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Genetic linkage maps of two apricot cultivars (*Prunus armeniaca* L.), and mapping of PPV (sharka) resistance

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Abstract Genetic linkage maps for two apricot cultivars have been constructed using AFLP, RAPD, RFLP and SSR markers in 81 F1 individuals from the cross 'Goldrich' × 'Valenciano'. This family segregated for resistance to 'plum pox virus' (PPV), the most-important virus affecting *Prunus* species. Of the 160 RAPD arbitrary primers screened a total of 44 were selected. Sixty one polymorphic RAPD markers were scored on the mapping population: 30 heterozygous in 'Goldrich', 19 heterozygous in 'Valenciano', segregating 1:1, and 12 markers heterozygous in both parents, segregating 3:1. A total of 33 and 19 RAPD markers were mapped on the 'Goldrich' and 'Valenciano' maps respectively. Forteen primer combinations were used for AFLPs and all of them detected polymorphism. Ninety five markers segregating 1:1 were identified, of which 62 were heterozygous in the female parent 'Goldrich' and 33 in the male parent 'Valenciano'. Forty five markers were present in both parents and segregated 3:1. A total of 82 and 48 AFLP markers were mapped on the 'Goldrich' and 'Valenciano' maps. Twelve RFLPs probes were screened in the population, resulting in five loci segregating in the family, one locus heterozygous for 'Valenciano' and four heterozygous for both, segregating 1:2:1. Of the 45 SSRs screened 17 segregated in the mapping family, resulting in seven loci heterozygous for the maternal parent and ten heterozygous for both, segregating 1:2:1 or 1:1:1:1. A total of 16 and 13 co-dominant markers were mapped in the female and male parent maps respectively. A total of 132 markers were placed into eight linkage groups on the 'Goldrich' map, defining 511 cM of the total map-length. The average distance between adjacent markers was 3.9 cM. A total of 80 markers were placed into seven linkage groups on the 'Valenciano' map, defining 467.2 cM of the total map-distance, with an average interval of 5.8 cM between adjacent markers. Thirty six marker loci heterozygous in both parents revealed straightforward homologies between five linkage groups in both maps. The sharka resistance trait mapped on linkage group 2. The region containing sharka resistance is flanked by two co-dominant markers that will be used for targeted SSR development employing a recently constructed complete apricot BAC library. SSRs tightly linked to sharka resistance will facilitate MAS in breeding for resistance in apricot.

Keywords Genetic linkage maps · *Prunus armeniaca* · Apricot cultivars 'Goldrich' and 'Valenciano' · Sharka resistance trait

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Introduction

Apricot is a member of the genus *Prunus*. Besides apricot, the genus comprises other economically important crops like peach, almond, cherry and plum. The apricot industry produced 2.3 millions tons in the world (being the third species from the stone fruit crops). The main growing areas are China, the Mediterranean European countries, Turkey and USA (FAO 2001). Since the spread of sharka (plum pox virus or PPV), the most economically important virus disease of fruit crops in Europe (Roy and Smith 1994; Llácer and Cambra 1998), all apricot breeding programs developed in the Mediterranean countries, and some initiated recently in the USA, have as their main objectives the introduction of sharka resistance into commercial cultivars (Audergon et al. 1994; Egea et al. 1999; Karayiannis et al. 1999). The sharka virus became the most limiting factor for the apricot crop and a severe problem for other *Prunus* species. Despite the number of apricot breeding programs, there is very little information available concerning the genet-

ics of apricot and the genetics, sources and mechanism of sharka resistance.

Perennial fruit crops are characterized by long generation times and large size, which has limited the development of genetic studies, resulting in less progress in tree-breeding programs compared to that of herbaceous crops. The genetic improvement of a species through artificial selection depends on the ability to distinguish genetic effects from environmental effects. DNA markers are potentially limitless in number and their identification and use are unaffected by the environment (Vogel et al. 1996). Genetic linkage mapping has proven to be a powerful tool for localizing and isolating genes controlling both simple and complex traits. Sharka disease is the most-important limiting factor for apricot cultivation, and molecular markers linked to this trait would be of great value for the identification and selection of resistant plant genotypes. Currently, selection for the sharka resistance trait is a very long and time-consuming procedure based on the biological test confirmed by ELISA and PCR analysis that needs a lot of space in the greenhouse and the management of many plants (Moustafa et al. 2001b). It is the most important limitation in the current apricot breeding program and only a few resistant cultivars from breeding programs are being released. Molecular markers are a potential tool for solving this problem by means of increasing selection efficiency.

Apricot is the least genetically characterized species of the genus *Prunus*. Inheritance of a few traits of interest have been determined as self-compatibility (Burgos et al. 1997) or male sterility (Burgos and Ledbetter 1994). Various studies based on allozymes (Badenes et al. 1996), morphological traits (Badenes et al. 1998a), AFLP (amplified length polymorphism) markers (Hurtado et al. 1999) or RFLP (restriction fragment length polymorphism) markers (De Vicente et al. 1998) have also been reported. Linkage maps based on molecular markers have been developed in *Prunus* species based on interspecific crosses of peach × almond (Joobeur et al. 1998) or intraspecific crosses of almond (Viruel et al. 1995; Joobeur et al. 2000), peach (Dirlewanger et al. 1998; Lu et al. 1998) and sour cherry (Wang et al. 1998). A genetic linkage map of apricot that included the sharka trait could be very valuable for apricot breeding and may provide tools for molecular marker-assisted selection.

The choice of the marker system to use for a particular application will depend on the type of genomic information required and its ability to detect polymorphism in a given population. Apricot has a medium level of heterozygosity among *Prunus* species, more than peach but less than almond, plums or cherries (Byrne and Littleton 1989). PCR-based genetic-marker techniques have reduced the cost of identifying genetic markers and allowed large-scale genotyping of individuals at any locus (Ferreira and Grattapaglia 1995). RAPD markers (random amplified polymorphic DNA) provide a source of large numbers of markers compared to RFLPs (Chaparro et al. 1994). AFLP (amplified fragment length polymorphism) samples a large number of loci in high-resolution sequencing gels. On the other hand, examination of co-dominant markers such as RFLPs or SSRs, either present or being mapped in other *Prunus* maps, would provide anchor loci for map comparison. Based on these reasons, PCR-based markers (RAPDs and AFLPs) and co-dominant markers (RFLPs and SSRs) were chosen for mapping a family from an intraspecific apricot cross.

We report here on the application of molecular markers to the genome-mapping of a family that segregated for sharka resistance. This research is a part of an ongoing breeding program aimed at improving apricot genetic knowledge and conducting marker-assisted selection in apricot.

Materials and methods

Mapping population

The mapping family in this study was a controlled intraspecific cross between: ‘Goldrich’ (female parent), a North American self-incompatible cultivar resistant to PPV (plum pox virus) or ‘sharka’, and ‘Valenciano’ (male parent), a Spanish self-compatible cultivar susceptible to sharka. The mapping population consisted of 81 F1 individuals in a pseudo test-cross configuration.

Screening for sharka resistance

Evaluation of sharka virus resistance on the progeny was based on the biological test described in Moustafa et al. (2001b). A peach seedling ‘GF-305’ was used as an indicator of susceptibility. ‘GF-305’ seedlings were obtained by germination of embryos after 20–30 days of chilling treatment. Then 10–12 weeks later when the peach seedling was the correct size for grafting they were inoculated with PPV, Dideron strain 3.3 RB described in Asensio (1996), by grafting buds from an already infected apricot. A minimum of six plants per genotype were screened. Simultaneously, the apricots for testing were grafed on to peach ‘GF-305’. Four weeks after grafting, when the bud has ‘taken’, a chilling treatment of 2 months was applied. The chilled plants were pruned to promote new shoots for scoring symptoms. The presence of the virus was analyzed by visual scoring of symptoms plus ELISA-DASI, performed as described by Lommel et al. (1982), and RT-PCR (Reverse Transcription-Polymerase Chain Reaction), as described in Wetzel et al. (1991), on the new shoots. Those plants that did not show symptoms and were ELISA negative were applied to a second chilling treatment. Two cycles of chilling and observations were made. Seedlings were classified as resistant if they did not show symptoms or replication of the virus after two chilling treatments in all plants assayed.

DNA isolation

Five grams of young expanded leaves of each individual were collected, rinsed with tap water, frozen, placed in plastic bags and kept at –80 °C before DNA isolation. Genomic DNA was isolated from the leaf samples following the CTAB method of Doyle and Doyle (1987). DNA concentrations were measured using a spectrophotometer, (UV-Visible spectrophotometer, UV-1601, Shimadzu Corp.).

Genotyping

RAPDs

A total of 160 10-base random primers (kits B, C, M, P, R, Y, Z and V; Operon Technologies, Alameda, Calif.) were screened

against the two parents and a progeny sample of six individuals, following Grattapaglia and Sederoff (1994). RAPD fragments which segregated in the progeny were detected. A total of 44 primers were selected during this step. Selected primers were used for mapping. RAPD analysis was performed as described by Badenes et al. (1998b).

AFLPs

This analysis was performed as described by Vos et al. (1995). Working solutions of genomic DNA at 50 ng/µl in TE buffer (10 mM of Tris–HCl, 1 mM of EDTA, pH 8.0) were prepared for AFLP analysis. Total genomic DNA (250 ng) was digested with 1.65 U of *Eco*RI and 1.65 U of *Mse*I in 16.5-µl reaction mixtures containing 10 mM of Tris-acetate buffer (pH 7.5), 10 mM of magnesium acetate, 50 mM of potassium acetate, and 5 mM of dithiothreitol for 2 h at 37 °C. Then, 16.5 µl of a mixture containing 2.5 pmol of *Eco*RI adapter, 25 pmol of *Mse*I adapter, 1 U of T_4 DNA ligase, 1 mM of ATP in 10 mM Tris-acetate (pH 7.5), 10 mM of magnesium acetate, 50 mM of potassium acetate and 5 mM of dithiothreitol was added, and the restriction-ligation reaction incubated at 37 °C for 2 h.

Core reagent and starter primer kits were purchased from Life Technologies (Gibco BRL, Gathersburg, Md., USA). The pre-amplification reaction was performed with 2.5 µl of template DNA (a 1:10 solution diluted from the restriction-ligation mixture), using a pair of primers based on the sequences of the *Eco*RI and *Mse*I adapters, EO1 and MO2. A 22.6-µl reaction mixture containing 20 mM of Tris HCl (pH 8.4), 50 mM of KCl, 1.5 mM of $MgCl_2$, 0.2 mM of dNTP, 15 ng of EO1, 15 ng of MO2 and 0.5 U of *Taq* DNA polymerase was used for the pre-amplification reaction as described by Vos et al. (1995). The selective amplification reaction was performed with two primers based on the same sequences as EO1 and MO2, but with 1–2 additional selective nucleotide(s) at the 3′ end of each primer. The *Eco*RI primers were labeled by phosphorylating the 5′ end with [γ^{-33}P] ATP for fragment detection.

The PCR products were mixed with an equal volume of tracking dye (98% formamide, 10 mM of EDTA, 0.05% bromophenol blue, and xylene cyanol), denatured at 90 °C for 3 min, and immediately cooled on ice. Aliquots (4 µl) of each reaction were loaded onto a denaturing 6.0% polyacrylamide gel (acrylamide-bisacrylamide 20:1) in 1 × TBE buffer (50 mM of Tris, 50 mM of boric acid, 1 mM of EDTA, pH 8.0) and 7.5 urea; 1 × TBE was used as an electrophoresis buffer. Gels were run at a constant power (70 W, equivalent to 40–50 V/cm), fixed, dried, and exposed to Kodak BioMax X-ray film for 2–4 days before being developed.

RFLPs

For RFLP analysis, 8 µg of DNA from each cultivar was digested separately with *Eco*RI and *Bam*HI, then electrophoresed in 0.8% agarose gels and Southern-blotted to Hybond N+ filters (Amersham Pharmacia Biotech). Twelve almond probes (kindly supplied by IRTA Cabrils, Barcelona, Spain) were labeled with dig-dUTP (Boehringer Mannheim) by PCR amplification per the manufacturer's protocol and used to probe the filters. Hybridization was detected per the manufacturer's protocol.

SSRs

Forty five microsatellite sequences from peach libraries were screened. PCR reactions were performed in a volume of 25 µl containing 20 mM of Tris–HCl pH 8.4, 50 mM of KCl, 2.5 mM of $MgCl_2$, 5% (v/v) DMSO (dimethyl sulphoxide), 0.2 mM of each dNTP, 0.2 µM of each primer, 30 ng of genomic DNA and 1 unit of *Taq* DNA polymerase (Life Technologies) using the following temperature profile: 94 °C for 2 min, then 35 cycles of (94 °C for 45 s, 50 °C for 60 s and 72 °C for 1 min and 15 s), with a final extension step of 72 °C for 5 min. All PCR reactions were performed with a Perkin Elmer model GeneAmpl 9700 thermocycler. PCR products were separated by electrophoresis in 3% Metaphor-agarose gels stained with etidium bromide (0.8 mg/ml) and visualized using UV light.

Marker criteria

Mapping data were obtained by visual scoring of gels (RAPDs and SSRs) and autoradiograms (AFLPs and RFLPs), independently by two readers. Segregating markers were scored for presence (1) or absence (0) of the amplified band from dominant markers. Only clear, unambiguous bands were scored. For co-dominant markers segregation was coded according to the alleles present, following Joinmap 3.0 data file coding (Stam and Ooigen 1995). Markers were defined as polymorphic fragments that did not significantly depart from the Mendelian ratio at the $\alpha = 0.05$ level.

Map construction

Loci were separated into three types: (1) those showing segregation for the female parent ('Goldrich' was heterozygous and 'Valenciano' was homozygous), (2) those loci showing segregation for the male parent ('Valenciano' was heterozygous and 'Goldrich' was homozygous), and (3) those loci heterozygous in both of them. Heterozygous genetic markers present in one parent but not in the other, plus markers heterozygous for both parents, were used to construct separate genetic linkage maps for the female (Goldrich) and male (Valenciano) parents, using the two-way pseudo-testcross strategy (Grattapaglia and Sederoff 1994). Two appoaches were used for mapping construction. Markers that showed segregation-ratio distortion (which departed from the Mendelian ratio at the $\alpha = 0.05$ level) were added to the maps after mapping the markers that did not depart from the Mendelian ratio. A second approach consisted in including in the analysis both types of markers (Mendelian and skewed segregation). The software Joinmap 3.0 (Stam and Ooigen 1995), setting cross-pollination type of data, was used for map calculation. The phase was determined internally by the software based on the recombination frecuency. A LOD score of 10.0 was set as a linkage threshold for grouping markers. A subset of high-confidence evenly spaced markers was selected at LOD > 8, and additional markers were added into the framework map with support for the order > LOD 5.0. Additional candidate markers were incorporated into the framework at LOD > 3, and markers that could not be ordered into the framework were excluded from the map. The maximun recombination frecuency used to construct the two maps was $\theta = 0.4$. Map distances in centimorgans were calculated using Kosambi's mapping function.

Results

RAPDs

Of the 160 RAPD arbitrary primers screened a total of 44 (27.5%) were selected. The rest of the primers did not yield any amplified polymorphic product. An average of nine bands were amplified per reaction. The fragment size of the RAPDs ranged from 200 to 1,600 pb. The number of polymorphic bands obtained per primer ranged between 1 and 3 (Fig. 1). A total of 61 RAPD markers were scored in the population; 30 heterozygous in 'Goldrich', 19 heterozygous in 'Valenciano' and 12 markers heterozygous in both parents. Segregation ratios that departed significantly from the expected were observed in six loci. A total of 33 RAPD markers were

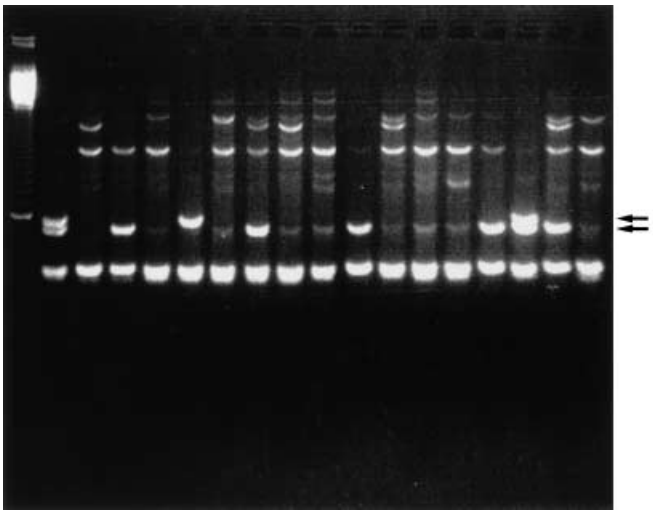


Fig. 1 RAPDs obtained with primer B8. Two markers heterozygous for 'Goldrich' and homozygous recessive for 'Valenciano' were obtained: B8-700 and B8-800. From left to right: 100 bp marker, 'Goldrich', 'Valenciano', seedlings (*S1–S15*)

mapped on 'Goldrich', nine remained unlinked, 19 on 'Valenciano' and 12 remained unlinked.

AFLPs

Using Eco +2/Mse +3 selective nucleotides, an average of 132 bands were amplified per reaction. All 14 primer combinations used for AFLPs detected polymorphism (Fig. 2), an average of ten AFLP markers per primer combination were identified. A total of 140 AFLP markers were scored in the population. Of the 95 markers segregating 1:1, 62 were heterozygous in the female parent (Goldrich) and 33 in the male parent (Valenciano). Forty five markers were present in both parents and segregated 3:1. Nineteen markers showed skewed segregation. A total of 82 AFLP markers were mapped on 'Goldrich', 25 remained unlinked. A total of 48 AFLP markers were mapped on 'Valenciano' and 30 remained unlinked.

Co-dominant markers

Twelve RFLPs probes were screened, resulting in five loci segregating in the family studied. One was heterozygous for 'Valenciano' and four were heterozygous for both parents, segregating 1:2:1 (Table 1). All of them fit Mendelian ratios. Those heterozygous for both parents were mapped and the one heterozygous for 'Valenciano' remained unlinked.

Forty five sequences flanking microsatellites in peach were screened for polymorphism in the family. Forty one of them amplified in apricot. In our experimental conditions 20 did not segregate in the family studied. Four were polymorphic with a confused pattern and were not included in the analysis. Fourteen polymorphic SSRs

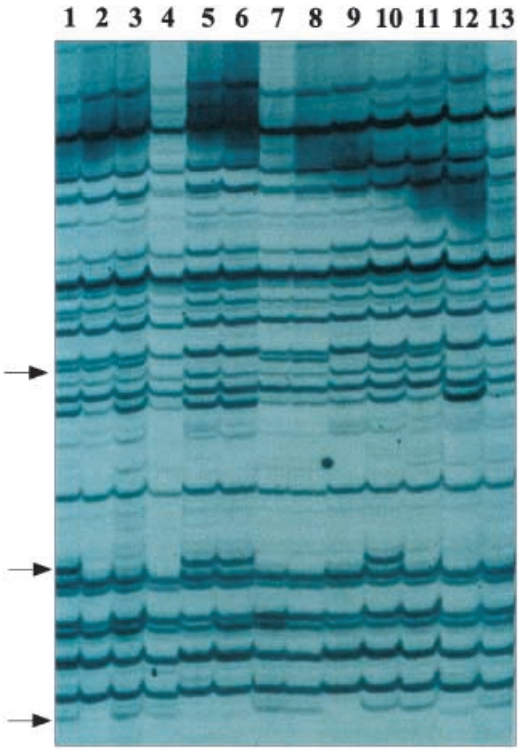


Fig. 2 AFLPs obtained with primer *Eco*RI AA/*Mse*I CCA: from left to right: 'Goldrich', ' Valenciano' seedlings (*S1–S11*)

Table 1 Co-dominant markers mapped

Marker	Segregation	Marker	Segregation
AG 6[a]	1:2:1	UDP 96018[d]	1:1
AC27[a]	1:2:1	UDP 96005[d]	1:2:1
AG56[a]	1:2:1	UDP 98409[d]	1:1
AG 7[a]	1:2:1	UDP 96010[d]	1:1
CPPCT 13[b]	1:2:1	UDP 98407[d]	1:2:1
pchgms5[c]	1:1	UDP 98410[d]	1:1:1:1
pchcms5[c]	1:1:1:1	UDP 98406[d]	1:2:1
pchcms1[c]	1:1	UDP 98411[d]	1:1:1:1
pchgms4[c]	1:2:1	UDP 96013[d]	1:2:1

[a] RFLP probes from the Dr. Arús laboratory (Viruel et al. 1995). Seven additional probes screened did not segregate in the family studied and one did not map
[b] SSR sequence from Aranzana et al. (2000b), five additional sequences screened did not segregate
[c] Sequences from Sosinski et al. (2000), ten additional sequences did not segregate in the family studied
[d] Sequences from Cipriani et al. (1999), three additional sequences segregating in the family did not map and five did not segregate in the family studied

corresponding to 17 loci were included in the mapping data (Table 1). Seven were heterozygous in one parent and ten heterozygous for both parents. A total of 13 SSR markers were mapped on the 'Goldrich' map, only three markers heterozygous for the maternal parent and one heterozygous for both remained unlinked. Nine markers heterozygous for both parents were mapped on the 'Valenciano' map and reveled homologies between both

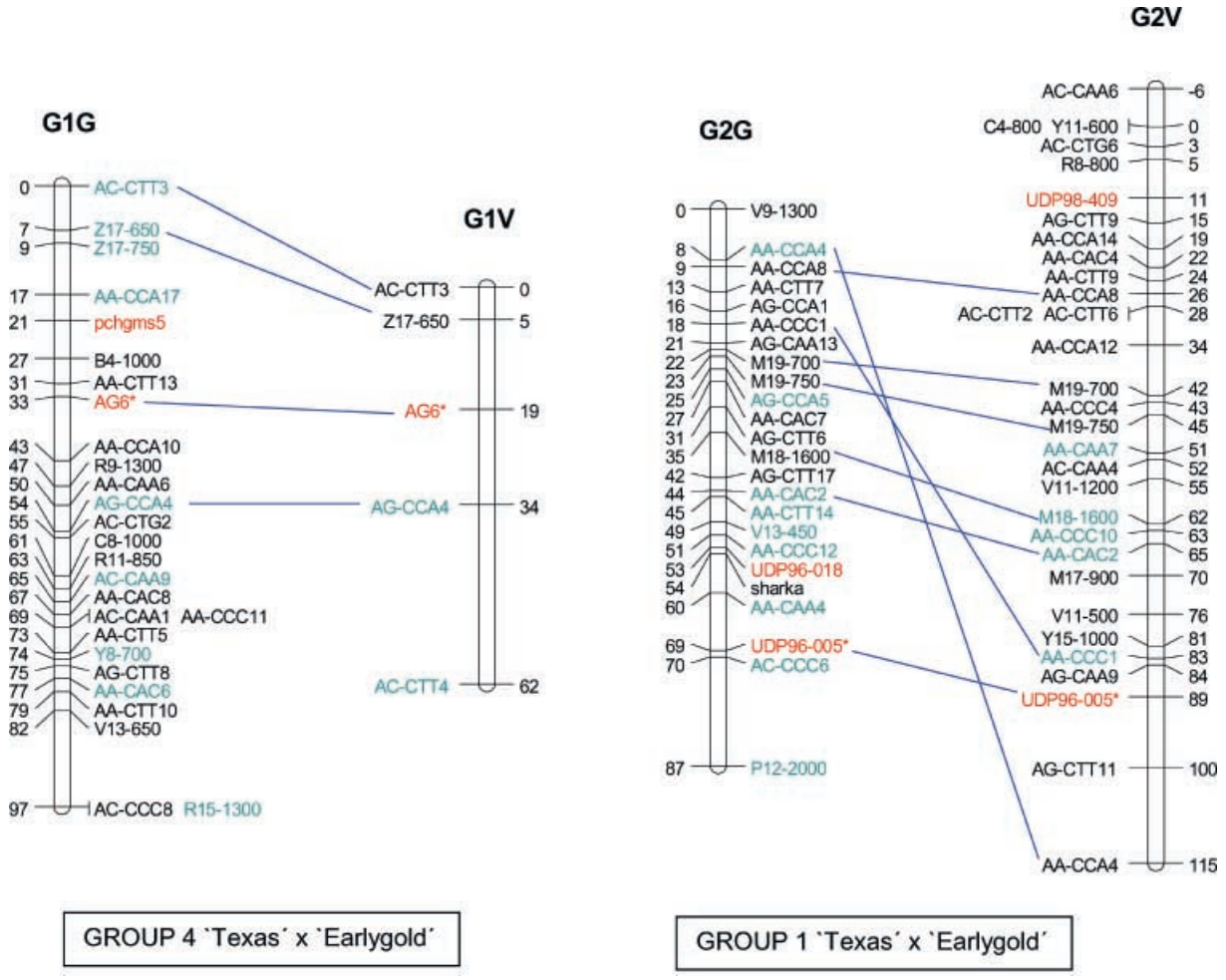


Fig. 3 Genetic linkage maps of 'Goldrich'and 'Valenciano'. Based on anchor loci, homologies between linkage groups from both apricot maps and the ones from 'Texas' × 'Early Gold' (Aranzana et al. 2001b) were identified in five groups. Those groups where homology could not be identified were labeled as *A*, *B*, *C*. Anchor loci that established homologies among the 'Goldrich', 'Valenciano' and 'Texas × 'Early Gold' maps are labeled by an *asterisk*. *G1G*. Linkage groups from 'Goldrich', *G1V*. Linkage groups from 'Valenciano'. Blue lettering: Markers added at LOD 3. Red lettering: Co-dominant markers

maps. Three microsatellites presented skewed segregation, and mapped in the same region of linkage group 4.

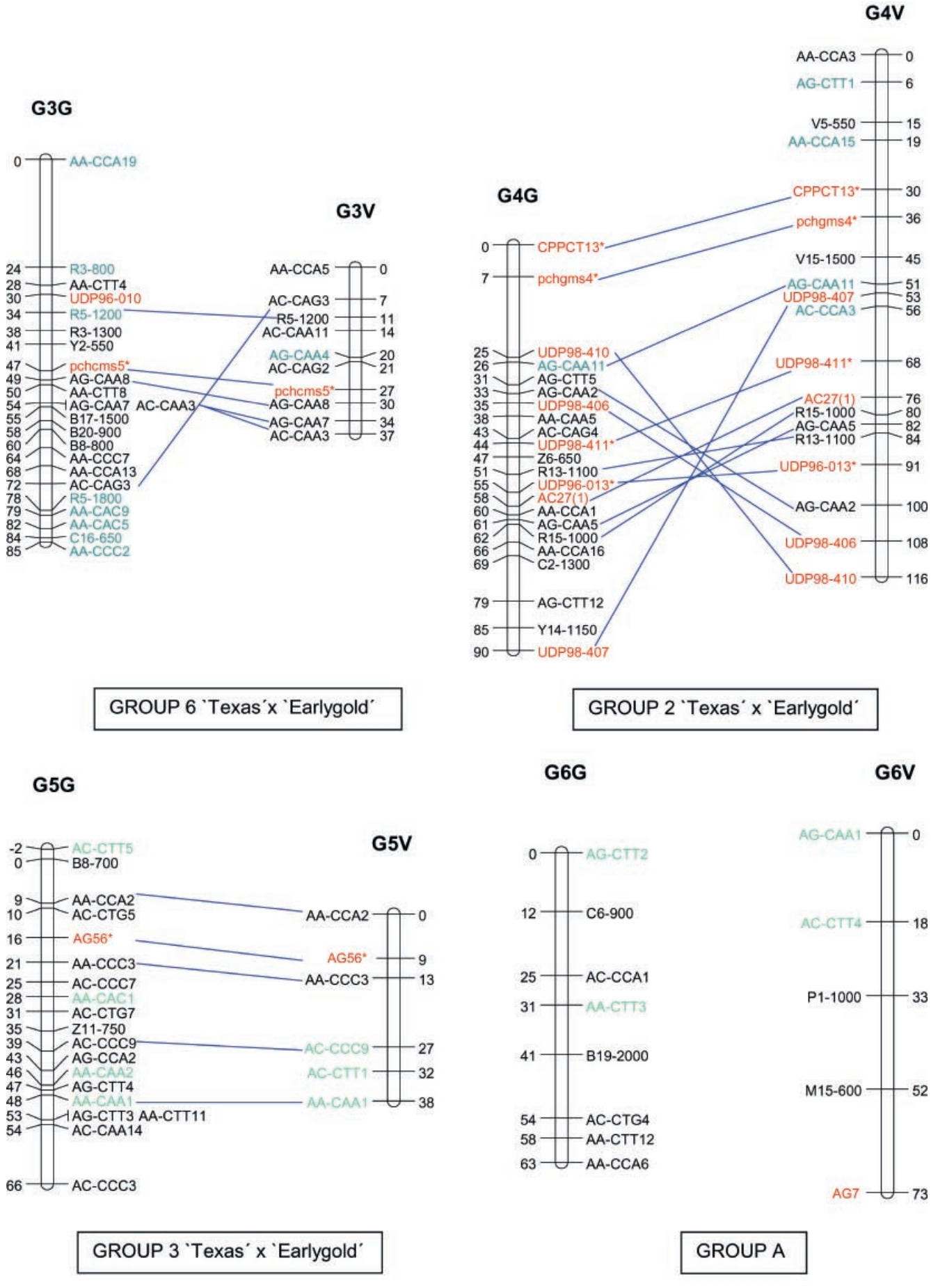
Map construction

Linkage was established at a LOD score range of 10 to 8. At an 8-LOD score eight linkage groups with more than three markers were obtained for 'Goldrich' and seven groups for 'Valenciano'. Apricot has n = 8 chromosomes, and therefore eight linkage groups were expected in each map. This subset of high-confidence evenly spaced markers was selected, and at a first step were added and re-ordered into the framework map with a support order > LOD 5. In these conditions the maternal 'Goldrich' has a total of 96 markers in eight linkage groups, the paternal Valenciano has 61 markers in seven linkage groups. Pairwise recombination between framework markers and other markers in each group was calculated using Joinmap (Stam and Ooigen 1995) to identify additional candidate markers that could be ordered into the framework at LOD >3. At the end of this process 36 markers were added to the 'Goldrich' map and 19 to the 'Valenciano' map. Those markers that could not be ordered into the framework were excluded.

Dominant and co-dominant markers, heterozygous in both parents, placed on the maps established homologies between linkage groups of both male and female maps. Forty five AFLPs, 12 RAPDs segregating 3:1 and 14 co-dominant markers segregating 1:2:1 or 1:1:1:1 were included in the analysis. Thirty six of them were mapped in both maps revealing straightforward homologies between the five linkage groups of 'Goldrich' and 'Valenciano' (Fig. 3).

Map length

A total of 132 markers were placed on the 'Goldrich' map, defining 511 cM of total map distance. The average distance between adjacent markers was 3.9 cM; only one interval was 24-cM long in linkage group 3. In the rest of the linkage groups the maximum interval ranged be-



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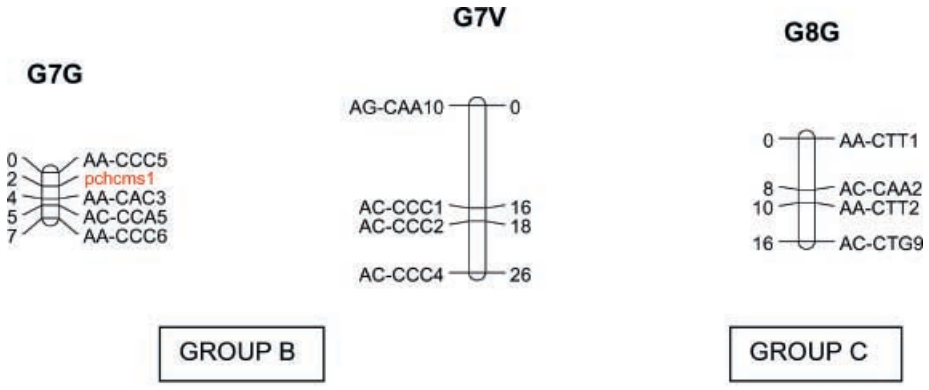

tween 10 and 15 cM. Six of the eight linkage groups were classified as "major" containing from 27 to 8 markers, and ranged from 97 to 63 cM. The other two "minor" linkage groups containing four and five markers each ranged from 6.0 to 16 cM. The 'Goldrich' map had 65% more markers than the 'Valenciano' map. A total of 80 markers were placed on the 'Valenciano' map, defining 467 cM of total map distance, with an average interval of 5.8 cM between adjacent markers. Only one interval was 28-cM large. Four "major" linkage groups containing from 6 to 31 markers were evident in 'Valenciano' and ranged from 116 to 73 cM. The other three linkage groups had 4–5 markers, and ranged from 62 to 26 cM.

Sharka Resistance

Screening of seedlings resulted in a segregation rate of 40/41 resistant versus susceptible after the first chilling treatment. However, after a second chilling cycle 19 genotypes classified as resistant became susceptible. The trait was coded in the mapping analysis according to the phenotypes observed: resistant versus susceptible. Resistant individuals were coded as heterozygous for the trait and those susceptible were coded as homozygous recessives. The trait was added into the framework map with support for the order > LOD 3.0 and mapped in linkage group 2.

Discussion

Markers

Sixty one RAPD markers were scored in the mapping populations. As expected, polymorphism found in apricot (27.5% of the primers screened revealed polymorphic loci) was higher than that found in peach: 17% and 16% was obtained by Dirlewanger et al. (1998) and Chaparro et al. (1994), respectively. However in peach, the average of markers per primer selected was 1.5, similar to that obtained in our study. When families from crosses between two closely related European cultivars were used for mapping, the level of detected polymorphism dropped to comparable rates described in peach (unpublished results). Therefore, the use of RAPD markers for mapping in families from crosses between closely related European cultivars would require extensive primer screening.

In contrast to the RAPD results, the yield of information achieved with AFLP markers was very high, demonstrating its utility for the mapping analysis of families in a test-cross configuration. With application of the AFLP technique on the apricot mapping population, we found an average of 132 fragments/reaction and ten polymorphic markers per primer combination. Similar ratios were reported in *Asparagus officinalis* by Spade et al. (1998). However, a lower rate of polymorphism was found in peach (Dirlewanger et al. 1998) and a greater rate was obtained in *Eucalyptus* (39%) (Marques et al. 1998) and in *Pinus tadea* L. (24.8%) by Remington et al. (1999). These results indicate that AFLPs are highly informative and are efficient markers for genome mapping in all species, even those with inherently low polymorphism. This technique is robust and reliable in apricot.

The proportion of distorted segregation-ratio fragments was 10% with RAPDs, which is higher than that reported by Verhaegen and Plomion (1996) and Byrne et al. (1995) in *Eucalyptus*, but lower than reported by Chaparro et al. (1994) in peach. On the other hand, a segregation ratio of distorted fragments of 13% was found from AFLP markers, which is lower than the one reported in *Eucalyptus* (15%) by Marques et al. (1998), but much lower than the one reported by Kuang et al. (1999) in *Pinus* (34%). In apricot we found a very low proportion of distorted markers heterozygous in one parent. Only three loci heterozygous in 'Goldrich' and four heterozygous in 'Valenciano' showed skewed segregation. The remaining distorted loci were heterozygous in both parents. By definition, we expect 5% of markers to deviate by chance alone, so there is no evidence of segregation distortion at all in the 1:1 markers. However, polymorphic bands that have distorted segregation-ratios may still be useful even though there is not an exact correspondence between expected and observed inheritance (Marques et al. 1998). Among co-dominant markers, only three SSRs showed skewed segregation, and clustered in linkage group 4.

Similar results were reported in an intraspecific cross of almond (Viruel et al. 1995); based on RFLPs, these authors reported 16% of loci showing skewed segregation that clustered in two linkage groups. However, in interspecific crosses of *Prunus* species, the proportion of markers displaying skewed segregation was much higher. For example, Joober et al. (1998) in an almond × peach cross, found a large proportion of loci (46%) that showed skewed segregation. Similar results were reported by Foolad et al. (1995), who reported 37% marker distortion and attributed this to an excess of heterozygotes. In the present apricot maps from an intraspecific cross a low proportion of distorted markers was found.

Heterozygosity

We have a 1.6-fold greater marker density in 'Goldrich' than 'Valenciano'. Our results agree with the origin of the cultivars. 'Goldrich' is a self-incompatible cultivar from the North American group, which according to Byrne and Littleton (1989) may have a hybrid origin between Asian and European apricots. On the other hand, 'Valenciano' is a self-compatible cultivar that belongs to the European group, the least diverse among the apricot groups. Additionally, a difference in heterozygosity level between these two cultivars was previously reported for allozymes (Badenes et al. 1996).

Sharka resistance trait

The genetic determinism of sharka resistance is not very well known and different genetic controls have been published. The procedure to determine the trait is lengthy. Every step of the grafting-inocu1ation-expression of the symptoms procedure implies a response of the plant; a failure in one of those steps results in no symptoms and cou1d lead to wrong conclusions about the trait. A standard procedure that allows us to compare the results between different laboratories has not been established. This may be the reason for the lack of agreement in the results already published.

Dosba et al. (1991) studied a family of 76 individuals from the cross of 'Screara', a French cultivar susceptible to PPV, by 'Stark Early Orange', which is resistant to the virus. They found a segregation of 3:1 susceptible/resistant, which did not fit monogenic control. The authors suggested a two-gene control of the trait. Dicenta et al. (2000) analyzed 291 seedlings from 20 crosses where the donor for resistance was 'Goldrich' or 'Stark Early Orange', resulting in a segregation of 1:1 susceptible/resistant, which fits the hypothesis of the monogenic control of the trait, the donor of resistance being heterozygous for the locus. However, Moustafa et al. (2001a), from crosses between resistant and susceptible cultivars, obtained similar results to those of Dosba et al. (1991). Guillet-Bellanger and Audergon (2001) reported a control of the resistance by at least three loci; however, the hypothesis was based on progenies with a low number of individuals and one of the families was obtained by selfing the 'Stark Early Orange'cultivar, which according to Burgos et al. (1997) is self-incompatible. Dicenta and Audergon (1998) evaluated seedlings from 'Stella' which resulted in 100% of resistant seedlings, indicating that the donor of resistance must be homozygous for the locus or loci involved. Recent results obtained from the self-pollination of 'Lito', a resistant cultivar from the cross 'Stark Early Orange' × Tyrintos, suggested that the genetic control relies in two dominant genes with epistatic effects (Badenes et al. 2001). Determination of sharka resistance involves steps of grafting, inoculation of plants, growth of plants in greenhouses, chilling treatments, and pruning to promote new shoots, etc. Every step should be accomplished successfully in order to follow the whole procedure. This is why many plants die, do not reach the optimum size, or bud-grafting fails. Our results suggest that it is necessary to screen at least six plants per genotype tested, and a second chilling treatment followed by the observation of symptoms in the new shoots is also necessary. The need for two chilling cycles might be explained because peach 'GF-305', although a good indicator of the virus, did not produce high titers; two cycles of chilling and breaking would produce the higher inoculation pressure needed to produce a pathogen–plant response. The correct determination of the trait is the key to establishing an hypothesis for the inheritance. Progenies with larger number of individuals and a wider range of progenitor combinations used as parentals are needed in order to determine the inheritance.

The 'Goldrich' cultivar has been used as a donor of sharka resistance in our breeding program; however, Polak et al. (1997) classified the cultivar as susceptible because, after grafting the apricot on an adult infected tree, some symtoms appeared in the leaves. We observed similar results when grafting 'Goldrich' and resistant seedlings onto an heavily infected apricot that was used as a source of inoculum. Once the new shoots grow the symptoms disapear, indicating that a minimum of leaves are needed for a plant-pathogen response to overcoming the virus multiplication (data not published). On the other hand, Fuchs et al. (1998) observed symptoms on leaves using chips as an inoculation method, which in out studies never happens. In another study carrried out under greenhouse conditions with five isolates Fuchs et al. (2001) detected PPV in some leaves, 24 weeks after double grafting, from cultivars that they classified as 'qualitatively resistant,' which included 'Goldrich' and 'Stark Early Orange'. These results did not contradict the classification of 'Goldrich' as a cultivar 'resistant' to PPV (Dosba et al. 1991; Karayiannis and Mainou 1994). Only under some stringent conditions has a limited multiplication of PPV been observed; never in field conditions and/or by natural spreading in those regions where the PPV is endemic.

Linkage map

Eight linkage groups were constructed with 132 loci heterozygous in 'Goldrich' and seven groups with 80 loci heterozygous in 'Valenciano'. For five linkage groups of both maps it was possible to establish homologues by the presence of anchor loci. These results suggest that the eight linkage groups found in 'Goldrich' correspond to the eight chromosomes of the haploid complement of *Prunus armeniaca*. More markers heterozygous in 'Valenciano' would be needed to obtain eight linkage groups. The distribution of markers along the linkage groups was quite uniform in the five major groups. Only those regions at the edge of some groups had a lower density of markers. This has also been reported for other species and marker systems (Foolad et al. 1995; Viruel et al. 1995; Marques et al. 1998; Boivin et al. 1999). The proportion of unlinked markers was similar to that reported by Lu et al. (1998) and lower than the one reported by Eujayl et al. (1998).

Estimates of total genome map size have been calculated for other *Prunus* species: almond 393 cM (Viruel et al. 1995), peach 396 cM (Chaparro et al. 1994) and peach × almond 491 cM (Joobeur et al. 1998). Independent construction of male and female maps for apricot produced a total map length of 511 cM for the female and 467 cM for the male, which agreed with previous maps constructed in other species of the genus.

Map comparison

Homologous linkage groups between both maps were recognized using markers heterozygous in both parents. Of the markers mapped, 36 revealed homologies between five linkage groups of 'Goldrich' and 'Valenciano'. There is a complete co-linearity between both maps in linkage groups 1 and 5. In linkage group 2, six markers showed complete co-linearity but two mapped on opposite sides of the linkage group, possibly due to a rearrangement of the region. Group 4 has 13 markers that established co-linearity between the maps. Eight out of them were co-dominant markers. In this linkage group, there was co-linearity in the order of all the markers except in the region delimited by the marker 98–410 to 98–406 of 'Goldrich' that mapped at the end of the group in 'Valenciano'. As with linkage group 2, this non-concurrence of marker position suggests a possible chromosomal rearrangement of this region. To establish homologies between the remaining groups more segregating markers are needed.

Co-dominant markers mapped in 'Goldrich' and 'Valenciano' as anchor loci can establish homologies with other *Prunus* maps. From these results, homologies between five 'major' linkage groups of the apricot maps and the map of an interspecific cross of almond by peach, 'Texas' × 'Earlygold' (Aranzana et al. 2001a), were established. The homologies based on the anchor loci mapped are indicated in the Fig. 3. Since more microsatellites are being mapped in the *Prunus* species families, these anchor loci will enable comparison of genome organization between apricot and other species in this genus, and a consensus linkage map could be established for the genus.

The construction of linkage maps covering the entire genome with markers distributed at short intervals, is required for some of the applications of molecular markers in plant breeding (Tanksley et al. 1989). Genetic linkage maps described in this study are the first ones reported for apricot. These maps provide basic information for geneticists and breeders. Resistance to sharka has been mapped in linkage group 2. The region containing sharka resistance is flanked by two co-dominant markers that will be used for targeted SSR development which will employ a recently constructed complete apricot BAC library (Badenes and Abbott, unpublished results). These SSRs, tightly linked to sharka resistance, will facilitate MAS in breeding for resistance in apricot. An additional task would be to test the AFLP markers obtained with BSA by Savala et al. (2001)

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