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Characterization of Cherimoya Germplasm by Isozyme Markers

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Abstract

Isozymes have been used as genetic markers to characterize more than 200 cherimoya and atemoya (*A. cherimola* x *A. squamosa*) accessions from the worldwide collection of cherimoya (*Annona cherimola* Mill) germplasm at the C.S.I.C. Estación Experimental "La Mayora" (Spain). These accessions have been incorporated into this collection from both the original species range (Peru and Ecuador) and the main producing regions (Bolivia, California, Chile, Israel, Madeira, Spain). We studied 13 enzyme systems encoded by 23 loci. Fifteen loci displayed polymorphism. The allozymes identified allowed us to genotype the cultivars, to differentiate 95% of them, and to address the possible origins of those cultivars with identical isozyme profiles. The atemoya and cherimoya cultivars showed clear isozyme differences based on alleles specific to atemoya.

Introduction

The cherimoya (*Annona cherimola* Mill.) is a small fruit tree which bears a valuable crop. The species is Andean in origin (14) but is now cultivated in several areas of the world, including Bolivia, California, Chile, Ecuador, Israel, Peru and Spain. The cherimoya belongs to the Annonaceae family, which includes both tropical and subtropical species, some of which are of commercial interest. Hybrids between species of this family, such as atemoya (*A. cherimola* x *A. squamosa*), have been obtained from controlled crosses since 1908 (19).

The increasing interest in cherimoya cultivation in various regions of the world has resulted in a proliferation of cultivars, distinguished primarily by their morphological and pomological characteristics (15). The limited resolution power of these techniques hinders appropriate identification of cultivars. Correct identification of cherimoya cultivars could benefit the study of such important polymorphic horticultural characteristics as cold tolerance, surface morphology, flowering and fruit ripening time, and seed to fruit ratio (6).

Isozymes have been used for varietal identification and linkage studies of a large number of herbaceous (18) and tree (16) crops, such as avocado (17), citrus (7), olive (11), almond (3), walnut (1), apple (19), hazelnut (13), and chestnut (5). Isozymes as genetic markers have been used in several studies of cultivated plants, including varietal certification, breeding system determinations, determination of effective pollen in fertilization, parental analysis, degree of heterozygosity, as well as an understanding of the possible relationships between isozymes and pomological and commercial characteristics (16, 18). In cherimoya, Ellstrand and Lee (4) studied the isozyme variation of eight isozyme systems, genotyping 15 cherimoya cultivars (most of Californian origin) and one atemoya cultivar for 17 loci. Lee and Ellstrand (6) studied the linkage relationships of some of these loci, and Pascual et al. (9) characterized the Spanish cherimoya cultivars.

The collection of cherimoya cultivars from the C.S.I.C. Estación Experimental "La Mayora" (Algarrobo Costa, Málaga, Spain) is the most inclusive worldwide cherimoya germplasm bank. It currently maintains about 250 cherimoya cultivars from diverse origins, and of particular importance, accessions from Peru and Ecuador, the center of origin of this species. This kind of collection is essential to the improvement of cultivated plants (2). In cherimoya it would be use-

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ful to obtain new cultivars with such valuable pomological characteristics as resistance to low temperatures, reduced susceptibility to infection by *Ceratitis,* and increased flesh sugar content. Genetic cultivar identification via isozyme analysis would lead to wider knowledge about this collection and, hence, to more precise use.

Materials and Methods

We analyzed 206 cultivars of cherimoya (*Annona cherimoya*) and four atemoya (*A. cherimoya* x *A. squamosa*) cultivars that come from the major producing countries: 122 from Peru, 39 from Ecuador, 10 from California, 10 from Chile, 10 from Madeira, eight from Spain, three from Bolivia, and the four atemoyas from other countries. All of the cultivars were sampled from the subtropical tree collection of the C.S.I.C. Estación Experimental "La Mayora" (Algarrobo Costa, Málaga, Spain).

We principally used leaf and stamen extracts; however for alcohol dehydrogenase (ADH), which is not expressed in these organs, we analyzed seeds produced from self-fertilization. Leaf extracts were obtained using a homogenizer (Polytron; Kinematica, Luzern, Switzerland); seed and stamen extracts, with a mortar. Crude extracts were centrifuged at 4000x g, 4°C, for 20 min. The supernatant was either used immediately for electrophoresis or stored at -80°C. The extraction buffer was a Tris-HCl buffer including 12% polyvinylpyrrolidone-40 (14). Aliquots of the supernatants were loaded on polyacrylamide gels or absorbed in 6 x 11 mm Whatman filter-paper wicks for horizontal starch gels. The compositions of the gels and buffer systems used for resolving the different enzyme systems have been described elsewhere (9). We made specific stains for the following enzymes: acid phosphatase (ACPH), alcohol dehydrogenase (ADH), diaphorase (DIA), glutamate oxalacetate transaminase (GOT), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucose isomerase (PGI), phosphoglucose mutase (PGM), 6-phosphogluconate dehydrogenase (6PGDH), shikimate dehydrogenase (SKDH), superoxide dismutase (SOD) and triose phosphate isomerase (TPI); stains were prepared as described by Perfectti and Pascual (10). Genetic control for these isozyme systems has been previously established (4, 9, 10). The loci were named according to the relative mobility of their electromorphs, with numbers reflecting their relative migration in the electrophoretic gel, as proposed by Lee and Ellstrand (6) and Pascual et al. (9). A similar system was used for alleles. Null alleles were named as '*n*'. Alleles present only in atemoya were given a number followed by a apostrophe (').

We have used Nevo and Beiles's (8) criterion to classify alleles, based on allele frequency in populations, but taking into account countries instead of populations. Alleles were classified into one of the following classes: (I) *common,* at least one sample with a frequency of ≥ 10%: (a) *widespread,* common occurrence in more than two countries; (b) *sporadic,* common occurrence in two countries; (c) *localized,* common occurrence in only one region. (II) *Rare,* never occurs with frequency ≥ 0.1: (d) *widespread,* rare occurrence in more than one country; and (e) *localized,* rare occurrence in only one country.

Results and Discusion

We studied 13 different isozyme systems encoded by 23 loci. *Acp-2, Dia-1, Got-3, Mdh-2, 6Pgd-2, Pgi-2, Skd-2* and *Sod-4* appeared as monomorphic loci, and were not helpful for distinguishing among cultivars. Table 1 shows the genotypes of all the cultivars for all of the polymorphic loci studied, arranging cultivars by their countries of origin. *Adh-1* has not been studied in all the cultivars because this locus is only expressed in seed tissues, and only in a subset of the cultivars were self-fertilizations to obtain seeds accomphished.

The high number of polymorphic loci (65.2%) and the high number of alleles (mean 3 alleles/polymorphic locus) have allowed us to characterize the majority of the cultivars thoroughly. From 210 genotyped cultivars we found 200 different genotypes. Only nine sets of cultivars, involving 19 cultivars, showed identical genotypes:

–*'Campas' and 'Campas Mejorada'*. These Spanish cultivars showed the same isozyme profile for the 23 loci studied, and for four other loci (*Lap-1, Lap-2, Lap-3* and *Aco-1*) analyzed previously (9). Both cultivars show similar morphological and physiological characteristics when they are grown in the same plantation and exposed to identical environmental influences. Consequently, 'Campas' and 'Campas Mejorada' are probably the same cultivar, or, less likely, 'Campas Mejorada' may be a 'Campas' seedling or a sport from a 'Campas' tree.

–*'Fino de Jete' and 'Hill'*. 'Fino de Jete' is the most widely cultivated cv. in Spain. 'Hill' comes from Chile, but there are some doubts as to its origin (J.M. Farré, personal communication). These cultivars share genotypes at some individual loci that are very rare in the collection, such as the '24' heterozygote for *Got-1,* which appears in fewer than 20 cultivars, or the '12' heterozygote for *Pgm-2* which appears in only 14 cultivars. These results suggest that they may be considered as the same cultivar.

–*'Concha', 'Concha lisa'* and *'Azapa-II'*. These accessions came originally from Chile but were incorporated into the collection from different places, 'Concha' and 'Azapa-II' coming from different Chilean experimental stations, and 'Concha lisa' from California. They are almost certainly the same cultivar.

–*'Selección Madeira 5' and 'Selección Madeira 23'*. As their names indicate, they come from Madeira and may represent a duplicated accession.

–*'Selección Peru 67' and 'Selección Peru 68'* were collected from the same location in the region of San Pablo (Cajamarca, Peru) after an expedition to sample the cherimoya germsplasm of Peru and Ecuador, and they are probably a duplicated accession.

–*'Selección Peru 39' and 'Selección Peru 60'*. These cultivars came from two adjacent sites (Marra and Chuanche) in the Asunción area (Cajamarca, Peru), and are probably the same cultivar.

–*'Selección Peru 4' and 'Selección Ecuador 39'*. In this case the cultivars derived from different, well separated places. For 22 analyzed genes they are identical, but it is possible that they differ in other loci.

–*'Selección Peru 75' and 'Selección Ecuador 37'*. Both cultivars share the same isozyme profile. They were sampled in different places.

–*'Selección Ecuador 10' and 'Chilena'*. The first cv. came from the Puellaro region of Ecuador. 'Chilena' came from Chile although it has been incorporated into the collection from California.

In several of the previous cases the cultivars show "typical" genotypes, understanding "typical" to be the most frequent genotype for each studied gene. Some of these duplications may be explained as being the product of confusions in the designation and origin of certain cultivars, a problem which is exacerbated if morphologic characters, which are clearly influenced by the environment, are used for varietal characterization (4). The cultivars 'Campas' and 'Campas mejorada'; 'Concha', 'Concha lisa' and 'Azapa-II'; 'Selección Madeira 5' and 'Selección Madeira 23'; 'Selección Peru 67' and 'Selección Peru 68' would be an example of this. Other accessions with identical isozyme profiles ('Selección Ecuador 10' and 'Chilena'; 'Selección Peru 75' and 'Selección Ecuador 37'; 'Selección Peru 39' and 'Selección Peru 60') were obtained from distant places. This fact suggests that similar cherimoya cultivars have been transported over long distances and cultivated in different places. Of course, these cultivars could be different at other loci not sampled in our analysis.

Atemoya cultivars have several isozymic characteristics that differentiate them from cherimoya cultivars. One of these characteristics is the presence of specific alleles, such as allele 3 of *Got-2,*

Table 1. Genotypes for 14 polymorphic loci in 206 cherimoya cultivars and four atemoya cultivars. Cherimoya cultivars are grouped by country of origin.

Cultivar, abbreviation and origin		*Adh-1*	*Got-1*	*Got-2*	*Idh-1*	*Idh-2*	*Mdh-1*	*Me-1*	*Pgi-1*	*Pgm-1*	*Pgm-2*	*Skd-1*	*Spd-6*	*Tpi-1*	*Tpi-2*	*Tpi-3*
Atemoyas																
Atemoya African	AA	22	33	13	11	23	23	22	35	11	12'	12	12	11	12	1'2
Atemoya Gefner	AG	22	34	13	11	23	23	22	35	11	12'	12	12	11	12	1'2
Atemoya Pink Mamout	AP	22	33	24	11	23	12	22	36	11	12	12	11	22	12	1'2
Joy	JOY		33	13	12	23	23	22	35	11	12'	12	12	11	12	22
Spain																
Campas	CA	12	34	22	22	22	11	11	45	11	11	12	22	11	11	12
Campas Mejorada	CM	12	34	22	22	22	11	11	45	11	11	12	22	11	11	12
Fino de Jete	FI	12	24	22	22	22	11	11	44	11	12	12	22	11	11	12
Manteca	MA	12	24	12	22	22	11	11	25	11	12	22	22	11	11	11
Negrito	NE	12	44	22	22	24	11	11	44	11	11	12	22	11	12	12
Pinchudo	PC	12	24	22	22	24	12	11	44	11	12	12	22	12	12	12
Piña	PÑ	22	44	22	22	22	11	11	26	11	11	22	22	11	11	11
Pazicas	PAZ		44	22	22	22	11	11	44	11	12	12	22	11	11	11
California (United States)																
Bays	BA	12	34	22	22	24	11	12	5n	11	11	22	22	11	12	22
Bonita	BO	12	33	22	22	22	12	22	46	12	12	22	22	11	22	12
Booth	BH	22	13	12	22	22	12	22	56	11	22	22	22	11	22	22
Chaffey Riverside	CH	12	33	22	22	44	12	12	44	11	11	22	12	11	12	22
C. Ott	CT	22	13	24	22	24	11	12	25	11	12	22	22	12	22	22
Loma	LO	22	34	22	22	22	11	12	45	11	11	22	22	11	11	11
Pierce	PI	22	24	22	22	24	11	11	46	11	11	22	22	11	12	22
Salmon	SA	22	34	12	22	22	13	12	44	11	12	22	22	11	22	22
Spain	SP	11	34	22	22	14	12	11	26	11	11	12	22	11	22	12
White	WH	12	23	22	22	12	11	11	46	12	11	22	22	12	22	12
Bolivia																
Bolivia seedling #1	B1	11	13	12	22	22	11	11	66	11	11	22	22	22	22	11
Bolivia seedling #2	B2	12	13	12	22	22	11	22	66	12	12	22	22	12	22	12
Bolivia seedling #3	B3	11	13	11	22	22	11	11	66	12	12	22	22	22	22	11
Chile																
Bronce suave	BS	12	33	22	22	44	11	12	44	11	11	22	22	12	22	22
Chilena	CL	11	33	22	22	24	11	12	66	11	11	22	12	11	22	22
Concha	CO	12	33	22	22	44	11	22	44	11	11	22	22	11	22	22
Concha Lisa	CN	12	33	22	22	44	11	22	44	11	11	22	22	11	22	22
Corazón	CR	22	33	22	22	24	11	12	66	11	11	22	22	22	22	22
Espinosa M	EM	12	13	22	22	24	12	22	66	11	11	22	11	11	12	22
Espinosa N	EN	12	34	22	22	22	12	12	66	11	11	22	12	11	11	22
Hill	HI	12	24	22	22	22	11	11	44	11	12	12	22	11	11	12
Serena	SE	12	34	22	22	22	11	11	44	11	12	12	22	11	11	12
Azapa-II	AZ		33	22	22	44	11	22	44	11	11	22	22	11	22	22
Other countries																
Zarzero	ZA	22	34	22	22	22	11	11	44	11	11	22	22	11	12	12

Cultivar, abbreviation and origin		Adh-1	Got-1	Got-2	Idh-1	Idh-2	Mdh-1	Me-1	Pgi-1	Pgm-1	Pgm-2	Skd-1	Spd-6	Tpi-1	Tpi-2	Tpi-3
Bronceada	BRON		33	22	22	24	12	22	66	11	11	22	11	11	12	22
Burtons	BURT		44	22	22	24	11	11	45	11	11	22	22	11	11	12
Mossman	MOS		34	12	22	44	13	11	46	11	11	12	22	11	12	22
Madeira (Portugal)																
Selección Madeira 4	M4		44	12	22	22	13	22	44	11	11	22	22	11	12	22
Selección Madeira 5	M5		24	11	22	22	33	22	66	11	11	22	22	11	22	12
Selección Madeira 7	M7		24	11	22	22	33	12	26	11	11	22	22	11	22	12
Selección Madeira 10	M10		22	12	22	24	33	12	66	11	11	22	12	11	12	12
Selección Madeira 14	M14		24	12	22	22	13	22	66	11	11	22	12	11	22	22
Selección Madeira 16	M16		22	11	22	22	13	12	66	11	11	22	12	11	22	22
Selección Madeira 17	M17		22	11	22	22	33	22	25	11	11	22	22	11	22	12
Selección Madeira 19	M19		44	11	22	22	33	12	66	11	11	22	22	11	22	12
Selección Madeira 23	M23		24	11	22	22	33	22	66	11	11	22	22	11	22	12
Selección Madeira 25	M25		22	11	22	22	33	11	66	11	11	22	12	11	12	22
Ecuador																
Lisa de Puellaro Lisa	LPL		33	22	22	24	11	11	26	11	11	22	22	nn	22	22
Lisa de Puellaro Pinchudo	LPP		44	22	22	22	11	12	24	11	11	22	12	11	12	22
Selección Ecuador 1	E1		34	22	22	44	11	12	24	11	11	22	12	11	12	22
Selección Ecuador 2	E2		34	22	22	24	11	12	45	11	11	22	12	11	11	22
Selección Ecuador 3	E3		33	nn	22	44	11	12	66	12	11	22	12	12	22	12
Selección Ecuador 4	E4		33	22	22	24	11	11	26	12	11	22	22	12	12	22
Selección Ecuador 5	E5		33	22	22	24	11	11	66	11	11	22	12	11	12	12
Selección Ecuador 6	E6		23	22	22	24	11	11	66	11	11	22	12	11	12	11
Selección Ecuador 8	E8		23	22	22	44	11	11	66	11	11	22	22	11	12	22
Selección Ecuador 9	E9		33	22	22	22	11	11	66	12	11	22	12	11	12	22
Selección Ecuador 10	E10		33	22	22	24	11	12	66	11	11	22	12	11	22	22
Selección Ecuador 11	E11		34	22	22	24	11	22	45	11	11	22	12	11	12	22
Selección Ecuador 12	E12		34	22	22	24	11	11	66	11	11	22	22	11	12	22
Selección Ecuador 13	E13		33	22	22	22	12	11	25	11	11	22	12	11	12	12
Selección Ecuador 14	E14		33	22	22	44	11	11	25	11	11	22	12	11	12	22
Selección Ecuador 15	E15		33	22	22	24	11	11	46	11	11	22	12	11	12	22
Selección Ecuador 16	E16		33	22	22	24	11	11	55	11	11	22	12	11	12	12
Selección Ecuador 17	E17		24	22	22	22	11	11	46	11	11	22	22	11	12	12
Selección Ecuador 18	E18		33	22	22	24	11	11	66	11	11	22	12	11	12	22
Selección Ecuador 20	E20		34	22	22	22	11	12	46	11	11	22	12	11	11	22
Selección Ecuador 21	E21		34	22	22	22	11	11	46	11	11	22	22	11	12	22
Selección Ecuador 22	E22		34	22	22	24	11	12	46	11	11	22	12	11	12	11
Selección Ecuador 23	E23		33	22	22	24	11	22	25	11	11	22	22	11	12	22
Selección Ecuador 24	E24		33	24	22	24	12	11	26	11	11	22	22	11	22	12
Selección Ecuador 25	E25		33	22	22	24	11	11	66	11	11	22	12	11	22	12
Selección Ecuador 26	E26		34	22	22	22	11	11	66	11	11	22	12	11	12	22
Selección Ecuador 27	E27		33	22	22	22	12	11	46	11	11	22	22	11	12	22
Selección Ecuador 28	E28		33	22	22	22	11	11	26	11	11	22	12	11	22	22
Selección Ecuador 29	E29		34	22	22	24	12	11	66	11	11	22	12	11	12	22
Selección Ecuador 30	E30		34	22	22	22	11	11	66	11	11	22	12	11	11	22
Selección Ecuador 31	E31		33	22	22	24	11	12	66	11	11	22	22	11	22	22
Selección Ecuador 32	E32		33	12	22	24	23	11	66	11	11	22	12	11	11	11
Selección Ecuador 33	E33		33	22	22	24	11	12	45	11	11	22	12	11	12	22
Selección Ecuador 34	E34		34	22	22	22	11	11	46	11	11	22	12	11	11	22

Cultivar, abbreviation and origin		*Adh-1*	*Got-1*	*Got-2*	*Idh-1*	*Idh-2*	*Mdh-1*	*Me-1*	*Pgi-1*	*Pgm-1*	*Pgm-2*	*Skd-1*	*Spd-6*	*Tpi-1*	*Tpi-2*	*Tpi-3*
Selección Ecuador 35	E35		33	22	22	22	12	11	24	11	11	22	12	nn	22	22
Selección Ecuador 36	E36		44	22	22	22	11	11	24	11	11	22	11	11	11	22
Selección Ecuador 37	E37		34	22	22	44	11	11	46	11	11	22	22	11	11	22
Selección Ecuador 38	E38		34	22	22	24	11	11	46	11	11	22	22	11	12	22
Selección Ecuador 39	E39		34	22	22	24	11	11	46	11	11	22	12	11	12	22
Peru																
Selección Peru 410-16	P410	12	12	22	22	22	11	11	44	11	12	12	22	11	11	12
Selección Peru 604	P604	22	12	22	22	24	11	12	66	12	11	22	22	11	22	22
Selección Peru 606	P606	12	14	22	22	22	11	11	44	11	12	12	22	11	11	12
Chiuna-1	C1		33	22	22	22	12	12	66	11	11	22	22	11	22	22
Chiuna-2	C2		33	22	22	24	11	12	46	11	11	22	22	11	12	22
Chiuna-3 Tardía	C3Td	12	33	22	22	44	11	12	25	11	11	22	22	11	12	22
Chiuna-3 Temprana	C3Te	22	33	22	22	24	12	12	65	11	11	22	22	11	22	22
Chiuna-4	C4	12	33	12	22	24	23	12	46	11	11	22	12	11	22	22
Cumbe	CU	22	33	22	22	24	12	12	56	11	11	22	22	11	22	22
Peru seed 24	PE	12	23	12	22	24	13	12	44	11	11	22	22	11	12	22
Selección Peru 7752	SP77	22	33	22	22	24	12	11	56	11	11	22	12	11	11	22
Selección Peru 78 (p 29)	SP78	12	23	22	22	24	11	12	44	11	11	22	12	11	12	12
Selección Peru 1	P1		33	22	22	24	12	11	44	11	11	22	12	11	12	22
Selección Peru 2	P2		33	22	22	24	11	12	46	11	11	22	22	11	22	22
Selección Peru 3	P3		33	22	22	22	11	11	44	11	11	22	12	11	11	22
Selección Peru 4	P4		34	22	22	24	11	11	46	11	11	22	12	11	12	22
Selección Peru 5	P5		34	12	22	44	13	22	44	11	11	22	12	11	11	22
Selección Peru 6	P6		33	22	22	44	12	11	44	11	11	22	12	11	11	22
Selección Peru 7	P7		33	22	22	24	11	12	44	11	11	22	12	11	12	22
Selección Peru 8	P8		33	22	22	44	11	11	44	11	11	22	12	11	12	22
Selección Peru 10	P10		33	22	22	24	13	22	44	11	11	22	12	11	12	22
Selección Peru 11	P11		33	11	22	22	33	12	44	11	11	22	22	11	12	22
Selección Peru 12	P12		33	12	22	24	13	22	46	11	11	22	12	11	12	22
Selección Peru 13	P13		33	22	22	44	11	11	66	11	11	22	12	22	12	22
Selección Peru 15	P15		33	22	22	24	11	11	44	11	11	22	12	11	11	22
Selección Peru 17	P17		33	22	22	44	11	22	46	11	11	22	22	12	22	22
Selección Peru 18	P18		33	22	22	44	22	12	46	11	11	22	22	11	12	22
Selección Peru 19	P19		34	22	22	24	11	11	44	11	11	22	22	11	12	22
Selección Peru 20	P20		34	44	22	24	11	11	44	22	11	22	12	11	12	12
Selección Peru 22	P22		33	nn	22	24	11	11	44	12	11	22	22	11	11	22
Selección Peru 23	P23		22	11	22	22	33	22	44	11	11	22	12	11	12	22
Selección Peru 25	P25		34	22	22	24	12	12	46	11	11	22	22	11	11	22
Selección Peru 26	P26		33	22	22	24	11	11	44	11	11	22	22	11	22	22
Selección Peru 27	P27		33	22	22	44	22	22	44	11	11	22	22	11	12	22
Selección Peru 29	P29		33	22	22	24	12	11	46	11	11	22	22	11	12	12
Selección Peru 33	P33		22	22	22	44	11	12	44	11	12	22	12	11	12	22
Selección Peru 34	P34		33	24	22	22	11	11	24	11	11	22	22	11	11	12
Selección Peru 35	P35		34	22	22	24	12	12	44	11	11	22	12	11	12	11
Selección Peru 38	P38		22	22	22	24	11	11	46	12	11	22	12	11	12	12
Selección Peru 39	P39		34	22	22	22	11	11	46	11	11	22	12	11	22	12
Selección Peru 40	P40		34	22	22	22	11	11	44	11	11	22	22	11	12	22
Selección Peru 41	P41		33	22	22	22	11	11	44	11	11	22	12	12	11	12
Selección Peru 42	P42		33	44	22	22	11	11	24	11	11	22	22	11	11	12
Selección Peru 43	P43		33	22	22	24	11	11	44	11	11	22	22	11	11	12

Cultivar, abbreviation and origin		Adh-1	Got-1	Got-2	Idh-1	Idh-2	Mdh-1	Me-1	Pgi-1	Pgm-1	Pgm-2	Skd-1	Spd-6	Tpi-1	Tpi-2	Tpi-3
Selección Peru 44	P44	22	22	22	22	11	11	44	11	11	22	22	11	12	22	
Selección Peru 45	P45	33	22	22	24	11	12	46	11	11	22	12	11	12	22	
Selección Peru 46	P46	33	22	22	24	11	22	46	11	11	22	12	nn	22	22	
Selección Peru 47	P47	34	22	22	24	11	12	44	11	11	22	12	11	11	22	
Selección Peru 48	P48	34	22	22	22	11	22	44	12	11	22	22	11	12	12	
Selección Peru 49	P49	34	24	22	44	11	11	44	12	11	22	22	11	11	12	
Selección Peru 50	P50	44	24	22	22	11	12	24	11	11	22	22	12	12	12	
Selección Peru 51	P51	34	22	22	22	11	11	44	11	11	22	22	11	11	22	
Selección Peru 52	P52	33	22	22	24	12	12	44	11	11	22	22	22	12	22	
Selección Peru 53	P53	34	22	22	22	11	11	25	11	11	22	12	11	11	12	
Selección Peru 54	P54	34	22	22	44	11	11	46	11	11	22	22	12	22	22	
Selección Peru 55	P55	33	22	22	44	13	11	24	11	11	22	22	22	12	12	
Selección Peru 56	P56	44	22	22	24	11	12	44	11	11	22	12	11	12	12	
Selección Peru 57	P57	34	22	22	22	11	12	44	11	11	22	12	11	12	22	
Selección Peru 58	P58	33	24	22	44	11	12	56	11	11	22	22	12	12	12	
Selección Peru 59	P59	34	22	22	24	11	11	25	11	11	22	12	22	11	12	
Selección Peru 60	P60	34	22	22	22	11	11	46	11	11	22	12	11	22	12	
Selección Peru 61	P61	24	22	22	22	11	11	46	11	11	22	22	11	11	22	
Selección Peru 62	P62	33	22	22	22	11	12	24	11	11	22	22	11	11	22	
Selección Peru 64	P64	24	24	22	24	11	11	24	12	11	22	22	11	11	22	
Selección Peru 66	P66	24	22	22	24	11	11	44	11	11	22	22	11	12	22	
Selección Peru 67	P67	33	22	22	24	11	11	24	11	11	22	12	11	12	22	
Selección Peru 68	P68	33	22	22	24	11	11	24	11	11	22	12	11	12	22	
Selección Peru 69	P69	24	22	22	22	11	12	44	11	11	22	12	nn	22	12	
Selección Peru 71	P71	34	22	22	24	11	11	24	11	11	22	11	11	12	12	
Selección Peru 72	P72	33	22	22	22	11	22	66	11	11	22	22	11	12	12	
Selección Peru 73	P73	34	24	22	24	11	11	44	12	11	22	12	11	12	12	
Selección Peru 74	P74	44	22	22	22	11	11	44	11	11	22	22	nn	22	12	
Selección Peru 75	P75	34	22	22	44	11	11	46	11	11	22	22	11	11	22	
Selección Peru 76	P76	34	22	22	44	11	12	44	11	11	22	12	11	12	12	
Selección Peru 77	P77	34	24	22	44	11	11	46	11	11	22	22	11	11	12	
Selección Peru 78	P78	33	22	22	24	11	11	44	11	11	22	12	12	12	12	
Selección Peru 79	P79	34	22	22	24	11	11	25	12	11	22	12	11	12	22	
Selección Peru 80	P80	33	22	22	24	11	11	24	11	11	22	11	11	12	22	
Selección Peru 81	P81	33	22	22	22	11	12	46	11	11	22	22	11	12	12	
Selección Peru 83	P83	33	22	22	24	11	12	44	11	11	22	12	12	12	12	
Selección Peru 84	P84	33	22	22	44	11	12	44	11	11	22	22	11	12	22	
Selección Peru 85	P85	33	22	22	24	11	11	44	11	11	22	22	11	12	11	
Selección Peru 86	P86	33	22	22	44	11	22	44	11	11	22	22	11	12	12	
Selección Peru 87	P87	34	22	22	22	11	12	44	11	11	22	22	11	12	22	
Selección Peru 88	P88	33	22	22	44	11	11	44	11	11	22	12	11	12	12	
Selección Peru 89	P89	23	22	22	44	11	22	46	11	11	22	12	11	12	12	
Selección Peru 90	P90	33	24	22	24	11	11	46	11	11	22	12	11	11	12	
Selección Peru 91	P91	33	24	22	24	11	12	25	11	11	22	12	12	12	22	
Selección Peru 92	P92	44	22	22	22	11	11	24	12	11	22	22	nn	22	22	
Selección Peru 93	P93	34	22	22	24	11	12	24	12	11	22	22	11	12	12	
Selección Peru 94	P94	23	22	22	22	11	11	24	11	11	22	12	11	12	12	
Selección Peru 95	P95	34	24	22	44	11	12	46	11	11	22	22	11	22	22	
Selección Peru 96	P96	34	22	22	44	11	11	44	22	11	22	11	22	11	22	
Selección Peru 102	P102	23	22	22	24	11	11	44	11	11	22	11	11	12	22	
Selección Peru 104	P104	23	24	22	44	11	11	44	11	11	22	22	11	12	22	

Cultivar, abbreviation and origin		Adh-1	Got-1	Got-2	Idh-1	Idh-2	Mdh-1	Me-1	Pgi-1	Pgm-1	Pgm-2	Skd-1	Spd-6	Tpi-1	Tpi-2	Tpi-3
Selección Peru 105	P105		33	44	22	22	11	11	44	11	11	22	22	11	12	22
Selección Peru 106	P106		33	22	22	44	11	12	44	11	11	22	11	11	12	11
Selección Peru 107	P107		33	22	22	24	11	11	44	11	11	22	22	12	11	22
Selección Peru 108	P108		33	12	22	24	23	12	66	11	11	22	12	12	11	22
Selección Peru 114	P114		33	12	22	44	13	12	46	11	11	22	22	11	22	22
Selección Peru 115	P115		34	22	22	24	12	12	46	11	11	22	22	12	22	22
Selección Peru 116	P116		44	11	22	22	33	22	44	11	11	22	22	11	12	22
Selección Peru 117	P117		34	22	22	44	11	22	46	11	11	22	12	12	11	22
Selección Peru 118	P118		33	12	22	24	23	22	44	11	11	22	12	11	22	22
Selección Peru 119	P119		33	22	22	44	11	22	66	11	11	22	22	11	22	22
Selección Peru 120	P120		34	22	22	24	11	12	46	11	11	22	22	11	22	22
Selección Peru 121	P121		33	12	22	44	33	22	46	11	11	22	22	12	12	22
Selección Peru 122	P122		33	12	22	44	23	22	66	11	11	22	22	11	12	22
Selección Peru 123	P123		33	22	22	22	11	11	44	11	11	12	22	11	12	12
Selección Peru 125	P125		33	12	22	44	23	22	44	11	11	22	22	22	22	22
Selección Peru 126	P126		33	12	22	22	13	12	56	11	11	22	22	12	22	22
Selección Peru 127	P127		34	11	22	44	23	12	56	11	11	22	12	12	22	22
Selección Peru 128	P128		33	22	22	44	11	12	44	11	11	22	22	11	22	12
Selección Peru 129	P129		33	22	22	24	12	12	44	11	11	22	22	11	22	22
Selección Peru 130	P130		33	22	22	22	22	11	44	11	11	22	22	11	22	22
Selección Peru 131	P131		33	22	22	22	12	12	56	11	11	22	22	11	22	22
Selección Peru 132	P132		33	12	22	22	11	12	44	11	11	22	22	11	11	12
Selección Peru 133	P133		23	22	22	24	13	11	56	11	11	22	12	12	12	22
Selección Peru 135	P135		33	22	22	24	12	22	44	11	11	22	12	11	22	22
Selección Peru 136	P136		33	22	22	44	22	12	44	11	11	22	22	11	11	22
Selección Peru 137	P137		33	22	22	24	12	12	44	11	11	22	22	11	12	22
Selección Peru 138	P138		33	22	22	24	12	11	44	11	11	22	12	11	11	22

ADH was only studied in 43 cultivars

allele 1 of *Idh-1,* allele 3 of *Idh-2,* allele 3 of *Pgi-1,* allele 2' of *Pgm-2,* and allele 1' of *Tpi-3.* These alleles, which probably derived from the other progenitor of this hybrid, the species *A. squamosa,* may be useful as markers at the specific level. Another characteristic is the non-detection of some heterodimers which we might expect in some dimeric enzymes encoded by *Got-1, Got-2* and *Pgi-1.* This may be explained by the divergence between polypeptidic subunits encoded by *A. cherimola* and *A. squamosa* genes, or also by the presence of a nule allele in these hybrids.

New cherimoya alleles were found. This is the case with alleles 4 and n of *Got-2,* allele 2 of *Pgm-1* and allele n of *Tpi-1.* These alleles appeared at very low frequencies (lower than 0.05) and, thus, are unlikely to be found when a low number of cultivars is studied (4, 9). Eleven cherimoya alleles were observed at a frequency less than or equal to 10%. These alleles account for 25% (30% if we only consider the polymorphic loci) of all the alleles found in cherimoya. If the two *Adh-1* alleles are not taken into consideration, because they have not been studied in all the cultivars, 22 out of the 51 alleles show frequencies lower than 0.2. This implies that some alleles appear in most of the cultivars and thus large differences (measured as χ^2 distances; unpublished data) do not exist between cultivars, with the exception of atemoya cultivars. Furthermore, one or two genotypes for each locus appear in most of the cultivars.

If the alleles are classified according to Nevo and Beiles (8), 89% (86% if monomorphic loci are not considered) are widespread common alleles. Only five alleles (11%, or 14% if monomorphic genes are not taken into account) showed more restricted geographic distributions and could be called rare alleles. These included *Got-2*:4, *Got-2*:n, *Tpi-1*:n, *Idh-2*:1 and *Pgi-1*:n. Most of these rare alleles are null and this defect might explain their limited presence in the collection. *Got-2*:4, *Got-2*:n and *Tpi-1*:n may be considered as rare alleles of wide distribution. These alleles (except *Got-2*:4) were only found in accessions from Peru and Ecuador, perhaps due to the larger quantity of cultivars from these countries or else due to these countries being the region of origin for this species. Allele *1 of Idh-2* and allele *n of Pgi-1* appeared only in California cultivars, and are considered to be local alleles. The United States is the only country that showed alleles in this category, in spite of there being only ten cultivars. The United States presented more than 90% of the alleles found in cherimoya. This country and Peru showed the highest percentage of different cherimoya alleles.

Finally, the power of isozyme analysis in the genotyping and identifying of cherimoya cultivars must be emphasized, leading as it has done to the precise classification of more than 95% of the cultivars studied. More than eighty billion possible genotypes may be assembled with the alleles found in this study (in cherimoya cultivars alone the number decreases to approximately two billions). Therefore it is no surprise that less than 10% of the cultivars studied present identical genotypes.

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Silver Dollar a Tribute to McIntosh

Those McIntosh trees in your orchard can trace their roots back almost 200 years, to a farm located in Dundas County in Ontario.

The Royal Canadian Mint is celebrating one of Canada's most commonly grown fruits with a 1996 commemorative Silver Dollar. McIntosh accounts for about half the 17 million bushels of apples produced each year.

One side of the coin features a stylized design of a McIntosh apple and an apple orchard; the other bears the Queen's portrait.

John McIntosh was born in the Mohawk Valley of New York State in 1777. He moved to Canada in 1796 and married Hannah Doran in 1801. By 1811, the McIntosh family established themselves near Dundela in Matilda Township.

While clearing his land, John McIntosh came across some young apple trees which he transplanted, starting an orchard near the family house.

John soon discovered only one tree produced a superior fruit. In the hopes of reproducing the fruit of the one tree, McIntosh began a seed orchard nursery. However, the results of this work was not as expected.

His son, Allan, began research on how to reproduce the single tree. In 1835, he learned the technique of grafting and, realizing the potential market for the apple, the McIntosh family began to promote their special variety to local farmers in Ontario and as far away as Vermont.

Sandy McIntosh, younger brother of Allan, also learned the grafting technique and travelled widely to promote and sell the grafts.

Harvey McIntosh followed in the footsteps of his father, Allan, and it was during his lifetime the McIntosh evolved from a locally celebrated fruit into Canada's most important commercial apple.

The variety is grown in all major apple producing provinces, including Ontario, Quebec, Nova Scotia, New Brunswick and British Columbia. It is also grown in parts of the U.S.

Prices are $29.95 for Proof Silver Dollars, and $19.95 for Brilliant Uncirculated Silver Dollars. The coins are available from coin dealers or by contacting the Mint at 1-800-267-1871.

Apple Flower Characteristics–Bee Pollination

Over 10 years 160 apple cultivars were evaluated and the following affect bee visits: color, nectar and pollen production as well as some morphological factors and pollen releasing capacity. However the most important factor was nectar productivity of the flowers rather than the sugar concentration.

From Benedek and Nyeki. 1994. ISHS Hort Congress Abstracts P-23-5, p. 253.