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NOTES ON NEGLECTED AND UNDERUTILIZED CROPS

Diversity and genetic structure of the husk tomato (*Physalis philadelphica* Lam.) in Western Mexico

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Abstract *Physalis* is an American plant genus that includes species of economic importance for their edible fruit. Consumption of this fruit is a historic tradition in Mexico. *Physalis philadelphica* is one of the most abundant species, which can grow under wild, weedy or cultivated conditions. It presents high morphological variability in terms of vegetative and reproductive traits; however, no study has been made of its genetic diversity or the impact of human activity on its diversity and differentiation patterns. We determined genetic parameters in nine populations representing a management gradient, including three wild, three weedy and three cultivated populations, using 88 inter-simple sequence repeat markers. The diversity of the total gene pool was high ($Ht = 0.292$, $HT_B = 0.319$) and did not decrease with the intensity degree of management. Reproductive system, life form and the wide distribution determined the genetic variation of the taxon. AMOVA revealed high variation within the total gene pool (44.3 %) and among populations (46.7 %). This was influenced by pollinator behavior, dispersal form, geographic discontinuity of the studied populations and human selection. Variation among population management categories was lower (9 %), indicating that this variable has little effect, most likely due to the broad gene pool of the taxon. However, analysis of genetic distance and Bayesian assignment distinguished two groups: cultivated and wild, with weedy populations interspersed between. This result suggests that selection for agricultural and morphological attributes of *P. philadelphica* contributes to this differentiation. Future studies could address the evolutionary dynamics of the wild–weedy–domesticated complex.

Keywords Annual crops · Domestication · Genetic resources · Human management · Landraces · *Physalis philadelphica*

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Introduction

Physalis is a diverse genus of plants that includes around 90 species, some of which are of economic significance and grown for their edible fruit or as ornamental plants. Mexico has around 70 species and is considered to be their center of origin (D'Arcy 1991; Vargas-Ponce et al. 2011). There has been a significant tradition of *Physalis* fruit consumption in Mexico since the preHispanic period (Montes-Hernández et al. 1991; Kindscher et al. 2012). This is favored by the species richness of the genus and its distribution throughout most of the territory (Vargas-Ponce et al. 2003). Sixteen taxa are used as food or for medicinal

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Fig. 1 Husk tomato (*Physalis philadelphica* Lam.). Commercial plantation of CG population studied (**A**); weedy population growing in the milpa agroecosystem in Yahualica, Jalisco (**B**); details of cultivated landrace maize, wild potato *Solanum cardiophyllum* Lindl., and husk tomato plants (**C**); fruits of the purple landrace (tomate morado) (**D**); corolla size variation (**E**); details of packing and sale: cultivated husk tomato sold in wholesale market (**F**), green and purple varieties cultivated in Cuquío, Jalisco (**G**); fruits of wild and weedy plants (known as milperos) in a small traditional market (**H**, **I**)

or ornamental purposes (Martínez 1998). The most common use is for preparing sauces with chili and in dishes that are widely consumed in Mexico throughout the year.

Physalis philadelphica Lam., commonly known as the husk tomato, originated in Mesoamerica (Engels et al. 2006). It is an annual herb, glabrous or sparsely vestite with short appressed hairs. It has yellowish corolla, rotate-reflexed when fully extended with distinctive blue anthers, convoluted after dehiscence and a clavate stigma. The fruiting calyx is globose, 10-ribbed. The berry is 1.5 cm in diameter when wild to 6 cm or more when cultivated (Nee 1986) (Fig. 1). Often the name *Physalis ixocarpa* Brot. ex Hornem. is used to refer to cultivated husk tomato. However, despite the morphological similarities between *P. ixocarpa* and *P. philadelphica*, these are different species. The former is distinguished from the latter by a capitate stigma, and relatively smaller flower and fruit (Fernandes 1974; Hanelt and IPK 2001).

Physalis philadelphica is one of the most abundant species of this genus, and is the most studied and used. It is wild or ruderal in much of Mexico (Nee 1986; Vargas-Ponce et al. 2003), but also grows as weedy populations in human-made environments; i.e., wild individuals growing by chance in agricultural fields (Montes-Hernández et al. 1991). The farmers have allowed the development of these individuals with useful characteristics (Fig. 1). It is often promoted in the Mesoamerican agroecosystem known as the milpa, where corn, beans and squash are grown together (Montes-Hernández et al. 1991). This entails managing the reintroduction of husk tomato seeds that are scattered between rows to increase the density of

plants when corn production drops due to rain shortage. Moreover, husk tomato can be grown alongside other species or as a monoculture. Fruits are gathered from plants obtained in any of these environments and from the wild, and used for both auto-consumption and sale. Sales take place locally in small regional markets and in wholesale markets that function as centers of storage and distribution to other parts of the country [e.g. Guadalajara, Monterrey, Mexico City (Fig. 1)]. The local names for *P. philadelphica* in Mexico are Miltomatl, miltomate, tomate de milpa, tomatillo, tomate de cáscara, tomate de hoja, and tomate verde (Montes-Hernández et al. 1991). In English, it is commonly known as the husk tomato, jamberry, husk cherry and Mexican tomato (Hudson 1986; Hanelt and IPK 2001).

Husk tomato is one of the main vegetables grown in Mexico for domestic sale and export. Domestic production in 2004 was 723,000 tons (Calyecac-Cortero et al. 2007), and rose to 805,271 tons in 2006 with a market value close to US$ 259 million (Gámez-Jiménez et al. 2009). Since the 1980s, it has been exported to the US and more recently to Canada as both a fresh and processed product, with growing demand from the Latino community (Díaz Pérez et al. 2005). The area under cultivation has increased by 71 % between 1982 and 2008 (Ponce Valerio et al. 2012) and, in this regard, it ranked third to fifth in Mexico among vegetables in 2010 and 2011 (SIAP 2012). The areas with the highest production in the spring–summer agricultural cycle, which takes advantage of the rainy season, are Jalisco, Puebla, Mexico State, Michoacán, Morelos and Hidalgo. In the autumn–winter cycle, these areas are Sinaloa, Puebla, Sonora, Michoacán and Mexico State. In some regions, there is a preference for consumption of small fruits of 0.8–1.5 cm in diameter, such as those produced by the wild and weedy populations (Hidalgo, Veracruz, Michoacán and Morelos). To meet this demand, local husk tomato populations with these characteristics (commonly called milperos) are grown or immature fruits are harvested (Montes-Hernández et al. 1991) from other cultivated populations. In other regions, there is a preference for and cultivation of local varieties with large fruits of 2–8 cm in diameter. Throughout the country, there is also a predilection for different fruit (color, sweetness, acidity) and agronomic attributes (precocity, yield) that has contributed to the diversification of the landraces of this species. This species has been introduced to America (USA), Europe and Asia for experimental greenhouse and small-scale cultivation (Mulato-Brito and Peña Lomeli 2007).

Physalis philadelphica presents the great morphological variability typical of domesticated plants (Hudson 1986) as well as self-incompatible species that require cross-pollination for reproduction, and those with broad distribution (Olsen and Wendel 2013). Montes-Hernández et al. (1991) states that the phenotypic plasticity of this species is influenced by soil fertility. There are clear differences between wild and commercially cultivated forms; however, wild and cultivated individuals frequently exhibit characteristics that are intermediate between both extremes (Hudson 1986). The characteristics with greatest variation are size, color, taste, shape, firmness of fruit, fruiting calyx features such as color and length, flower size (Fig. 1), growth habit, reproductive cycle and number of seeds per fruit (Hudson 1986; Montes-Hernández et al. 1991). Husk tomato berries can be 1–10 cm in diameter with whitish-yellow, yellow, green, purple or green with purple hues (Hudson 1986; Mulato-Brito and Peña Lomeli 2007) (Fig. 1). Berry flavor varies from acidic to sweet and sour. This variability is apparent in the eight races recognized by Peña Lomelí et al. (2008) and as well as in other landraces, and wild and weedy varieties recognized locally by traditional farmers.

The morphological variability observed in *P. philadelphica* is an indicator of its genetic diversity. However, species domestication and population management have direct effects on diversity levels, which may be reduced even if the number of landraces with different morphological features increases (Ladizinsky 1998). Genetic diversity can be estimated using molecular techniques that allow us to observe and quantify more precisely the degree of variation that exists in a species (Hedrick 2000). Inter-simple sequence repeats (ISSRs) are molecular markers that are highly sensitive to polymorphisms. They segregate on a Mendelian basis and can be amplified with astringent alignment temperatures (Zietkiewicz et al. 1994), thus providing a source of reproducible and informative data. Inter-simple sequence repeats have been successfully used to evaluate genetic diversity and differentiation in plants (Wakte et al. 2012; Ding et al. 2013). This technique has proved useful in the assessment of diversity and genetic relationships

Table 1 Genetic diversity of (A) populations, (B) pool management type, and (C) total pool of *Physalis philadelphica* studied

Analysis levels	Num[a]	Code[b]	Collection site	N	*He*	*P*	He_B
A) Populations							
Wild	1	AGS	Aguascalientes[1]*	17	0.187	48	0.182
	2	VAL	Valparaiso[2]*	5	0.131	28	0.140
	3	ZAP	Zapotlanejo[3]*	20	0.187	42	0.174
Weedy	4	UT	Unión de Tula[3]	17	0.163	40	0.170
	5	YAH	Yahualica[3]	20	0.214	50	0.207
	6	CUA	Cuautla[3]*	24	0.163	41	0.159
Cultivated	7	CG	Cuquío[3]	18	0.207	51	0.215
	8	CR	Cuquío[3]	22	0.215	50	0.207
	9	CO	Cuquío[3]	20	0.203	48	0.197
B) Management category							
					Ht	*P*	Ht_B
Wild				42	0.247	68	0.332
Weedy				61	0.268	81	0.296
Cultivated				60	0.275	77	0.298
C) Species							
					HT	*P*	HT_B
P. philadelphica			163	0.292	91	0.319	

N, number of sampled individuals; *He*, expected heterozygosity (Nei 1972); *P*, percentage of polymorphic loci assuming Hardy–Weinber equilibrium (HWE); *Ht*, expected heterozygosity by management category; *HT*, species total expected heterozygosity; He_B, Ht_B, and HT_B, Bayesian expected panmictic heterozygosity by population, by management pool and species respectively

* Individual plants are obtained from seed germination

[a] Numerical codes as in Fig. 1

[b] Alphabetical population codes used in text

State of origin: [1]Aguascalientes, [2]Zacatecas, [3]Jalisco

among *Physalis* species (Vargas-Ponce et al. 2011). Genetic diversity in *P. philadelphica* has not previously been evaluated, nor has the influence of human management on patterns of genetic diversity and differentiation. In this study, we therefore measured genetic parameters of *P. philadelphica* populations grown under different intensities of management levels using ISSR markers to determine genetic status and assess the effect of population management. The husk tomato is under a process of domestication, which is apparent in the high variation of morphological traits. Since this process could also have an adverse effect on genetic variation, and cause an increase in genetic differentiation levels, we hypothesized that: (1) wild populations would present more genetic variation than cultivated populations; (2) lower management intensity in weedy populations could allow more genetic variation than in cultivated, but less than in wild populations; (3) the wild genetic pool could be genetically differentiated from the cultivated genetic pool, but would have less differentiation with the weedy populations, and (4), given their geographical proximity, cultivated populations would present lower levels of differentiation among themselves.

Materials and methods

Plant material

Nine populations of *P. philadelphica* were selected with different management levels. These consisted of three wild populations, three weedy populations and three commercially grown (Table 1). The wild plants came from the states of Aguascalientes, Jalisco and Zacatecas. The tolerated weeds plants came from the municipalities of Cuautla, Unión de Tula and

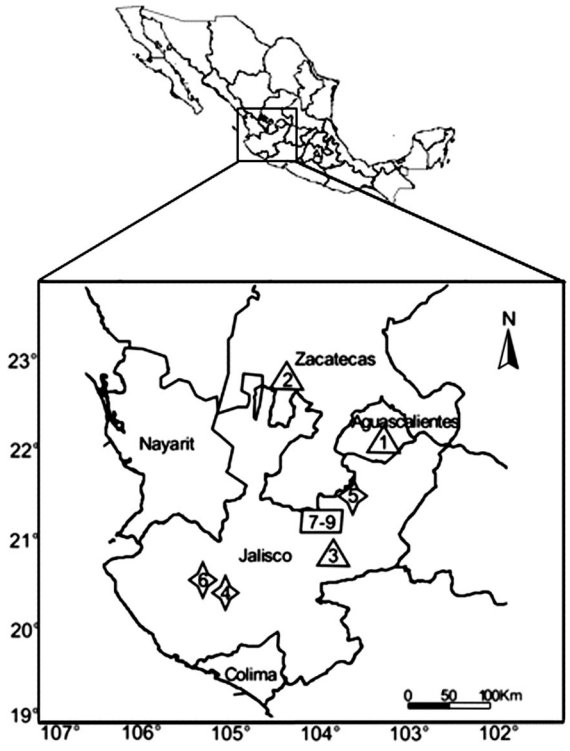

Fig. 2 Study populations of *Physalis philadelphica*: wild (*triangle*), weedy (*star*) and cultivated (*rectangle*). Numerical codes of populations are as in Table 1

Yahualica in Jalisco. Cultivated plants were obtained from different plots in Cuquío Township, Jalisco, one of the main seasonal (spring–summer) husk tomato-producing areas in the country (Fig. 2).

In five of the populations, the three cultivated ones and two tolerated weeds, leaves were collected directly in the field. Plant material from the other four studied populations, the three wild and the weedy from Cuautla, obtained from seed germination was acquired in previous collections during 2009–2011 and deposited in the Seed Laboratory of the University Center for Biological and Agricultural Sciences (CUCBA). For this, at each locality, several fruits of all plants that make up the populations were obtained; the seeds were extracted from these fruits and mixed to form a representative germplasm collection. Seedlings were grown in a greenhouse until reproductive maturity. Three to five leaves were taken from 18 to 32 individuals of each studied population. The leaves were placed in foil envelopes, which were labeled and stored on ice for laboratory processing.

DNA extraction and amplification

DNA was extracted using the Doyle and Doyle (1987) method. The quality and quantity of DNA were measured through spectrophotometry. Eleven ISSR markers were evaluated, out of which three were selected: the dinucleotides I.$(GA)_8$RG and II.$(CA)_6$RG and the tetranucleotide III.$(GACA)_6$YR, which showed more polymorphic banding patterns and reproducibility in amplification. Polymerase chain reaction (PCR) was performed in a final volume of 20 μL. The reaction mixture consisted of 80 ng of DNA, 10 mM amplification buffer, 2.5 mM $MgCl_2$, 0.3 mM dNTPs, 0.2 mM primer, 0.5 U *Taq* DNA polymerase and high-performance liquid chromatography–grade water. The amplification protocol consisted of denaturation at 95 °C for 3 min, followed by 40 cycles of 45 s at 94 °C, 45 s at the annealing temperature (56.5 °C for primer I and 47 °C for primers II and III) and 90 s at 72 °C and one final extension of 10 min at 72 °C. Amplification products were separated by vertical electrophoresis in 6 % polyacrylamide gels with 7 M urea 1× TBE buffer. Electrophoresis was performed in a dual electrophoresis chamber (CBS Scientific) at 200 V (constant) for 5 h or until the loading buffer indicated a 20 cm migration distance. Silver nitrate staining was used to visualize amplification products (Sanguinetti et al. 1994).

Data analysis

The above gels were captured as images in a digital photodocumentation system (Gel Logic 100, Kodak). The amplified bands were documented using the analysis software Phoretix 1D. Analyzed images included two reference columns of 100 bp molecular markers for estimating band weights. The bands that reached the same migration distance or molecular weight between individuals were considered homologous fragments. The bands observed for each initiator by individuals and populations were recorded on a data matrix considering their presence (1) or absence (0). From these data, diversity and differentiation parameters were calculated at three levels: (a) species; in which the nine studied populations were grouped; (b) management category, considering the wild, weedy and cultivated populations as three special pools; and (c) population.

Genetic diversity

Four parameters were used to measure genetic diversity: (1) the proportion of polymorphic loci (*P*) and (2) expected heterozygosity (*He*) according to Nei (1972) by applying the Lynch and Milligan (1994) correction for dominant markers using the TFPGA v1.3 (Miller 2000), (3) the Shannon diversity index (*I*) with Popgene v1.32 (Yeh et al. 1999) and 4) the expected heterozygosity with a Bayesian estimator (He_B) using the f-free model with Hickory v1.1 (Holsinger 1999; Holsinger and Lewis 2007), and a burn-in period of 50,000 iterations, followed by 250,000 Markov chain sampling iterations and retention values every 50 iterations.

Genetic differentiation

Genetic differentiation was evaluated using three parameters: (1) the Wright (1951) differentiation index (*Fst*), also called θ, using TFPGA v1.3 (Miller 2000), (2) the Bayesian differentiation index (θ_B) with Hickory v1.1 (Holsinger and Lewis 2007) with the same conditions above to estimate genetic diversity, and (3) The differentiation coefficient (ϕst) by analysis of molecular variance with Arlequin 3.5 (AMOVA, Excoffier et al. 1992). In addition, Fisher's non-parametric test (Raymond and Rousset 1995) of combined probability was used to assess whether there were significant differences in allele frequencies between pairs of populations considering a random distribution of individuals with Arlequin 3.5 (Excoffier et al. 2005). In a complementary manner to our estimation of genetic structure, the indirect measure of gene flow (*Nm*) was estimated according to Crow and Aoki (1984). *Nm* reflects the degree of connectedness among populations preventing population differentiation caused by genetic drift (Hedrick 2000).

Genetic similarity

To identify affinity between populations, Nei's (1972) genetic distance (*D*) was estimated, and a cluster analysis was performed with the average group method with unweighted arithmetic means (UPGMA) using the matrix of gene distances between pairs of populations (Popgene v1.32, Yeh et al. 1999). To determine the likely number of populations, a Bayesian analysis was used by assigning individuals in Structure v2.3.1 (Pritchard et al. 2000) with 50,000 burn-in trees, 250,000 replicates and retention values every 10 iterations. To do this, one to nine populations were assumed (K = 1 to K = 9), and 10 simulation runs were performed for each K value. The results of the 90 simulations were analyzed with the HARVESTER software package (Earl and vonHoltd 2012), which used the method of Evanno et al. (2005) to obtain the most probable K value.

Results

On average, 19 individuals were evaluated per population by amplification with all three primers. A total of 88 bands were generated, and each was considered a locus. Out of all bands, 36 were amplified with primer I with a molecular weight between 200 and 900 bp, 28 with primer II with a molecular weight between 220 and 1,400 bp and 23 with primer III with a molecular weight between 260 and 1,400 bp.

Genetic diversity

The percentage of polymorphic loci for the total *P. philadelphica* population was 91 %. The wild gene pool showed 68 % of polymorphic loci, the weedy pool 81 % and the cultivated pool 77 %. The percentage of polymorphic loci in the wild populations ranged from 28 to 48 %, in the weedy populations from 40 to 50 % and in the cultivated populations from 48 to 51 %. In addition, the total expected heterozygosity (*Ht*) of the nine husk tomato populations studied was $Ht = 0.292$. Heterozygosity values by management category were $Ht = 0.247$ in the wild pool, $Ht = 0.268$ in the weedy pool and $Ht = 0.275$ in the cultivated pool. Expected heterozygosity at the population level (*He*) showed values of $He = 0.131$–0.187 in the wild populations, $He = 0.163$–0.214 in the weedy populations and $He = 0.203$–0.215 in the cultivated populations (Table 1). The estimated diversity for the species using Shannon's diversity index was $I = 0.492$. For the three population management groups, these values were $I = 0.379$ for the wild, $I = 0.430$ for the weedy and $I = 0.426$ for the cultivated. With this estimator, wild populations had values ranging from $I = 0.173$ to 0.266, the weedy population had values from $I = 0.231$ to 0.301 and the cultivated population had values from

Table 2 Genetic differentiation estimators, analogous to Wright' s (1951) *F* statistics, in the total species pool, between groups of populations for management category (wild, weedy, cultivated) and populations within groups of *Physalis philadelphica*

	AMOVA Excoffier et al. (1992)						Bayesian estimator, Holsinger (1999)			Weir and Cockerham (1984)		
	df	SS	VC	%V	ϕsc	CI	θ^B	SD	CrI	θ *Fst*	SD	CI
Between individual plants within populations (total pool)	154	1218.966	7.915	44.35	0.513 ϕct	0.472, 0.555	0.544	0.020	0.504, 0.583	0.449	0.031	0.111, 0.50
Between groups of populations per management category	2	505.128	1.577	8.84	0.088* ϕst	0.040, 0.132	0.220	0.023	0.173, 0.226	0.061	0.027	0.009, 0.389
Between populations within categories	6	925.734	8.354	46.81	0.556*	0.512, 0.600						
Pool management category												
Wild							0.458	0.037	0.385, 0.531	0.350	0.038	0.279, 0.425
Weedy							0.539	0.031	0.475, 0.599	0.477	0.041	0.394, 0.554
Cultivated							0.478	0.035	0.406, 0.546	0.406	0.053	0.302, 0.505

df degrees of freedom, *SS* Sum of squares, *VC* variance components, *%V* percentage of variation, *SD* standard deviation, *CrI* credibility interval, *CI* confidence intervals

$I = 0.287$ to 0.296. However, the expected heterozygosity with the Bayesian estimator (He_B) was slightly higher than that obtained with *He*, which at the species level reached $HT_B = 0.347$. The expected heterozygosity was similar in all populations to the estimated *He* values and varied from $He_B = 0.140$ to $He_B = 0.215$.

Genetic differentiation

Genetic differentiation among the nine *P. philadelphica* populations was estimated to be $Fst = 0.449$, while the number of migrants per generation at this level was $Nm = 0.642$. Between the three population management categories, the index of differentiation was low, with $Fst = 0.061$ and $Nm = 3.308$. Differentiation indices for each category were $Fst = 0.350$ and $Nm = 0.938$ for the wild pool, $Fst = 0.477$ and $Nm = 0.774$ for the weedy pool and $Fst = 0.406$ and $Nm = 1.273$ for the cultivated pool (Table 2). The Bayesian estimator of differentiation at the species level was $\theta_B = 0.544$, consistent with values shown by *Fst*. Between managed categories, differentiation was $\theta_B = 0.220$. Treating each population management category as a pool, wild populations had differentiation of $\theta_B = 0.458$, the weedy had $\theta_B = 0.539$ and the cultivated had $\theta_B = 0.478$ (Table 2). The percentage of variation (AMOVA) within the total species pool was 44.3 %, while for management categories, the variation was 8.84 %. Between populations, the variation was 46.81 % (Table 2).

Genetic similarity

Genetic distance between the nine analyzed populations ranged from $D = 0.1003$ to $D = 0.3096$. Distance values ranged from $D = 0.1003$ to 0.1893 for the wild populations, from $D = 0.1895$ to 0.2981 for the weedy populations and from $D = 0.1102$ to 0.1989 for the cultivated populations (Table 3). A generated dendrogram indicated the formation of two groups. The first included two subgroups: one that meets two Cuquío cultivated populations (CG and CR) and another that consisted of the cultivated (CO) and the weedy population (UT) (Fig. 3). The second group consisted of five populations, three wild and two weedy. The nearest populations were the weedy (CUA) and wild (VAL). Wild ZAP was associated with this population pair, followed by another wild group (AGS) and a weedy group (YAH).

Individual assignment analysis indicated two genetic groups as the most probable population

Table 3 Matrix of genetic distances among *Physalis philadelphica* populations obtained with ISSRs markers (Nei 1972)

Num[a]		1	2	3	4	5	6	7	8	9
1	AGS[1]									
2	VAL[1]	0.13								
3	ZAP[1]	0.1691	0.1893							
4	UT[2]	0.1926	0.1899	0.1728						
5	YAH[2]	0.3096	0.2444	0.2216	0.1895					
6	CUA[2]	0.2567	0.2391	0.2208	0.2981	0.2046				
7	CG[3]	0.2166	0.2455	0.2796	0.2667	0.2295	0.1672			
8	CR[3]	0.2328	0.2696	0.1874	0.2463	0.1939	0.1003	0.1989		
9	CO[3]	0.2409	0.2607	0.2122	0.2455	0.1975	0.1628	0.1876	0.1102	

[a] Numerical codes as in Table 1

[1] Wild, [2]Weedy, [3]Cultivated

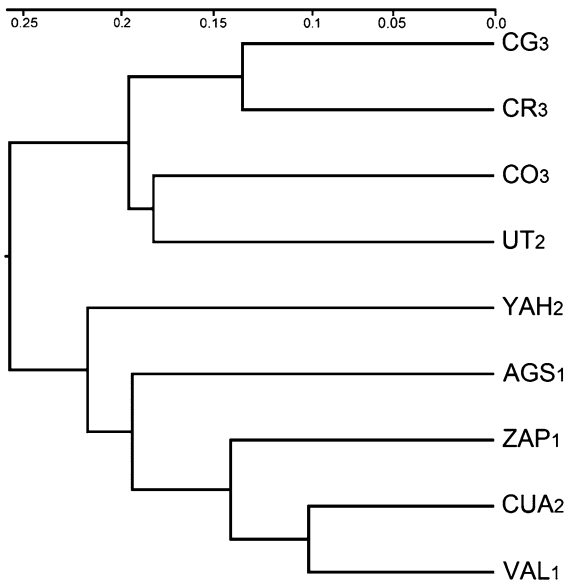

Fig. 3 Dendrogram of genetic relationships among the study *Physalis philadelphica* populations, based on Neis distances (1972) obtained with ISSRs markers. Wild[1],Weedy[2], Cultivated[3]

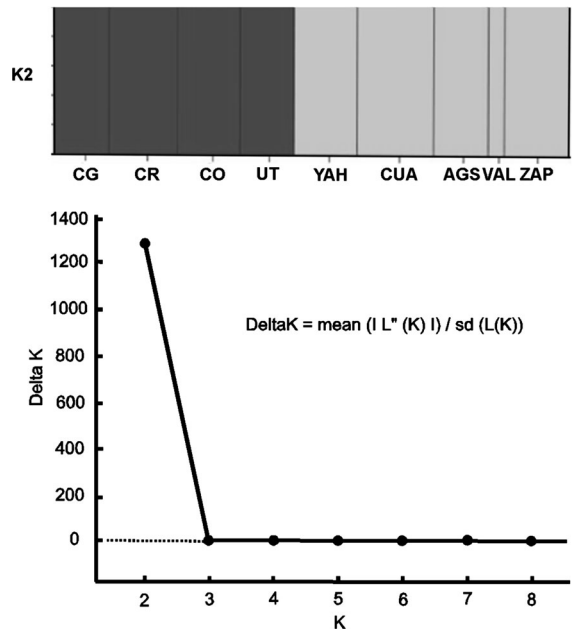

Fig. 4 Hierarchical organization of genetic relatedness of nine *Physalis philadelphica* populations, analyzed by STRUCTURE software (Pritchard et al. 2000). The optimal clustering number ($K = 2$) was determined based on the ad hoc statistic ΔK following Evanno et al. (2005) using the Harvester program (Earl and vonHoltd 2012)

number (K = 2). The first group included the three cultivated populations and the weedy UT group, while the second was made up by the remaining populations. This result is consistent with those found with Nei's genetic distances (Figs. 3, 4).

Discussion

Genetic diversity

The four estimators used showed that *P. philadelphica* has high levels of genetic diversity ($p = 91$ %, $Ht = 0.292$, $HT_B = 0.319$, $I = 0.492$). The values of these parameters in the husk tomato are characteristic of annual dicotyledons, with cross-pollination and wide distribution ($He = 0.186$, $p = 59$ %), according to studies conducted with dominant molecular markers (Nybom and Bartish 2000) and isozymes (Hamrick and Godt 1996). These values are also similar to those found with ISSR markers in *Swertia tetraptera* ($He = 0.222$, $p = 98.9$ %, Yang et al. 2011) and

Sisyrinchium micranthum ($He = 0.240$, $p = 69$ %, Tacuatia et al. 2012), two species with habits and reproductive systems similar to those of *P. philadelphica*.

Generally, genetic diversity is expected to decrease with increased intensity of plant population management (Doebley et al. 2006; Ladizinsky 1998). In the husk tomato, the pool of cultivated populations showed slightly higher diversity ($He = 0.275$) compared to the weedy ($He = 0.268$) and the wild ($He = 0.247$) pools. Plants used for cultivation that have been subject to strong selection pressure during domestication have lower levels of diversity compared to wild pools (Wright et al. 2005; Bitocchi et al. 2013). However, certain traditional farming practices can influence the maintenance or expansion of crop genetic diversity relative to wild populations. In the husk tomato, this could be the result of gene pool management integrated by germplasm accession from several original populations (e.g. *Stenocereus stellatus*, Casas et al. 2006; Cruse-Sanders et al. 2013; *Agave angustifolia* and *A. rhodacantha*, Vargas-Ponce et al. 2009). Alternatively, this observation could be explained by the development of new individuals from a seed bank within the site (*Anemone altaica*, Xu et al. 2013; *Solanum trilobatum*, Shilpha et al. 2013).

In this sense, traditional farming practices of some plant species can be very efficient at maintaining diversity *in situ* (Jarvis et al. 2007). This seems to be the case for the cultivated populations of *P. philadelphica* under study. In general, the exchange of seeds or seedlings of husk tomato occurs with a low frequency and each producer selects seed from their own harvest for use as a reservoir for the next cropping season. This practice is common in many areas of Mexico (Montes-Hernández et al. 1991); however, the selection pressures on husk tomato fruit are not so rigorous and producers maintain morphological variants through the selection of characteristics that reflect popular demand. These can vary widely, and include fruit size, fruit color, adhesion and coverage of the calyx of the fruit, life post-harvest, etc. Furthermore, the cross-pollination required by the husk tomato for reproduction is a significant factor that favors gene exchange, incorporation of new alleles and maintenance of high levels of genetic diversity (Hamrick and Godt 1996). The morphological diversity of materials under cultivation and gene flow via human handling or natural pollinators could then act to mitigate the bottleneck generated by artificial selection, which reduces genetic diversity of crops (Ladizinsky 1998, Doebley et al. 2006).

The pool of weedy populations in the traditional farming system showed high values of diversity ($Ht = 0.268$, $p = 81$ %); these values were slightly lower than in the cultivated pool but higher than in the wild pool. Particular cases exist that can help us explain these diversity patterns in the weedy populations. The Union of Tula (UT) population grows on a plot cultivated with chickpeas that germinate after the corn harvest, taking advantage of the residual moisture during the autumn–winter. The diversity value of the UT population is lower than the studied wild populations. This could be the result of genotype removal through the use of herbicides or because some individuals fail to germinate due to existing moisture conditions or competition with the chickpea plants. In addition, the direct extraction and removal of individuals modifies gene composition by decreasing certain phenotypes or effective population sizes (Casas et al. 2007; Sebbenn et al. 2008). In contrast, the weedy population from Yahualica (YAH) develops among corn, beans and squash during the rainy season. This population is persistent in the plot, and its emergence has been observed consistently over four agricultural cycles. It presents a high diversity ($He = 0.214$, $p = 50$ %) that reflects the maize system and the adaptation of the population to the rainy season and its ability to establish itself in the presence of natural competitors (*Solanum cardiophyllum* Lindl., *Physalis angulata L.* and *Amaranthus* sp.) that grow on the site. In herbaceous annual plants with wide distribution and rustic traditional cultivation, high levels of diversity have been associated with adaptation and survival in diverse environments (e.g. *Salvia miltiorrhiza* by Li et al. 2008).

The pool of studied wild populations showed a high genetic diversity ($Ht = 0.247$), but which was slightly lower than those of the cultivated or weedy groups. This result was despite the fact that the population (VAL) was represented by five individuals, which could have had an impact on the index calculation. This diversity is comparable to that reported in other cross-pollinating herbaceous annuals with wide geographical distribution, as estimated by ISSRs in a larger number of populations. This is the case with five *Salvia miltiorrhiza* populations ($Ht = 0.195$, Song et al. 2010), 34 *Swertia tetraptera* populations

($Ht = 0.222$, Yang et al. 2011) and five *Sisyrinchium micranthum* populations ($He = 0.19$–0.25, Tacuatia et al. 2012).

Genetic differentiation

The differentiation index of the total *P. philadelphica* pool under study was high ($Fst = 0.449$). Gene flow, as measured indirectly through the number of migrants ($Nm = 0.642$), was low. This result contrasts with the values expected for cross-pollinating wild species of wide distribution, in which greater genetic homogeneity is expected ($Fst = 0.170, Nm = 1.38$, Hamrick and Godt 1996; Morjan and Rieseberg 2004). The geographic distance between populations and the dispersal distance of seeds, fruits or pollen are factors that could explain these results. The wild and weedy populations were more than 100 km apart, except for two weedy populations (UT and CUA), which were separated by approximately 50 km (Fig. 1). This distance is outside the forage limits for bees, the natural pollinators of *Physalis* (Sullivan 1984; Villanueva-Gutiérrez et al. 2013), which do not exceed 5 km (Jha and Kremen 2013). In *Sisyrinchium micranthum*, a bee-pollinated species with similar biological characteristics to *P. philadelphica*, high differentiation ($F_{ST} = 0.337$, Yang et al. 2011) has been found between populations in an area of less than a 4 km radius. This indicates that apparently short distances can affect an isolation process through limited displacement of pollinators (Eckert 1933; Wenner et al. 1991). In a complementary manner, the dispersion of natural *Physalis* populations can help explain the detected differentiation levels. Dispersion of individuals via seeds is limited since this occurs by gravity, run-off and zoochory (Martin et al. 1961; Cipollini and Levey 1997; Olea et al. 2007). In general, populations of *P. philadelphica* and other related species of the genus (*P. angulata* L., *P. ampla* Waterf., *P. microcarpa* Urban et Eckman, *P. solanaceus* Schltdl.) contain few dispersed individuals per site and are distributed in several distinct patches in an area. Thus, these populations could act as independent evolutionary lineages in the presence of limited gene flow.

In this sense, Morjan and Rieseberg (2004) postulate that while gene flow involves all genes, these could have contrasting effects in genetic differentiation between populations. While the alleles of some loci that are under selection pressure promote the more rapid homogenization and cohesion of the populations of a species, other neutral alleles may simultaneously contribute to differentiation due to genetic drift. This seems to be the case for wild and weedy pools of *P. philadelphica*, which had low numbers of migrants between populations within groups ($Nm = 0.938$ and $Nm = 0.774$, respectively). Wild populations are commonly ruderal and are formed by few individuals. The resulting low gene flow in this group can be an indicator of genetic drift due to mutations in the neutral loci analyzed in this study. In weedy populations, this value may be the result of isolation by distance, considering both the flight distance of bees and geographical distance apart. Similarly, these populations are favored by habitat disturbance (Vibrans 1999) when farmers establish their agroecosystem. In this way, disturbances act as a selection factor: For example, the YAH population contains many individuals and is resistant to predators and competition while the UT population presents low individual density and herbicide resistance.

It is likely that the high genetic differentiation in the cultivated populations within 1.5 km of each other analyzed in this study was due to the foraging behavior of pollinators in the area. This behavior can be influenced by mass synchrony of flowering in local plots where resource concentration provides pollinators with optimal conditions for foraging. This reduces the need for movement and incurs lower energy costs (Holzschuh et al. 2013). Flowering synchrony and a high density of individuals are common features in cultivated husk tomato populations. However, Sullivan (1984) recorded a low but constant frequency of bee visits in *P. cinerascens* (Dunal) Hitchc. populations and found that foraging behavior (e.g. *Perdita*) includes visits to flowers on the same plant and several plants within a small patch. This phenomenon involves high local structuring in plants (Domínguez et al. 2005). Additionally, the high genetic differentiation in cultivated populations could be related to high morphological variation in the husk tomato. It is common for producers in Cuquío to maintain morphological variants in the same plot. However, varieties in high demand predominate. In addition, fruits are selected and separated for marketing according to their organoleptic features. For example, purple fruits, which are sweeter, are packaged in purple bags, while green fruits, which are sour, are packaged in green bags. It is likely that the seeds stored from this

selection may increase the differentiation of populations. Although the ISSR markers evaluated in this study are neutral, morphological differentiation between populations of different species of plant, animals and fungi show a direct relationship to neutral genetic differentiation (50 spp., Leinonen et al. 2008).

Genetic similarity

Both genetic similarity and differentiation of populations reflect natural historic processes and those mediated by man, according to the wild or cultivated state (Ladizinsky 1998; Doebley et al. 2006). Exact differentiation analysis (Raymond and Rousset 1995) showed significant differences in allele frequencies ($p < 0.05$) between pairs of *P. philadelphica* populations, confirming that the processes mentioned above influenced genetic condition. Furthermore, there was a structure of ~ 9 % between population management categories (AMOVA, Table 2) that may have been influenced by anthropogenic management, as has been shown for other species (Oyama et al. 2006; Shi et al. 2008; Cruse-Sanders et al. 2013). Consistent with this result, our cluster analysis and Bayesian assignment analysis show the separation of two large genetic groups (Figs. 2, 3). The first group showed a genetic affinity for cultivated populations (CG, CR, CO) with the weedy (UT) group. The second group showed the genetic similarity of the wild populations (AGS, VAL, and ZAP) with two weedy populations (YAH and CUA). This result could indicate that the genetic composition of weedy populations is an intermediate mixture between two groups, which highlights in both analyses the separation of cultivated and wild groups. The estimated indirect gene flow between population management categories was high ($Nm = 3.308$). However, it was not possible in this study to accurately determine the proportion and direction of this flow and the role it plays in tolerated populations in this evolutionary dynamic. Nevertheless, man's preference for certain desirable characteristics in fruit (such as size, color, flavor and texture) and other agricultural traits (erect vs. prostrate habit, precocity, yield) of *P. philadelphica* may contribute to this emerging separation that is observed between both conditions (Hudson 1986; Montes-Hernández et al. 1991).

We conclude that the obtained diversity values indicate no apparent genetic erosion in *P. philadelphica* and that this species maintained historical patterns of genetic variation. The management intensity of *P. philadelphica* populations does not decrease genetic diversity but contrarily seems to increase it. This result indicates efficient management of morphological and genetic diversity by producers of this significant production zone of seasonal husk tomato in Mexico. Wild, weedy and cultivated populations retain high diversity values and high structuring. Cross-pollination, self-incompatibility and adaptation to different ecological niches contribute to the genetic diversity of *P. philadelphica*. The species has distinguished itself among populations by discontinuity of distribution, low gene flow between populations and artificial selection targeted towards maintaining distinct morphological varieties in crops. Future studies could focus on the reproductive biology of the species, its ecology of pollination and its seed dispersal. Moreover, it is important to extend the population-genetic study of this species to a larger number of populations within its geographic and evolutionary gradient to understand the evolutionary dynamics of the wild–weedy–domesticated complex of the husk tomato.

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