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Assessing Genetic Diversity in Mexican Husk Tomato Species

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Abstract Mexico is the center of diversity of the husk tomato (*Physalis* L., Solanaceae), which includes a number of commercially important edible and ornamental species. Taxonomic identification is presently based on morphological characteristics, but the presence of high inter- and intraspecific morphological variation makes this task difficult. Six ISSR primers were used on eight Mexican species of *Physalis* to determine their utility for interspecific taxonomic discrimination and to assess their potential for inferring interspecific relationships. The six ISSR primers amplified 101 bands, with 100% polymorphism across samples. The number of bands per primer varied from 10 to 21. All primers produced different fingerprint profiles for each species, confirming the ISSR value in taxonomic discrimination. Discrimination values based on Simpson's diversity index varied from 0.48 to 0.58. Genetic interspecific similarity values ranged from 0.20 to 0.57, and intraspecific similarity values were highest for *Physalis angulata* (0.71), followed by *Physalis philadelphica* (0.63) and *Physalis lagascae* (0.55). The UPGMA analysis grouped accessions of the same species together and clustered together *Physalis* species of similar morphological traits. Thus, ISSR markers are useful in estimating genetic relationships in *Physalis*.

Keywords *Physalis* · Husk tomato · Genetic diversity · Genetic relations · ISSR markers

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Introduction

Like other Solanaceae genera, *Physalis* L. includes a number of commercially important edible and ornamental species. With the exception of *P. alkekengi* L., most of the 90 species in the genus are native to the Americas, and their distribution ranges from the USA through Mexico and into South America, including the Antilles. Many weedy and cultivated species have also been introduced to Old World tropical regions (Martínez 1998; Whitson and Manos 2005). With 50 native species, Mexico is the center of diversity (D'Arcy 1991; Waterfall 1967). In natural areas and traditional agroecosystems, the fruit of at least ten wild species are collected for consumption, but only *Physalis philadelphica* Lam. has been domesticated and cultivated in the region for centuries.

The high diversity of *Physalis* species observed in Mexico parallels the presence of high inter- and intraspecific morphological variation. This variation is observed mostly in widely distributed species or those that are managed and cultivated, making taxonomic identification more challenging.

Physalis species are currently identified by morphological characteristics. A number of studies have been done using morphological data to characterize species, to study the systematics of some sections (Martínez 1998, 1999a; Seithe and Sullivan 1990; Sullivan 1985; Vargas-Ponce et al. 1999, 2001, 2003), to determine the infrageneric taxonomy (Martínez 1999b), and to understand the phylogenetic relationships between *Physalis* and the genera related to the physaloid group (Axelius 1996; Estrada and Martínez 1999). Sequence data from part of the nuclear *Waxy* gene, the internal transcribed spacer of the nrDNA (ITS) and chloroplast regions (Martínez 1998; Whitson and Manos 2005) have also been used to address the phylogeny

of *Physalis*, related genera, and the Solanaceae (Olmstead et al. 2008).

The PCR-based technique using inter-simple sequence repeats (ISSR) is widely used because of its technical simplicity and reproducibility and the high polymorphism of the markers (Arif et al. 2009; Bornet and Branchard 2001; Reedy et al. 2002; Zietkiewicz et al. 1994). ISSRs provide extremely useful data for estimating genetic diversity (Ge et al. 2005; Nan et al. 2003; Petros et al. 2007; Gomes et al. 2009; Vargas-Ponce et al. 2009), phylogenetic analyses (Matos et al. 2001; Mort et al. 2003; Wolfe and Randle 2001) for studying the inter- and intraspecific relations among plants (Archibald et al. 2006; Petros et al. 2008; Wolfe and Randle 2001). The objective of the present study was to determine whether ISSR markers provide sufficient resolution for interspecific discrimination of Mexican species of *Physalis* and to assess their utility for inferring interspecific relationships.

Materials and Methods

Plant Material

Samples were taken from 12 wild *Physalis* populations, representing eight species and five taxonomic sections of the genus. The plants were collected in the states of Aguascalientes, Coahuila, Colima, and Jalisco, Mexico (Table 1). Three species are perennial, and five are annual. Leaf material was collected and dried in silica gel from 11 to 20 individuals per population. *Physalis virginiana* Miller, *Physalis cordata* Miller, *Physalis cinerascens* (Dunal) Hitch., *Physalis lignescens* Waterfall, and *Physalis sulphurea* Waterfall were represented by a single population, while *Physalis angulata* L. and *Physalis lagascae* Roem. and Schult. were represented by two populations each. The highly morphologically variable *P. philadelphica* Lam. was represented by three populations (Table 1).

DNA Extraction and PCR Amplification

DNA was extracted from 20 mg of leaf samples following the CTAB method of Doyle and Doyle (1987). The extracted DNA of five individual plants from the same population was mixed to create a genetic pool representing each population using the approach of Martínez et al. (2008), and Gilbert et al. (1998). Eleven primers were used in the initial screening; six were selected because they showed the highest potential to distinguish between species (Table 2). PCR was carried out twice by each ISSR primer with all *Physalis* samples included in the study. Only those bands that were present in both runs were scored and included in the data set. Each 20-µl PCR reaction mixture contained 60-ng genomic DNA, 10 mM buffer, 2.5 mM $MgCl_2$, 0.3 mM dNTPs, 0.2 µM primer, 0.5 unit *Taq* polymerase, and HPLC water. The PCR reactions were performed in a PTC-100 thermal cycler (MJ Research, Inc.) programmed for an initial step of 4 min at 95°C; followed by 40 cycles of 1 min at 95°C, 1 min annealing temperature (see Table 2), 2 min extension at 72°C; and a final 15 min extension at 72°C.

Amplification products were separated in 6% 19:1 bis-acrylamide gels containing 7 M urea and TBE buffer IX. A 100-bp molecular marker standard was included in each gel to estimate band sizes. Electrophoresis was run at 70 V in a dual adjustable slab gel unit (CBS Scientific) until the

Table 1 Eight *Physalis* species and 12 populations included in the study

Species	Section	Geographic locations: latitude, longitude		Voucher specimen
Physalis virginiana [a]	Lanceolatae	Saltillo, Coahuila	25°25′37″,100°59′50″	JS 301
Physalis cordata[b]	Epeteiorhiza	Tomatlán, Jalisco	19°56′30″,105°14′52″	JS 70
Physalis sulphurea[b]	Angulatae	Tizapan, Jalisco	20°09′43″,103°02′44″	JS 19
Physalis cinerascens[a]	Stellatae	Saltillo, Coahuila	25°25′37″,100°59′50″	JS 302
Physalis lignescens[a]	Coztomatae	V.Carranza, Jalisco	19°44′47″,103°46′90″	OVP 456
Physalis philadelphica-1[b]	Angulatae	Aguascalientes	21°52′55″,102°17′29″	JS 246
Physalis philadelphica-2[b]	Angulatae	Atemajac, Jalisco	20°08′19″,103°43′34″	JS sn
Physalis philadelphica-3[b]	Angulatae	Cuquío, Jalisco	20°25′05″,104°19′08″	JS 334
Physalis angulata-1[b]	Angulatae	Juchitlán, Jalisco	20°05′02″,104°06′03″	OVP 2022
Physalis angulata-2[b]	Angulatae	Techaluta, Jalisco	20°04′32″,103°33′12″	OVP 2017
Physalis lagascae-1[b]	Angulatae	Cuyutlán, Colima	18°55′46″,104°03′56″	OVP 445
Physalis lagascae-2[b]	Angulatae	Acatic, Jalisco	20°46′50″,102°54′34″	OVP 917

Each population was represented with pooling ADN from five individuals

[a] Perennial herbaceous

[b] Annual herbaceous

Table 2 ISSR primers used in the analysis, number of polymorphic loci, and discriminatory resolution of each primer (Simpson's Index)

Primer sequence	Annealing temperature (°C)	Fragment size (bp)	Number of loci analyzed	Polymorphic loci (%)	Simpson's index
$(GA)_8$ YG	56.5	280–1500	21	100	0.487
$(CA)_6$ RY	50	280–1500	17	100	0.526
$(CA)_6$ RG	47	200–1400	19	100	0.489
RY $(GACA)_3$	47	200–1000	17	100	0.483
$(CT)_8$ RG	47	350–800	10	100	0.500
$(CT)_8$ RC	47	350–1600	17	100	0.579

R=A, G; *Y*=G, T

bromophenol blue indicator dye had traveled 20 cm. The products were visualized with the silver staining technique of Sanguinetti et al. (1994).

Data Analysis

A digital imaging system (Kodak Gel Logic 100) was used to record gel images as TIFF files. ISSR banding patterns were analyzed with Phoretix 1D image analysis software (TotalLab). Bands were automatically assigned, then manually adjusted based on the resulting images. Each unique fragment size, considered as a locus for each primer, was scored as present (1) or absent (0) for each sample. The polymorphic loci percentage (*Pl*) by primer across samples was calculated using the POPGENE v.1.31 program (Yeh and Boyle 1999). Each primer's discrimination potential was expressed in terms of the Simpson's diversity index $\left[h_j = \sum (1 - \sum p_i^2)/n\right]$, where p_i is the frequency of the *i*th allele, and *n* corresponds to the number of loci detected by each primer (Hunter and Gaston 1988; Lüdtke et al. 2009). A value of 1.0 indicates that the primer is able to discriminate between all samples, and a value of 0.0 indicates that all samples are identical. From the 101 polymorphic bands, only 74 were chosen for the genetic relation analysis because of their clarity and constant amplification in all samples. These bands were generated with the primers $(GA)_8$YG, $(CA)_6$RY, $(CA)_6$RG, and RY $(GACA)_3$. Genetic similarity among all samples was calculated using the Nei and Li coefficient (Nei and Li 1979), which excludes absent bands from the analyses. Genetic similarity values were used to estimate genetic relations, and then represented with a dendrogram generated by the unweighted pair-group mean analysis method using the Freetree (Pavlicek et al. 1999). Relative support for the relations was assessed with bootstrap analyses (1,000 replicates; Felsenstein 1985) using the Freetree (Pavlicek et al. 1999) and Tree View (Page 1996) programs. Another genetic relation analysis was applied to a small subset of *Physalis* samples of *Physalis* section *Angulatae* (four species/eight populations, see Table 1) to assess the potential utility of ISSR data for inferring fine relations among taxa showing close genetic relations.

Results and Discussion

The six ISSR primers amplified 101 scorable bands (Table 2). The number of bands per primer varied from 10 to 21, with an average of 16.8 fragments per primer. The amplified product ranged from 200 to 1,600 bp, while the number of products amplified per primer varied from 10 [$(CT)_8$RG] to 21 [$(GA)_8$YG]. All primers produced 100% polymorphic bands, a very high interspecific percentage that has also been reported for *Polygala* (Polygalaceae), *Guizotia* (Asteraceae), *Morus* (Moraceae), and *Agave* (Agavaceae) (Lüdtke et al. 2009; Petros et al. 2007, 2008; Prasanta et al. 2008; Vargas-Ponce et al. 2009).

ISSR—Taxonomic Discrimination Among Physalis Species

All six primers produced different fingerprinting profiles for each species (Fig. 1). In addition, four of the primers generated species-specific bands. Primer $(CA)_6$RG generated a unique band for *P. philadelphica* (580 bp), and primer $(CA)_6$RY produced a specific band for *P. cordata* (1,100 bp). Likewise, primer RY$(GACA)_3$ yielded a band for *P. cinerascens* (590 bp) and another for *P. sulphurea* (700 bp). Last, the primer $(CT)_8$RC resulted in a unique band for *P. cinerascens* (700 bp), *P. philadelphica* (1,350 bp), and *P. sulphurea* (1,400 bp). Thus, ISSR markers can be useful in generating ISSR genetic fingerprints specific to each *Physalis* species. Indeed, ISSRs have been useful in the genomic fingerprinting of diverse plant groups Reedy et al. (2002).

Banding patterns showed clear interspecific differentiation and intraspecific similarity, demonstrating the value of ISSRs in taxonomic discrimination of *Physalis* species (Fig. 1). Simpson's index values varied from 0.483 (primer RY$(GACA)_3$) to 0.579 (primer $(CT)_8$RC; Table 2) showing moderate to high potential to discriminate between samples.

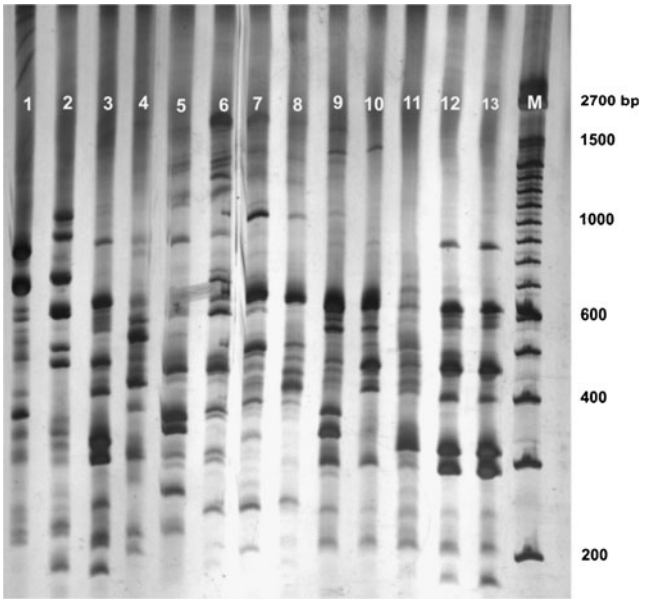

Fig. 1 Inter-simple sequence repeat (ISSR) banding pattern obtained on acrylamide gels for 12 populations representing eight *Physalis* species with the primer. $(GA)_8$ YGM=DNA Molecular size marker (100 bp ladder) in the *last lane*. Lane/s: *1, P. virginiana*; *2*, *P. cordata*; *3*, *P.sp*; *4*, *P. cinerascens*; *5*, *P. lignescens*; *6*, *P. sulphurea*; *7–9*, *P. philadelphica*; *10–11*, *P. angulata*; *12–13*, *P.lagascae*

Because Simpson's diversity index is sensitive to the number of groups or samples and size of the group, the variation may be attributed to the unequal number of individuals per sample in the study (Dillon et al. 1993). However, Simpson's index has been successfully used to discriminate among microorganisms (Dillon et al. 1993; Harth-Chu et al. 2009) and plants (Agostini et al. 2008; Lüdtke et al. 2009), with values slightly greater than those obtained here for *Physalis*.

Genetic Similarity and Relations

Genetic similarities, as shown by ISSRs, did not parallel the morphological variation and ecological preferences observed for the *Physalis species* included in this study. Genetic interspecific similarity values, as calculated with the Nei and Li coefficient, ranged from 0.20 to 0.57. The highest values were observed for the *P. lignescens/P. sulphurea* comparison (0.57) and the lowest for the *P. cinerascens*/*P. virginiana* comparison (0.20). The genetic similarity between *P. lignescens* and *P. sulphurea* contrasts with their growing habits and habitat preferences. *Physalis lignescens* is a perennial herb growing in coniferous forests, whereas *P. sulphurea* is an annual herb found in flood plains (Vargas-Ponce et al. 2003). On the contrary, *P. cinerascens* and *P. virginiana*, although morphologically different, had a very low genetic similarity value, maybe reflecting that both species are perennial herbs growing in a tropical, dry forest. In future studies, sampling must be widened to better represent the genus *Physalis*, all of its taxonomical sections, and the diversity in biological form and gradients in its ecological and geographical habitats.

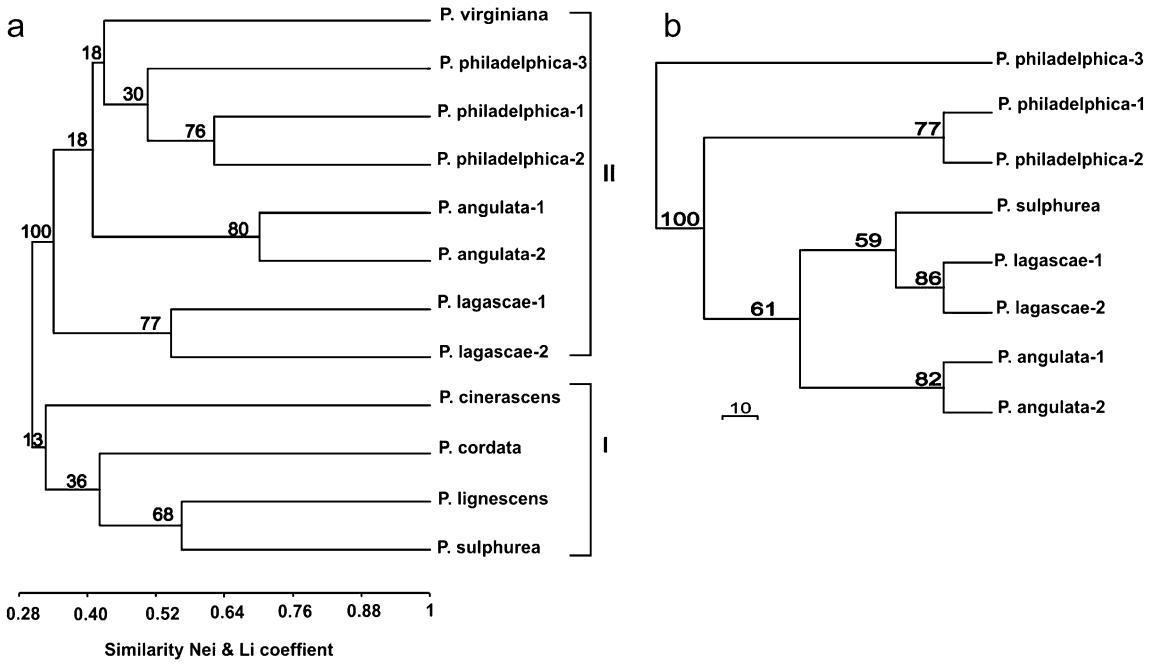

Fig. 2 Genetic similarity relationship among eight *Physalis* species (**a**) and among *Physalis* species of *Section Angulatae* (**b**). The bootstrap values are shown on the *branches* (1,000 permutations)

The genetic similarity values between species pairs in *Physalis* section *Angulatae* ranged from 0.24 to 0.59: *P. philadelphica/P. angulata* (0.32–0.59), *P. philadelphica*/*P. lagascae* (0.24–0.39), *P. philadelphica*/*P. sulphurea* (0.25), and *P. lagascae*/*P. angulata* (0.36–0.45). Intraspecific similarity values were highest for the three species of the *Physalis* section *Angulatae*: *P. angulata* (0.71), *P. philadelphica* (0.63), and *P. lagascae* (0.55). Similar values have been reported for *Polygala* (Polygalaceae), *Cunila* (Lamiaceacea), *Guizotia* (Asteraceae), and other genera (Reedy et al. 2002; Agostini et al. 2008; Petros et al. 2008; Lüdtke et al. 2009).

The genetic relations phenogram (Fig. 2) that included all samples showed two principal clusters diverging at the 0.31 phenon level. Cluster one (I) included *P. cinerascens* (*Physalis* sect. *Stellatae*), *P. sulphurea* (*Physalis* sect. *Angulatae*), *P. lignescens* (*Physalis* sect. *Coztomatae*), and *P. cordata* (*Physalis* sect. *Epeteiorhiza*). Cluster two (II) included *P. angulata*, *P. lagascae*, *and P. philadelphica* of *Physalis* sect. *Angulatae*, and one population of *P. virginiana* (*Physalis* sect. *Lanceolatae*). Neither cluster corresponds to the presently accepted intrageneric taxonomic classification (Martínez 1999b). As mentioned already, cluster II encompasses *P. angulata*, *P. lagascae*, *and P. philadelphica*, but excluded *P. sulphurea*, which belongs to the same section (Fig. 2a).

The analysis of distantly related lineages (e.g., *P. cinerascens, P. lignescens,* and *P. virginiana*) might generate incongruence between genetic similarity values and taxonomic section affiliation. As a result, high bootstrap values support the intraspecific nodes but not the interspecific ones. The cluster analysis of *Physalis* sect. *Angulatae* had better resolution and higher boostrap values (Fig. 2b), suggesting an advantage to including closely related species in the analyses. Two important aspects of the genetic similarity analyses were found in the phenograms. First, samples corresponding to the same species grouped together. Second, the genetic differentiation among *Physalis* species corresponded to the observed morphological variation between them, indicating that distinctive morphological traits can be used to differentiate the species. For example, the small corolla and the small yellow anthers of *P. angulata* separate it from *P. philadelphica* (Vargas-Ponce et al. 2003).

The results demonstrate that ISSR markers are useful in estimating genetic relations in *Physalis*. The markers are also an effective tool for documenting interspecific variability, discriminating species, and assessing intraspecific similarity and differentiation. Moreover, the development of this highly versatile marker for *Physalis* opens the way for implementing a wide range of studies needed to understand, use, and conserve species of husk tomato.

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References

Agostini G, Echeverrigaray S, Souza-Chies TT (2008) Genetic relationships among South American species of *Cunila* D. Royenex L. based on ISSR. Plant Syst Evol 274:135–141

Archibald JK, Crawford DJ, Santos-Guerra A, Mort ME (2006) The utility of automated analysis of Inter-Simple Sequence Repeat (ISSR) loci for resolving relationships in the Canary Island species of *Tolpis* (Asteraceae). Am J Bot 93:1154–1162

Arif M, Zaidi NW, Singh YP, Rizwanul-Haq QM, Singh US (2009) A comparative analysis of ISSR and RAPDS markers for study of genetic diversity in Shisham (*Dalbergia sissoo*). Plant Mol Biol Report 27:488–495

Axelius B (1996) The phylogenetic relationships of the physaloid genera (Solanaceae) based on morphological data. Am J Bot 83:118–124

Bornet B, Branchard M (2001) Nonanchored Inter Simple Sequence Repeat (ISSR) marker: reproducible and specific tools for genome fingerprinting. Plant Mol Biol Report 19:209–215

D'Arcy WG (1991) The Solanaceae since 1976, with a Review of its Biogeography. In: Hawkes JG, Lester RN, Nee M, Estrada N (eds) Solanaceae III: taxonomy, chemistry and evolution. Royal Botanic Gardens, Kew, pp 75–137

Dillon JR, Rahman M, Yeung K (1993) Discriminatory power of typing schemes based on Simpson's index of diversity for *Neisseria gonorrhoeae*. J Clin Microbiol 31:2831–2833

Doyle J, Doyle J (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Estrada E, Martínez M (1999) *Physalis* (Solanoideae: Solaneae) and allied genera: I. A morphology-based cladistic analysis. In: Nee M, Lester RN, Jessop JP (eds) Solanaceae IV: advances in biology and utilization. The Royal Botanic Gardens, Kew, pp 139–160

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Ge XJ, Zhang LB, Yuan YM, Hao G, Chiang TY (2005) Strong genetic differentiation of the East-Himalayan *Megacodon stylophorus* (Gentianaceae) detected by Inter-Simple Sequence Repeats (ISSR). Biodivers Conserv 14:849–861

Gilbert JE, Lewis RV, Wilkinson MJ, Caligari PDS (1998) Developing an appropriate strategy to assess genetic variability in plant germplasm collections. Theor Appl Genet 98:1125–1131

Gomes S, Martins-Lopes P, Lopes J, Guedes-Pinto H (2009) Assessing genetic diversity in Olea europaea L. using ISSR and SSR markers. Plant Mol Biol Report 27:365–373

Harth-Chu E, Espejo RT, Christen R, Guzmán CA, Höfle MG (2009) Multiple-locus variable-number tandem-repeat analysis for clonal identification of *Vibrio parahaemolyticus* isolates by using capillary electrophoresis. Appl Environ Microbiol 75:4079–4088

Hunter PR, Gaston MA (1988) Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol 26:2465–2466

Lüdtke R, Agostini G, Sfoggia Miotto ST, Souza-Chies TT (2009) Characterizing *Polygala* L. (Polygalaceae) species in Southern Brazil using ISSR. Plant Mol Biol Report 28:317–323

Martínez M (1998) Revisión de *Physalis* sección *Epeteiorhiza* (Solanaceae). Ann Ins Biol Bot 69:71–117

Martínez M (1999a) *Physalis hunzikeriana* (Solanaceae: Solanoideae), una nueva especie del norte de México. Kurtziana 27:383–385

Martínez M (1999b) Infrageneric taxonomy of *Physalis*. In: Nee M, Lester RN, Jessop JP (eds) Solanaceae IV: Advances in biology and utilization. Royal Botanic Gardens, Kew, pp 275–284

Martínez J, Colunga-GarcíaMarín P, Zizumbo-Villarreal D (2008) Genetic erosion and in situ conservation of Lima bean (*Phaseolus lunatus* L.) landraces in its Mesoamerican diversity center. Genet Resour Crop Evol. doi:10.1007/s10722-008-9314-1

Matos M, Pinto-Carnide O, Benito C (2001) Phylogenetic relationships among Portuguese rye based on isozyme, RAPD and ISSR markers. Hereditas 134:229–236

Mort ME, Crawfordl DJ, Santos-Guerra A, Francisco-Ortega J, Esselman EJ, Wolfe AD (2003) Relationships among the Macaronesian members of *Tolpis* (Asteraceae: Lactuceae) based upon analyses of Inter Simple Sequence Repeat (ISSR) markers. Taxon 52:511–518

Nan P, Shi S, Peng S, Tiang C, Zhong Y (2003) Genetic diversity in *Primula obconica* (Primulaceae) from central and South-West China as revelead by ISSR Markers. Ann Bot (Lond) 91:329–333

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restrictions endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Olmstead EG, Bohs L, Migid HA, Santiago-Valentin E, García VF, Collier SM (2008) A molecular phylogeny of the Solanaceae. Taxon 57:1159–1181

Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358

Pavlicek A, Hrda S, Flegr J (1999) FreeTree—Freeware program for construction of phylogenetic trees. Folia Biol (Praha) 45:97–99

Petros Y, Merker A, Zeleke H (2007) Analysis of genetic diversity of *Guizotia abyssinica* from Ethiopia using inter simple sequence repeat markers. Hereditas 144:18–24

Petros Y, Merker A, Zeleke H (2008) Analysis of genetic diversity and relationships of wild *Guizotia* species from Ethiopia using ISSR markers. Genet Resour Crop Evol 55:451–458

Prasanta KK, Srivastava PP, Awasthi AK, Raje US (2008) Genetic variability and association of ISSR markers with some biochemical traits in mulberry (*Morus* spp.) genetic resources available in India. Tree Genet Genomes 4:75–83

Reedy MP, Sarla N, Sidiq EA (2002) Inter Simple Sequence Repeat (ISSR) polymorphism and its aplicattion in plant breeding. Euphytica 128:9–17

Sanguinetti C, Diaz Nieto F, Simpson AJ (1994) Rapid silver staining and recovery of PCR products separated on acrylamide gels. Biotechniques 17:915–918

Seithe A, Sullivan J (1990) Hair morphology and systematic of *Physalis*. Plant Syst Evol 170:193–204

Sullivan JR (1985) Systematics of the *Physalis viscosa* complex. Syst Bot 10:426–444

Vargas-Ponce O, Martínez M, Dávila P (1999) *Physalis waterfallii* (Solanaceae), una especie nueva de los estados de Jalisco y Michoacán. Acta Bot Mex 48:21–26

Vargas-Ponce O, Martínez M, Dávila P (2001) Two new species of *Physalis* (Solanaceae) endemic to Jalisco, México. Brittonia 53:505–510

Vargas-Ponce O, Martínez M, Dávila P (2003) La familia Solanaceae en Jalisco: el género *Physalis*. Colección Flora de Jalisco16. Universidad de Guadalajara, Guadalajara, pp 1–127 pp

Vargas-Ponce O, Zizumbo-Villarreal D, Martínez-Castillo J, Coello-Coello J, Colunga-GarcíaMarín P (2009) Diversity and structure of landraces of agave grown for spirits under traditional agriculture: a comparison with wild populations of *A. angustifolia* (Agavaceae) and commercial plantations of *A. tequilana*. Am J Bot 96:448–457

Waterfall UT, Waterfall UT (1967) *Physalis* in Mexico, Central America, and the West Indies. Rhodora 69:82–120, pp. 203–239; 319–329

Whitson M, Manos PS (2005) Untangling *Physalis* (Solanaceae) from the physaloids: a two-genes phylogeny of the Physalinaeae. Syst Bot 30:216–230

Wolfe AD, Randle CP (2001) Relationships within and among species of the holoparasitic genus *Hyobanche* (Orobanchaceae) inferred from ISSR banding patterns and nucleotide sequences. Syst Bot 26:120–130

Yeh FC, Boyle TJB (1999) Popgene v. 1.31. Microsoft Windows-based freeware for population analysis. University of Alberta and Centre for International Forestry Research, Edmonton

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176–183