Theor Appl Genet (1995) 90:503–510

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Genetic linkage mapping in peach using morphological, RFLP and RAPD markers

Received: 8 March 1994 / Accepted: 28 June 1994

Abstract We have constructed a genetic linkage map of peach [*Prunus persica* (L.) Batsch] consisting of RFLP, RAPD and morphological markers, based on 71 F_2 individuals derived from the self-fertilization of four F_1 individuals of a cross between 'New Jersey Pillar' and KV 77119. This progeny, designated as the West Virginia (WV) family, segregates for genes controlling canopy shape, fruit flesh color, and flower petal color, size and number. The segregation of 65 markers, comprising 46 RFLP loci, 12 RAPD loci and seven morphological loci, was analyzed. Low-copy genomic and cDNA probes were used in the RFLP analysis. The current genetic map for the WV family contains 47 markers assigned to eight linkage groups covering 332 centiMorgans (cM) of the peach nuclear genome. The average distance between two adjacent markers is 8 cM. Linkage was detected between Pillar (*Pi*) and double flowers (*Dl*). RFLP markers linked to *Pi* and flesh color (*y*) loci were also found. Eighteen markers remain unassigned. The individuals analyzed for linkage were not a random sample of all F_2 trees, as an excess of pillar trees were chosen for analysis. Because of this, *Pi* and eight other markers that deviated significantly from the expected Mendelian ratios (e.g., 1:2:1 or 3:1) were not eliminated from the linkage analysis. Genomic clones that detect RFLPs in the WV family also detect significant levels of polymorphism among the 34 peach cultivars examined. Unique fingerprint patterns were created for all the cultivars using only six clones detecting nine RFLP fragments. This suggests that RFLP markers from the WV family have a high probability of being polymorphic in crosses generated with other peach cultivars, making them ideal for anchor loci. This possibility was examined by testing RFLP markers developed with the WV family in three other unrelated peach families. In each of these three peach families respectively 43%, 54% and 36% of RFLP loci detected in the WV family were also polymorphic. This finding supports the possibility that these RFLP markers may serve as anchor loci in many other peach crosses.

Key words *Prunus persica* · Restriction fragment length polymorphism · Random amplified polymorphic DNA · Linkage mapping · Cultivar identification

Communicated by G. E. Hart

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Introduction

The Rosaceae ranks third in plant families of economic importance in temperate regions. In addition to species of ornamental significance (e.g., rose, flowering cherry and quince), many important temperate fruit crop species (e.g., stone fruits, apple, pear and strawberry) are found within the Rosaceae. Of these, the stone fruits of the genus *Prunus* (e.g., peach and nectarine, almond, apricot, cherry and plum) represent a large and impor-

tant portion of this family. Although peach [*Prunus persica* (L.) Batsch] is considered the best genetically characterized species of the genus, the structure and organization of the nuclear genome of *Prunus* species are poorly understood. In peach breeding programs, to select for fruit-specific characters, trees must be maintained until fruiting begins, usually after 3–4 years (Sherman and Lyrene 1983). This process is expensive and time-consuming. The ability to select very early in plant development and to select at the gene level, rather than at the gene expression level, saves time and the expense of cultivating trees lacking the traits of interest. For these reasons, molecular markers linked to these traits have great utility for the identification of the plant genotype well before the traits are expressed.

Peach is a self-pollinated diploid (2n = 16) and has a small genome. Using flow cytometry, Baird et al. (1994) estimated the size of the peach genome to be approximately 0.59×10^9 bp or 0.61 pg/diploid nucleus. This is consistent with an earlier estimate contained in a survey of nuclear sizes in more than 100 species by Arumuganathan and Earle (1991) who reported a value of 0.55 pg/diploid peach nucleus. These studies indicate that the peach genome is only slightly larger than that of *Arabidopsis thaliana*, which has a genome size of 0.3 pg/diploid nuclei (Arumuganathan and Earle 1991). Both a low base chromosome number and a small genome size makes peach a suitable model to study genome organization, and to develop a genetic data base for map-based cloning of important genes in perennial tree species.

Commercial peaches depend on a narrow genetic base, as most cultivars grown in the U. S. have been derived from a handful of seedlings imported directly from China (Schery 1972). Approximately 25 morphological (Monet et al. 1985) and ten biochemical (Monet 1989; Mowrey et al. 1990) single-gene traits have been described in peach. Previously, we reported our initial results of RFLP analysis and its utility for linkage mapping in peach (Eldredge et al. 1992). This work demonstrated that even though the level of genetic variability was low, probably due to the narrow genetic base of peach germplasm, it is sufficient for RFLP mapping. We also found sufficient RAPD polymorphism in peach for genetic mapping. We have, therefore, used a combination of RFLP and RAPD markers to construct a genetic linkage map in peaches. Genetic linkage maps based on RAPD markers have been developed in peach (Chaparro et al. 1994) and sweet cherry (*Prunus avium*) (A. Iezzoni, personal communication). Additionally, Messeguer et al. (1994) are currently using RFLP markers to map almond (*Prunus amygdalus*).

Our long-term objectives are to develop a saturated genetic linkage map in peach that can serve as a permanent and practical resource for peach breeders, and to identify genetic markers linked to agronomically important traits, such as canopy shape, fruit flesh color and fruit quality, for use in marker-assisted selection programs.

Materials and methods

Peach progeny

We have studied 71 F_2 trees resulting from the self-fertilization of four F_1 individuals. These F_1s originated from a cross between a 'New Jersey Pillar' tree and a KV7719 tree. This progeny, designated as the West Virginia (WV) family, is maintained at the USDA's Appalachian Fruit Research Station, Kearneysville, West Virginia.

'New Jersey Pillar' is a pillar (*Pi*; also known as *Br*)-type tree while KV 77119 is a compact (*Ct*) tree heterozygous for the recessive brachytic dwarfing (*Dw*) gene (Scorza et al. 1989). Compared to the standard spreading type, the pillar form has a distinctive columnar growth habit, and, therefore, an increased potential for high-density production systems that can significantly increase the yield per unit area of orchard. Pillar appears to be controlled by an incompletely dominant gene, the heterozygote having an intermediate upright habit (Yamazaki et al. 1987; Scorza et al. 1989). In the brachytic dwarf (*Dw*), which has short internodes and large leaves, the homozygous condition is recessive to the standard type (Lammerts 1945). The densely branching compact (*Ct*) type is dominant to the standard type (Mehlenbacher and Scorza 1986). In addition to segregating for *Pi*, *Ct* and *Dw* characters, this progeny also segregates for the following morphological characters controlled by single genes: non-showy/showy flowers (*Sh*), colored/white flowers (*W*), pink/red flowers (*R*), dark pink/light flowers (*P*), single/double flowers (*Dl*), and white/yellow flesh color (γ). Non-showy flowers, which have small petals, are dominant over the showy type with large petals (Bailey and French 1942; Weinberger 1944; Lammerts 1945). Colored flowers are dominant over white, pink flowers are dominant over red, dark flowers are dominant over light pink, and single flowers consisting of one whirl of petals are dominant overt double (Lammerts 1945). White fruit flesh is dominant over yellow (Connors 1920a,b).

DNA isolation and library construction

Genomic DNA was isolated from leaf tissue using a CTAB method (Eldredge et al. 1992). Probes for RFLP analyses were of two types: (1) randomly selected genomic clones and (2) fruit-specific cDNA clones. Genomic and cDNA libraries were prepared as previously described (Eldredge et al. 1992). The cDNA libraries were screened by differential hybridization; the nine isolated clones represented genes whose transcript accumulation was regulated during fruit development. These clones were converted to pBluescript plasmids following the manufacturer's instructions (Stratagene, La Jolla, Calif.)

Detection of RFLPs

Genomic clones were pre-screened to determine sequence type as previously described (Eldredge et al. 1992). Only clones judged to carry low-copy sequences were further analyzed as potential RFLP probes. Genomic DNA from individual trees (parents, F_1s and F_2s) was digested with *Hind* III, and samples of each were electrophoresed on a 0.8% agarose gel for approximately 16h at 45V. Phage lambda DNA digested with *Hind* III was included as a molecular-size standard. Gels were treated and blotted onto nylon membranes using the manufacturer's instructions. Membranes were hybridized with either pre-screened genomic DNA or cDNA probes. To prepare the probes, recombinant plasmids were purified and digested to release the inserts, wich were then isolated by electroelution from agarose gels (Maniatis et al. 1982). Genomic probes were labeled by priming with random hexamers to incorporate the radioactive signal (Feinberg and Vogelstein 1983). Isolated cDNA inserts were random primed using a kit from BRL (Gaithersburg M.). Filters were hybridized and, following autoradiography, were stripped of the radioactive probe for re-use (Eldredge et al. 1992).

RAPD analysis

DNA concentrations in samples were measured using a mini fluorometer (Hoefer, San Francisco, Calif.) and working solutions of 10 ng/μl were prepared. DNA amplifications were done in a volume of 25 μl containing 15 ng of tempelate DNA, 0.2 μM of 10-mer primer (Operon Technologies, Almeda Calif.), 0.75 U of *Taq* DNA polymerase (Perkin-Elmer/Cetus, Norwalk, Conn.), 5 mM of $MgCl_2$ and 200 μM of dNTP (Perkin-Elmer/Cetus) in reaction buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl]. Amplification was carried out in a thermal cycler (Perkin-Elmer/Cetus 480) for 45 cycles of 1 min at 94 °C, 1 min at 36 °C, and 2 min at 72 °C. Amplification products were separated by electrophoresis in 1.2% agarose gels and visualized by ethidium bromide staining.

Data analysis

Linkage was analyzed using MAPMAKER/EXP version 3.0 (Lincoln et al. 1992). To combine RFLP and RAPD data for linkage analysis and to combine the coupling- and repulsion-phase markers, the following genotype symbols were employed to identify the allele combinations at one locus (Lincoln et al. 1992). Homozygous for allele *A* originating from the Pillar parent, A; homozygous for allele *a* originating from the KV77119 parent, B; heterozygous for alleles *A* and *a*, H; not homozygous for allele *A* (either *Aa* or *aa*), C; not homozygous for allele *a* (either *Aa* or *AA*), D. Co-dominant markers were scored A, H or B depending on the origin of the bands. Dominant markers were scored D for presence and B for absence when the marker originated from the Pillar parent, and C for presence and A for absence when the marker originated from the KV 77119 parent.

The "group" command of MAPMAKER was used to identify the linkage groups at a minimum LOD (logarithm of odds) score of 3.0 and recombination fraction of 0.3. To determine the most likely order of markers within a linkage group, the "compare" command was used with up to six markers at a time. The best order determined by maximum likelihood was selected. When finding the best order for a linkage group which has more than six markers, subsets of six markers were analyzed separately and those subsets were put together in the order that gave the lowest total recombination value. The position of morphological markers on linkage groups was verified using "try" command. "Map" command was then used to obtain the distances between markers.

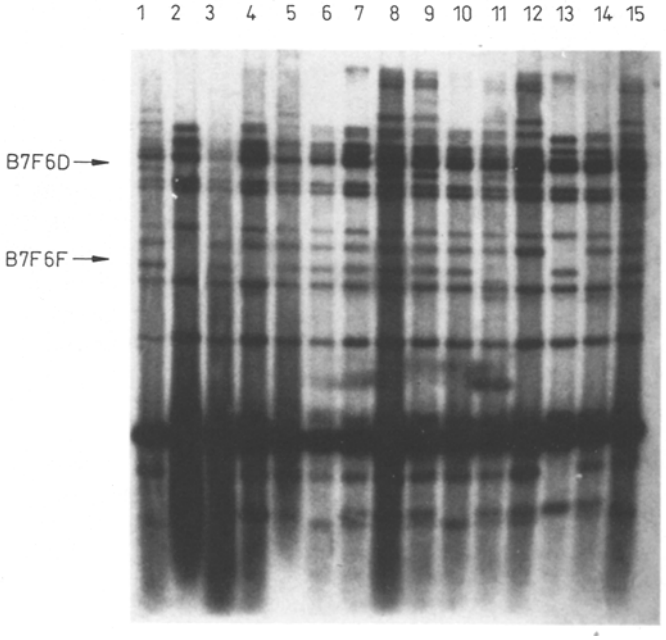
Results and discussion

RFLP markers

We initiated our mapping program using RFLP markers. More than 150 low-copy genomic peach clones and nine cDNA clones were evaluated in the WV family. Forty-seven RFLP markers were obtained from 28 genomic clones, while two RFLP markers were obtained from cDNA clones. One cDNA clone was not included in the segregation analysis as clear scorings were not obtained. RFLP markers were scored as dominant when more than two segregating bands were observed for a single probe, since allelic relationships were too complex to be analyzed in these situations. Examples of such markers are illustrated in Fig. 1 where several polymorphic bands were observed with genomic clone B7F6. About 50% of RFLP loci are scored as co-dominant markers because of the simple band patterns observed in the autoradiograms, as shown in Fig. 2. Co-segregating polymorphic bands obtained from two linked loci are presented in Fig. 2.

Many genomic clones that identify RFLPs in the WV family also detected polymorphism among a large number of peach cultivars, making them useful for the identification of peach cultivars. Unique fingerprint patterns were created for 34 cultivars using only six genomic clones which detected nine RFLP fragments representing eight loci (Table 1). Cultivars were scored for the presence (1) or absence (0) of particular fragments using RFLP data with *Hin*d III-digested DNA. All but B2F12A and B3C2 clones were polymorphic in the WV family. The last four cultivars of Table 1 were not a part of the cultivar identification study, but were included since they are the parents of two additional progeny screened with genomic clones from the same library. Two of these four cultivars, 'Jalhousia' and 'White Glory', also had unique fingerprints. As calculated in the last three rows of Table 1, a minimum of 21% of cultivars (for B3C2) to a maximum of 50% of cultivars (for B6D1) exhibited one allelic form of particular loci. This illustrates the significant level of polymorphism for these loci among a large number of peach cultivars and suggests that RFLP markers from the WV family have a high probability of being polymorphic in crosses generated from other peach cultivars, making them ideal for anchor loci. This possibility was tested by searching for polymorphism in three other unrelated crosses between the cultivars: (1) 'Jalhousia' × 'Summergrand', (2) 'Marsun' × 'White Glogy', and (3) 'Bailey' × 'Sun Crest'. The results of this analysis are summarized in Table 2. In the three peach families, respectively 43%, 54% and 36% of RFLP loci detected in the WV family were also poly-

Fig. 1 A complex RFLP profile detected by hybridizing genomic clone B7F6 with genomic DNA digested with *Hind* III. *Lanes 1* and *2*, parents 'New Jersey Pillar' and KV 77119, respectively; *lane 3*, F_1 offspring; *lanes 4–15*, F_2 offspring. Two of many polymorphic bands are indicated with *arrows*



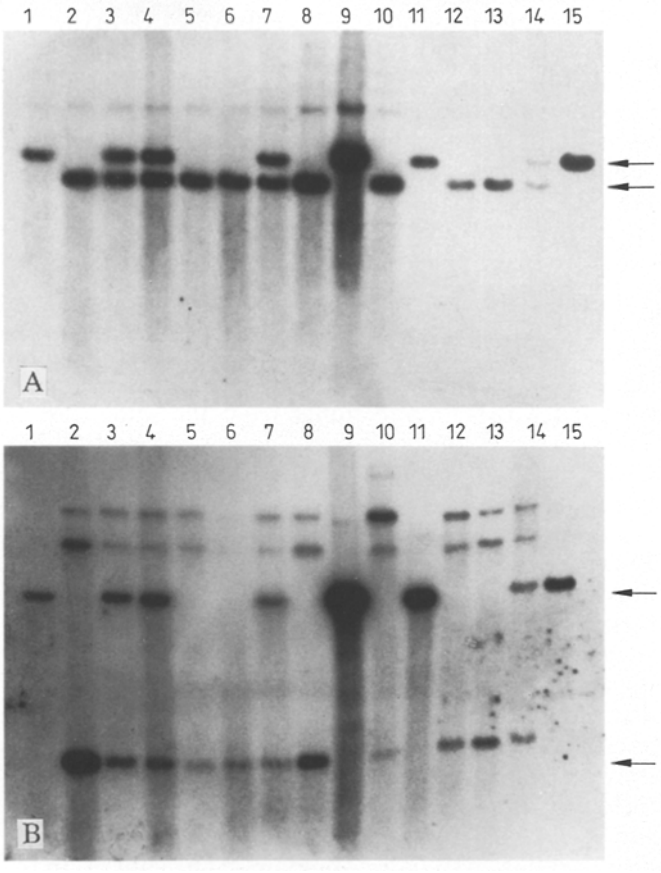


Fig. 2A,B Southern hybridization patterns of two RFLP probes of peach detected by autoradiography. Genomic DNA was digested with *Hind* III and probed with low-copy genomic probe B7A5 (**A**) and B4A9 (**B**). *Lanes 1* and *2*, parents 'New Jersey Pillar' and KV 77119, respectively; *lane 3*, F_1 offspring; *lanes 4–15*, F_2 offspring. Note that for corresponding individuals in **A** and **B**, the fragments detected by the arrows in two probes co-segregate. The autoradiogram reveals that DNA in *lane 10* is incompletely digested

morphic. This finding supports the possibility that these RFLP markers may serve as anchor loci in many other peach crosses.

RAPD markers

Approximately 50% of the primers screened in the WV family amplified one or more polymorphic fragments. The 22 primers produced a total of 25 RAPDs, and most of the polymorphic fragments were between 0.5 and 3.0 kb in size. The level of RAPD polymorphism in peaches is greater than the level of RFLP polymorphism. Examples of two RAPD markers obtained with primer OPC-06 are illustrated in Fig. 3.

Using primer OPC-04, a RAPD band that was not present in either parent was observed in all four F_1s. This non-parental RAPD band appears to be segregating in all of the F_2 progenies. The occurrence of this type of aberrant RAPD band seems to be widespread as it has been observed in peach (M. Pooler, personal communication) and in other species, e.g., tobacco bud worm (D. Heckel, personal communication), honey bee (Hunt and Page 1992) and baboon (Riedy et al. 1992). This RAPD marker, C4A, was not used in the linkage analysis.

Linkage analysis

Segregation of markers in 71 F_2 plants derived from four F_1s were analyzed. All four F_1s were produced from the same two parent trees. Prior to combining data from the four F_1 progenies, the following tests were carried out to ensure that no violation of mapping assumptions was made..

First, only those markers known to be in the same phase (i.e., parental type or recombination) in all four F_1s were selected and used in the linkage analysis. One of the assumptions in the MAPMAKER program is that all the data have been derived from a single F_1 individual. Since we analyzed four different F_1 families, all the markers were carefully scrutinized to be certain that F_2 marker data from F_1s that are of parental and recombinant types were not mismatched. When comparing the linkage of two loci (e.g., A and B), data from different F_1 progenies cannot be combined when both parents are heterozygous for both loci (e.g., AaBb × AaBb) or when one parent is heterozygous for both loci and the other parent is heterozygous for one locus and homozygous for the other (e.g., AaBb × AaBB). In these two situations, parental types and recombinant types could not be identified effectively. Although peach is largely a self-pollinating crop, we have observed that parent peach trees are not homozygous for all loci. Therefore, we eliminated all markers (two RFLP, 11 RAPD, and one morphological) in which both parents are heterozygous.

Second, before combining the segregation data of the four F_1 families for any marker, homogeneity of the segegation ratios for the four families was tested by chi-square analysis. This test is a measure of the likeness or unlikeness of the different samples or progenies. It is independent of the actual segregation ratio (i.e., 3:1 or 1:2:1) in that the individual progenies can depart from this ratio without increasing heterogeneity χ^2, provided that they depart in the same direction and approximately to the same extent. The segregation data were combined if no significant deviation from homogeneity was detected. The four F_2 families differed in segregation ratios for seven dominant markers. The deviation from homogeneity in six out of these seven markers was due to a single F_2 progeny. When marker data from the suspected F_2 progeny was removed and the remaining data re-tested, chi-square values were not significant. Also, on RAPD marker (C6B), in which two F_2 progenies segregated differently from the other two F_2 progenies, was not included in the linkage analysis.

Third, data from four F_2 progenies were analyzed separately for linkage and map order to determine if there is any significant difference in linkage and order when the map is generated with data combined from the

Table 1 Peach cultivar fingerprinting by RFLP analysis. Refer to text for description

Cultivar	B2A10		B2F12A	B2G2	B3C2		B4A9	B6D1	
	5.7kb	4.3kb	8.6kb	1.9kb	4.0kb	3.3kb	1.6kb	3.9kb	1.7kb
Cresthaven	1	1	0	0	1	1	0	0	1
Babygold 5	1	1	0	1	1	0	1	0	0
Babygold 6	1	1	0	1	0	1	1	1	1
Babygold 7	0	1	0	1	0	1	1	1	0
Babygold 8	1	1	0	1	0	1	1	1	0
Babygold 9	0	1	0	1	1	0	1	1	1
Rangger	1	0	0	0	0	1	0	0	0
Redglobe	0	1	1	0	1	1	0	0	1
KV 77119	1	1	0	0	0	1	0	0	1
New Jersy Pillar	0	1	0	1	0	1	1	1	1
Carogem	1	1	0	0	1	1	0	0	0
Bicentennial	1	0	1	1	1	1	0	0	0
Biscoe	1	1	0	1	0	1	0	0	0
G. Belle	1	1	0	1	1	1	0	0	1
Majestic	0	1	0	0	1	1	0	0	0
ROG	0	1	0	1	1	1	1	1	1
Glohaven	1	1	0	1	0	1	0	0	1
Jayhaven	1	0	0	0	0	1	1	1	0
Sweethaven	1	0	0	0	0	1	1	0	1
Springcrest	1	1	0	1	1	1	1	1	1
Camden	0	1	0	0	1	0	0	1	1
Chinese Blood	1	0	1	1	1	0	0	0	0
Fireprince	0	1	1	1	1	1	1	1	0
Newhaven	1	0	0	1	0	1	0	0	1
Ouachita G.	1	1	1	1	1	0	0	0	1
Polly	1	1	1	0	0	1	1	1	1
Prairie D	1	1	0	0	0	1	0	1	1
Sentry	1	0	0	1	0	1	0	1	1
Sparton Cling	1	1	1	1	0	1	1	0	1
Suncrest	0	1	0	0	1	0	1	1	0
Topaz	1	0	0	0	0	1	0	0	1
Chinese Cling	1	0	1	1	1	0	0	0	1
Champion	1	1	1	0	1	0	1	1	0
Garnet Beauty	1	1	0	1	0	1	0	1	1
Jalhousia	1	0	0	1	1	1	–[a]	1	0
Summergrand	1	1	0	1	0	1	–	0	1
Marsun	0	1	0	1	0	1	1	1	1
White Glory	0	1	1	1	0	1	1	1	1
% Cultivars exhibiting fragment		29[b]	26	63	47	79	47	50	63
% Cultivars not exhibiting fragment or having alternate allele		26	74	37	53	21	53	50	37
% Cultivars exhibiting both alleles		45	na	na	na	na	na	na	na

[a] Not screened
[b] Two fragments in B2A10 represent a single locus which was scored as codominant for mapping

Table 2 Number of RFLP loci in the WV family detecting polymorphism in three other peach families

Family	Total number of loci examined out of 46 RFLP loci in the WV family	Number of polymorphic loci	Percentage of polymorphic loci
'Jalhousia' × 'Summergrand'	37	16	43%
'Marsun' × 'White Glory'	26	14	54%
'Bailey' × 'Sun Crest'	45	16	36%

four progenies. Linkages and map orders between individual F_2 progeny maps and the combined data agree for all markers.

A total of 65 markers, consisting of 46 RFLP loci, 12 RAPD loci and seven morphological loci, were scored. Out of these, 47 markers (72%) were placed in eight linkage groups covering 332 centiMorgans (cM) of the peach nuclear genome (Fig. 4), and 18 markers remain unlinked. In linkage group 1, three markers, B4C9U, B7F6B and B8F10, which are linked by the two-point

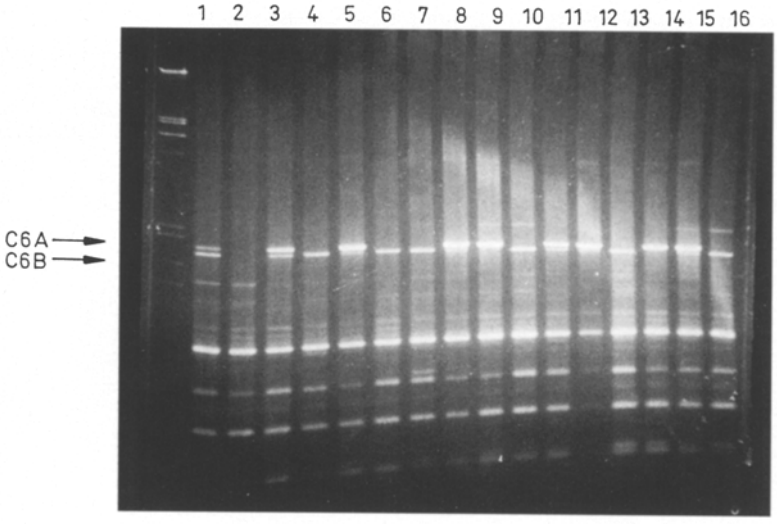


Fig. 3 RAPD analysis of peach. Peach DNA was amplified using primer OPC-06. *Lanes 1* and *2*, parents 'Pillar' and KV 77119, respectively; *lane 3*, F_1 offspring; *lanes 4–16*, F_2 offspring. Polymorphic bands are indicated by *arrows*. Molecular-weight marker on the left is Phage lambda DNA digested with *Hin*dIII and *Eco*RI

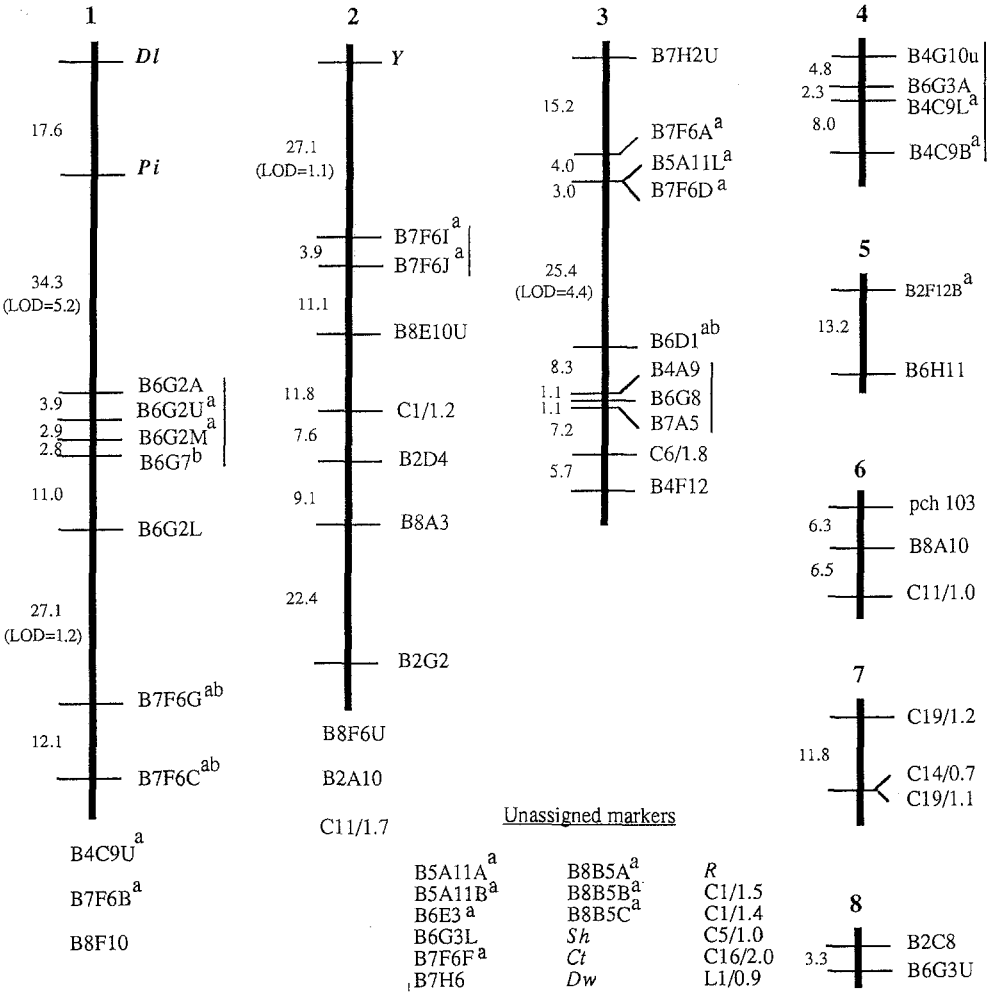


Fig. 4 Current genetic linkage map for peach. Linkage group numbers are indicated on top. Map distances are indicated at the left side of each interval between two markers. Six markers in groups one and two did not order well. These markers are, therefore, listed below their respective linkage groups. Adjacent markers with LOD scores less than 3.0 are identified on the map. LOD scores between markers with distances more than 25 cM are also indicated on the map. All RFLP markers on the map start with the letter *B* and are identified by the clone numbers (e.g., B7F6) that have given rise to them. When more than one marker is generated from a single clone, different letters are used at the end of clone identification (e.g., B7F6A and B7F6B). RAPD markers begin with the letter *C* or *L*. Marker from cDNA clone is indicated as *pch*. Morphological markers are in *italics*. RFLP markers scored as dominant markers are indicated by an "*a*". Markers identified with "*b*" are placed temporarily. Group of markers with a *vertical bar* to the right of the marker name are ordered tentatively

analysis to the other markers in this group, did not order well. Similarly, in linkage group 2, B8F6U, B2A10 and C11/1.7 markers, which are linked by the two-point analysis to the other markers in this group, did not order well. These markers are, therefore, listed below their respective linkage groups. In linkage groups with more than six markers, a few markers were located in different places in the linkage group when different subsets of markers were compared for the best order. These are identified by a "b" on the map (Fig. 4). For most of the

orders obtained the LOD score was greater than 3.0. However, in two places on the map LOD scores for adjacent markers were less than 2.0 but more than 1.0. A low LOD score was often a result of adjacent markers not being informative in all four F_1 progenies, hence the number of individuals informative for both loci are low. All markers that have linkages with LOD scores less than 3.0 with adjacent markers have linkages with LOD scores over 3.0 to a non-adjacent markers. Clear recombinants were observed between several loci detected with a single genomic clone that were mapped very close to each other (e.g., three B6G2 loci linkage group 1); therefore, these markers do not reflect multiple alleles sharing similar mobility (Fig. 4).

In four places on the map, indicated with a vertical bar to the right of the marker name, the most likely order determined by the compare command is only marginally more likely than the second most likely order (Fig. 4). For example, for three closely mapped markers, B4A9, B6G8 and B7A5, in linkage group three the most likely order given in the map is only log 1.10- or 12.6-fold more likely than the next best order. Therefore, the orders are given tentatively for these markers.

The average distance between adjacent markers was 8 cM; only one interval was larger than 30 cM. Among linkage groups, the average distance between two adjacent markers varied from 3.3 cM in linkage group 8 to 12.4 cM in linkage group1. In a survey of genetic maps in 12 plant species, the average distance between two markers varied from 2.0 cM in *Arabidopsis thaliana* and tomato to 14.0 cM in *Brassica napus* (Nodari et al. 1993). In the peach map presented here, three of the linkage groups have ten or more markers, while the other five groups consist of two or three markers each.

Eighteen markers (27% of total) consisting of nine RFLP, five RAPD and four morphological loci remain unassigned. This proportion is higher than that reported for potato (10%) and *B. napus* (13%) (Nodari et al. 1993) and may reflect the low number of scored markers on our map. We expect this proportion to decrease as the number of mapped markers increases. The significant difference in the proportion of unassigned markers among RFLP (20% of total RFLP markers), RAPD (42%) and morphological markers (57%) could be due to either random chance or the low number of mapped markers.

Linkage was detected in this study between two morphological characters, Pillar (*Pi*) and double flowers (*Dl*), and this linkage was also observed in another peach mapping project (Chaparro et al. 1994). Four and two RFLP markers were found linked to *Pi* and flesh color (γ) loci, respectively. Although these molecular markers are linked to the above loci, their potential use as molecular tags is limited by their relatively large genetic distance from the loci.

The most likely map order obtained using the "compare" command of MAPMAKER placed all three morphological markers, γ, *Pi* and *Dl*, at the terminals of linkage groups. The map positions of these markers were also verified using the "try" command. For each of the three morphological markers, strong evidence (for γ, log 2.7-fold more likelihood; for *Pi*, log 4.7; for *Dl*, log 4.4) supports the position indicated on the map compared to the second best position in the linkage group. Placement of all three morphological marker to ends of linkage groups and their loose linkage associations with adjacent molecular markers are two interesting features of this map. *Pi* and *Dl* loci were also mapped to the ends of linkage groups in the peach genetic map developed by Chaparro et al. (1994). The 71 F_2 individuals used in our study do not represent the entire F_2 population obtained from selfing the four F_1s of the cross between 'New Jersey Pillar' and KV 77119 since a greater number of pillar trees was chosen. Therefore, although chi-square tests were conducted to find the goodness of fit for the 3:1 and 1:2:1 segregation ratios, those markers that deviated from test ratios were not removed from linkage analysis since they could be linked to *Pi*. One such marker, *Dl*, which deviated significantly from the test ratio was indeed found to be linked to *Pi*. Only 9 markers out of 65 deviated significantly from the test ratio at the 0.05 level of significance.

The genetic length of the peach nuclear genome covered by this map is approximately 332 cM. The total recombinational length of the peach genome is not known. However, we can estimate it using the parameters outlined by Meagher et al. (1988) who concluded that the number of map units per chromosome would range from 50–300 cM regardless of the physical size of the chromosome. This rule holds true for plant species as diverse as tomato (1 600 cM, $n = 12$, Paterson et al. 1988), rice (1 600 cM, $n = 12$, McCouch et al. 1988), soybean (2000 cM, $n = 20$, Keim et al. 1990), *Arabidopsis* (630 cM, $n = 5$, Reiter et al. 1992), and maize (1 800 cM, $n = 10$, Coe et al. 1990), where 100–150 cM per linkage groups is a consistent estimate. Applying these values to peach, with $n = 8$, we would estimate the size of the peach genome to be between 800 and 1 200 cM. Therefore, our present linkage map may cover less than half of the total nuclear genome, with an average spacing of less than 10 cM.

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