 85% respectively) but higher than the one reported in *Rosa* (63%) with the same restriction enzyme combination by Rajapakse et al. (2001).

The cross-species transportability of SSRs within the *Prunus* genus (Hormaza et al. 2002; Dirlewanger et al. 2002) allowed the detection of microsatellite markers in the L × L apricot population with primer sequences developed in peach and cherry (Cipriani et al. 1999; Testolin et al. 2000; Sosinski et al. 2000; Aranzana et al. 2002a). Polymorphism detected in SSRs (42%) has been similar to that obtained in apricot (46%) by Hurtado et al. (2002) and Hormaza et al. (2002), but lower than those obtained in peach by Dettori et al. (2001) and Joobeur et al. (2000) (65% and 75% respectively). This is not unexpected since the Joobeur and Dettori maps are based on interspecies crosses, thus more likely to be exhibit polymorphism. In addition, some markers coming from other *Prunus* species gave no amplification (7%) (Cipriani et al. 1999) and the L × L progeny analyzed is a self-pollinated F_2 population with the consequent decrease in variability. SSR loci were evenly distributed throughout the map and 29 loci were present in ten out of the 11 linkage groups. The number of microsatellites varied from zero to six per group and no clusters were evident. These results are consistent with the uniform distribution and the suitable genome coverage of SSR loci previously observed in different species with more saturated SSR based maps, in peach (Aranzana et al. 2002b), kiwifruit (Testolin et al. 2001) and wheat (Roder et al. 1998).

Self-incompatibility and sharka resistance traits

In the *Rosaceae* self-incompatibility is attributed to the multi-allelic *S*-locus expressed gametophytically (Sonneveld et al. 2001). Molecular typing of *S*-alleles has been achieved in cherry (Tao et al. 1999; Sonneveld et al. 2001; Wiersma et al. 2001) and almond (Tao et al. 1997; Ushijima et al. 1998; Ma and Oliveira 2001) through the identification and characterization of cDNAs corresponding to stylar RNases. In apricot *S*-alleles have not been yet identified and stylar ribonuclease assays have been used to detect self-compatible seedlings in apricot (Burgos et al. 1998) and other fruit tree species (Sassa et al. 1992; Boskovic and Tobutt 1996; Boskovic et al. 1997; Boskovic and Tobutt 1999). However, ribonuclease assays require flowering trees for collection of a substantial amount of pistils for protein extractions and separation by polyacrylamide gels, and this is very time consuming. The identification of apricot *S*-alleles would provide an easy way to characterize progenies by means of PCR techniques. In this work we used primers based on conserved regions of *Prunus S*-RNase sequences (Tao et al. 1999) in order to amplify segregating fragments corresponding to *S*-alleles in the L × L population. 'Stark Early Orange' is an SI cultivar with unknown S-allele genotype and 'Thyrintos' is an SC cultivar. The F_1 ('Lito') obtained from the cross between 'Stark Early Orange' as female parent and 'Thyrintos' as male parent is putatively heterozygous at the *S*-locus having a self-compatibility allele (*Sc*) from the male parent and a self-incompatibility allele (*Si*) from the female parent. PCR amplification produced one band around 1.5 kb in 'Stark Early Orange' and 'Lito' cultivars, but 'Thyrintos' did not show any fragment suggesting that the amplified band corresponds to the *Si* allele. The L × L F_2 population originated from selfing of an individual of the F_1 progeny ('Lito') in a semi-compatible cross, as the *Si* pollen is unable to fertilize the ovule, and only *SiSc* and *ScSc* individuals in a 1:1 proportion can be produced. Following this model the amplified fragment corresponding to the *Si* allele segregated 1:1 in the L × L progeny (χ^2 = 0.26), deviating significantly ($\chi^2 = 19.6$ at $P < 0.01$) from the expected segregation ratio for a dominant marker (3:1). The *Si* locus was mapped at a LOD score of 4.0 in the G6 linkage group as was previously reported by Ballester et al. (1998) for almond. These authors evaluated the self-compatibility in an almond cross measuring fruit set, pollen-tube growth and stylar ribonuclease activity. More recently Bliss et al. (2002) also mapped the *Si* locus at the same linkage group in an almond × peach population. In this case self-incompatibility was determined by the PCR products obtained using primers developed from *S*-alleles controlling this trait in almond.

The inheritance of resistance to PPV in apricot has been studied by several authors but there is currently no

genetic model for this resistance. Currently, there are three different published hypothesis suggesting that the resistance trait is controlled by one (Dicenta et al. 2000; Martínez-Gómez and Dicenta 2000), two (Moustafa et al. 2000a, b) or three genes (Guillet-Bellanguer and Audergon 2000). Due to the difficulty of the evaluation of this trait on a large-scale, only families with a limited number of individuals have been studied until now. This fact could explain the lack of agreement in the hypothesis of inheritance. In this work we have evaluated the PPV resistance in an F_2 population (76 individuals) derived from selfing of the cultivar 'Lito' by means of the improved methodology of Moustafa et al. (2000a; 2001). 'Lito' originated from the cross between 'Stark Early Orange', a North American sharka resistant cultivar, and 'Thyrintos', a Greek sharka susceptible cultivar (Syrgianidis and Mainou 1991). The L × L population segregated for this trait and therefore 'Lito' is assumed to be heterozygous for the resistance locus/loci. From the 76 individuals analyzed by grafting, ELISA and RT-PCR, 46 were found resistant and 30 susceptible. This segregation ratio deviated significantly ($\chi^2 = 8.5$ at $P < 0.01$) from the expected for a single dominant locus (3:1) but fits ($\chi^2 = 0.5$) with a model for two dominant loci (9:7) supporting the model of inheritance previously reported by Moustafa et al. (2000a, b). The sharka resistance was tentatively mapped as a single-locus controlled trait at a LOD score of 4.0 in the G1 linkage group. This result is in agreement with that previously reported in apricot by Hurtado et al. (2002) using an F_1 population derived from the cross between 'Goldrich', the source of PPV resistance, and 'Valenciano' cultivars, suggesting that sharka resistance has been assigned to the correct group.

Genetic linkage map

This map is the first one based on an F_2 apricot population and incorporates 211 loci into 11 linkage groups. The presence of seven gaps between 10 and 20 cM, the high number of markers that remain independent (52) and the identification of 11 linkage groups, three more than the expected basic number of chromosomes of *Prunus* ($x = 8$), suggest that an increase in the number of mapped markers would improve the coverage of the genome. However, this map covers 602 cM with an average distance between markers of 3.28 (cM/marker) lower than that obtained for most of the *Prunus* maps published (Rajapakse et al. 1995; Viruel et al. 1995; Joobeur et al. 2000; Dettori et al. 2001; Bliss et al. 2002; Hurtado et al. 2002) except Joobeur et al. (1998) and Dirlewanger et al. (1998). In addition, an average of 1.3 gaps of $\geq$10 cM per Morgan was calculated and this result also indicates a good coverage of the genome. The apricot genome length estimated using the method of Meagher et al. (1988) was between 800 and 1,200 cM and following Hulbert et al. (1988) the methodology was 1,362 cM; therefore this linkage map may cover from the 44% to 75% of the total nuclear genome depending on the estimator. G1 and G6 were the largest linkage groups and this result is in agreement with those observed in most *Prunus* maps (Joobeur et al. 1998; Aranzana et al. 2002b; Dettori et al. 2001; Bliss et al. 2002) and with cytogenetic data suggesting the existence of a chromosome (G1) larger than the rest in *Prunus* (Jelenkovic and Harrington 1972).

Twenty two SSR markers act as anchor loci between this map and other *Prunus* maps (Joobeur et al. 2000, Dettori et al. 2001; Aranzana et al. 2002b; Hurtado et al. 2002) and they are distributed rather uniformly in the genome. These common loci allowed the integration of the apricot map with the general *Prunus* map (Joobeur et al. 2000). We also detected seven new SSR loci that were not placed in previous *Prunus* maps and could be useful as anchor loci in the future. On the basis of the 15 common SSR loci the best correspondence was established between this map and the T × E map (Aranzana et al. 2002b). Most of the SSR common markers displayed the same order in the different *Prunus* maps.

The percentage of markers showing distorted segregation (14%) is similar to those obtained in peach [15% in Lu et al. (1998) and 18.5% in Dettori et al. (2001)], and apricot [11% in Hurtado et al. (2002)] but much lower than those in peach × almond maps [37% in Foolad et al. (1995), 46% in Joobeur et al. (1998) and 45% in Bliss et al. (2002)] probably because of the interspecific nature of this cross (Bliss et al. 2002). Most of the distorted loci were located on the G1 (55%) and G6 (14%) linkage groups, and these regions with skewed segregations were similar to those found by Dettori et al. (2001) in peach and Joobeur et al. (1998) in almond × peach. In the G6 linkage group, distortion may be attributed to the self-incompatibility allele as in the T × E map (Ballester et al. 1998; Joobeur et al. 1998). In fact, a strong selection against the 'Stark Early Orange' alleles in the neighbourhood of the *Si* locus was observed and most of these markers showed aberrant segregations. On the other hand, more than a half of the distorted loci were located on G1 and they can be separated in two main clusters. The first one includes markers of the female parent ('Stark Early Orange') with a significant departure towards a lack of recessive homozygotes, indicating that selection favoured these alleles. The second one groups markers of the male parent ('Thyrintos') showing an excess of recessive homozygotes, indicating that selection in this case was against these markers. As no plants from the L × L population were lost in the field, distortions should not be related to this kind of selection as was reported in almond × peach by Joobeur et al. (1998). More probably the selection occurred at the pre- or post-zygotic level against lethal or sublethal genes linked to the markers under selection.

The backbone of this linkage map is composed of AFLPs that cover the apricot genome uniformly. To complement this structure we used SSR markers that can be easily transferred to other populations. SSR loci in common with other *Prunus* maps enable integration of different species map data and will facilitate construction of a consensus map for the genus in the future. Two

important agronomical traits were included in this map, self-incompatibility, located on the G6 group, and sharka resistance, tentatively placed on the G1 group. Both of these are main objectives in the apricot breeding programs developed since the beginning of the nineties, illustrating the relevance of these traits in future research. Saturation with additional codominant markers should be pursued in the future to facilitate the implemention of this map for MAS of these two important traits, and to develop physical mapping strategies to identify and characterize Sharka resistance genes.

Acknowledgements This research was supported by a grant from the Ministerio de Ciencia y Tecnología (AGL2001-1122-C02-02). CCPCT primer sequences were kindly provided by Dr. P. Arús IRTA, Cabrils, Spain. We thank Dr. L. Burgos from the CEBAS-CSIC, Murcia, Spain, for his advice and technical assistance in the self-incompatibility trait mapping. AFLP radioactive labelling was performed in Dr. Ricardo Flores laboratory (IBMCP, UPV-CSIC, Valencia, Spain). The authors thank José Martínez, Dolores Archelós and José Miguel Soriano, for their technical contributions. S.V. was funded by a fellowship from the Ministerio de Ciencia y Tecnología of Spain.

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