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RESEARCH ARTICLE

Genetic characterization of a collection of chayote, *Sechium edule* (Jacq.) Swartz, in Costa Rica by using isozyme markers

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Abstract We established protocols for the analysis of genetic diversity in chayote (*Sechium edule*) by using isozyme markers, thereby determining the level of genetic diversity present in 42 accessions of chayote from Costa Rica. We obtained clear and reproducible zymograms for eight enzyme staining systems: PGM, 6-PGD, PGI, IDH, MDH, SOD, SKD, and EST, and were able to score 14 putative loci. Eight of the 14 loci examined were polymorphic. We found 35 distinct multilocus genotypes among these accessions. Five of these multilocus genotypes were homozygous for all loci. In addition, our data also revealed that most of the multilocus genotypes (24) were heterozygous for only one of the eight loci, and the rest were heterozygous for two or three loci (9 and 4 accessions, respectively). Seven multilocus genotypes were found in two different accessions. Dice similarity coefficient was used to study the relationship between accessions. This analysis, based on the presence and absence of alleles, revealed that accessions collected in the same location seldom shared the same multilocus genotype. The value of isozyme polymorphisms as tools to continue studies on the characterization of chayote is discussed.

Keywords Cucurbitaceae · *Ex situ* conservation · Genetic diversity · Genetic differentiation · Germplasm characterization

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Introduction

Sechium edule (chayote) is an ancient vegetable crop native to Mesoamerica (Newstrom 1985, 1990, 1991; Hawkes 1991). Historical records, linguistics, the occurrence of wild forms, and the distribution of related wild species support the notion that the crop was domesticated in Mexico and Central America (Newstrom 1990; Hawkes 1991, Lira 1996). Furthermore, Engels (1983) showed significant genetic variation for eleven different traits in fruits of Central American chayotes, and proposed that Costa Rica should be considered part of the center of diversity of this species.

Sechium edule (Cucurbitaceae) is an herbaceous, monoecious, self-compatible, perennial vine (Newstrom 1990; Lira 1996). The male and female flowers are almost identical, have rotated corollas and ten nectaries at the base of the hypanthium, and are pollinated by insects (Wille et al. 1983). However,

male flowers are borne in racemes, while female flowers are solitary. Under cultivation, chayote flowers are pollinated by *Apis mellifera* L., bees of the genus *Trigona*, and various other taxa of large bees (Newstrom 1990). The single-seeded fruits are viviparous and variable in size, shape, color, pulp texture, and density of spines (Engels 1983).

Chayote is an important staple food for low-income groups in Latin America, and it is most commonly grown in homegardens for family consumption (Lira 1995). However, commercial production of chayote is important in several countries, including Costa Rica, Mexico, Brazil, and Puerto Rico (Lira 1996; Hord et al. 1997) where fruit commercialization and export represent a significant source of revenue.

Costa Rica has become the leading exporter of chayote (Lira 1996; Hord et al. 1997). In this country, vegetative propagation has been extensively used to produce uniform fruits that fulfill the requirements of the international market (Newstrom 1989; Lira 1996; Hord et al. 1997). It has been argued that this intense selection of fruit phenotypes by farmers may be contributing to increased genetic erosion in some areas, thus, jeopardizing genetic diversity of chayote (Brenes et al. 1996). In addition, the loss of traditional agricultural practices in Costa Rica, particularly homegardens, is also likely to reduce genetic diversity (Hodel and Gessler 1999; Lamont et al. 1999). Because the largest genetic diversity of chayote is found in Mesoamerica (Engels 1983; Newstrom 1985, 1989, 1991), there is an urgent need to establish *ex situ* germplasm collections in this region (Lira 1996).

Genetic analysis using genetic markers is a useful tool for ex situ conservation, as it may contribute to the characterization and evaluation of similar accessions to avoid duplication of plant material. Isozyme markers have been extensively used to examine the levels of genetic diversity and differentiation of crop plants in the Neotropics, both in the field and in materials kept in *ex situ* collections (Newbury and Ford-Lloyd 1997). For example, there are multiple studies that examine genetic diversity of *Manihot esculenta* Crantz (cassava) in the Neotropics (Hawkes 1991; Zaldivar et al. 2004). Other studies have successfully used isozyme markers in *Capsicum annuum* to determine genetic differentiation between accessions from different geographical areas within their range (Conicella et al. 1990). In Cucurbitaceae, isozyme markers have been extensively used to study genetic variation in the *Cucurbita pepo* complex (Decker and Wilson 1987; Decker-Walters et al. 1990) and in *Cucumis sativa* (Meglic and Staub 1996; Meglic et al. 1996; Staub et al. 1997a, b, 1999). Currently, other molecular markers are more frequently used to characterize genetic variation in Cucurbitaceae (Dijkhuizen et al. 1996; Staub et al. 1997a; Horejsi and Staub 1999); however, the use of isozyme markers is a very useful tool to measure the genetic diversity of plants (Hamrick and Godt 1990).

The goal of this study is to establish protocols for isozyme analysis of *Sechium edule* (chayote), and to determine levels of genetic diversity of a field collection of this crop maintained by the National University of Costa Rica in Heredia, Costa Rica.

Materials and methods

Plant material

Leaf tissue from a living collection of chayote maintained by the School of Agricultural Sciences of the National University of Costa Rica was used as source of plant material. A total of 42 accessions from different locations in Costa Rica were considered in this study (Table 1). Young leaves were collected and temporarily stored at −40°C at the School of Biology of the University of Costa Rica.

Most of the accessions considered in this study are from the two most important inter-montane valleys in Costa Rica, i.e., the Central and Guarco Valleys. However, other accessions are from the Atlantic lowlands (Guapiles and La Suiza), the Pacific lowlands (Perez Zeledón and Buenos Aires), and from the Tilaran and Talamanca mountain ranges (Tilarán and Agua Buena, respectively) (Fig. 1).

Establishment of conditions for starch electrophoresis analysis

Leaf samples were hand ground and homogenized in 1.5 ml Eppendorf tubes, at a 1:2 ratio by volume of tissue to buffer, which consisted of 10 mM KCl, 20 mM $MgCl_2$, 17 mM Sodium metasulfite, 1 mM EDTA, 0.03 mM NAD, 0.1 % Mercaptoethanol, 4 % PVP, 10 % Glycerol, 0.1 M Tris–HCl pH 7.5, 0.1 %

Table 1 Location of origin of the 42 accessions included in this study

Accession	Elevation	Location	Abbreviation
UNA-275	1110	San Felipe de Alajuelita, San José	ALA1
UNA-289	998	Ajenjal de Ujarrás, Cartago	UJA1
UNA-292	570	La Suiza de Turrialba, Cartago	LST1
UNA-295	570	La Suiza de Turrialba, Cartago	LST2
UNA-299	1180	Coral de Agua Buena, Puntarenas	COR1
UNA-300	1180	Coral de Agua Buena, Puntarenas	COR2
UNA-302	1090	San Pedro de Santa Bárbara, Heredia	SBR4
UNA-303	1090	San Pedro de Santa Bárbara, Heredia	SBR5
UNA-305	1440	Desamparados, San José	DES1
UNA-306	1110	San Juan de Santa Bárbara, Heredia	SBR1
UNA-307	1110	San Juan de Santa Bárbara, Heredia	SBR2
UNA-325	1300	Zetillal, Guadalupe, San José	GDP2
UNA-354	1560	Santa Cruz de Turrialba, Cartago	SCT1
UNA-366	1200	Santa Lucía de Barba, Heredia	HER5
UNA-370	1330	Cristo Rey de Desamparados, San José	DES2
UNA-376	620	Tilarán, Guanacaste	TIL1
UNA-377	690	El Roble, Tilarán, Guanacaste	TIL2
UNA-397	1320	Puruba de Santa Bárbara, Heredia	HER3
UNA-398	1220	Palmital de Miramar, Puntarenas	MIR
UNA-399	1250	San Juan de Naranjo, Alajuela	NAR2
UNA-400	1580	San José de Naranjo, Alajuela	NAR1
UNA-403	998	Ajenjal de Ujarrás, Cartago	UJA2
UNA-407	950	Ajenjal de Ujarrás, Cartago	UJA3
UNA-408	950	Ajenjal de Ujarrás, Cartago	UJA4
UNA-409	950	Ajenjal de Ujarrás, Cartago	UJA5
UNA-410	950	Ajenjal de Ujarrás, Cartago	UJA6
UNA-411	950	Ajenjal de Ujarrás, Cartago	UJA7
UNA-430	1435	Cartago Centro,Cartago	CAR1
UNA-432	500	Guápiles, Limón	GUA
UNA-434	1080	San Isidro de Peréz Zeledón , San José	PEZ
UNA-437	437	Buenos Aires, Puntarenas	BAP3
UNA-444	620	Río Cajón, Buenos Aires, Puntarenas	BAP1
UNA-446	620	Río Cajón, Buenos Aires, Puntarenas	BAP2
UNA-451	1450	Cartago Centro, Cartago	CAR2
UNA-523	1110	San Juan de Santa Barbara, Heredia	SBR3
UNA-532	1300	Concepción, Heredia	HER4
UNA-563	1200	El Yas de Paraíso, Cartago	YAS3
UNA-564	1200	El Yas de Paraíso, Cartago	YAS1
UNA-565	1200	El Yas de Paraíso, Cartago	YAS2
UNA-628	1150	Guadalupe Centro, San José	GDP1
UNA-629	1110	Heredia Centro, Heredia	HER2
UNA-630	1110	Heredia Centro, Heredia	HER1

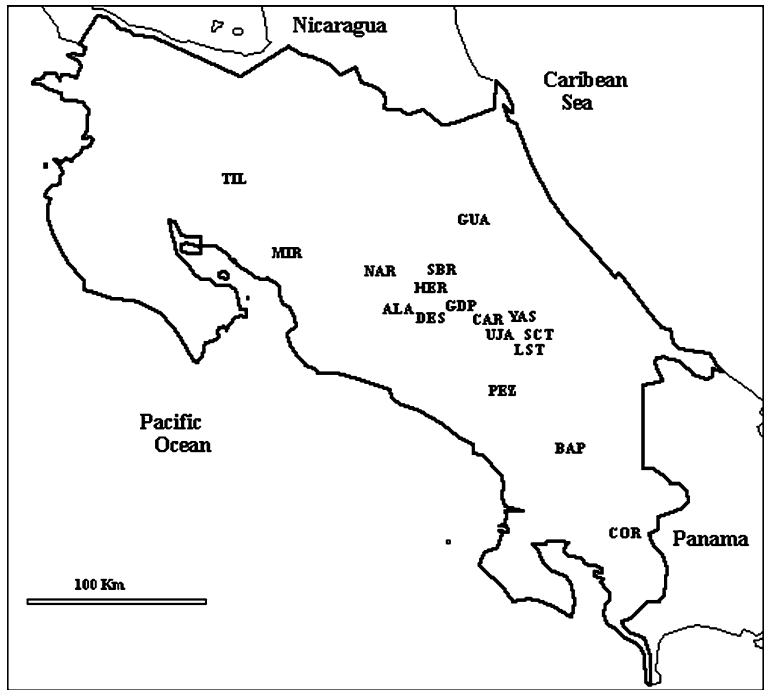

Fig. 1 Geographical distribution of the main locations of origin of the 42 accessions of chayote (*Sechium edule*) considered in this study. Most accessions were located in the inter-montane valleys located in the center of Costa Rica. For abbreviations see Table 1

Triton X, and 20 mM DTT (Hall et al. 1994). Crude extracts were centrifuged at 12,000 × *g* for 15 min at 5°C. The supernatant was stored at −20°C in a clean tube. Horizontal electrophoresis was conducted in a 10% starch and 3.5% sucrose gel (Maquet et al. 1996).

The separation and resolution of isozymes was evaluated with five buffer systems (Histidine-citrate pH 6, Histidine-citrate pH 6.5, Tris-citrate pH 7.5, Poulik pH 8, and Citrate-histidine pH 7), and several running conditions (constant voltage from 115 to 250 V and various running periods) (Table 1). Enzyme staining was performed following protocols described by Soltis and Soltis (1989) and Kephart (1990).

For genetic analysis, we determined the multilocus genotype of each accession. In addition, we also recorded the number of polymorphic (heterozygous) loci observed in each accession. We used the presence and absence of alleles to estimate genetic similarities for all pair-wise comparisons among populations. Genetic similarity was calculated on basis of the number of alleles common to both populations according to the following equation proposed by Dice (1945), where

$$GS_{xy} = 2a/(2a + b + c),$$

where a is the number of alleles common for populations x and y, b the number of alleles present only in population x, and c the number of alleles present only in population y.

The relationship between the populations was revealed by a cluster analysis based on sequential, agglomerative, hierarchical, and nested clustering methods (SAHN, UPGMA; NTSYS-pc-p package; Rohlf 1993).

Results and discussion

Electrophoretic analysis

Clear and reproducible zymograms were obtained for eight enzyme-staining systems; namely, phosphoglucomutase (PGM), glucose phosphate isomerase (PGI), shikimate deshydrogenase (SKD), esterase (EST), phosphogluconate dehydrogenase (PGD), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH-NAD), and superoxide dehydrogenase (SOD). The optimal separation conditions were obtained by using the buffer systems: histidine-citrate pH 6.5, POULIK pH 8.0, and citrate-histidine pH 7.0 (Table 2). The histidine-citrate system allowed visualization of PGM, PGD, IDH, MDH, SOD, and EST.

Table 2 Buffers, running conditions and enzymatic systems visualized in starch electrophoresis of extracts from young leaf tissue of chayote (*Sechium edule*).

Electrode buffer	Gel buffer	Running conditions	Enzymatic system visualized
Histidine-citrate			
0.065 M L-Histidine	0.065 M L-Histidine	150V constant,	PGM, PGD, IDH,
0.019 M citric acid	0.019 M citric acid	for 5 h	MDH, SOD, EST
pH 6.5			
POULIK system			
0.3 M boric acid	0.083 M Tris	150 V constant,	PGI, SKD
0.05 M NaOH	0.005 M citric acid	for 5 h	
pH 8.0	pH 8.6		
Citrate–Histidine			
0.410 M citric acid Na_3	0.005 M L-Histidine	50 Ma constant,	EST, PGI, IDH,
salt	pH 7.0	for 5 h	SKD
pH 7.0			

In contrast, an excellent separation of PGI and SKD was obtained using the POULIK system. And the citrate-histidine system also produced good results for EST, PGI, IDH and SKD. Zymograms for the isozyme systems are illustrated in Fig. 2. We found two loci for phosphoglucomutase and three for esterase; however, in both cases only one locus was polymorphic. Other enzyme systems such as aconitase (ACO) and peroxidase (PER), were clearly visualized but were not polymorphic, while aspartate aminotransferase (AAT) and malate dehydrogenase (ME-NADP) exhibited low activity or were not visible for all individuals.

Description of enzymatic patterns

The patterns of variation observed in the eight polymorphic loci are shown in Fig. 2. According to their banding patterns four isozymes; namely, PGM, PGI, EST, and SKD, appeared to be monomeric. The enzymatic systems PGD, IDH, MDH, and SOD, only showed one zone of activity, and according to the banding patterns of heterozygotes, all of them appeared to be dimeric. Even though more than one loci were observed for PGM and EST (two and three, respectively), only one of them was polymorphic.

Genetic analysis

Our analysis revealed significant variation among the 42 accessions in the chayote field collection of Costa Rica. We found that eight of the fourteen loci examined were polymorphic. All polymorphic loci had only two alleles.

Also, we observed 35 distinct multilocus genotypes. Five of these multilocus genotypes were homozygous for all fourteen loci, 24 were heterozygous for only one locus, and the rest were heterozy-

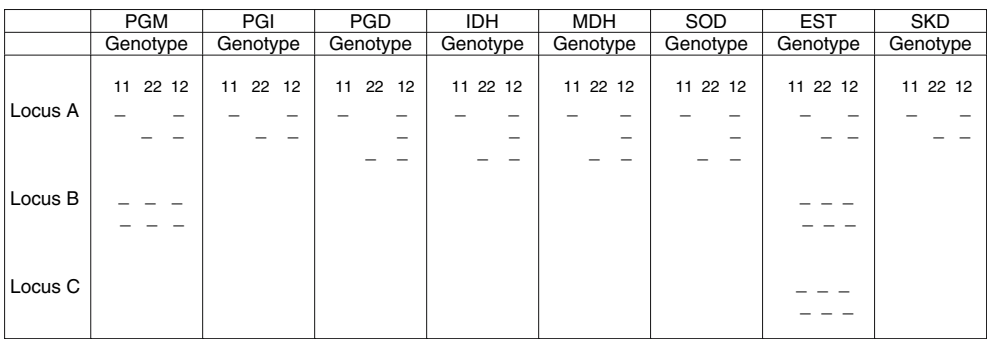

Fig. 2 Schematic representation of the zymograms obtained for the eight polymorphic isozyme systems examined in tissue from young leaf of chayote (*Sechium edule*)

Table 3 Number of accessions and accession ID number with zero, one, two and three heterozygous loci in their multilocus locus genotype

Number of heterozygous loci	Frequency	Accession ID number
0	5	289, 302, 354, 409, 434
1	24	275, 295, 299, 300, 303, 305, 307, 325, 370, 377, 397, 398, 399, 407, 408, 410, 411, 432, 446, 451, 563, 628, 629, 630
2	9	292, 366, 400, 403, 430, 437, 444, 532, 564
3	4	306, 376, 523, 565

gous for two or three loci (9 and 4 accessions respectively) (Table 3). In seven cases, two accessions shared the same multilocus genotype. In one case, both accessions were from the same location; namely San Juan de Santa Barbara, Heredia (accessions 306 and 523). Our analysis suggests that, unless these accessions are morphologically distinct, it is likely that they represent duplication in the collection. Three pairs of accessions sharing multilocus genotypes were from different locations in the Central Valley of Costa Rica; namely, accessions 532 and 628, accessions 629 and 275, and accessions 325 and 630 (Table 1). Three pairs of accessions with the same multilocus genotype were from separate geographical regions; namely, accessions 305, from the Central Valley, and 563, from Guarco Valley, accessions 366, from Central Valley, and 444 from the Pacific lowlands, and accessions 411 from Guarco Valley, and 432 from the Atlantic lowlands.

The high number of homozygous loci among the multilocus genotypes observed in the 42 accessions may be explained by two main factors. First, it has been claimed that chayote is self-compatible, as self-crosses are likely to set fruit (Newstrom 1990). Second, one or two seeds from the same plant are typically grown in homegardens. Thus, chayote vines growing in the same garden are closely related (OJR, personal observations). Selfing and mating between close relatives may contribute to the high level of homozygosity observed.

Figure 3 illustrates the relationship among the 42 accessions considered in this study on basis of the presence or absence of alleles. There were at least

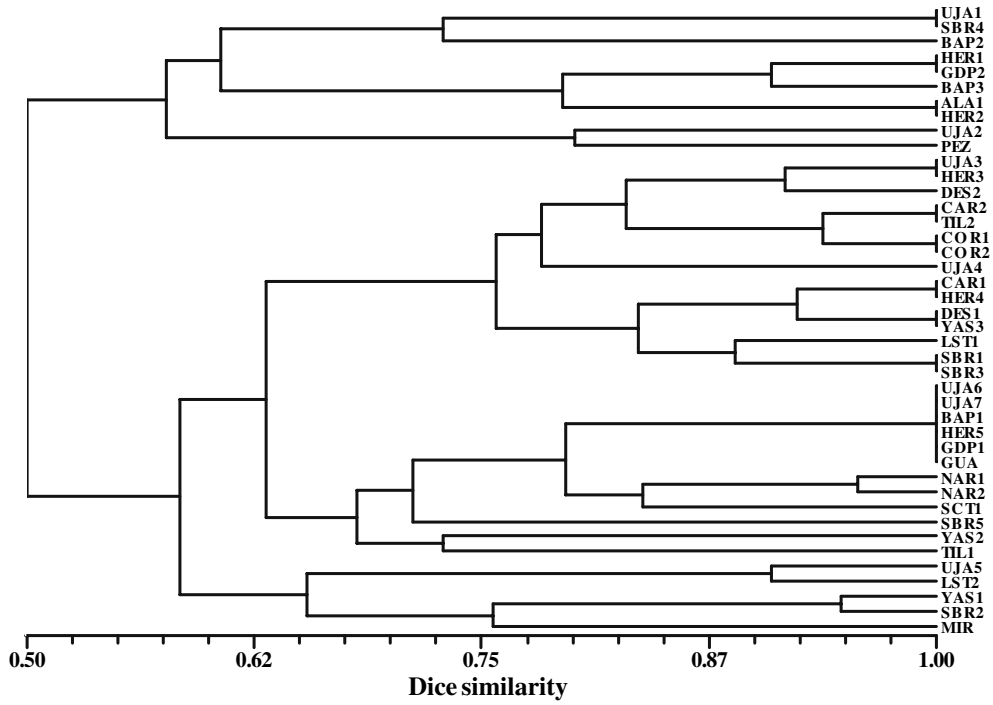

Fig. 3 Dendrogram of the 42 accessions of chayote (*Sechium edule*) based on Dice similarity coefficient (Dice 1945)

two well defined groups among the accessions, with one of them likely to be subdivided in three groups. Each group included accessions from different geographical locations.

In summary, our results revealed a high degree of genetic diversity in the chayote accessions in the field collection maintained by the National University of Costa Rica. Our data also showed that isozyme markers are a valuable tool for the characterization of this field collection. In addition, it may contribute to the detection of redundant materials and selective acquisition of unique new accessions.

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