Hereditas 128: 87–90 (1998)

Brief report
Genetic linkage of isozyme loci in *Annona cherimola*

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(Received May 26, 1997. Accepted December 10, 1997)

Cherimoya tree (*Annona cherimola* Mill) is a small fruit tree that bears a commercially valuable fruit. A native tree of Ecuador and Peru, it is now cultivated in several areas of the world, including California, Chile, Ecuador, Israel, Peru, and Spain. Several aspects of the crop, including cold tolerance, fruit quality, resistance to insects of the genus *Ceratitis*, and the number of seeds per fruit, are open for improvement. Tight linkage between loci coding for these important traits and genetic markers could accelerate the breeding in this plant, because marker-assisted selection may allow for earlier selection and reduce plant population size during breeding (STAUB et al. 1996). Linkage analysis of isozymes could be the first step in developing a genetic map, which will aid future breeding by identifying potential markers for commercial traits. In addition, the information about linkage groups could be useful in population genetic studies of species of this primitive angiosperm family.

Only one linkage study has been reported for this crop (LEE and ELLSTRAND 1987). This initial map was made up of only two linkage groups involving nine isozyme loci, although the cherimoya karyotype consists of seven chromosomes (WALKER 1972).

The purpose of this paper is to report the linkage relationships among 13 isozyme loci in *Annona cherimola*.

MATERIALS AND METHODS

Seeds were produced by hand self-fertilization of 13 cultivars of cherimoya (*Annona cherimola*). The self-fertilizations were carried out in one tree for each cultivar. Genotypes of these cultivars, extraction, electrophoresis, and staining procedures have been described elsewhere (PERFECTTI and PASCUAL 1996).

For nomenclature of loci, the criteria of ELLSTRAND and LEE (1987) and PASCUAL et al. (1993) were followed, i.e., the loci were named according to the mobility of their electromorphs, with numbers reflecting their relative advance in the electrophoretic gel (the slower the mobility, the smaller the number). A similar system was used for alleles. There is some ambiguity in the loci nomenclature used in cherimoya. LEE and ELLSTRAND (1987) designated three *Tpi* loci (*Tpi-1*, *Tpi-z* and *Tpi-2*). We use *Tpi-2* to name the former *Tpi-z* and *Tpi-3* for the former *Tpi-2* according to their mobility (PASCUAL et al. 1993). In addition LEE and ELLSTRAND (1987) studied the linkage of *Idh-1*, but since *Idh-1* is monomorphic in cherimoya (ELLSTRAND and LEE 1987), they are probably referring to *Idh-2*.

For each locus under study, the observed segregations were tested against the expected Mendelian ratio (1:2:1) for the progeny of a heterozygous individual by means of χ^2 tests for goodness-of-fit. After PHAM et al. (1990), progenies showing strong one-locus distortions ($p < 0.01$) were rejected for linkage calculations.

The linkage phases of the parents were unknown, and were inferred by examining the frequencies of the four double-homozygote phenotypic classes. We grouped different crosses when parentals had similar heterozygous genotypes at the two loci, or when parentals were different in one allele at these loci. The data were corrected for linkage phase differences when necessary. Pooled and heterogeneity statistics were calculated for progeny segregating at each pair of loci using the computer program RXC, provided by Dr. G. Carmody (Carleston University, Canada). Data from different progenies, segregating for the two markers, were pooled if homogeneous. Linkages were tested using both pooled data and data from individual cultivars. Distances were calculated from the pooled data or, in cases of heterogeneity among cultivars, as weighted arithmetic means.

Contingency χ^2 tests were used to detect linkage. The computer program LINKAGE-1 (SUITER et al. 1983) was used to calculate single gene segregation tests, contingency χ^2, the recombination fraction (r) and its standard error. To reduce type I errors (i.e., to accept as linked two loci, when they are not linked) two loci were considered linked only if the contingency χ^2 test was significant with $P < 0.01$. The order of the markers was established based on the r values showed among pairs of loci.

RESULTS AND DISCUSSION

We tested 64 of the 78 possible pairwise combinations of loci (Table 1). Several loci showed large deviations ($p < 0.01$) from Mendelian proportions in some pro-

Table 1. *Number of seeds and cultivars tested for each pair of loci (upper right half) and results of linkage analysis (lower left half). The number of progenies that showed strong non-Mendelian segregation in at least one locus is showed between parentheses*

	Adh-1	*Got-1*	*Got-2*	*Idh-2*	*Mdh-1*	*Me-1*	*Pgi-1*	*Pgm-1*	*Pgm-2*	*Skd-1*	*Tpi-1*	*Tpi-2*	*Tpi-3*
Adh-1		328 5(1)	161 2	327 4(1)	258 4(1)	286 3(1)	216 3	144 2(1)	207 4(2)	99 2	135 2	261 4(2)	91 2
Got-1	ns		151 3(1)	169 3(1)	119 3(1)	19 2(1)	173 2	120 2(1)	202 5(2)	94 2	130 2	71 2	91 2
Got-2	ns	ns		105 1	113 2	115 2	67 1	–	77 2	–	–	53 1	–
Idh-2	ns	ns	ns		296 4	370 4	94 2	70 1(1)	32 1(1)	30 1	104 2	214 3	54 1
Mdh-1	**	ns	**	ns		343 5(1)	74 2	45 1	99 3(2)	31 1	41 1	111 2(1)	–
Me-1	ns	ns	ns	**	ns		24 1	–	20 1	–	–	163 2(1)	–
Pgi-1	ns	**	ns	ns	ns	ns		146 2(1)	115 2	–	105 1	–	54 1
Pgm-1	ns	ns		?	ns		ns		160 3	–	101 1(1)	–	54 1(1)
Pgm-2	ns	**	ns	?	ns	ns	**	ns		70 2(2)	33 1(1)	32 1(1)	38 1(1)
Skd-1	**	ns		ns	ns						30 1	31 1	38 1
Tpi-1	**	ns		ns	ns		ns	?	?	ns		33 1	54 1
Tpi-2	ns	**	ns	**	ns	ns			?	ns	ns		–
Tpi-3	ns	ns		ns			ns	?	?	ns	ns		

ns = not significant
** = significant ($p < 0.01$) in at least one cultivar or at pooled data
– = Not tested
? = Not studied due to strong segregation distortion

genies (PERFECTTI and PASCUAL 1996). These progenies were not used to study linkage, as indicated in Table 1. Linkage to deleterious factors which prevent the development of some gametes, seems to explain these non-Mendelian segregations in cherimoya (PERFECTTI and PASCUAL 1996). These factors reduced the number of gene pairs tested for linkage to 56. Among these gene pairs, we found deviation from independence in ten gene pairs (Table 1 and Table 2).

LEE and ELLSTRAND (1987) made a primary analysis of linkages in the cherimoya, finding two linkage groups: the first was composed of *Got-2*, *Mdh-1*, *Adh-1*, *Tpi-1*, and *Aco-2*, the second was composed of *Tpi-3* (they named it *Tpi-2*), *Pgi-1*, *Got-1*, and *Aco-1*. We also found those two and another new linkage group (Fig. 1). Furthermore, the *Skd-1*, *Tpi-3*, and *Pgm-1* loci did not appear to be linked to any of the other markers.

The first linkage group (Fig. 1) corresponds well with that of LEE and ELLSTRAND (1987). The order of the markers was the same, with some different distances between loci. LEE and ELLSTRAND (1987) showed *Mdh-1* and *Adh-1* with $r = 0.27 \pm 0.04$, whereas the present data shows $r = 0.39 \pm 0.03$. *Got-2* and *Mdh-1* were described by LEE and ELLSTRAND (1987) with $r = 0.03 \pm 0.01$ and $r = 0.13 \pm 0.03$ by us. These differences in r values could be due to differing recombination frequencies in the different cultivars used.

In the second linkage group, *Got-1* and *Pgi-1* appeared to be linked, with $r = 0.295$, similar to LEE and ELLSTRAND's (1987) data. In addition, we found linkage between *Got-1* and *Pgm-2* ($r = 0.281$), and between *Pgi-1* and *Pgm-2* ($r = 0.216$). These three loci are clearly linked. However, our results do not confirm the order *Tpi-3–Pgi-1–Got-1* presented by LEE and ELLSTRAND (1987), who found $r = 0.30$ between *Pgi-1* and *Tpi-3*, with $P = 0.03$, after analyzing 30 seeds of the cultivar White. In the present work, 54 seeds of the same cultivar (White) were studied for these loci, but we did not find linkage ($P = 0.417$).

The third linkage group (Fig. 1) is composed of loci *Me-1*, *Idh-2*, and *Tpi-2*, separated by 35.2 and 42.1 cM, respectively. Neither *Me-1* locus nor the relationship between *Tpi-2* and *Idh-2* was studied by LEE and ELLSTRAND (1987). High values of r could question these linkages. However, the low P-values obtained with a high number of seeds studied (370

Table 2. *Linkage test for pairs of loci showing linkage in individual progenies or after pooling. Recombination values are shown for pooled data and for individual progenies when heterogeneous*

Loci	cv	n	Observed genotypic frequencies									χ^2	P	r ± SE
			S/S	S/H	S/F	H/S	H/H	H/F	F/S	F/H	F/F			
Adh-1–Mdh-1	BO	50	5	8	2	4	13	4	1	9	4	3.524	0.474	
	PC	32	3	2	1	5	8	2	2	6	3	2.384	0.666	
	PE	119	2	22	10	20	31	7	9	15	3	12.442	0.014	
		pooled (χ^2h = 12.303, P = 0.749)										13.581	0.009	0.387 ± 0.033
Adh-1–Skd-1	SE	70	5	10	5	8	14	9	5	12	2	2.581	0.630	
	PC	29	4	0	1	1	7	6	1	3	6	14.268	0.006	
		pooled (χ^2h = 12.844, P = 0.115)										5.208	0.267	
Adh-1–Tpi-1	PC	32	4	2	0	6	8	1	3	3	5	9.120	0.058	
	WH	103	27	8	0	5	39	2	0	3	19	119.424	<0.001	0.091 ± 0.021
Got-1–Pgi-1	MA	69	14	6	2	7	22	8	0	5	5	20.917	<0.001	0.249 ± 0.044
	WH	104	10	10	2	16	25	7	3	15	16	19.005	0.001	0.324 ± 0.042
Got-1–Pgm-2	B3	20	0	3	1	2	6	1	4	0	3	9.577	0.048	
	MA	69	5	5	12	7	28	2	7	1	2	33.660	<0.001	
		pooled (χ^2h = 8.838, P = 0.367)										37.870	<0.001	0.281 ± 0.041
	SA	19	3	3	2	5	3	0	1	1	1	2.965	0.563	
Got1–Tpi-2	PC	25	4	1	4	2	9	0	3	2	0	14.299	0.007	
	PE	46	2	2	2	6	13	7	8	3	3	5.242	0.263	
		pooled (χ^2h = 11.677, P = 0.171)										12.936	0.012	
Got-2–Mdh-1	PE	103	1	8	14	6	45	2	21	6	0	88.973	<0.001	
	SA	10	0	2	1	0	3	0	3	1	0	8.472	0.076	
		pooled (χ^2h = 3.558, P = 0.744)										96.170	<0.001	0.129 ± 0.024
Idh-2–Me-1	C3te	29	4	3	0	1	6	5	2	3	5	8.296	0.081	
	CU	105	16	6	5	13	29	3	5	16	12	24.233	<0.001	
	SP78	107	11	11	6	10	26	16	4	13	10	5.870	0.209	
	PE	129	4	11	14	20	26	18	20	12	4	16.791	0.002	
		pooled (χ^2h = 24.752, P = 0.462)										41.603	<0.001	0.353 ± 0.023
Idh-2–Tpi-2	PC	31	1	5	1	5	5	4	4	4	2	2.809	0.590	
	SP78	107	10	13	5	13	26	13	4	9	14	9.629	0.047	
	PE	76	6	9	2	10	14	15	11	5	4	8.800	0.066	
		(pooled (χ^2h = 19.287, P = 0.248)										13.631	0.009	0.421 ± 0.033
Pgi-1–Pgm-2	BO	45	7	2	0	8	9	3	1	4	11	21.726	<0.001	
	MA	70	2	7	12	7	24	3	11	3	1	36.312	<0.001	
		pooled (χ^2h = 4.968, P = 0.819)										53.660	<0.001	0.216 ± 0.031

cv = cultivar
n = number of seeds
χ^2 = linkage test
P = probability
χ^2h = homogeneity test
r = frequency of recombination
SE = standard error

for *Idh-2:Me-1* and 214 for *Idh-2:Tpi-2*) support the hypothesis of linked loci (Table 2).

We found high variability in the frequency of recombination among different cultivars, and between cultivars and the pooled data. Differences in the frequency of recombination among progenies have been reported in previous studies. For example, PHAM et al. (1990) found contradictory results, either linkage or independence, depending upon the progenies studied in different crosses in rice. The same results have also been reported in other crops (KRUEGER and KNAPP 1990; VAILLANCOURT and SLINKARD 1993; EICKMEYER et al. 1990) and in numerous coniferous species (LEWANDOWSKI and MEJNARTOWICZ 1991; SZMIDT and MUONA 1989). SAYLOR and SMITH (1966) suggested that these variations in linkage may be due to meiotic irregularities, whereas ANDERSON et al. (1969) and NIEBLING et al. (1987) suggested that factors such as differences in cross-over intensity among different trees, or environ-

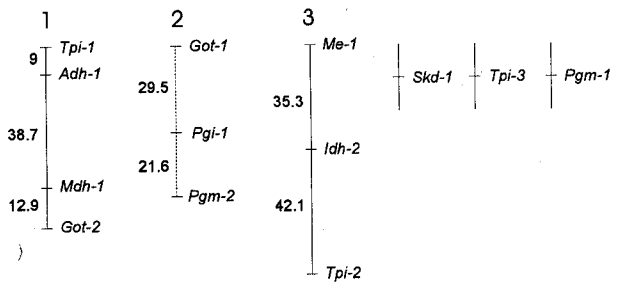

Fig. 1. Linkage map of cherimoya deduced from segregation data. Linkage groups are designated by large numbers. Locus names and map distances as recombination fractions (× 100) between adjacent markers are listed for each linkage group. *Skd-1*, *Tpi-3*, and *Pgm-1* did not appear to be linked to any other markers studied. The order of the second linkage group was not firmly established. The distance between *Got-1* and *Pgi-1* was calculated as a weighted mean of the distances obtained from cultivars PC and WH.

mental variations during gamete development may be responsible for these phenomena.

In conclusion, the linkage map of the cherimoya, bearing in mind the *Aco* loci mapped by LEE and ELLSTRAND (1987), would have three linkage groups, the first with five loci: *Got-2*, *Mdh-1*, *Adh-1*, *Tpi-1* and *Aco-2*; the second with four loci: *Pgm-2*, *Pgi-1*, *Got-1*, and *Aco-1*; and the third with three loci: *Tpi-2*, *Idh-2*, and *Me-1*. *Skd-1*, *Tpi-3*, and *Pgm-1* were not linked to any of the other loci studied. However, more studies are required to confirm the order of the loci in some of these linkage groups.

ACKNOWLEDGMENTS

We thank Dr J.M. Farré and J.M. Hermoso for providing the material, and S.R. Bordenstein for his help in correcting the English. Financial support for this work was provided by the Programa Nacional de Investigación Agrícola, project AGR89-0419 (Comisión Interministerial de Ciencia y Technología, Spain), and a grant from the Spanish Ministerio de Educación y Ciencia to F.P.

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