Genetic Resources and Crop Evolution **51:** 837–843, 2004.

Geographic variation for isozymes in cherimoya (*Annona cherimola* Mill.)

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Received 10 February 2003; accepted in revised form 9 May 2003

Key words: Annona, Cherimoya, Genetic diversity, Isozyme variation

Abstract

Cherimoya (*Anonna cherimola* Mill.) is a fruit tree which originated in Peru and Ecuador and is now cultivated in several subtropical areas of the world. The characterization of cherimoya cultivars at allozyme level has been previously reported, but the geographic distribution and organization of this variation have not been fully characterized. In this study, we assessed the relationships among 206 cherimoya and four atemoya (*A. cherimola* × *A. squamosa*) cultivars based on allozyme polymorphism. We have confirmed the genetic differences between atemoya and cherimoya cultivars, and showed that cherimoya accessions from Madeira, Bolivia and Spain form homogeneous groups of cultivars. Accessions from Chile and California form heterogeneous groups, probably due to their mixed origins. Cultivars from Peru and Ecuador showed a wide range of allelic variation, as is expected for accessions from the center of origin of this species.

Introduction

The conservation, analysis and evaluation of plant germplasm are essential for a better use of plant resources for agricultural improvement. A component of these analyses is the study of the differences among cultivars, or groups of cultivars, that may arise during domestication and cultivation in distant places. Some genes or gene complexes, responsible for interesting agronomic characteristics, could be favoured at different locations, promoting population differences at morphological and genetic levels. Genetics markers such as isozymes are used to describe and analyse these genetic differences, and to characterize and manage germplasm collections (Bretting and Widrlechner 1995). Allozymes have also been used for varietal identification and linkage studies of a large number of herbaceous (Weeden 1989) and tree (Torres 1990) crops.

The cherimoya tree (*Annona cherimola* Mill.) is a semi-deciduous fruit tree of Andean origin (Thomson 1970) now cultivated in several subtropical areas of the world, including California, Chile, Ecuador, Israel, Peru and southern Spain. The fruit is a pulpy, edible, valuable crop weighing about 0.25–0.5 Kg. Although rapid ripening affects negatively its commercialisation, cherimoya is a promising fruit that is gathering major interest (Chatrou 1999). In cherimoya, isozymes have been used to characterize genetic resources (Ellstrand and Lee 1987; Pascual et al. 1993; Perfectti and Pascual 1998a), to determine genetic linkage (Lee and Ellstrand 1987; Perfectti and Pascual 1998b), to investigate non-Mendelian segregation (Perfectti and Pascual 1996), to analyse developmental stability (Perfectti and Camacho 1999), and to study genetic diversity (Perfectti and Pascual, MS submitted).

The characterization of cherimoya cultivars at allozyme level has been previously reported (Perfectti and Pascual 1998a), but the geographic distribution and organization of this variation within *A. cherimola* have not been fully

characterized, being the analysis of this variation the aim of the present article.

Materials and methods

Germplasm

We analysed 206 cultivars of cherimoya (*Annona cherimola* Mill.) and four atemoya (*A. cherimola* Mill. × *A. squamosa* L.) cultivars from the major producing countries: Peru (122), Ecuador (39), USA (California, 10), Chile (10), Portugal (Madeira, 10), Spain (8), Bolivia (3), four additional cultivars with diverse origin (two cultivars from Australia, one from New Zealand and one putatively from Costa Rica, grouped under the label 'other') and four atemoyas from other countries. A complete list of the cultivars can be found in Perfectti and Pascual (1998a). 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), where an extensive worldwide cherimoya collection is maintained (Hermoso et al. 1999).

Isozyme analysis

For isozyme analysis, we used leaf and stamen extracts obtained using a homogeniser (Polytron; Kinematica, Luzern, Switzerland) for leaf, and a mortar for stamens. The 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 including 12% polyvinylpyrrolidone-40 (Soltis et al. 1983). Aliquots of the supernatants were loaded on polyacrylamide gels or absorbed in 6 × 11 mm Whatman filter-paper wicks for horizontal starch gels. The gel composition and buffer systems used for resolving the different enzyme systems have been reported elsewhere (Pascual et al. 1993). We made specific stains for the following enzymes: acid phosphatase, diaphorase, glutamate oxalacetate transaminase, isocitrate dehydrogenase, malate dehydrogenase, malic enzyme, phosphoglucose isomerase, phosphoglucose mutase, 6-phosphogluconate dehydrogenase, shikimate dehydrogenase, superoxide dismutase and triose phosphate isomerase. Stains have been described in Perfectti and Pascual (1996). Genetic control for these isozyme systems has been 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). A similar system was used for alleles.

The genotype of all the cultivars where established for 22 isozyme loci (see table in Perfectti and Pascual 1998a), 14 of them being polymorphic.

Data analysis

To obtain an index of dissimilarity between cultivars, we calculated a matrix of χ^2 distances (Balakrishnan and Sanghvi 1968; Benzecri 1979) from the table of allelic frequencies for each cultivar, using the program BMDP 2M. Mean values of distances between cultivars were computed for each region of origin, and a matrix of mean distances was obtained. The distances grouped by origin were compared by Kruskal–Wallis tests and the significances were corrected by the sequential Bonferroni method (Rice 1989), to show the homogeneity or heterogeneity of cultivar groups.

The matrix of mean χ^2 distances was used to construct a dendrogram showing the relationships among different geographical groups of accessions, using the UPGMA (unweighted pair-group method of arithmetic averages) method of amalgamation (Sneath and Sokal 1973).

To represent the genetic relationship among cultivars, we performed a factorial correspondence analysis of the table of cultivars × allelic frequencies. To produce this table, each allele was considered a character with three estates: zero if the allele is not present, one if the cultivar is heterozygous, and two if the cultivar is homozygous for that allele (Bennaceur et al. 1991). The correspondence analysis was performed with the BMDP CA program.

We also conduced a discriminat analysis to determine which variables (alleles) discriminate between different cultivar groups, using the BMDP 7M program. Atemoyas were not included in this analysis, because they are easily identifiable by exclusive alleles (Perfectti and Pascual 1998a).

Table 1. Mean distances and their standard errors among cultivars grouped by geographic origins. Minimum and maximum distances are between parentheses. Atemoyas were grouped together. ATE = atemoyas, SPA = Spain, USA = cultivars from California, BOL = Bolivia, CHI = Chile, OTH = other origins, MAD = Madeira, ECU = Ecuador, PER = Peru.

χ^2	ATE	SPA	USA	BOL	CHI	OTH	MAD	ECU	PER
ATE	2.19 ± 0.37 (0.82–3.21)								
SPA	3.82 ± 0.04 (3.32–4.20)	1.85 ± 0.10 (0.00–2.71)							
USA	3.55 ± 0.05 (2.89–4.12)	2.69 ± 0.05 (1.41–3.56)	2.59 ± 0.05 (1.91–3.16)						
BOL	3.86 ± 0.09 (3.37–4.24)	3.19 ± 0.04 (2.71–3.46)	2.92 ± 0.08 (1.83–3.83)	1.86 ± 0.23 (1.41–2.16)					
CHI	3.56 ± 0.05 (2.94–4.00)	2.76 ± 0.08 (0.00–3.65)	2.52 ± 0.04 (1.63–3.37)	3.11 ± 0.08 (2.16–3.74)	2.33 ± 0.13 (0.00–3.46)				
OTH	3.55 ± 0.09 (2.83–4.16)	2.37 ± 0.13 (1.41–3.65)	2.53 ± 0.08 (1.63–3.42)	3.20 ± 0.09 (2.65–3.74)	2.47 ± 0.08 (0.82–3.46)	2.55 ± 0.23 (1.63–3.16)			
MAD	3.43 ± 0.06 (2.89–4.20)	3.18 ± 0.03 (2.31–3.74)	2.97 ± 0.03 (1.63–3.56)	3.04 ± 0.05 (2.45–3.65)	3.10 ± 0.03 (2.16–3.65)	2.98 ± 0.05 (2.16–3.46)	1.91 ± 0.08 (0.00–2.83)		
ECU	3.55 ± 0.02 (2.83–4.00)	2.60 ± 0.02 (1.63–3.83)	2.47 ± 0.02 (1.00–3.65)	3.02 ± 0.03 (2.31–3.92)	2.39 ± 0.02 (0.00–3.74)	2.29 ± 0.03 (1.15–3.37)	2.98 ± 0.02 (1.83–3.83)	2.07 ± 0.02 (0.82–3.65)	
PER	3.57 ± 0.01 (2.45–4.47)	2.65 ± 0.02 (1.00–4.08)	2.52 ± 0.01 (0.82–4.28)	3.15 ± 0.02 (2.00–4.08)	2.41 ± 0.01 (1.00–3.87)	2.38 ± 0.02 (0.82–3.56)	3.04 ± 0.01 (1.15–4.55)	2.31 ± 0.01 (0.00–3.92)	2.34 ± 0.01 (0.00–4.00)

In addition, the four cultivar grouped under 'other' were also excluded from this analysis.

Results and discussion

We have calculated a 210 × 210 matrix of χ^2 distances among the cultivars to show the genetic relationship among the cultivars grouped by their origins. The minimum distance was zero, and was found between various cultivars than show identical isozyme pattern for the 22 loci analysed. Nine sets of accessions, involving a total of 19 cultivars, showed identical genotypes (Perfectti and Pascual 1998a). These cultivar sets were mainly of similar origin (e.g., the set of three cultivars composed by '*Concha*', '*Concha lisa*' and '*Azapa-II*' showed identical genotype and the three cultivars came originally from Chile). The maximum distance observed was 4.55, between a cultivar from Madeira and one from Peru. Mean distance among all the cultivars was 2.49. Intermediate distance values were common and implying that cherimoya cultivars are genetically very similar. Only atemoyas and cherimoya cultivars from Bolivia and Madeira showed marked differences when compared with the other cultivars.

To ascertain the relationship between groups of cultivars, we obtained a matrix of mean distances among cultivars grouped by origin (Table 1). These were compared using non-parametric Kruskal–Wallis tests (Table 2). Atemoyas showed the highest distance values when compared with cherimoyas, highlighting the genetic differences among the cherimoya and its hybrid. In addition, atemoya intra-group distances were lower than the inter-group distances with the cherimoyas (Table 1), indicating that atemoya cultivars present distinct characteristics (exclusive alleles) and are clearly different from cherimoyas. Spanish cherimoyas also formed a homogeneous group, with intra-group distances significantly lower than inter-group distances (see Table 2). The accessions from California, however, showed a different pattern: intra-group distances were similar to inter-group distances, except for the comparison with atemoyas and Madeira cultivars (Table 2). Similar patterns were shown by Chilean accessions and the cultivars grouped under 'others'. Bolivian accessions are genetically homogeneous, with a reduced intra-group distance. However, they did not show significant differences with the other origins, except with Spain and the atemoya cultivars, probably due to the smaller sample of Bolivian cultivars (3). Cultivars from the island of Madeira showed a similar pattern: low intra-group distances and significant differences for inter-group distances.

Table 2. Significance, after sequential Bonferroni correction, of Kruskal–Wallis tests for comparison between intra-group and inter-group distances. Each file represents an analysis of the intragroup distance (first column) with the inter-group distances (additional columns). * = significant difference. Ns = no significant difference.

	Inter group distances								
Intra-group distances	ATE	SPA	USA	BOL	CHI	OTH	MAD	ECU	PER
ATE-ATE	–	*	*	*	*	*	*	*	*
SPA-SPA	*	–	*	*	*	*	*	*	*
USA-USA	*	ns	–	ns	ns	ns	*	ns	ns
BOL-BOL	*	*	ns	–	ns	ns	ns	ns	ns
CHI-CHI	*	*	ns	*	–	ns	*	ns	ns
OTH-OTH	ns	ns	ns	ns	ns	–	ns	ns	ns
MAD-MAD	*	*	*	*	*	*	–	*	*
ECU-ECU	*	*	*	*	*	*	*	–	*
PER-PER	*	*	*	*	*	ns	*	ns	–

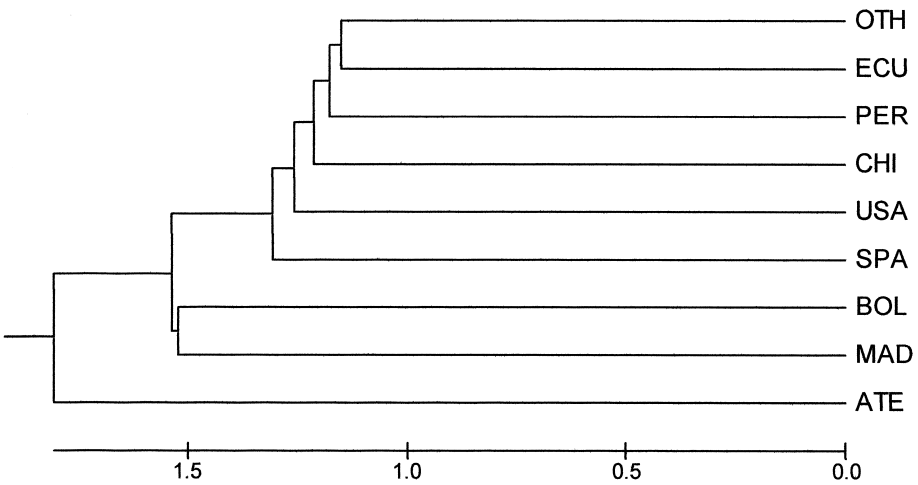

Figure 1. Cluster analysis by the UPGMA method of cherimoya cultivars grouped by origin (except for atemoyas and the 'other' group). χ^2 distances were calculated from genotypes for 22 isoenzymatic loci, and the mean distances matrix were used to construct the dendrogram. ATE = atemoyas, SPA = Spain, USA = cultivars from California, BOL = Bolivia, CHI = Chile, OTH = other origins, MAD = Madeira, ECU = Ecuador, PER = Peru.

Madeira cultivars seem to be a very homogeneous group, probably the descendant of a small number of seeds or plants imported to the island. Intra-group distances of Ecuadorian cultivars were lower than inter-group distances with other origins, showing also the homogeneity of the accessions from this country. Peruvian accessions showed a wide range of intra-group distances (0–4.0, see Table 1), with a mean distance similar to that of Ecuador, but significantly lower than the inter-group distances with the other origins.

A cluster analysis of the mean distances matrix also showed that atemoyas were clearly separated of the cherimoya accessions. In addition, Bolivian cultivars and accessions from Madeira were also positioned in different cluster, showing that this group of cultivars have particular genetic characteristics (Figure 1).

The factorial correspondence analysis was used to detect genetic relationship among accessions (Figure 2). The first axis had an inertia value of 18.7%, the second one 9.6% and the third 7.4%. These low inertia values prevent the representation of the entire genetic variation in a bi or tridimensional space. Low inertia values (<50%) are a common problem found in this type of analysis. For instance, Smith et al. (1985) found that the two first axis in a principal component analysis of

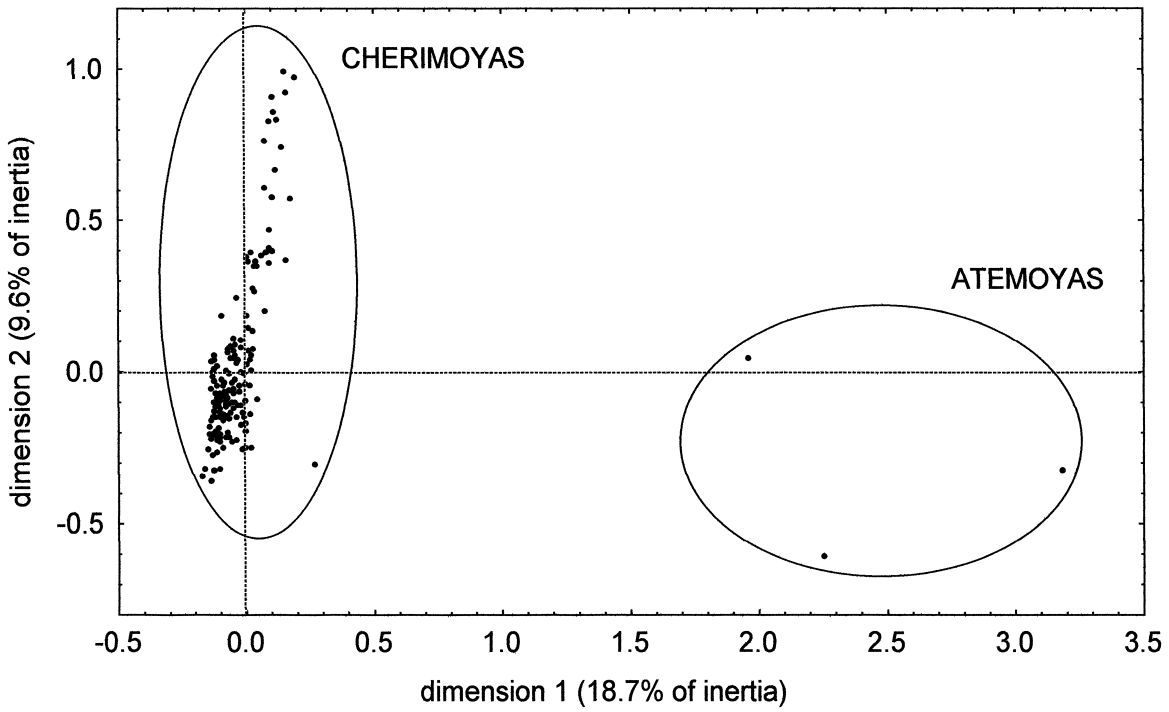

Figure 2. Graphical representation of the genetic relationship among atemoya and cherimoya cultivars using factorial correspondence analysis of genotype data. Space formed by the first and second dimension (28.3% of inertia).

maize and teosinte explained only 38.5% of the variation; Wendel et al. (1992) found that the two first dimension accounted for only 36% of the variation in a collection of cotton cultivars, and Liu et al. (2001) found that the first two axes only accounted for 31% of the total variation in a core collection of barley cultivars. In cherimoya, in spite of this low inertia, dimension one clearly separated atemoyas and cherimoya cultivars (see Figure 2), and the second dimension separated the cultivars from Madeira from the other cherimoya accessions. It is noteworthy that accessions from Peru and Ecuador, the countries of origin of the species, are disseminated throughout the space represented by these dimensions. To obtain better resolution, we repeated the analysis excluding atemoya cultivars (Figure 3). In this case, the first axis had an inertia value of 12.2%, the second one 9.3% and the third 8.4%, values not enough to clearly separate group of cultivars by country of origin.

Since atemoyas are easily identifiable by exclusive alleles (Perfectti and Pascual 1998a), they were not included in the discriminant analysis. This analytical procedure finds the variables (alleles) that best classify cultivars in their respective *a priori* groups (geography origin). Ten alleles of eight loci were found to best classify the cultivars in their countries of origin, namely Got-1 alleles 1 and 3, Got-2 allele 1, Skd-1 allele 2, Pgi-1 alleles 4 and n, Idh-2 allele 1, Mdh-1 allele 3, Pgm-2 allele 2, and Tpi-1 allele 2. The classification functions derived from these alleles classified correctly 71.8% of the cherimoya cultivars to their countries of origin (Spain: 75%, USA 70%, Bolivia 100%, Chile 10%, Madeira 100%, Ecuador 89.7% and Peru 68%). The origins with more homogeneous cultivars (i.e., Madeira, Bolivia, Spain and, to a lesser extent, Ecuador) showed higher values of correct classification. The cultivars from USA, Chile and Peru showed lower values of correct classification, showing the heterogeneous genetic nature of these accessions.

Isoenzymatic data clearly differentiated between cherimoya and atemoya cultivars and showed that particular alleles are present at high frequency in cultivars from some origins. A relationship between country or region of origin and genotypic or allelic frequencies has been observed in a wide

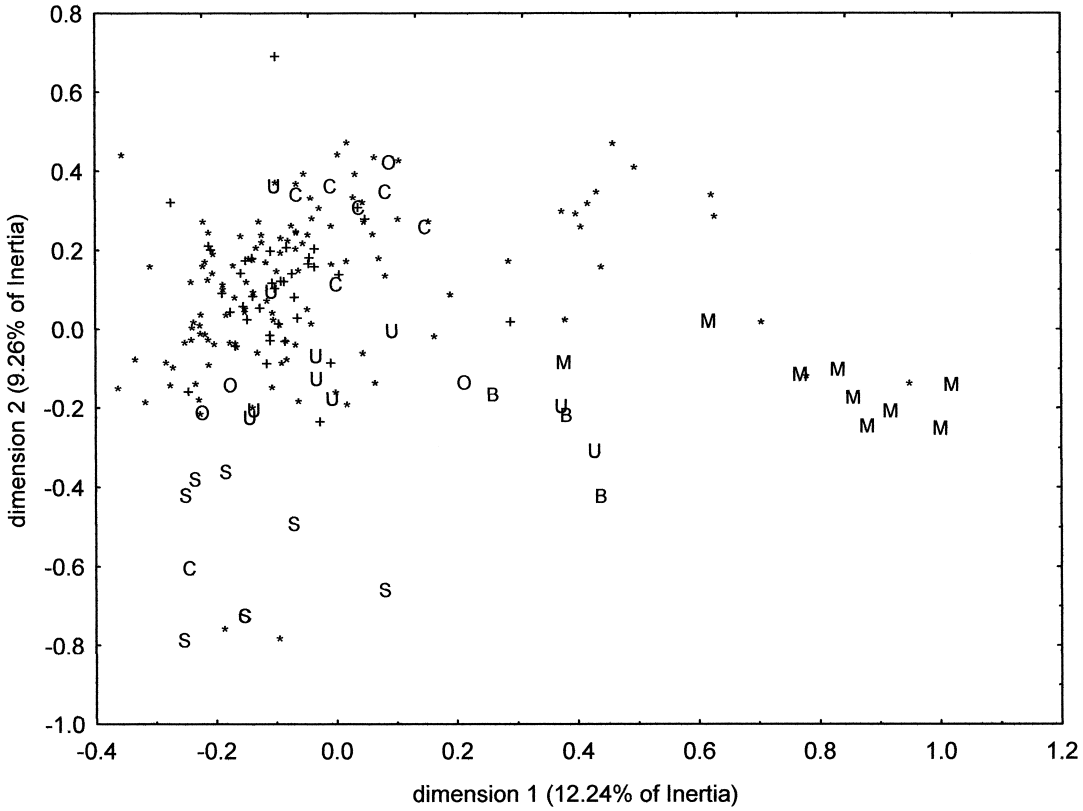

Figure 3. Depiction of the genetic relationship among cherimoya cultivars using factorial correspondence analysis of genotype data, after excluding atemoya cultivars. Space formed by the first and second dimension (21.5% of inertia). Abbreviations: A = atemoyas, S = Spain, U = cultivars from California, B = Bolivia, C = Chile, O = other origins, M = Madeira, + = Ecuador, * = Peru accessions.

range of plants, both cultivated and wild, for example, in sorghum (Morden et al. 1990), lentil (Erkine and Muehlbauer 1991), wheat (Asins and Carbonell 1989), chickpea (Tuwafe et al. 1988), cotton (Percy and Wendel 1990), sunflower (Tersac et al. 1994) and trees such as chestnut (Huang et al. 1994), date palm (Bennaceur et al. 1991) and beech (Comps et al. 1990). The causes of these relationships are subject of debate. Nevo and Beiles (1989) found that environmental and ecologic factors are more important that geographic distances. Li and Rutger (2000) found that epistasis and selection of multiple gene complexes was responsible for macro-geographic differentiation at isozyme loci in rice. However, other studies have shown that geography is the main factor explaining these relationships (e.g., Parker and Hamrick 1992). However, this kind of geographic relationship is not always found. For instance, Leford-Buson et al. (1991) found no relationship between the geographic origin of several populations of maize and their allelic frequencies, and in *Avena sterilis*, Beer et al. (1993) also found no relationship between genetic and geographic distances.

To conclude, the results of this study confirm the genetic differences between atemoya and cherimoya cultivars, and show that cherimoya accessions from Madeira, Bolivia and Spain form homogeneous groups. Accessions from Chile and California are heterogeneous, probably due to a mixed origin. Cultivars from Peru and Ecuador show a wide range of allelic variation as is expected for accessions of the center of origin of this species.

Acknowledgements

We express our thanks to J.M. Farré and J.M. Hermoso for kindly providing access to the

cherimoya collection. Financial support was provided the Programa Nacional de Investigación Agrícola. F.P. was supported by MEC and Junta de Andalucia (Grupo CVI-165).

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