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Original article

Variation in genetic, morphological, colorimetric and flavor traits of two traditional *Spondias purpurea* L. variants: 'Tuspana abal' and 'Tuspeña abal'

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Summary

Introduction – ***Spondias purpurea* is a fruit tree cultivated since prehispanic times by ethnic groups like Mayan Yucatec. Two *S. purpurea* variants received very similar names in the Yucatan Peninsula: 'Tuspana abal' and 'Tuspeña abal'. The resemblance in name and uses between these two variants could indicate likeness in fruit morphology and even genetic similarity.** *Materials and methods* – **Thirty trees distributed in 18 backyards in Yucatan and another 30 in two commercial orchards in Campeche were sampled to obtain biological material of 'Tuspana abal' and 'Tuspeña abal', respectively. Morphological, colorimetric and flavor data were recorded for unripe and ripe fruits. ISSR DNA fragments were amplified from purified genomic DNA of foliar tissue; and its absence/presence used to estimate the genetic diversity (He) and the polymorphic loci percentage (PLP) for each variant.** *Results and discussion* – **The colorimetric traits were correlated with variation among unripe and ripe fruits, with larger phenotypic variation of 'Tuspana abal' fruits. Genetic differentiation between the two variants was θ = 0.1509. Only ripe fruits 'Tuspana abal' and 'Tuspeña abal' were clearly grouped apart.** *Conclusion* – **The traditional 'Tuspana abal' and 'Tuspeña abal' differed genetically and phenotypically, suggesting that these two variants originate from different genetic lineages and constitute distinct phenotypical entities, distinguishing mainly by colorimetric traits of ripe fruits.**

Keywords
Mexico, Mexican plum, red mombin, *Spondias purpurea*, genetic resource management, fruit quality

Résumé

Variation des caractères génétiques, morphologiques, colorimétriques et aromatiques de deux variantes traditionnels de *Spondias purpurea* L.: 'Tuspana abal' and 'Tuspeña abal'.

Introduction – ***Spondias purpurea* est un arbre fruitier cultivé depuis l'époque préhispanique par des groupes ethniques tels les mayas yucatèques.**

Significance of this study

What is already known on this subject?
- Two *Spondias purpurea* variants cultivated by the Mayan ethnic group received very similar names suggesting likeness in fruit morphology or genetic similarity.

What are the new findings?
- The two variants originate from different genetic lineages and constitute distinct phenotypical entities, differing mainly by colorimetric traits of the ripe fruit.

What is the expected impact on horticulture?
- Inclusion of colorimetric traits as test guidelines for legal protection of *S. purpurea* variants is advisable. Nutritional traits associated with differences in color matrix may further enhance the commercial value of the two variants.

Deux variantes de *S. purpurea* ont reçu des noms très semblables dans la péninsule du Yucatan: 'Tuspana abal' et 'Tuspeña abal'. La ressemblance de nom et d'utilisation entre ces deux variantes pourrait indiquer une similarité morphologique du fruit et une même génétique. *Matériel et méthodes* – **Trente arbres distribués dans 18 arrière-cours du Yucatan et 30 autres dans deux vergers commerciaux à Campeche ont été échantillonnés pour obtenir le matériel biologique 'Tuspana abal' et 'Tuspeña abal', respectivement. Les données morphologiques, colorimétriques et gustatives ont été enregistrées pour les fruits mûrs et avant maturité. Les fragments d'ADN ISSR ont été amplifiés à partir d'ADN génomique purifié de tissu foliaire et leur absence/présence utilisée pour estimer la diversité génétique (He) et le pourcentage de loci polymorphes (PLP) de chaque variant.** *Résultats et discussion* – **Les caractères colorimétriques ont été corrélés à la variation entre les fruits mûrs et non mûrs, avec une plus grande variation phénotypique des fruits de 'Tuspana abal'. La différenciation génétique entre les deux variantes était θ = 0,1509. Seuls les fruits mûrs de 'Tuspana abal' et 'Tuspeña abal' étaient clairement groupés.** *Conclusion* – **Les variantes traditionnels 'Tuspana abal' et 'Tuspeña abal' diffèrent génétiquement et phénotypiquement, suggérant qu'ils proviennent**

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de lignées génétiques différentes et constituent des entités phénotypiques distinctes, se distinguant principalement par des traits colorimétriques sur fruits mûrs.

Mots-clés
Mexique, prunier d'Espagne, mombin rouge, *Spondias purpurea*, gestion des ressources génétiques, qualité du fruit

Introduction

The Mexican plum (*Spondias purpurea*) is a fruit tree native to the Neotropics where it has been cultivated for centuries (Miller and Spondias, 2011; Ruenes-Morales *et al*., 2010). Known by a myriad common names (*e.g.,* ciruela Mexicana, jocote, red mombin, hog plum, *etc*.), it is found throughout Mesoamerica and South America, where it is generally cultivated for its fruit in traditional agroecosystems such as backyards and living fences (Miller and Spondias, 2011; Miller and Knouft, 2006). In rare cases, it is grown in large parcels (Miller and Spondias, 2011). Mexican plum accounts for less than 1% of the area planted in fruit trees in Mexico, while in the state of Yucatan the corresponding area is less than 0.1% (SIACON, 2010).

Propagation of Mexican plum is clonal, using cuttings (Miller and Spondias, 2011; Ruenes-Morales *et al*., 2010; Cuevas, 1994). As a result, very few genotypes are used in a given cultivation area, leading to low genetic diversity within a system and high genetic differentiation between systems (Miller and Schaal, 2006). Variation in fruit size, color and flavor between cultivated variants has been well studied (Miller and Spondias, 2011; Ruenes-Morales *et al*., 2010; Cuevas, 1994; Miller and Schaal, 2006; Mitchell and Daly, 2015; Avitia-González *et al*., 2003; Macía and Barfod, 2000). Quantitative studies of fruit morphology, colorimetry and flavor have identified wide variation among variants grown in different regions and states of Mexico: central-west – Jalisco and Colima (Ramírez-Hernández *et al*., 2008); central-south and southwest – Morelos, Guerrero, Oaxaca and Chiapas (Alia-Tejacal *et al*., 2012; Pérez-Arias *et al*., 2008); southeast – Tabasco (Vargas-Simón *et al*., 2011); and east – Veracruz (Nava-Kuri and Uscanga, 1979).

The Yucatan Peninsula has the highest diversity of traditional *S. purpurea* variants, although these have received only limited attention (Ruenes-Morales *et al*., 2010; Cuevas, 1994; García de Miguel, 2004). Sixteen variants have been identified to date in the region; most identified using traditional Maya nomenclature. Maya ethnobotanical nomenclature is a complex classification system utilizing morphemes of a species' (or even subspecies') characteristic variables, and lexemes that allow taxonomic classification. Indeed, a species' Mayan name often corresponds to a current botanical classification (Barrera *et al*., 1976). An example of this correspondence at the genus level is the lexeme *abal*, containing the morphemes *ab* ("becomes limp") and *al* (an abstraction suffix), which matches the *Spondias* genus (Ruenes-Morales *et al*., 2010; Barrera *et al*., 1976). Classifying morphemes are mainly associated with the *Chakoob* morphemes (*i.e.*, the sacred colors the Maya associated with the four cardinal points), which can consist of lexemes at the species or variant level (Barrera *et al*., 1976). For example, the three *S. purpurea* variants 'Chak abal', 'Ek' abal' and 'K'an abal' correspond respectively to the sacred colors of red, black and yellow (Ruenes-Morales *et al*., 2010; Barrera *et al*., 1976). The Maya terms *nuc abal*, *ycan abal*, *yxhouen abal* and *zabac abal* refer to green, yellow, red and purple fruit variants (Morales, 2006). Morphemes not associated with colors are used to indicate sub-genus taxa (Barrera *et al*., 1976); for example, *Chi abal, Keken abal* and *Campech abal* are variants of *S. purpurea* (Ruenes-Morales *et al*., 2010, 2016). Variants with different classificatory lexemes within the *abal*s have varying fruit color, flavor and shape traits that inform Maya speakers of the type of *abal*s they are growing, buying and/or eating (Ruenes-Morales *et al*., 2010, 2016).

The Yucatec Maya have given to the *S. purpurea* variants very similar names: 'Tuspana abal' and 'Tuspeña abal'. The resemblance in name and uses between these two variants could indicate likeness in fruit morphology and even genetic similarity. The 'Tuspana abal' variant, also known as *Tuspana de Yucatan*, is grown in the "Mayan solar" (backyards which are maintained by the family unit) largely for home use, although excess production is sold on the local and regional markets either fresh or processed as preserves. The 'Tuspeña abal' variant, also known as *Tuspeña campechana* or *Tuxpeña abal*, is grown in commercial orchards for sale in regional markets. Both belong to the group of *abal*s of intermediate fruit size. They are eaten, like most Mexican plum fruit, when physiologically immature (a condition known as *dzipon* in Maya), and are often preserved in sugar or alcohol (Ruenes-Morales *et al*., 2010; García de Miguel, 2004). The similarity in their Mayan names can cause confusion in identifying and marketing these two variants, especially because when unripe the fruit from both are quite similar in color, only manifesting clear differences once ripe.

Mexican plum cultivation is widespread on the Yucatan Peninsula, where it is grown in family backyards and commercial orchards. This raises the possibility of differentiation between potentially closely related variants such as 'Tuspana abal' and 'Tuspeña abal', and, if cutting exchange is limited to the local level, among the same variant at different locations. In contrast, variation among the 'Tuspeña abal' variant cultivated commercially in the Camino Real de Campeche region is probably much more limited because commercial orchards commonly use a limited pool of mother plants. Morphological, colorimetric and total soluble solids values may exhibit similar patterns between 'Tuspana abal' and 'Tuspeña abal', although 'Tuspana abal' is more likely to exhibit higher variability since management practices are much more heterogeneous in backyards than on commercial plantations. The present study objective was to identify traits useful in distinguishing between these two variants on the Yucatan Peninsula. To this end genetic diversity was compared between the variants using ISSRs DNA markers; while variation in morphological, colorimetric and flavor traits was evaluated in unripe and ripe Mexican plum fruit collected in two commercial plantations and in eighteen backyards from six localities from Yucatan Peninsula.

Materials and methods

Study area and sampling

Samples were collected of unripe and ripe fruit, and young leaves. Sampling sites were selected in the states of Campeche and Yucatan, Mexico, based on three criteria: 1) parcel or household owner granted permission to collect samples; 2) presence of at least three individuals of one of the studied variants per sample site; and 3) variants present that were identified by local people as 'Tuspana abal',

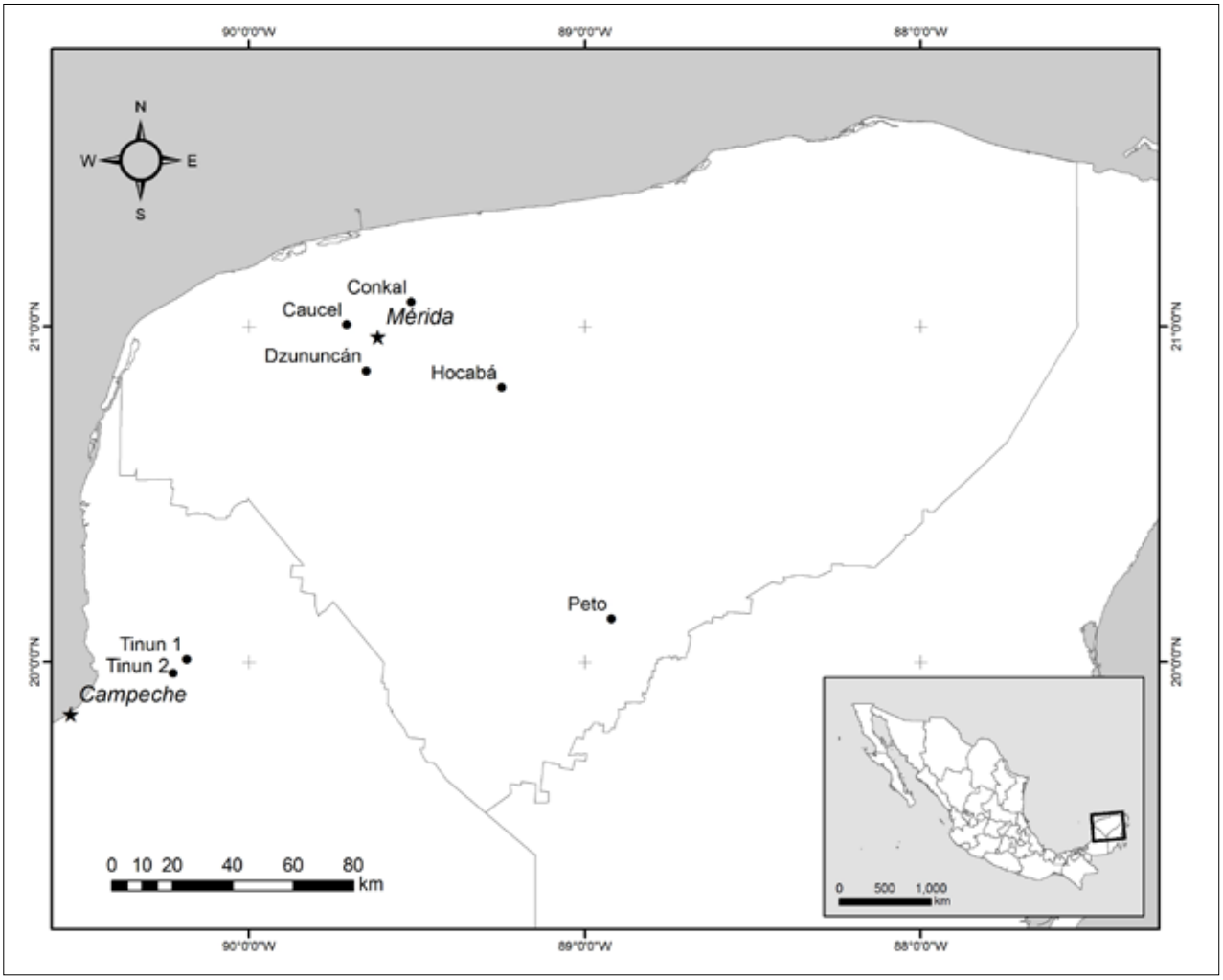

Figure 1. 'Tuspana abal' and 'Tuspeña abal' (*Spondias purpurea*) sample site locations in the states of Campeche and Yucatan, Mexico.

Tuspana or *Tuspana yucateca*, and 'Tuspeña abal', *Tuxpeña abal* or *Tuspana campechana*. Using these criteria, two commercial orchards in Campeche, and eighteen backyards in Yucatan were chosen for sampling (Figure 1; Table 1). The two commercial orchards in Campeche were Tinún 1 and Tinún 2, and the backyards were located in the state of Yucatan in Caucel, Conkal, Dzununcan-San José Tzal (Dzununcan hereafter), Hocabá and Peto (Table 1). Thirty trees were identified and sampled in Campeche and another thirty in Yucatan. Three collections were done: twelve unripe fruit per tree in May–June 2015; six to twelve ripe fruit per tree collected three weeks after initial visit (variability due to predation by birds and weather); and young leaves (leaflets < 1 cm long) collected in July 2016. All collected samples were placed in sealed plastic bags for transport and then stored at -20 °C in the Ecophysiology and Biodiversity Laboratory of the Autonomous University of Yucatan.

Table 1. Number of backyards/orchards, trees and fruits sampled per location and per *Spondias purpurea* variant.

Variants Locations	Backyards/ orchards	Unripe fruits		Ripe fruits	
		Trees	Fruits	Trees	Fruits
'Tuspana abal'	18	30	359	23	264
Caucel	3	5	60	5	60
Conkal	4	6	72	1	12
Dzununcan	4	7	83	7	84
Hocabá	3	5	60	3	24
Peto	4	7	84	7	84
'Tuspeña abal'	2	30	360	29	285
Tinún 1	1	19	228	19	183
Tinún 2	1	11	132	10	102
Total	20	60	719	52	549

Genetic variation among and within 'Tuspana abal' and 'Tuspeña abal' variants

Leaves from each sampled tree were ground separately using liquid nitrogen. Fifty milligram portions of this lyophilized leaf tissue were used to isolate genomic DNA with the DNeasy Plant Mini kit (No. Cat. 69104, QIAGEN), following manufacturer instructions. Using material from ten of the studied individual trees, ISSR amplification was done using nine primers (Primers UBC 808, 810, 818, 842, 857, 854, 872, 881, 891; Biotechnology Laboratory, University of British Columbia, Canada). Because primers 818 and 891 produced consistent band patterns they were used to amplify all individuals. Reactions for PCR amplification (15 µL total volume) were 20 ng DNA, 6.8 µL GoTaq (2X; Promega®), 1.5 µL primer (35 mM) and 5.3 µL ultrapure water. A thermocycler (Select Cycler II, Select BioProducts®) was used to run the PCR amplification under these conditions: 94 °C for 4 min; 40 cycles at 94 °C for 30 sec; 56 °C for 30 sec; 72 °C for 4 min; and 72 °C for 10 min. Four negative controls (containing water instead of genomic DNA) were run for each primer to evaluate dimeric amplification and sample contamination. The amplified products were separated in 6% CE polyacrylamide gels under constant voltage (85 V) electrophoresis with TBE 0.5 X buffer for approximately 1 h 15 min. After staining with a silver nitrate/benzenic-sulphuric acid solution, the DNA fragments were viewed (Westermeier, 2016). Fragment molecular weights were calculated using the 100 bp DNA ladder (BioLabs) as a reference. Presence and absence of fragments for each individual was documented with a binary data matrix. Variation in genetic data was reduced with a principal coordinates analysis using the Simpson similarity index and a quadratic transformation. Analyses were run with the PAST v. 3.0 program (Hammer *et al.*, 2001). The values for the first two coordinates were analyzed heuristically to evaluate grouping of fruit based on the two variants ('Tuspana abal' and 'Tuspeña abal') and place of origin (Tinún 1, Tinún 2, Caucel, Conkal, Dzununcan, Hocabá and Peto). Values were also calculated for Nei genetic diversity (He), polymorphic loci percentage (PLP) at a 95% criterion per variant. Genetic differentiation (θ) between variants was obtained and confidence interval values were obtained from 1,000 bootstrap replicates between loci. Analyses were run with the TFPGA program (Miller, 1997).

Morphological, colorimetric and flavor variations in fruit

Morphological, colorimetric and flavor data were recorded for unripe and ripe fruit, including four traits for the whole fruit; fifteen for the epicarp: two for the mesocarp and five for the endocarp (Table 2). To create an image database of the collected fruit, the fruit from each tree were photographed (SONY® DSLR-α230) on a white background with a scale. They were then weighed on an analytical scale, mesocarp width measured with a manual Vernier, and total soluble solids (°Brix) measured with a manual refractometer (ATAGO®, Master Refractometer 0.0–33%). Fruit pulp was removed, and the endocarp weighed and photographed. The resulting digital images were used to estimate length, width and area for the fruit, endocarp and endocarp protuberance. Estimates were made using the ImageJ program (Schindelin *et al.*, 2015) (see Figure 2).

Epicarp colorimetry was analyzed in the red + green + blue luminous color composition space. The ImageJ program was used to estimate minimum, maximum and mean values

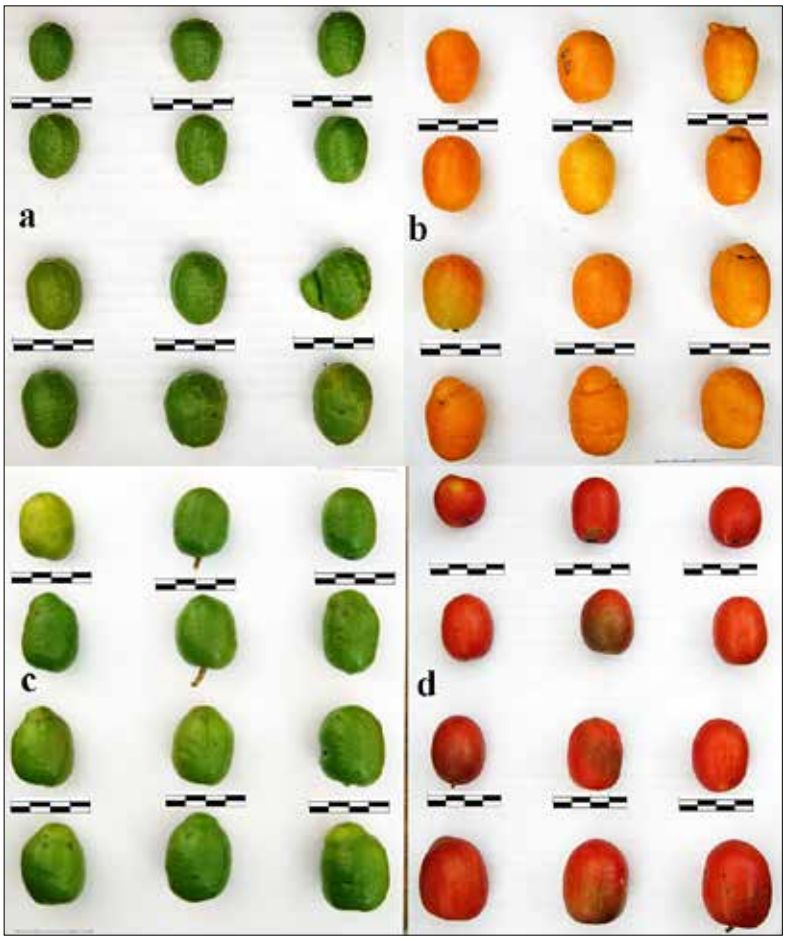

Figure 2. Examples of unripe (a and c) and ripe fruits (b and d) from the *Spondias purpurea* variants 'Tuspana abal' (a and b) and 'Tuspeña abal' (c and d).

Table 2. Means and standard errors (SE) of morphological, flavor and color traits evaluated for unripe and ripe fruits of the *Spondias purpurea* variants 'Tuspana abal' and 'Tuspeña abal'.

	Unripe fruits						Ripe fruits					
	'Tuspana abal'			'Tuspeña abal'			'Tuspana abal'			'Tuspeña abal'		
Fruit traits	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE
Weight	30	14.40	0.45	30	13.94	0.38	22	15.31	0.69	29	16.87	0.68
Length	30	3.69	0.05	30	4.11	0.06	22	3.81	0.07	29	3.75	0.05
Width	30	2.77	0.03	30	3.00	0.05	22	2.87	0.04	29	2.93	0.04
Area	30	7.61	0.16	30	7.68	0.24	22	7.95	0.29	29	8.34	0.22
Pericarp traits	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE
Red mean	30	62.39	4.29	30	37.75	1.07	22	167.89	4.33	29	72.00	2.16
Red minimum	30	18.36	2.43	30	5.68	0.48	22	57.46	2.62	29	20.06	1.46
Red maximum	30	150.95	10.28	30	84.14	1.71	22	239.85	4.89	29	115.87	2.93
Green mean	30	66.16	3.91	30	54.18	0.92	22	88.73	3.11	29	21.02	0.71
Green minimum	30	19.18	1.84	30	19.06	0.57	22	23.41	1.99	29	0.89	0.15
Green maximum	30	147.23	10.08	30	91.27	1.39	22	211.89	5.32	29	100.94	2.43
Blue mean	30	21.90	3.32	30	9.38	0.42	22	27.51	1.55	29	7.46	0.72
Blue minimum	30	3.19	0.82	30	0.00	0.00	22	0.51	0.21	29	0.00	0.00
Blue maximum	30	130.43	11.13	30	58.76	1.54	22	192.23	5.22	29	93.51	2.73
Mean color value	30	50.15	3.77	30	33.77	0.67	22	94.71	2.66	29	32.75	1.21
Minimum color value	30	15.68	1.93	30	9.67	0.32	22	33.13	1.64	29	9.55	0.70
Maximum color value	30	141.23	10.56	30	75.32	1.42	22	212.00	5.31	29	99.30	2.63
Mean brightness	30	59.98	3.92	30	44.16	0.82	22	105.43	2.96	29	34.63	1.04
Minimum brightness	30	18.60	1.99	30	14.48	0.44	22	34.95	1.80	29	9.88	0.65
Maximum brightness	30	145.31	10.32	30	83.53	1.44	22	216.16	5.30	29	101.42	2.55
Mesocarp traits	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE
Width	30	2.23	0.71	30	0.78	0.02	22	0.75	0.03	29	0.77	0.05
Soluble solids	30	11.41	0.84	30	9.70	0.19	22	18.72	0.33	29	15.73	0.33
Endocarp traits	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE
Weight	30	1.40	0.06	29	1.60	0.05	22	1.46	0.07	29	1.60	0.05
Length	30	2.51	0.03	19	2.74	0.07	22	2.52	0.04	19	2.74	0.07
Width	30	1.36	0.02	19	1.66	0.03	22	1.36	0.02	19	1.66	0.03
Protuberance length	22	0.11	0.02	19	0.13	0.04	20	0.47	0.02	12	0.54	0.02
Protuberance width	22	0.11	0.02	19	0.15	0.04	20	0.50	0.03	12	0.62	0.02

for red, green and blue, color value [(red + green + blue)/3], and color brightness (luma; 0.299 red + 0.587 green + 0.114 blue) (Schindelin *et al*., 2015).

Variation within the analyzed traits was reduced with a principal component analysis (PCA) run separately for unripe and ripe fruit using the variance-covariance matrix and Euclidean distances. Analyses were run with the PAST v. 3.0 program (Hammer *et al*., 2001). The values of the first two components were analyzed heuristically to evaluate grouping of fruit by variant ('Tuspana abal' and 'Tuspeña abal') and place of origin (Tinún 1, Tinún 2, Caucel, Conkal, Dzununcan, Hocabá and Peto). To determine if the analyzed traits differed between the 'Tuspana abal' and 'Tuspeña abal' variants, the first principal components for the unripe and ripe fruit were analyzed separately considering independent variables in a nested ANOVA. This analysis was run with the JMP v 5.1.2 program (SAS Institute, 1989–2007). Individual trees and places of origin were considered random effects while the variants were treated as fixed effects. Trees were nested within place of origin and place of origin within variants. Variation of the set of traits with correlations > 3.0 or < -0.3 were analyzed with a MANOVA run separately for unripe and ripe fruit; again, trees were nested within place of origin and place of origin within variants. This analysis was run with the JMP v 5.1.2 program (SAS Institute, 1989–2007).

Genetic and phenotypical variation between 'Tuspana abal' and 'Tuspeña abal'

Averages for each of the analyzed traits, by tree, were organized into a new data matrix and joined with the genetic data. Using this matrix, a principal coordinates analysis was run using Euclidean distances to reduce observed variation within 'Tuspana abal' and 'Tuspeña abal' trees. This analysis was run with the PAST v. 3.0 program (Hammer *et al*., 2001). The heuristic analysis of grouping by variant ('Tuspana abal' and 'Tuspeña abal') and place of origin (Tinún 1, Tinún 2, Caucel, Conkal, Dzununcan, Hocabá and Peto) was done by graphing the principal coordinate values.

Results and discussion

Genetic variation between 'Tuspana abal' and 'Tuspeña abal' trees

The first two axes explained 30.34% of the variation, while the first ten axes explained 61.50% (Table 3). The first axis separated most of the 'Tuspana abal' trees from the 'Tuspeña abal' trees. The first two axes grouped the 'Tuspana abal' trees in quadrants I, II and III, and the 'Tus-

Table 3. Eigenvalues and percentage variation for component or principal axis (variation) and cumulative for principal component analysis of the *Spondias purpurea* variants 'Tuspana abal' and 'Tuspeña abal', for unripe and ripe fruit morphological traits, for values by tree (average), and for principal coordinates analysis for genetic data, data averages and joint genetic data.

Analyses		Component or principal axis									
		1	2	3	4	5	6	7	8	9	10
Genetic	Eigenvalue	4.10	3.37	2.06	1.36	1.00	0.84	0.80	0.59	0.53	0.50
	Variation	16.65	13.69	8.34	5.52	4.07	3.39	3.25	2.40	2.15	2.04
	Cumulative	16.65	30.34	38.67	44.20	48.27	51.66	54.91	57.31	59.46	61.50
Unripe fruits	Eigenvalue	16798.60	447.16	153.58	77.22	68.33	24.20	19.04	16.84	9.94	6.45
	Variation	95.26	2.54	0.87	0.44	0.39	0.14	0.11	0.10	0.06	0.04
	Cumulative	95.26	97.80	98.67	99.11	99.49	99.63	99.74	99.83	99.89	99.93
Ripe fruits	Eigenvalue	24752.30	954.70	481.24	243.73	142.95	54.75	39.19	33.88	18.16	9.58
	Variation	92.53	3.57	1.80	0.91	0.53	0.20	0.15	0.13	0.07	0.04
	Cumulative	92.53	96.10	97.89	98.81	99.34	99.54	99.69	99.82	99.89	99.92
Joint	Eigenvalue	1690100.00	626990.00	48234.00	27910.00	13214.00	8298.30	5538.70	3467.60	2044.50	1698.90
	Variation	63.33	23.50	1.81	1.05	0.50	0.31	0.21	0.13	0.08	0.06
	Cumulative	63.33	86.83	88.64	89.68	90.18	90.49	90.70	90.83	90.90	90.97

peña abal' trees in quadrants III and IV. Quadrant I contained the 'Tuspana abal' from Conkal, five from Dzununcan and two from Hocabá. Quadrants II and III included one 'Tuspana abal' from Hocabá and all the 'Tuspeña abal' from Tinún 1 and 2. Quadrant IV contained the 'Tuspana abal' from Caucel, Peto, and one each from Dzununcan and Hocabá (Figure 3).

The Nei genetic diversity index (He) was 0.08 for 'Tuspana abal' and 0.10 for 'Tuspeña abal', while the polymorphic loci percentage (PLP) was 33.33% for 'Tuspana abal' and 40% for 'Tuspeña abal'. Genetic differentiation (θ) between these two variants was 0.1509, (lower confidence interval = 0.0470; upper confidence interval = 0.2491).

Genetic diversity was similar within the two variants, which were genetically differentiated. Values for PLP and He observed in the present results were similar to previously reported value ranges for *S. purpurea* in Central America, Chiapas and western Mexico for backyards (6.5–57.5% and 0.033–0.198, respectively) and orchards (10.0–38.5% and 0.031–0.121, respectively) (Cuevas, 1994). This low genetic variation is linked to the vegetative propagation of *S. purpurea*, a common practice in Mesoamerican agroecosystems (Miller and Schaal, 2006). In contrast, the Brazil plum (also known as Imbu; *S. tuberosa* Arruda) has much higher values (PLP = 80.95–91.67%; He = 0.27–0.34) because it propagates sexually in wild and cultivated populations in Brazil (de Freitas Lins Neto *et al.*, 2013). Even though the studied *S. purpurea* trees were clonally propagated, no identical genotypes were identified in the present results. Two factors may be mainly responsible for this: 1) cuttings are used from different trees in backyards in different locations and commercial orchards; and/or 2) somatic mutations may occur that lead to differentiation between genotypes. The latter is more plausible because producers at Tinún, Conkal, Hocabá, Peto and Caucel commented during sample collection that some of the sampled trees came from the same mother plant.

Genetic differentiation between 'Tuspana abal' and 'Tuspeña abal' was positive, although the value was lower than reported in a comparison of *S. purpurea* populations in ten backyards (0.289 ± 0.016) and six orchards (0.395 ± 0.026) (Miller and Schaal, 2006). This discrepancy can be attributed to the genetic diversity analysis scale used in the different studies; the present analyses were done at a regional scale whereas Miller and Schaal (2006) covered all of Mesoamerica. Nonetheless, the present genetic differentiation results

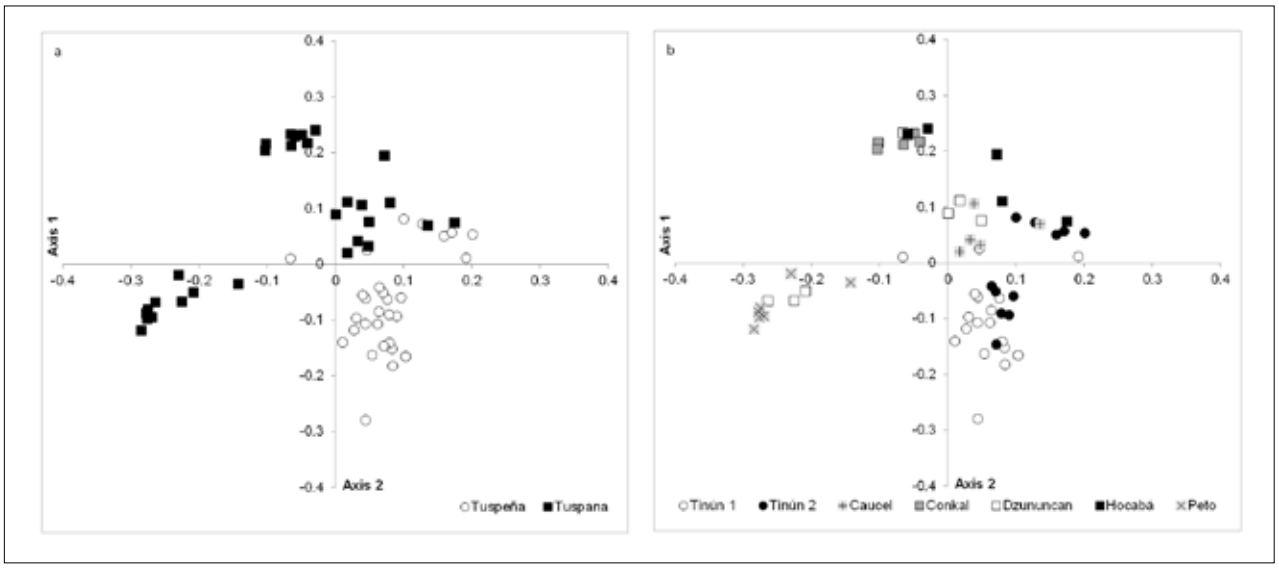

Figure 3. Principal coordinates analysis for genetic variation grouped by variant (a) and place of origin (b).

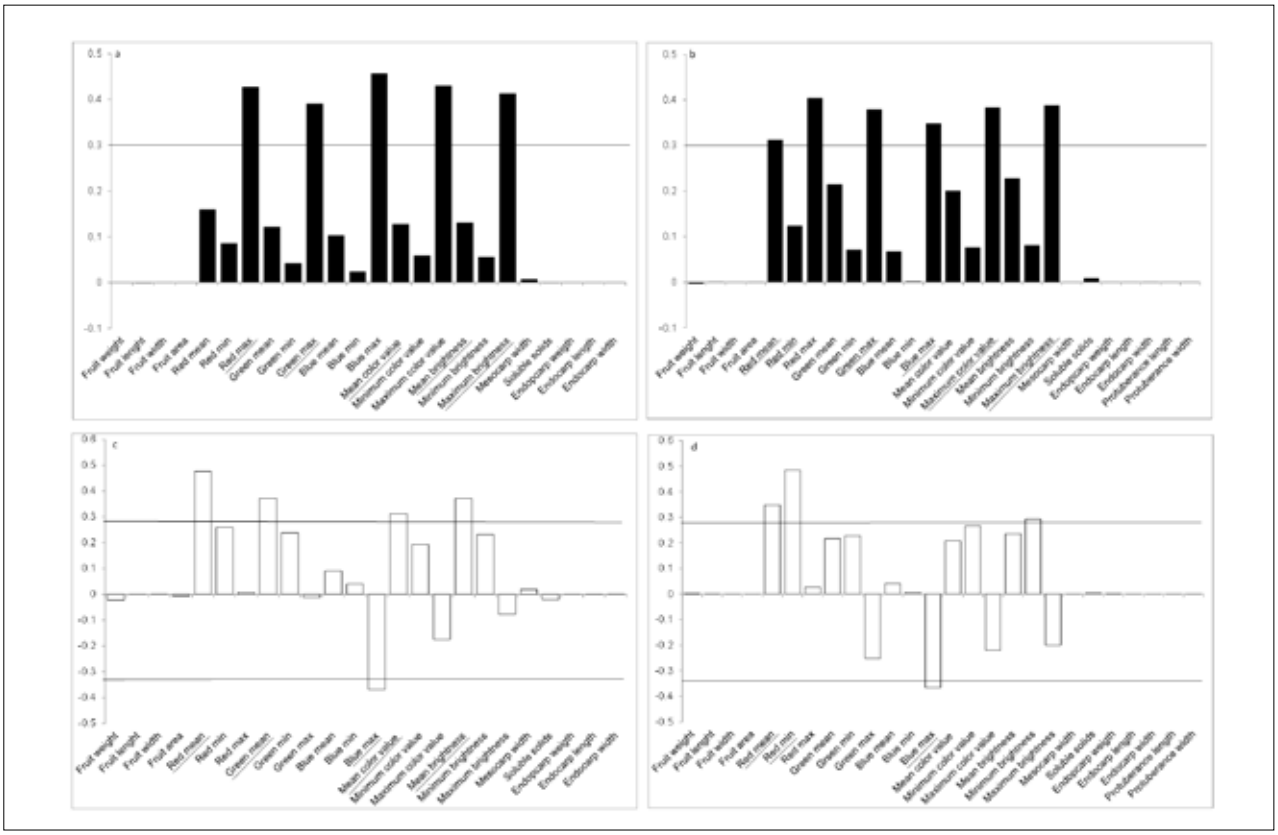

Figure 4. Correlation values for the variables reduced in first (a and b) and second (c and d) principal components for the morphological, colorimetric and flavor traits of unripe fruit (a and c) and ripe fruit (b and d) derived from the principal component analysis of the *Spondias purpurea* variants 'Tuspana abal' and 'Tuspeña abal'.

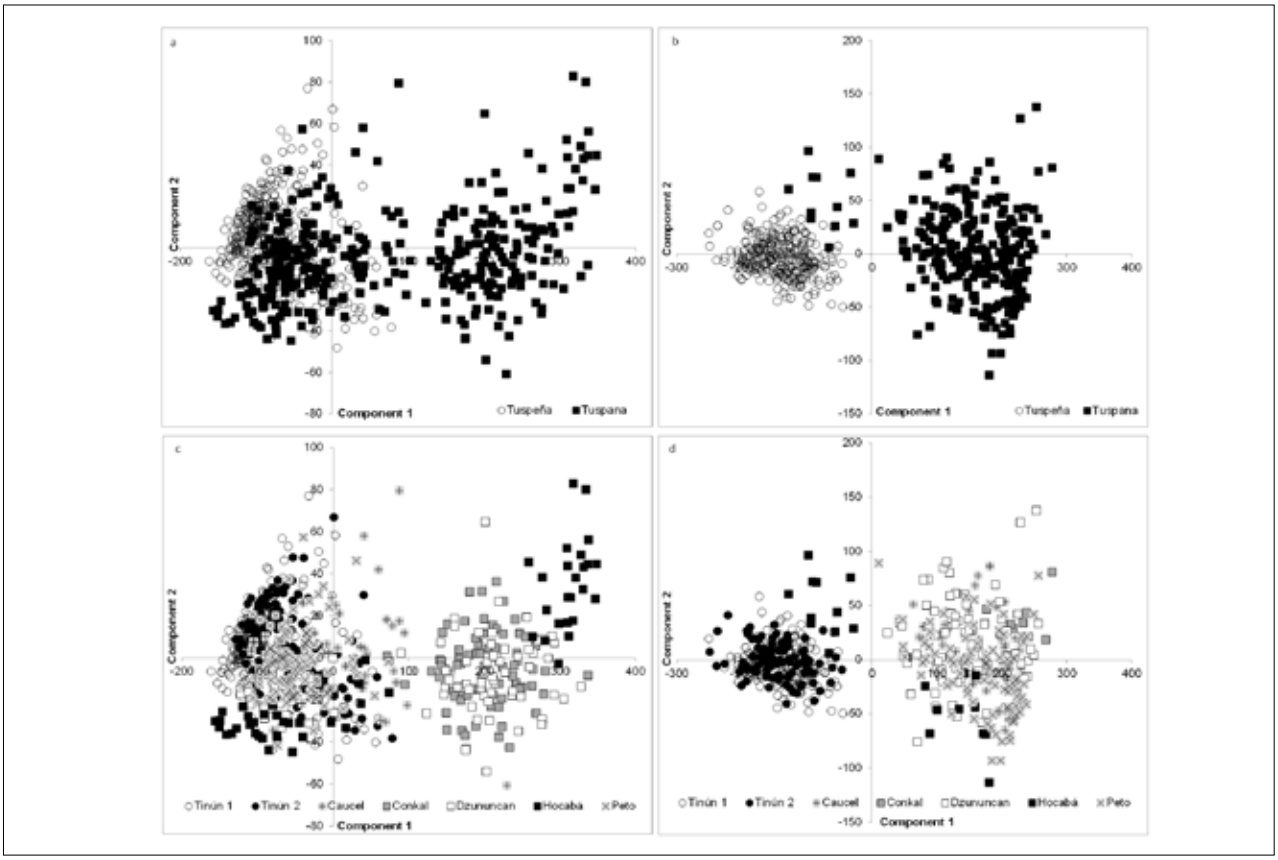

Figure 5. Principal component analysis of the *Spondias purpurea* variants 'Tuspana abal' and 'Tuspeña abal' for morphological, colorimetric and flavor traits variation in unripe fruit (a and c); ripe fruit (b and d); grouped by variant (a and b); and grouped by place of origin (c and d).

indicated that the variants are distinct genotypes. At times they are confused (a 'Tuspeña abal' tree in Hocabá was identified as 'Tuspana abal' by locals), but the genetic lineages of the two variants are different. The graphic analysis also suggested that the sampled 'Tuspana abal' lineages differ between locations (Figure 3). It may be the case that phenotypic variation between these two variants has a strong genetic component, which, particularly in the case of 'Tuspana abal', is augmented by the effects of local management practices.

Morphological, colorimetric and flavor variation in fruit

The variation explained by the first two components was 97.80% for the unripe fruit and 96.10% for the ripe fruit (Table 3). Only the colorimetric traits positively or negatively correlated to the first and second components in analysis of the unripe and ripe fruit (Figure 4). Grouping of the data for unripe and ripe fruit in the quadrants of the first two principal components suggested greater variation in 'Tuspana abal' than in 'Tuspeña abal' (Figures 5a and 5b). The unripe fruit were divided into two groups: 1) all 'Tuspana abal' fruit from Caucel and Peto, some from Dzununcan and Hocabá, and all 'Tuspeña abal' fruit; and 2) all 'Tuspana abal' fruit from Conkal and most of the fruit from Dzununcan and Hocabá (Figure 5c). Ripe fruit was also divided into two groups: 1) 'Tuspana abal' fruit from one tree in Hocabá and all 'Tuspeña abal' fruit; and 2) the remaining 'Tuspana abal' fruit from all the locations in Yucatan (Figure 5d).

Values for the first principal component differed between variants for ripe fruit ($F = 43.04$, df = 15, $P = 0.0012$), but not for unripe fruit ($F = 4.04$, df = 15, $P = 0.1007$). However, in the multivariate analysis, the 'Tuspana abal' variant had higher values for the colorimetric traits for both the unripe ($F = 607.63$, df = 9,650, $P < 0.0001$) and ripe fruits ($F = 1180.02$, df = 7,492, $P < 0.0001$).

Phenotypic variation between 'Tuspana abal' and 'Tuspeña abal' fruit was related to colorimetric traits, but not to morphological or flavor traits (*i.e.*, total soluble solids in the present study). This suggests that perception of ripe fruit color by the human population of the Yucatan Peninsula is important to cataloging these two *S. purpurea* variants. Epicarp color varies among cultivated *S. purpurea* variants, and is a trait used by people in different cultural areas throughout Mexico to distinguish between variants (Ramírez-Hernández *et al.*, 2008; Alia-Tejacal *et al.*, 2012; Vargas-Simón *et al.*, 2011; Nava-Kuri and Uscanga, 1979). When analyzed with the two principal components, variation in these traits was lower in the ripe fruit of 'Tuspeña abal'. This could reflect its management in homogeneous (mono-variety) orchards for commercial fruit production, which would explain the lower genetic variability in samples of this variant. Commercial producers select vegetative material for propagation from individuals that produce fruit with the most appealing traits (shape, color, flavor) and that ripen in the most appropriate season for marketing.

The multivariate analysis comparing the principal component values for morphological, colorimetric and flavor traits suggested that unripe fruit of the two variants could not be distinguished. However, analysis of the colorimetric traits showed the values for color and brightness to be higher in ripe 'Tuspana abal' fruit. Similar color results have been reported for *S. purpurea* variants in the state of Tabasco, Mexico, in which fruit weight and size did not differ, but fruit color did; as a result, variants were distinguished by fruit color, the red fruit variant was called Tuspana *roja* and that with yellow fruit Tuspana *amarilla* (Vargas-Simón *et al.*, 2011). To date, test guidelines for legal protection of varieties of *Spondias purpurea* have not been published within the frame of the International Union for the Protection of New Varieties of Plants (UPOV). Legal protection for this species portraying great commercial potential and an important phenotypic diversity with *ca.* 32 traditional variants identified in Mexico (Avitia-González *et al.*, 2003) should be considered as priority for custodian farmers of this resource. Colorimetric traits should be included, therefore, as guidelines in tests to the Examination of Distinctness, Uniformity and Stability required to protect the varieties of *S. purpurea* under UPOV and national laws of Mexico.

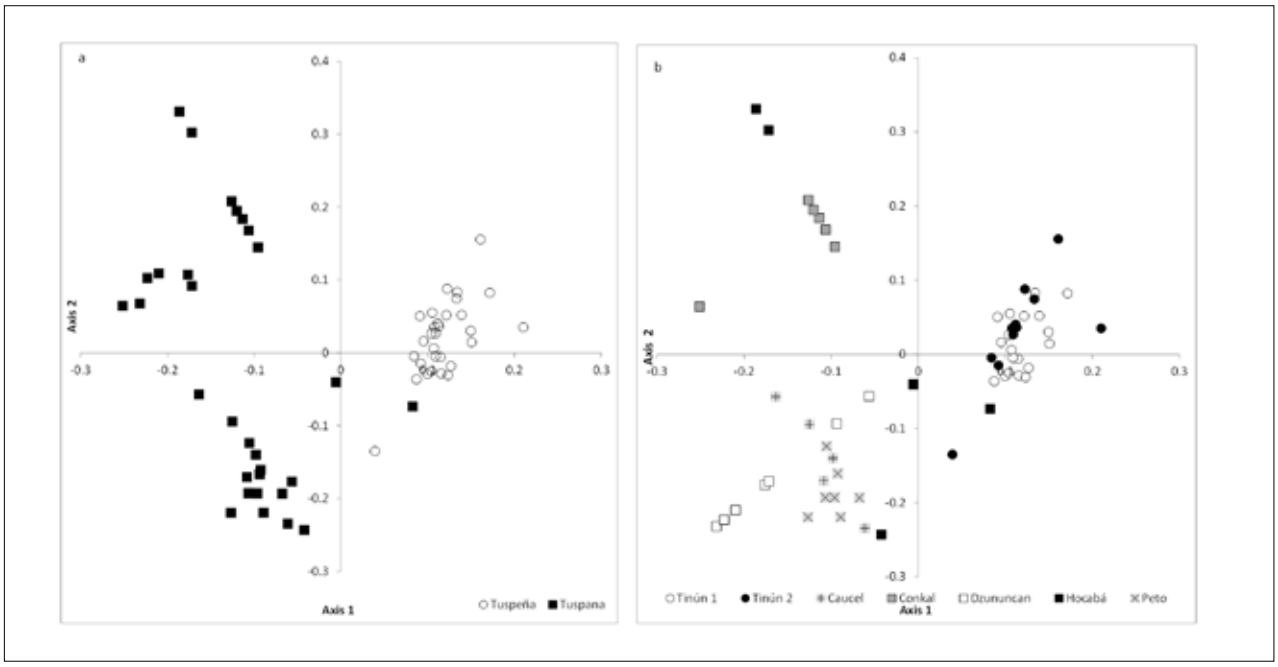

Figure 6. Principal coordinates analysis of the *Spondias purpurea* variants 'Tuspana abal' and 'Tuspeña abal' of joint variation grouped by variant (a) and place of origin (b).

Genetic and phenotypical variation between 'Tuspana abal' and 'Tuspeña abal' trees

In the joint analysis, the first two axes explained 86.83% of the variance; including the next two axes increased variation less than 1.81% (Table 3). The first axis separated all but one of the 'Tuspana abal' trees from the 'Tuspeña abal' trees (Figure 6a). The first two axes grouped all the 'Tuspeña abal' trees from Tinún 1 and 2, except for one Tinún 2 tree isolated in Quadrant IV near a 'Tuspana abal' tree from Hocabá. Quadrant II encompassed the 'Tuspana abal' trees from Conkal and Hocabá, while Quadrant III contained the 'Tuspana abal' trees from Caucel, Dzununcan and Peto (Figure 6b).

In the present study, the only analyzed flavor trait was total soluble solids, although flavor can also be influenced by sugar composition, acidity, and the contents of phenolic compounds, vitamin C and other antioxidant compounds. These chemical characteristics are positively correlated to fruit color values (Solorzano-Morán *et al*., 2015). On the other hand, susceptibility to fungus infection on post-harvested fruits differed among variants with different color fruits in three ecotypes from Morelos, Mexico (Bautista-Baños *et al*., 2000). There may be further differences between 'Tuspana abal' and 'Tuspeña abal' in terms of flavor, nutritional traits, and reduced susceptibility to fungus infection that were not analyzed here. Chemical composition and nutritional value have been shown useful in studying *S. purpurea* in cultivated varieties in Ecuador (Kozioł and Macía, 1998), and among fifteen cultivated ecotypes (Solorzano-Morán *et al*., 2015). Including these analyses in future studies of the 'Tuspana abal' and 'Tuspeña abal' variants could help to promote the commercial value of these traditional variants into the regional and national markets.

Conclusion

The *S. purpurea* variants 'Tuspana abal' and 'Tuspeña abal' differed genetically and phenotypically in the present study, suggesting that these two variants originate from different genetic lineages and constitute distinct phenotypical entities. Although genetic variation between the variants was low, it was significant. Phenotypic variation among unripe and ripe 'Tuspana abal' fruit was greater than within 'Tuspeña abal'.

These results suggest that the domestication of *Spondias purpurea* by the Mayan Yucatec comprises the selection of different genetic lineages, that were cultivated for their edible fruit, and that differ to date phenotypically, mainly in colorimetric traits. A practical protocol to identify the fruits of 'Tuspana abal' and 'Tuspeña abal' entails the size ranging from 3.7 to 4 cm length, 2.7 to 3 cm width and 14 to 17 g weight; and the color differentiation of ripened fruits, orange for the first and reddish for the second. These variants are maintained for their contribution as food resources. Identifying which traits contribute to their distinctness could help better understand the genetic diversity of this native fruit tree. Strengthening and maintaining the variants of this underutilized crop could increase its use in the regions where it grows and thus bolster regional food security and sovereignty.

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