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Morphometry of the organs of cherimoya (*Annona cherimola* Mill.) and analysis of fruit parameters for the characterization of cultivars, and Mexican germplasm selections

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Abstract

Three commercially available cherimoya cultivars (Campas, Burtons and White), one cultivar recently obtained and registered in Mexico (Cortés-II-31) and seven Mexican germplasm selections (S-196, S-256, S-260, S-266, S-9651, S-Selene and S-Carapan) were characterized by using the morphometric traits of various organs of the adult plant, together with several agronomical and chemical characteristics. The objectives were: (i) to seek an alternative approach to the definition of cherimoya cultivars through multivariate analysis, using commercial varieties and Mexican germplasm selections and (ii) to elucidate the grouping of cultivars and selections obtained by multivariate analysis on the basis of their origin and geographical distribution. Plant material was collected in 2002 from adult plants 3 years after grafting. Twenty-one morphometric characteristics (seven of leaves, nine of flowers, two of fruits and three of seeds), plus five fruit characteristics of agronomical importance and three chemical parameters of the fruit were selected for characterizing accessions. The intra-accession variability recorded for the traits selected made them suitable for identifying cultivars. All of the traits but one were capable of showing up differences between accessions at a significance level of 0.001. Principal Component Analysis (PCA) showed that the traits which yielded the maximum separation between accessions were: leaf blade form factor, angle of the fifth vein of leaves, upper angle of leaves, area of the cross-section of petals, sepal maximum projected area, weight of fruits, total soluble solids in fruits, resistance of the skin of fruits to a penetrometer and width-to-length ratio of the maximum projected area of seeds. Consequently, all of these characteristics may be of interest as descriptors for cherimoya varieties. Furthermore, four consistent groups of accessions were defined by the three-dimensional plot obtained through projecting the accessions onto the first three principal components and the tree-diagram, or dendrogram, obtained through Cluster Analysis (CA). Two of these groups were made up of accessions with a well-defined common origin. The first consisted of selections S-196, S-256, S-260 and S-266 obtained from Coatepec Harinas in Mexico State, while the second comprised selections S-Selene and S-Carapan from *Purépecha* native Indian communities in Michoacán State. For the other two groups the origin of the accessions forming them is not fully known. Hence, further studies, based on molecular markers, might be carried out in order to ascertain if these accessions are genetically related.

Keywords: Cherimoya germplasm; Multivariate analysis; Principal component analysis; Cluster analysis; Diversity analysis; Descriptors; Phenotypic characterization; Cultivar identification; Genetic resources for fruit

1. Introduction

The cherimoya (*Annona cherimola* Mill.) is a subtropical fruit tree, indigenous to Andean South America (Lee and Ellstrand, 1987), most probably originating in Ecuador and the neighbouring part of Peru (DeCandolle cited by Popenoe (1921)). Nevertheless, the presence of the fruit in Mexico and Central America has led botanists to assume that it might also be indigenous to the latter countries (Popenoe, 1921).

As for most of cultivated fruit–tree species, the identification of cherimoya cultivars is convoluted. Sometimes more than one genotype has been accidentally given the same cultivar name. Conversely, some cultivars have several synonymous names. The problem of identifying them is worsened by variation within cultivars, even when their name represents a single genotype (Ellstrand, 1997). In addition, the parentage of most

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cultivars is unknown and their phylogenetic relationships are not clear (Rahman et al., 1998). Moreover, the diversity of cherimoya germplasm, estimated from genetic parameters, is high in comparison with the average diversity of cultivated plants (Pascual et al., 1993b). The germplasm diversity in a cultivated crop depends on the pollination system, the method of propagation and the process of domestication. The mating system of cherimoya exhibits the phenomenon of protogynous dichogamy, a condition in which the receptivity of stigmas occurs prior to the release of pollen from a given flower. This increases diversity within natural populations because of cross mating (Ellstrand, 1997; George, 1984). A very large number of cherimoya cultivars trace their origin to open pollination populations (Richardson and Anderson, 1996) and have thereafter been sexually and asexually propagated. Furthermore, very few breeding programmes have been carried out on the cherimoya (Pascual et al., 1993b; Andrés-Agustín, 1997), with the development of cultivars based chiefly on selection. One particular problem is that classification of cherimoya cultivars has traditionally been based on characteristics of the surface of the fruit. These are in accordance with five botanical varieties, smooth, fingerprint, tuberculate, mamillate and umbonate, recorded long ago (Irazazabal, 1985), together with other pomological characteristics (Thomson, 1970). However, the surface and shape may vary substantially from fruit to fruit even on the same tree (Ellstrand and Lee, 1987). Thus, classification of cherimoya cultivars on the basis of skin type has been difficult (Perfectti et al., 1993; Rahman et al., 1998). In the light of this, there is a need for a reliable classification of cherimoya cultivars. Several approaches have been tried in recent years. These have involved isoenzymatic (Ellstrand and Lee, 1987; Perfectti et al., 1993; Pascual et al., 1993a,b; Perfectti and Pascual, 1998) or DNA-based molecular characterization: AFLP (Rahman et al., 1998), PCR-RFLP (Rahman et al., 1997), and RAPD (Ronning et al., 1995). Isoenzymatic and molecular characterization have the advantage that they are expressed in young material, making cultivar identification easy and reliable (Ellstrand and Lee, 1987). However, morphological characters are the most evident features. Hence, they are the basis for the description and identification of cultivars (Perrier, 1998), with crop descriptors mainly based upon morphology (i.e. IPGRI, 2004). In spite of the increasing interest in biochemical and molecular techniques for characterizing and identifying cultivars (Christie, 2001), it remains crucial to have adequate morphological traits. Morphological features have well-known problems for germplasm characterization. When used for assessing variability, they may lead to systematic errors. Several factors explain this. Chief among them are the polygenic inheritance of morphological features, their generally unknown hereditability (Perrier, 1998) and the influence of domestication on some of the morphological and agronomical parameters, which is a generalized phenomenon in cultivated species (Casas et al., 1999). In order to overcome these problems, morphological traits must fulfil certain requirements in order to be adequate for characterization (Perrier, 1998; González-Andrés, 2001). In principle, the set of traits must be as extensive as possible and drawn from various different organs of the plant. Other major considerations are (i) objectivity: qualitative characteristics need to have unambiguous and objective alternative expressions, whereas, quantitative characters derived from measurements are more objective in themselves; (ii) consistency: it is preferable for traits to be related to organs either unaffected or only slightly affected by selection and the environment. For metrical (or measurement-based) traits, ratios between measurements remain stabler than absolute measurements. In the present work morphometric characteristics of several organs of the adult plant (leaf, flower, fruit and seed) have been used, together with several agronomical and chemical traits of fruits, in order to characterize four commercial cherimoya cultivars, one of them obtained and registered in Mexico, and seven Mexican selections. The objectives were (i) to look for an alternative approach to the definition of cherimoya cultivars through multivariate analysis using commercial varieties and Mexican germplasm selections, and (ii) to explain the grouping of cultivars and selections obtained from multivariate analysis on the basis of their origin and geographical distribution.

2. Materials and methods

The cherimoya cultivars and selections under investigation (Table 1) were three foreign cultivars widely cultivated in Mexico, one cultivar obtained and registered in Mexico in 2003 and seven germplasm selections developed in this same country. Mexican germplasm came from landraces. The plant material, leaves, flowers, fruits and seeds, was obtained in part from open-air experimental plots with a randomized complete block design at the Fundación Salvador Sánchez Colín CICTAMEX, S.C., in Coatepec Harinas in Mexico State. This is located at latitude 18°46′38″N, longitude 99°46′38″W and at a height of 2240 m above sea level. It has an average annual rainfall of 1100 mm and an average temperature of 16 °C. Other locations yielding material were Jujacato, Tingambato and Carapan in Michoacán State, Mexico, lying at latitudes 19°26′–19°51′N, longitudes 101°49′–102°22′W and heights of 1900–2198 m above sea level. The average rainfall for these sites was between 1300 and 1800 mm yearly and their average temperature 19.9 °C.

Plant material was collected in 2002 from adult plants 3 years after grafting. Ten different plants were selected per accession and for each specimen one single leaf and flower, plus three fruits, were selected on the basis of the criteria given below. For leaves, a healthy and fully developed organ located between the eighth and ninth node was collected. This was scanned and the digital photograph used for morphometric measurements. One healthy fully developed flower was randomly selected per plant. It was dissected into the following parts: petals, receptacle, pedicle and sepals. The polar projection (maximum projected area) of each of these pieces, plus the cross-section of the petal, were scanned and the digital photograph used for measurements. For fruits, three healthy well-developed fruits were collected per specimen at the ripening stage, from three different places on the plant: upper, intermediate and lower. After collection they were weighed,