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Genetic diversity in a worldwide collection of cherimoya cultivars

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Abstract

Genetic diversity of 206 cherimoya (*Annona cherimola* Mill.) cultivars representing the major regions of production was determined from an analysis of 23 isozyme loci. Gene diversity in cherimoya was high, although not equally distributed among countries. Peru and Ecuador, the countries of origin of this species, showed the greatest diversity, and Spain and Madeira (Portugal) showed the least, probably due to genetics bottlenecks resulting from limited introduction. Cultivars from California showed high values for several genetic diversity measures, likely due to their diverse origins. The expansion of cherimoya cultivation out the area of origin has produced a change in the apportionment of genetic diversity, with an increase in the inter-populational components, but likely without a general erosion overall.

Introduction

Analysis and evaluation of plant germplasm are essential for better utilisation and conservation of plant resources. The domestication and cultivation of new plants may involve several processes, including human selection, dispersal and hybridisation, that frequently lead to genotypic changes, usually in the direction of a reduction in genetic diversity, potentially limiting future improvement. During domestication, different genes or gene complexes, responsible for interesting agronomic or physiological characteristics, may be favoured at different, and usually distant, locations, promoting genetic differentiation. Genetic markers, such as isozymes, have been widely used to analyse these populational differences and to describe and analyse cultivars of different geographic origin. Isozymes have also been used for varietal identification and linkage studies for a large number of herbaceous (Weeden 1989) and tree (Torres 1990) crops, to characterise and manage germplasm collections (Bretting and Widrlechner 1995), and as useful tools in plant biology (Soltis and Soltis 1989).

The cherimoya (*Annona cherimola* Mill.) is a semi-deciduous fruit tree that originated in Andean areas of Peru and Ecuador (Thomson 1970). Its pulpy fruit is edible, with considerable market demand. The cultivation of this tree and the harvest of its promising fruit are now gathering major interest (Van Damme et al. 1999). The cultivation of cherimoya has expanded from its area of origin to several subtropical areas of the world, including California, Chile, Ecuador, Israel, Peru and southern Spain and Italy.

In this species, isozymes have been used to determine genetic linkage (Lee and Ellstrand 1987; Perfectti and Pascual 1998b), analyse non-Mendelian segregations (Perfectti and Pascual 1996) and study developmental stability (Perfectti and Camacho 1999). The genetic characterization of cherimoya cultivars has been previously reported (Ellstrand and Lee 1987; Pascual et al.

1993; Perfectti and Pascual 1998a), and Perfectti and Pascual (in press) have analysed the geographic distribution and organisation of isozyme variation, but the genetic diversity (allelic richness, polymorphism, heterozygosity) of this fruit tree and the distribution of this diversity have not been previously analysed. The goal of this study was to evaluate genetic diversity of cherimoya, based on the allozyme analysis of an inclusive worldwide collection of cherimoya cultivars.

Material and methods

Plant material

We analysed 206 cherimoya cultivars from the major producing countries: 122 from Peru, 39 from Ecuador, 10 from California (USA), 10 from Chile, 10 from Madeira (Portugal), 8 from Spain, 3 from Bolivia and 4 additional cultivars of diverse origin (grouped under the label "other"). A complete and detailed list was reported in Perfectti and Pascual (1998a). All of the cultivars were sampled from the subtropical tree collection of the "Estación Experimental La Mayora" ("Consejo Superior de Investigaciones Científicas," Algarrobo Costa, Málaga, Spain), where an inclusive worldwide cherimoya collection is maintained (Hermoso et al. 1999).

Isozyme analysis

Leaf and stamen extracts were used for isozyme analysis. Leaf extracts were obtained by first using a homogeniser (Polytron; Kinematica, Luzern, Switzerland) and stamen extracts with a mortar. Crude extracts were centrifuged at 4000 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 (pH = 7.5) including 12% polyvinylpyrrolidone-40 (Soltis et al. 1983). Aliquots of the supernatant were loaded on polyacrylamide gels or absorbed in 6 × 11 mm Whatman filter-paper wicks for horizontal starch gels. The composition of the gels and buffer systems used for resolving the different enzyme systems have been described elsewhere (Pascual et al. 1993). Specific stains for the following enzymes: acid phosphatase (ACPH), diaphorase (DIA), glutamate oxalacetate transaminase (GOT), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucose isomerase (PGI), phosphoglucose mutase (PGM), 6-phosphogluconate dehydrogenase (PGDH), shikimate dehydrogenase (SKDH), superoxide dismutase (SOD) and triose phosphate isomerase (TPI), were made by following protocols previously described (Perfectti and Pascual 1996). Genetic control for these isozyme systems has been also previously established (Ellstrand and Lee 1987; Pascual et al. 1993; Perfectti and Pascual 1996). 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 (1987) and Pascual et al. (1993).

After the analysis of 12 enzyme staining systems, the genotypes of the cultivars were established for 22 isozyme loci, 14 of them being polymorphic. A genotypic table for the polymorphic loci is presented in Perfectti and Pascual (1998a).

Data analysis

We calculated several parameters related to the genetic diversity of the cherimoya collection: Polymorphism (P, calculated as the percentage of polymorphic loci at the 99% allele frequency criterion), number of alleles per locus (A), observed heterozygosity (H_o, calculated as the number of heterozygous loci in relation to total number of loci analysed per cultivar), and gene diversity or mean expected heterozygosity ($H_e = D$; Nei 1987), calculated as the arithmetic mean across loci of h, being $h = 1 - \sum_{i=1}^{i} x_i^2$ where x_i is the frequency of the ith allele of the xth locus). These parameters have been calculated for each cultivar and for cultivars grouped by country of origin, with the BIOSIS-1 software (Swofford and Selander 1989).

The distribution of gene diversity among countries was analysed following the variance method of Wright (1978) that can be also expressed as Nei's gene diversity indexes (Nei 1977). The gene diversity for polymorphic loci in the total collection (H_T) was partitioned into gene diversity within (H_S) and among (D_{ST}) countries of origin.

Table 1. Some measurements of genetic variability for the cherimoya collection.

	A	*P* (%)	*D*	H_o	*LH*/cv	*A*/*L*
Cherimoyas	2.05	59.09	0.199	0.162	3.53	1.16
	(0.25)		(0.046)	(0.039)	(0.11)	(0.01)
Bolivia	1.32	31.82	0.155	0.167	3.67	1.17
	(0.10)		(0.051)	(0.065)	(1.20)	(0.05)
Chile	1.59	50.00	0.173	0.105	2.30	1.10
	(0.16)		(0.029)	(0.029)	(0.56)	(0.03)
Ecuador	1.82	50.00	0.163	0.164	3.62	1.17
	(0.21)		(0.045)	(0.051)	(0.19)	(0.01)
Madeira	1.50	40.91	0.139	0.136	3.00	1.14
	(0.16)		(0.040)	(0.040)	(0.42)	(0.02)
Peru	1.95	59.09	0.188	0.162	3.53	1.16
	(0.22)		(0.044)	(0.040)	(0.15)	(0.01)
Spain	1.59	45.45	0.150	0.176	3.88	1.18
	(0.17)		(0.044)	(0.054)	(0.72)	(0.03)
USA	1.95	59.09	0.225	0.223	4.90	1.22
	(0.23)		(0.053)	(0.055)	(0.38)	(0.02)
Other	1.55	45.45	0.209	0.170	3.75	1.17
	(0.14)		(0.054)	(0.053)	(0.75)	(0.03)

A = mean number of alleles per locus; P = percent of polymorphism; D = gene diversity; H_o = observed heterozygosity; *LH*/cv = mean number of heterozygous loci per cultivar; A/L = mean number of alleles per locus per cultivar. Standard errors are between parentheses.

The relative degree of gene differentiation (G_{ST}) measures the portion of the gene variation that is attributable to genetic differentiation among subpopulations (countries), i.e. $G_{ST} = D_{ST}/H_T$ (Nei 1973). The null hypothesis ($G_{ST} = 0$) was tested following the method of Workman and Niswander (1970). The diversity values were compared with those reported by Hamrick and Godt (1990, 1997) and Doebley (1990) for cultivated and wild plants.

Results and discussion

Number of alleles per locus

The cherimoya collection presented a mean of 2.05 alleles per locus (average from 22 loci). Cultivars from two countries (Peru and USA) displayed the greatest allelic diversity with an identical mean of 1.95 alleles per locus (see Table 1), but from a very different number of sampled accessions (USA with 10 cultivars and Peru with 122 cultivars). Cultivars from Ecuador also displayed a high number of alleles per locus, and Bolivia was the country with the lowest mean (1.32 alleles per locus), probably because the low number of accessions sampled.

This measure of allelic richness has been used frequently in studies of genetic variability (Nevo 1978; Gottlieb 1981; Brown and Weir 1983) in spite of its high dependence on sample size (Nei 1978). The value of 2.05 ± 0.25 alleles per locus in cherimoya is similar to the mean for plants in general (1.96 ± 0.05; Hamrick and Godt 1990), for woody, long-lived, perennial plants (2.19 ± 0.09), and for temperate-tropical plants (2.53 ± 0.39).

Although cherimoya's floral cycle predisposes it to allogamy (Thompson 1970), in Spain, cherimoyas tend to be more autogamous (allogamy indexes between 0% to 31%, Manzano 1994), with an insect of the genus *Orius* acting as pollinator (Farré et al. 1999). However, the cherimoya's allelic richness is higher (but the difference is not significant) than the mean value for species with mixed pollination systems and animal pollinators ($A = 1.68 \pm 0.08$; Hamrick and Godt 1990) and similar to the mean for outcrossing species with animal pollinators (1.99 ± 0.07), which is probably the natural system of pollination of *A. cherimoya*.

Peru, Ecuador and USA showed the highest number of alleles per locus. Since Peru and Ecuador encompass the area of origin of this species (Popenoe 1921; Guzmán 1951), and the

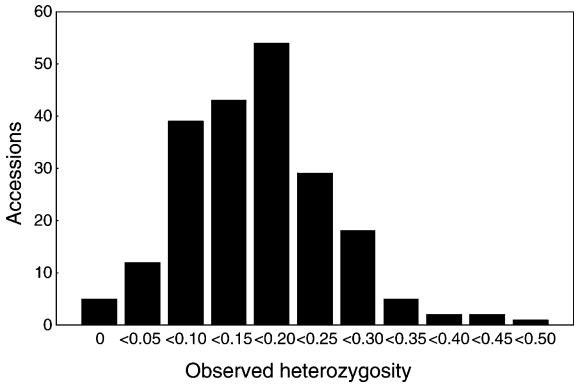

Figure 1. Number of sampled accessions for each observed-heterozygosity category.

number of accessions analysed from these countries was high, it is not remarkable that they showed the higher values of allelic richness. However, the case of cultivars from California is different: it is not a country of origin, only 10 cultivars were analysed, and the value of A is comparable to that of Peru and Ecuador cultivars. Cultivars from California present unique alleles (such as *Idh-2*: 1, *Pgi-1*: 1 and *Pgi-1*: *n*; see Ellstrand and Lee 1987; Perfectti and Pascual 1998a) not found in the cultivars sampled in Peru and Ecuador. These alleles could proceed from central America, since cherimoya arrived to USA through Mexico (Schroeder 1945), where hybridisation with other Annonaceae and subsequent introgression of some alleles could have occurred. Unfortunately, we do not have data from cherimoya cultivated in central America to test this hypothesis.

The low values shown by other countries sampled could be related to low sample size (as in the case of Bolivia) or the result of genetic bottlenecks and founder effects (Madeira, Spain, Chile).

Polymorphism

The proportion of polymorphic loci or polymorphism has been widely used as a measure of genetic diversity (Gottlieb 1981) despite P is subjected to large sampling error when a low number of individuals or loci are sampled (Nei 1978). The complete cherimoya collection showed $P = 59.09\%$. This percentage was also presented by the Peru and USA cultivars, while the minimum value was displayed by the Bolivian cultivars (31.82%). Nine polymorphic loci (*Adh-1*, *Lap-1*, *Lap-2*, *Lap-3*, *Aco-1*, *Aco-2*, *Aco-3*, *6Pgd-1* and *Mpi*), not included in the present study, were reported in Californian and Spanish cultivars (Pascual et al. 1993; Ellstrand and Lee 1987). If we also considered these loci, P could increase to 64.51% ± 2.17. This value is higher than the mean for cultivated plants (49%) obtained by Doebley (1990), similar to the estimation for all the crops (61.2%) of Hamrick and Godt (1997), but see the polymorphism reported by Hamrick and Godt (1997) for groups of cultivated crops (dicots, 52.6%; perennial, 62.5%; mixed mating breeding system, 58.4%). The polymorphism in cherimoya is higher than the P values presented for plants with similar biological characteristics to that of *A. cherimoya* (taxonomic status, geographic range, regional distribution or breeding system; see Hamrick and Godt 1990), and similar to the mean for perennial woody plants (64.7%; Hamrick and Godt 1990).

Observed heterozygosity

Cherimoya cultivars showed a mean value of 0.162, with a maximum of 0.223 (USA) and a minimum of 0.105 (Chile). The value for Chile was influenced by three cultivars of identical genotypes ("Concha," "Concha lisa" and "Azapa-II", see Perfectti and Pascual 1998a), completely homozygous for the analysed loci. Most cultivars (60%) showed values between 0.13 and 0.23 (see Figure 1), which corresponds to between 3 and 5 heterozygous loci out of the 22 loci analysed. A weak (no significant at 5% level) association between the number of heterozygous loci and country of origin was revealed (Kruskal-Wallis ANOVA, $H_7 = 13.77769$, $p = 0.0553$).

Gene diversity

The mean gene diversity or expected heterozygosity ($D = H_e$) is a widely used parameter of genetic diversity (Nei 1987). The values of gene diversity (range 0.139–0.225) were similar for different groups of cultivars (Table 1). The total for cherimoya was 0.199 ± 0.046, a value that is higher than the mean for cultivated plants (0.093; Doebley 1990; but compare with the estimation of Hamrick and Godt (1997): 0.189), probably

because the latter was obtained mainly from herbaceous plant studies that usually present low levels of gene diversity. Although cherimoya showed a high value of gene diversity, this value was not significantly different from mean values of D for perennial woody (0.177 ± 0.01), temperate–tropical (0.170 ± 0.026) or allogamous with animal pollinators (0.167 ± 0.01) plants. However, when cherimoya gene diversity is compared with a recent estimation of gene diversity in crop species of similar characteristics (Hamrick and Godt 1997), it appears as not showing high diversity (dicots, 0.159; perennial, 0.196; mixed mating breeding system, 0.147).

The gene diversity maximum (0.225) was presented by California cultivars, and the lowest values were obtained from Madeira (0.139), Spain (0.150) and Bolivia (0.155) cultivars, but differences among countries were not statistically significant (compare means and standard errors in Table 1).

Diversity distribution

The total gene diversity for polymorphic loci (H_T) is showed in Table 2. This parameter ranged from 0.652 at *Got-1* to 0.124 at *Pgm-1*, with an average across all polymorphic loci of 0.379 (Table 2), a value higher than the mean value for H_T in cultivated (0.19), woody (0.298 ± 0.012), temperate–tropical (0.301 ± 0.045) or allogamous with animal pollinators (0.310 ± 0.01) plants. The genetic diversity within countries (H_S) ranged from 0.514 at *Got-1* to 0.111 at *Pgm-1*, with an average value of 0.295. For genetic diversity (D_{ST}) among countries, the highest value was shown by *Got-2* (0.166) and the lowest by *Pgm-1* (0.012).

The relative degree of genetic differentiation among countries (G_{ST}) varied from 0.429 (*Tpi-1*) to 0.069 (*Sod-6*). All the G_{ST} values were significantly different from zero (tested by χ^2 tests, results not shown). The average over all polymorphic loci was 0.223 ± 0.032, meaning that 22.3% of genetic diversity was attributed to differences among countries of origin, and 77.7% of genetic diversity resided within countries. This value is similar to that of plants for temperate–tropical areas (0.223 ± 0.049), for allogamous plants with animal pollinators (0.197 ± 0.017), and for crop species with mixed-mating system (0.218), higher than G_{ST} for perennial woody plants (0.076 ± 0.01), and lower than G_{ST} for dicot crop species (0.475) and perennial cultivated plants (0.352).

An analysis of cherimoya cultivars collected from the region of origin showed that G_{ST} among provinces of Peru and Ecuador was 0.080 (F. Perfectti and L. Pascual, unpublished data). The higher value of G_{ST} in cherimoya cultivars organised by countries may be the consequence of such circumstances as founder effect, isolation and clonal habit (e.g. Sherman-Broyles et al. 1992). In general, an increase of G_{ST} with respect to the wild relative is usual in cultivated plants (Doebley 1990).

Domestication

The domestication of cherimoya probably occurred in Peru and Ecuador, the origin's areas of this species (thus implying that accessions from Peru and Ecuador showed high levels of genetic diversity) in pre-Columbian times (Schroeder 1945). From these areas, the cultivation expanded, possibly by both seed and vegetative propagation, to other places in South and Central America, arriving in 1871 to California from Mexico (Schroeder 1945), and earlier to Spain and Italy.

Domestication tends to erode genetic diversity, but this reduction in diversity depends on the type of domestication process (e.g. Morden et al. 1989; Gepts 1990). The domestication process usually implies a selection for agronomic characters that may produce genetic bottlenecks and, in some cases, episodes of founder effect (Ladizinsky 1985). However, the reduction in genetic diversity does not seem to equally affect herbaceous and tree species. Some fruit trees subjected to domestication for centuries, such as olive, date palm, mango or fig tree, seem to have not experienced great changes in their genetic characteristics (Spiegel-Roy 1986). Cherimoya may also be such an example of a species with its genetic diversity conserved, likely because cherimoya has not been subjected to planned breeding until recently. Popenoe (1921) found that cherimoyas cultivated in California were quite similar to wild cherimoyas in Ecuador, implying that, at least morphologically, cultivation had not produced ample changes. In addition, cultivars can be propagated

Table 2. Parameters of gene diversity for the 13 polymorphic loci analysed.

Locus	Allele	Frequency	H_T	H_S	D_{ST}	G_{ST}
Got-1	1	0.0828	0.0759	0.0560	0.0199	0.262
	2	0.1328	0.1151	0.0843	0.0308	0.267
	3	0.4820	0.2497	0.1946	0.0551	0.221
	4	0.3025	0.2110	0.1791	0.0319	0.151
	Total	–	0.6517	0.5140	0.1377	0.211
Got-2	1	0.2374	0.1810	0.0952	0.0859	0.474
	2	0.7418	0.1915	0.1121	0.0795	0.415
	4	0.0166	0.0163	0.0160	0.0003	0.019
	n	0.0042	0.0042	0.0042	0.0000	0.007
	Total	–	0.3930	0.2274	0.1657	0.421
Idh-2	1	0.0125	0.0123	0.0118	0.0005	0.043
	2	0.6852	0.2157	0.1816	0.0341	0.158
	4	0.3023	0.2109	0.1777	0.0333	0.158
	Total	–	0.4390	0.3711	0.0679	0.155
Mdh-1	1	0.7731	0.1754	0.1187	0.0567	0.323
	2	0.0859	0.0785	0.0785	0.0000	0.001
	3	0.1410	0.1211	0.0503	0.0709	0.585
	Total	–	0.3751	0.2474	0.1276	0.340
Me-1	1	0.6445	0.2291	0.1962	0.0329	0.144
	2	0.3555	0.2291	0.1962	0.0329	0.144
	Total	–	0.4583	0.3925	0.0658	0.144
Pgi-1	2	0.0675	0.0629	0.0621	0.0009	0.014
	4	0.3894	0.2378	0.1903	0.0474	0.199
	5	0.0898	0.0817	0.0808	0.0009	0.011
	6	0.4471	0.2472	0.1731	0.0741	0.300
	n	0.0063	0.0062	0.0062	0.0000	0.000
	Total	–	0.6358	0.5125	0.1233	0.194
Pgm-1	1	0.9339	0.0618	0.0557	0.0061	0.098
	2	0.0662	0.0618	0.0557	0.0061	0.098
	Total	–	0.1235	0.1114	0.0122	0.098
Pgm-2	1	0.8818	0.1042	0.0953	0.0090	0.086
	2	0.1182	0.1042	0.0953	0.0090	0.086
	Total	–	0.2085	0.1905	0.0180	0.086
Skd-1	1	0.0828	0.0759	0.0661	0.0099	0.130
	2	0.9172	0.0759	0.0661	0.0099	0.130
	Total	–	0.1519	0.1322	0.0197	0.130
Sod-6	1	0.1626	0.1362	0.1268	0.0094	0.069
	2	0.8374	0.1362	0.1268	0.0094	0.069
	Total	–	0.2723	0.2535	0.0188	0.069
Tpi-1	1	0.8287	0.1420	0.0814	0.0606	0.427
	2	0.1608	0.1349	0.0725	0.0624	0.463
	3	0.0105	0.0104	0.0101	0.0003	0.025
	Total	–	0.2873	0.1640	0.1233	0.429
Tpi-2	1	0.4057	0.2411	0.1817	0.0594	0.246
	2	0.5943	0.2411	0.1817	0.0594	0.246
	Total	–	0.4822	0.3634	0.1188	0.246
Tpi-3	1	0.3465	0.2264	0.1756	0.0508	0.224
	2	0.6535	0.2264	0.1756	0.0508	0.224
	Total	–	0.4529	0.3512	0.1017	0.224
All		–	0.3793	0.2947	0.0847	0.223

H_T = gene diversity for polymorphic loci in the total collection; H_S = gene diversity within countries of origin; D_{ST} = gene diversity among countries of origin; G_{ST} = relative degree of gene differentiation among countries.

by grafting (Sanewski 1988), maintaining allelic richness.

The genetic diversity in cherimoya is high, but not equally distributed among countries. The countries of origin of this species showed the highest values, and countries as Spain and Madeira (Portugal) showed low values probably due to founding effects. Cultivars from California showed relatively high values in several of the genetic diversity measures, probably due to the mixed origins of these cultivars. The distribution of cherimoya out of the area of origin has produced a change in the arrangement of the genetic diversity, with an increase in the inter-population components but probably without a general erosion of the genetic resources. The analysis of natural populations of this tree will be useful to test the previous hypothesis, i.e. that the domestication process have not significantly reduced the genetic diversity of cherimoya.

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