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ORIGINAL ARTICLE

Taxonomic implications of the morphological and genetic variation of cultivated and domesticated populations of the *Agave angustifolia* complex (Agavoideae, Asparagaceae) in Oaxaca, Mexico

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Abstract

The *Agave angustifolia* complex, distributed from Mexico to Costa Rica, comprises four species and five varieties, including three species used for mescal production. The complex is represented in the Mexican state of Oaxaca by two wild taxa, *A. angustifolia* var. *angustifolia* and *A. angustifolia* var. *rubescens*, the cultivated form *A. angustifolia* "Espadín" and the partially cultivated species *A. rodacantha*. The aims of this study were to investigate the morphological and genetic variation of the *A. angustifolia* complex in the state of Oaxaca and to identify traits useful for taxonomic delimitation. Four wild and three cultivated populations of *A. angustifolia* from Oaxaca, one population of *A. tequilana* from Guanajuato and one population of *A. angustifolia* from Sonora were sampled for morphological, genetic and cytometric analyses. We showed that cultivated populations of *A. angustifolia* "Espadin," *A. rhodacantha* and *A. tequilana* could be clearly differentiated from wild populations. Furthermore, the domesticated populations of *A. angustifolia*, known locally as "Espadin," had a higher ploidy level and lower genetic variation than their related wild populations. The population of *A. angustifolia* from Sonora could be recognized as a different entity. Populations of *A. rhodacantha* need to be studied throughout their entire distribution area to further evaluate their taxonomic delimitation.

Keywords AFLP · *Agave* · Agavoideae · Domestication · Espadín · Oaxaca

Introduction

The subfamily Agavoideae, which is endemic to the Americas, is found from southern Canada to Bolivia. According to Dahlgren et al. (1985), this subfamily is composed of nine genera containing approximately 330 species. Agavoideae is distributed in almost all ecosystems, except in the tundra, and is particularly diverse in semiarid and temperate zones (Dahlgren et al. 1985; García-Mendoza 2004). The genus *Agave* is the most diverse, with approximately 200 species. Of these, 159 (75%) are found in Mexico, with 74% of these being endemic species. Within Mexico, the state of Oaxaca with 39 species has the highest species richness (García-Mendoza 2011) representing 25% of the number of *Agave* species known for the country.

In Mexico, agaves have great economic and cultural importance for many indigenous people and mestizos, who have used these plants for centuries as a source of food, drink, medicine, fuel, decoration, hard fibers from leaves (*ixtle*) and fertilizer, among other uses. Agaves were among the first plants exploited by the residents of Mesoamerica (García-Mendoza 2007). The most extensively exploited species are: *Agave americana* L., *A. angustifolia* Haw., *A. cupreata* Trel. & A.Berger, *A. durangensis* Gentry, *A. funkiana* K.Koch & C.D.Bouché, *A. fourcroydes* Lem., *A. inaequidens* K.Koch, *A. karwinskii* Zucc., *A. lechuguilla*

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Torr., *A. marmorata* Roezl, *A. mapisaga* Trel., *A. maximiliana* Baker, *A. potatorum* Zucc., *A. rhodacantha* Trel., *A. salmiana* Otto ex Salm-Dyck, *A. sisalana* Perrine and *A. tequilana* F.A.C.Weber. Some species such as *A. tequilana* include commercial cultivars (e.g., *A. tequilana* "Azul") which are only known from cultivation; others are semi-domesticated or simply collected from natural populations.

According to Pickersgill (2007), domestication is the final stage of a process involving human selection, which starts with exploitation of wild plants, followed by cultivation of plants taken from natural populations but not yet genetically different from wild plants, and ending with the fixation of morphological and genetic differences that distinguish the domesticated form from its wild progenitor. These differences constitute the domestication syndrome that generally results in plants dependent on man for their growth and reproduction.

In relation to the process of domestication, the correlation between the level of selection applied to a species and the morphological variations found between domesticated and wild populations has been reported in groups of plants such as *Opuntia* (Colunga-GarcíaMarín et al. 1986) in addition to species of *Agave* (Colunga-GarcíaMarín et al. 1996; Figueredo-Urbina et al. 2017). In these studies populations with a higher degree of management and selection show very low morphological variation, whereas populations with scarce or no management/selection contain individuals with a wider range of morphological variation. Regarding genetic variation in populations under some degree of selection, studies in *A. tequilana* (Gil-Vega et al. 2006) and Chili pepper (*Capsicum annum*) (Aguilar-Meléndez et al. 2009) have shown low variation at both genetic and morphological levels within populations, whereas Vargas-Ponce et al. (2009) found that wild populations of the *A. angustifolia* complex show similar levels of genetic diversity in relation to populations under traditional management in keeping with the lower level of selection applied in traditional systems.

Levels of management and selection of *Agave* populations have evolved through a two-stage process from the pre-hispanic era when the first objective of selection was to identify germplasm optimal for the production of fibers or food (including pulque) or other basic necessities. With the introduction of the distillation process, these same populations were submitted to a second selection pressure to provide the best germplasm for the production of distilled spirits (Colunga-GarcíaMarín and Zizumbo-Villarreal 2007).

In modern times the commercial success of tequila and mescal has placed emphasis on this area of exploitation and currently *Agave* species are used for the production of distilled spirits in different regions of the country and at various levels of technification. Level 1 is simply to harvest wild agave plants to produce relatively small quantities of spirit mainly for local consumption as is the case in Zacatecas for example where naturally growing agaves (mainly *A. salmiana*) are harvested directly (Morales Carrillo et al. 2007). These plants have undergone no selection or agricultural management and can reproduce freely. The danger is over-exploitation leading to decimation of the wild populations. Level 2 or intermediate level is the case of *A. angustifolia* exploited to produce Bacanora (a local mescal). Until 1992, production of distilled spirits from agave was illegal in Sonora and done clandestinely by harvesting wild plants or from plants grown close to small farms (Salazar-Solano 2007). Since 1994, farmers have been able to develop plantations, implement agronomical practices and select genotypes with desired traits although a range of diverse germplasm is used. Although production in Sonora may be higher in comparison with other regions, it does not reach the scale of Level 3. This is the case for production of mescal from *A. angustifolia* in Oaxaca and especially for tequila production in Jalisco and neighboring states. In these states spirits are produced at an industrial scale for both internal consumption at the national level and to supply a growing international market. To meet the demands of the industry, thousands of hectares are committed to agave cultivation in these regions using standard agricultural practices and modern technology. Selection of desired genotypes has led to a system of monoculture of *Agave angustifolia* "Espadín" for mescal and *Agave tequilana* for tequila and a narrowing of the germplasm base in these highly cultivated varieties due to the typical vegetative propagation from suckers to renew plantations after harvesting. The different levels of domestication, management and exploitation of germplasm described above will influence the diversity of both cultivated and partially domesticated genotypes. In some regions such as Oaxaca, exploitation of agave species occurs at all three levels and involves different species.

Agave angustifolia is the species with the widest geographical distribution within the genus, as it is found from Sonora and Tamaulipas in Mexico to Costa Rica (Gentry 1982). Accordingly, *A. angustifolia* grows in diverse types of vegetation and climate, such as *Pinus-Quercus* forests, xeric scrubland and tropical deciduous forests and from sea level to 2500 m elevation (García-Mendoza and Chiang 2003). Within its wide distribution area, several populations have been subject to domestication for their hard fibers and as raw material for the production of distilled spirits.

Taxonomically, *Agave angustifolia* belongs, along with 12 other species, to *A.* section *Rigidae* (Gentry 1982). Species in this section are characterized by narrow rigid leaves, a small lax panicle with few branches, green or yellow flowers and quickly withering tepals. Using a morphological species concept, Gentry (1982) grouped into a single series of populations (varieties) plants that other authors (Salm-Dyck 1834; Hooker 1871; Trelease 1907, 1920; Berger 1915) had proposed as distinct species. However, it is difficult to consistently separate species and their intraspecific taxa based on the vegetative and reproductive traits of populations; in *A. angustifolia*, more than 20 synonyms are recognized. Gentry (1982) divided this species into three wild varieties, *A. angustifolia* var. *angustifolia, A. angustifolia* var. *rubescens* and *A. angustifolia* var. *sargentii*, as well as four cultivated varieties, *A. angustifolia* var. *deweyana, A. angustifolia* var. *letonae, A. angustifolia* var. *marginata* and *A. angustifolia* var. *nivea*. Gentry (1982) also claimed that *A. rhodacantha* and *A. tequilana* were distinct species. Nonetheless, this author indicated the difficulty in distinguishing these taxa using morphology and considered *A. rhodacantha* and *A. tequilana* to be part of the *A. angustifolia* taxonomic complex.

Recent studies have clarified the taxonomy of this complex, particularly the relationship between domesticated plants and certain wild populations. Colunga-GarcíaMarín et al. (1996); Colunga-GarcíaMarín and May-Pat (1997) and Keb-Llanes et al. (2002) discussed the morphological and genetic relationships of henequén (*Agave fourcroydes*) with wild populations of *A. angustifolia* in the Yucatán Peninsula, indicating that in general they form distinct groups with the exception of one cultivated genotype that was closer to the wild *A. angustifolia* genotypes. In the states of Jalisco and Guanajuato, which are considered centers for domestication of the complex, Gil-Vega et al. (2006, 2007), Vargas-Ponce et al. (2007, 2009) analyzed genetic variability and relationships between wild and cultivated taxa, focusing on *A. tequilana* showing also higher diversity within wild varieties and landraces in comparison with *Agave tequilana* "Azul."

This study aims to clarify the taxonomy of the *A. angustifolia* complex in the state of Oaxaca, a region where wild and domesticated populations of *A. angustifolia* coexist. Because the morphological evidence previously used by Trelease (1908, 1920) and Gentry (1982) is not sufficient to clearly delineate these taxa, it is necessary to employ new, recently proposed tools to interpret the variability that exists within the taxa and populations and thus to propose an objective and practical taxonomic delimitation.

Materials and method

An extensive review of the taxonomic literature on the *A. angustifolia* complex was conducted in order to define the range of taxa to include in the study. Initially, the taxonomy proposed by Gentry (1982) who recognizes the wild taxa *A. angustifolia* var. *angustifolia* and *A. angustifolia* var. *rubescens*, as well as cultivated plants recognized as *A. angustifolia* "Espadín," was considered. *A. rhodacantha* and *A. tequilana*, taxa that Gentry split from *A. angustifolia*, were also considered within the complex since Gentry did mention their morphological similarity with this complex.

Herbarium work

All accessions of taxa of the complex distributed in the state of Oaxaca deposited in MEXU were reviewed obtaining 120 records. In addition, 220 records were gathered from GBIF and UNIBIO. *Agave angustifolia* var. *angustifolia, A. angustifolia* var. *rubescens* and *A. rhodacantha* were confirmed for Oaxaca, as well as the cultivated variety *A. angustifolia* "Espadín." Populations of *A. tequilana* from the state of Guanajuato and *A. angustifolia* from Sonora were also reviewed. A database of 45 georeferenced localities was generated from these records. Nine populations were selected for field sampling (seven in the state of Oaxaca, one in the state of Sonora and one in the state of Guanajuato), including the type locality.

Field and laboratory work

In the nine selected localities (Table 1), fresh samples of vegetative and reproductive tissues were collected for morphometric analysis, and live plants were collected for genetic analysis. At least five individuals per taxon were collected. Voucher specimens were also collected and deposited in the herbarium MEXU. Live plants were deposited in the National Collection of Agavaceae at the Botanical Garden of the Institute of Biology of the Universidad Nacional Autónoma de México.

The following traits were recorded in the field for the morphological analysis of 46 individuals at reproductive stage: height and diameter of the rosette, number of leaves, length of the inflorescence, length of the peduncle, number of branches of the inflorescence and length of its longest branch. In addition, one mature leaf per plant and the third bract of the inflorescence were collected *in situ* for examination in the laboratory; flowers and fruits were stored in GAW (15% glycerin, 45% alcohol and 40% water).

Forty-six traits were recorded and evaluated: four qualitative and 42 quantitative. These traits were selected considering diagnostic characters used in previous taxonomic treatments (Gentry 1982; Trelease 1915). The traits length of the leaf, width of the leaf, size of the terminal spine and number of teeth were included because, according to Colunga-GarcíaMarín et al. (1996), they have changed with domestication. Vargas-Ponce et al. (2007) used 16 quantitative vegetative characters to analyze the morphological variability in *Agave* populations exploited to produce distilled beverages, and most of these characters were also evaluated in this study.

Statistical analysis

A data matrix consisting of 46 individuals and 46 traits was generated. A correlation analysis was performed using the

Table 1 Localities selected for the study

Locality	Municipality	Elevation (m)	Geographical coordinates	Taxon collected Wild/Cult	Individuals sampled (morphological/genetic/herbarium)
El Camarón	San Carlos Yautepec	900	16°33′11″N 96°0′10″ O	*Agave angustifolia rubescens* W	4/10/6
Las Sedas	San Francisco Telixtlahuaca	1860	17°17′36″ N 95°55′43″O	*Agave angustifolia rubescens* W	4/10/10
La Reforma	San Andrés Dinicuiti	1920	17°44′43″N 97°42′25″O	*Agave angustifolia angustifolia* W	3/10/4
La Luz Nagore	Huajolotitlán	1660	17°50′18″N 97°43′37″O	*Agave angustifolia angustifolia* W	3/10/4
Magdalena Teitipac	Magdalena Teitipac	1685	16°55′09″N 96°33′04″O	*Agave angustifolia* "Espadín" C	5/10/10
Los Coaches	San Pedro Totolapan	970	16°41′39″N 96°11′05″O	*Agave angustifolia* "Espadín" C	5/10/10
El Coyote	Yogana	1400	16°27′45″N 96°46′39″O	*Agave rhodacantha* C	5/10/10
Puerto interior	Silao de la Victoria	1800	21°00′51″N 101°29′46″O	*Agave tequilana* "Azul" C	10/10/16
Nácori Chico	Nácori Chico	845	29°41′00″N 108°59′22″O	*Agave angustifolia* "Sonora" C	7/8/0

C cultivated, *W* wild

program Stata® 9.0. This analysis allowed identification of traits with a high correlation coefficient and elimination of those traits from subsequent analyses. Canonical discriminant analysis (CDA) identified the traits with greater weight in variances (Cristofolini and Crema 2005; Cron et al. 2007; Strandby et al. 2009; Urbaniak 2010; Chiapella et al. 2011).

Amplified fragment length polymorphism (AFLP) analysis

Eighty-eight foliar tissue samples were collected from the six taxa studied, preserved in liquid nitrogen and stored at – 80 °C. The protocol of Vos et al. (1995) modified by Simpson (1997) for the Agavoideae subfamily was used to obtain amplified DNA fragments. Fluorescently labeled Eco R I + ACC and Eco R I + AGG and non-labeled Mse I + ACCC and Mse I + ACGT primers were used, to generate four distinct primer combinations. The amplified DNA fragments were analyzed using a LI-COR sequencer with the program SAGA^{MX} from AFLP Quantar. This program identifies the corresponding fragment by its fluorescence intensity and records it with a + sign; its absence is denoted by a – sign, and missing data are marked with the letter F. All designations determined by the software were revised and corrected manually. It was assumed that each fragment is a dominant allele at a particular polymorphic locus; data for fragment presence/absence were used to construct a basic data matrix. The software AFLP-SURV (Vekemans et al. 2002) was used to calculate the number and percentage of polymorphic loci and Nei's genetic diversity for each taxon (Nei 1978). Samples from individuals with run errors and those with more than 20% missing data were excluded from the matrix, and the software FreeTree was used to generate a similarity matrix using the Nei-Li\Dice coefficient (Pavlícek et al. 1999). This array was processed to generate a similarity tree with the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm and a Jackknife resampling analysis with 3000 repetitions.

Ploidy analysis

To analyze the effect of domestication on the cultivated populations and their level of ploidy, a comparative cytometric analysis was performed to determine the relative content of DNA in the identified populations. Nuclear DNA was stained with DAPI (4′,6-diamidino-2-phenylindole), which indicates A-T bonds, and quantified using a Partec® PA II model flow cytometer. To determine ploidy, five individuals were analyzed per population; five samples were taken from each individual, and each was tested in three repetitions. The level of ploidy was estimated using *Zea mays* and *Agave tequilana* plants as the standard because their DNA content is well established and because the taxa analyzed are likely to be within the range of these species (Dolezel et al. 1998; Palomino et al. 2003).

Table 2 Discriminant functions and the percentage of variance explained from the data analyzed

Function	Canonical correlation	Eigen value	Variance	
			Proportional	Accumulated
1	0.9924	65.3875	0.6241	0.6241
2	0.9721	17.1768	0.1640	0.7881
3	0.9570	10.8796	0.1038	0.8919
4	0.9420	7.87901	0.0752	0.9671
5	0.8804	3.44492	0.0329	1.0000

Table 3 Proportion of each of the variables in the first three discriminant functions

Variable	Function		
	1	2	3
Number of leaves	0.008	0.175	**0.615**
Leaf length	**−1.188**	0.323	0.153
Number of teeth	**0.927**	−0.303	**1.235**
Tooth length	**1.422**	−0.267	**1.209**
Thorn length	0.201	**0.672**	0.521
Inflorescence length	−0.001	0.290	**−0.850**
Peduncle/panicle length	0.109	0.153	−0.327
Sterile bract length	−0.454	**0.555**	0.157
Pedicel length	−0.060	−0.163	**0.648**
Ovary length	0.479	−0.033	0.456
Ovary diameter	0.317	0.471	0.443
Perianth tube width	−0.137	−0.198	0.471
External tepal length	0.213	**−0.676**	0.036
Anther length	0.011	**0.860**	0.008
Stylus length	−0.095	−0.260	0.223
Capsule length	−0.127	−0.060	0.397
Seed length	−0.333	**0.611**	−0.244
Proportion of mature seeds	**1.318**	0.220	0.023

Bold values represent the variables with more importance in the variance of each function

Results

Statistical analysis

Twenty-six traits with very low significance and high correlation values were discarded, as were two other continuous traits not considered by the canonical discriminant analysis, which uses only discrete traits. With five discriminant functions of this analysis, 100% of the variance in the data was explained (Table 2). The first function explained 62.5% of the variance and was positively related to the length of teeth halfway up the leaf, the proportion of mature seeds in each fruit and the number of teeth; it was negatively correlated with the length of the leaf. The second function, which explained 16.4% of the variance, was positively related to the lengths of anther, spine and seed and was negatively related to the length of the external tepal. The third function, explaining 10% of the variance, was positively correlated with the number of teeth and their length and also with the length of the pedicel; it was negatively correlated with the length of the inflorescence (Table 3). Contrasting the standardized coefficients of the first two functions yielded a phenotypic scatter plot of the data analyzed, which shows how the taxa are clearly grouped according to level of cultivation and human management (Fig. 1).

Ploidy analysis

Diploid and triploid populations were found in the analyzed samples. The diploids genotypes are *Agave angustifolia* var. *angustifolia* from Oaxaca and Sonora, *A. angustifolia* var. *rubescens* and *A. rhodacantha* from Oaxaca and *A. tequilana* from Guanajuato. *A. angustifolia* "Espadín" commonly used for the production of mescal in Oaxaca is triploid (Online Resource 1).

Genetic diversity

A total of 289 fragments were obtained using the four combinations of primers. The genetic diversity index indicated a large number of polymorphisms and a high percentage of polymorphic loci among the taxa studied. *Agave tequilana* "Azul" and *A. angustifolia* "Sonora" showed the lowest levels of polymorphism, consistent with a high level of management that includes clonal propagation of selected individuals to produce a homogeneous crop. *A. angustifolia* var. *angustifolia* and *A. angustifolia* var. *rubescens* exhibited comparatively higher levels of polymorphism, consistent with the fact that they are wild populations which have not undergone selection by direct human intervention (Table 4).

The grouping of populations in the phenogram (Online Resource 2) is consistent with the results obtained in the canonical discriminant analysis. Two groups are observed in the phenogram. One group consisted of *Agave tequilana* "Azul," *A. rhodacantha, A. angustifolia* "Sonora" and *A. angustifolia* "Espadín," which are all taxa subject to a gradient of artificial selection. Of these, *A. tequilana* "Azul" and *A. angustifolia* "Espadín," are populations dependent on the human management of the environment in which they are grown, while *A. rhodacantha* and *A. angustifolia* "Sonora" are thought to be populations with incipient domestication. Wild

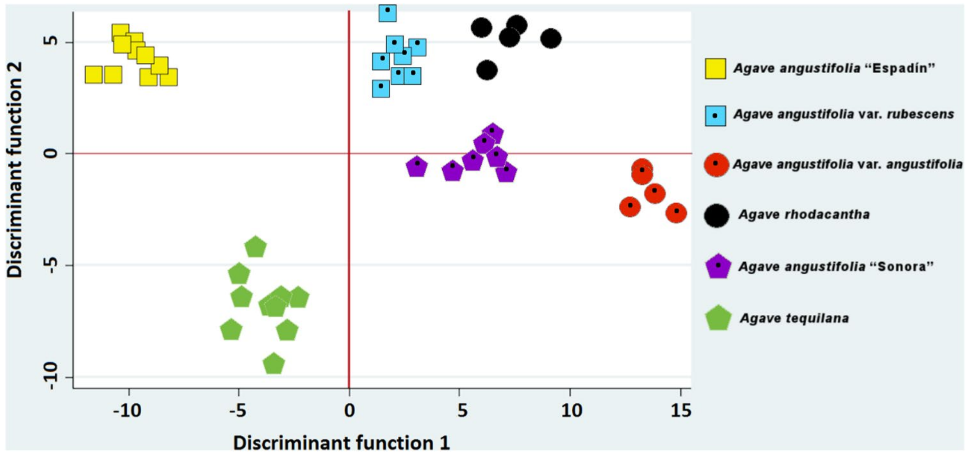

Fig. 1 Scatter plot of the taxa based on the first two canonical discriminant functions

Table 4 Genetic diversity obtained by AFLP analysis

Taxon	*N*	NLE	NPL	PPL	He	SDHe
Agave angustifolia var. *angustifolia*	14	289	281	97.2	0.31371	0.00873
A. angustifolia var. *rubescens*	10	289	280	96.9	0.31064	0.00886
A. angustifolia "Espadin"	13	289	277	95.8	0.31034	0.00854
A. rhodacantha	7	289	238	82.4	0.23402	0.01019
A. tequilana "Azul"	5	289	231	79.9	0.20550	0.00983
A. angustifolia "Sonora"	6	289	226	78.2	0.26062	0.01003

N number of individuals, *NLE* number of loci, *NPL* number of polymorphic loci, *PPL* percentage of polymorphic loci, *He* Nei's genetic diversity, *SDHe* standard deviation

populations of *A. angustifolia* var. *angustifolia* and *A. angustifolia* var. *rubescens* comprised the other large group (Fig. 2).

Discussion

In a previous study of *Agave* the number of individuals analyzed was higher (Vargas-Ponce et al. 2007). However, that study only collected and analyzed plants in their vegetative form, whereas this study includes individuals at the reproductive stage allowing detailed analysis of floral morphology, which is essential to obtain a more robust classification of the genotypes sampled. For the genus *Agave*, this is a complex endeavor due to the intrinsic reproductive characteristics of these plants (e.g., long life cycle, monocarpic flowering, non-synchronous flowering within populations, low reproductive success and reversion to bulbil formation). Despite these difficulties, we collected at least five individuals per taxon specifically for morphological analysis, a sufficient number for the statistical analyses.

The CDA analysis proved to be useful for the *A. angustifolia* complex, as it explained 80% of the variance in two functions. This analysis identified the traits that best explain the morphological variation among the taxa and populations analyzed: length of the leaf, number of teeth, length of teeth, length of the spine, length of the anther, length of the seed and proportion of mature seeds. The combination of these traits allows for morphological delimitation of these taxa.

Agave angustifolia "Espadín" is clearly distinguishable from the wild populations of Oaxaca by its larger leaves, larger inflorescences and very low proportion of mature seeds. The type variety of *A. angustifolia* is distinguishable from *A. angustifolia* var. *rubescens* by the greater number of leaves, fewer and smaller teeth and smaller terminal thorn. *A. rhodacantha* is similar to *Agave angustifolia* "Espadín" but differs by the bright green color of its leaves, greater number of leaves and more numerous and larger teeth. On the other hand, the population of *A. angustifolia* "Sonora" is morphologically distinguished from the Oaxaca population by the much greater length of leaves and more numerous and smaller teeth. It also differs from the Oaxaca populations in the size of the inflorescence and the sterile bract. In both cases, these exceed the size of those from Oaxaca. Finally, *A. tequilana* "Azul" is the taxon that shows the lowest number of leaves, presents very small teeth and has the smallest terminal spine.

With regard to the level of ploidy in the populations studied, some morphological and physiological traits that have been indicated as distinctive of polyploid plants were identified *a priori*, including fewer fruits and mature seeds as

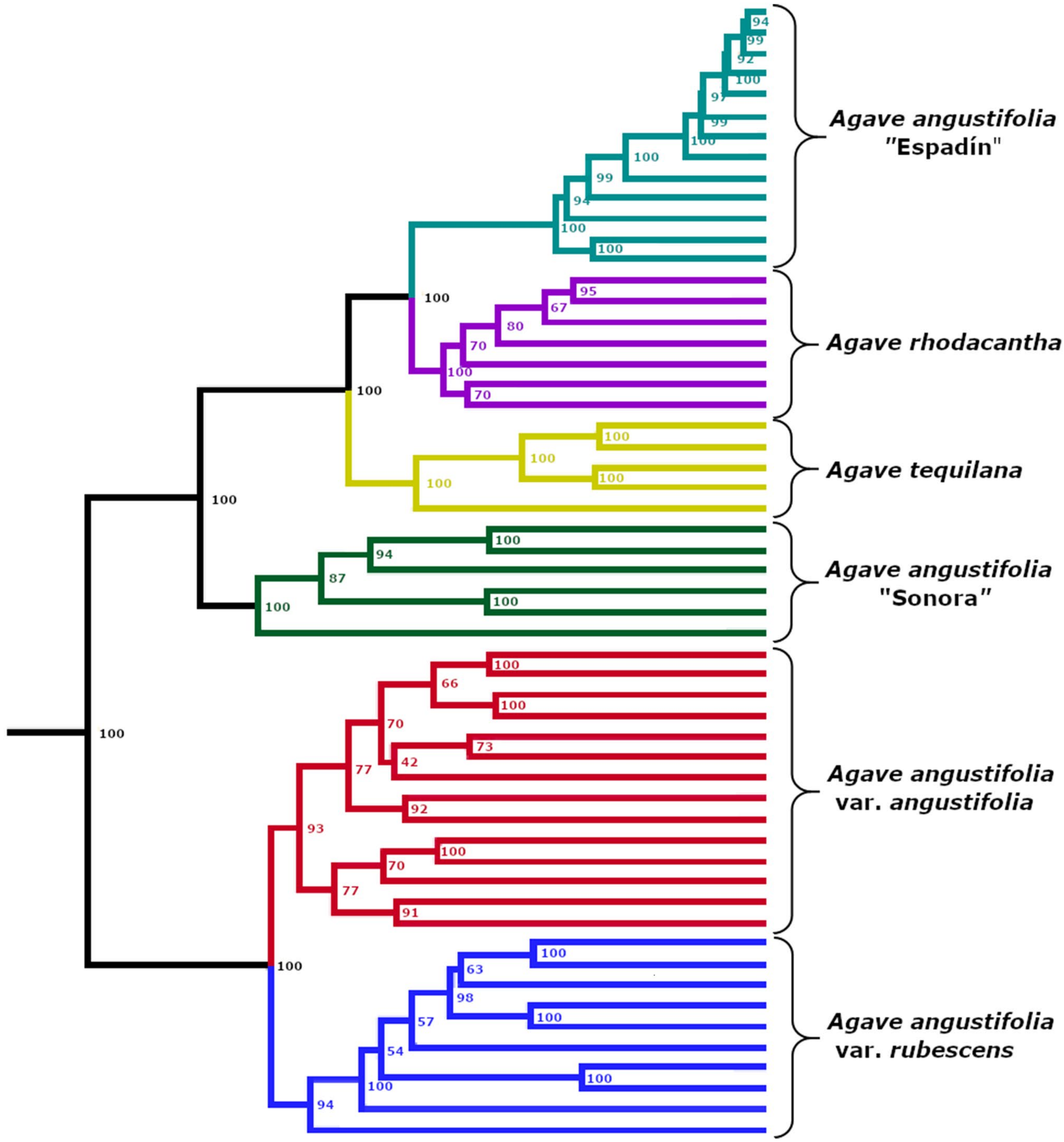

Fig. 2 Phenogram of genetic similarity of the taxa analyzed using AFLP. The numbers on each node represent jackknife support values. Samples with substantial missing data or run errors were eliminated before statistical analysis

well as gigantism. Levin (2002) described this phenomenon in several groups of plants, and Colunga-GarcíaMarín et al. (1996) observed this behavior in *Agave fourcroydes*. These observations were confirmed by cytometric analysis, which indicated triploidy in populations of *Agave angustifolia* "Espadín," confirming the results of Palomino et al. (2005) who indicated the same level of ploidy. Zeven (1980) comments that formation of polyploids in natural populations may be disadvantageous in terms of reproduction or lead to competition with individuals of the same species found in close proximity. The size and robustness of the triploid *Agave angustifolia* "Espadín" may have been an attractive trait that was selected during the domestication process, therefore favoring the survival and expansion of the use of this genotype.

Analysis of molecular polymorphisms confirmed the clear grouping for each genotype obtained by the CDA of morphological traits and also clearly shows two large groups comprising either the wild (*A. angustifolia* var. angustifolia and *A. angustifolia* var. rubescens) or domesticated (*A. angustifolia* "Sonora," *A. tequilana* "Azul," *A. rhodacantha* and *A. angustifolia* "Espadín") genotypes. This supports the idea of Gentry that *A. tequilana* and *A. rhodacantha* are distinct from *A. angustifolia* var. *angustifolia* and *A. angustifolia* var. *rubescens* but within the Angustifolia complex. Within the "domesticated" group two main subgroups are observed, one containing only *A. angustifolia* "Sonora" and the other containing *A. tequilana*, *A. rhodacantha* and *A. angustifolia* "Espadín." *Agave rhodacantha* is a taxon that has not been well studied. According to Gentry (1982), it is distributed from Sonora to Nayarit, with isolated populations in Durango, Jalisco and Oaxaca, where the plants are used to produce mescal (Vargas-Ponce 2007; Vargas-Ponce et al. 2007; González-Elizondo et al. 2009). The present study examined the only population described in the state of Oaxaca and compared it morphologically with the original description by Trelease (1920). It is necessary to analyze populations of this species throughout its area of distribution to establish differences and similarities among populations and to decide whether it is one species or separate populations of distinct taxonomic entities. Our results indicate that the *Agave rhodacantha* genotypes cultivated in at least one municipality of Oaxaca are closely related to *A. tequilana*.

Two hypotheses have been proposed to explain the biological/geographical origin of *A. angustifolia* "Espadín" in the central valleys of Oaxaca. Genetic and morphological evidence, as well as information obtained in the field from different growers in the districts of Ejutla, Miahuatlan and Tlacolula, Oaxaca, indicates the introduction of "Espadín" from western Mexico via individuals related to *A. tequilana* to produce mescal during the first half of the twentieth century. Walton (1977) reported that in the valley of Oaxaca the exploitation of the "tequilana" variety was recent. The domesticated populations in Oaxaca are under a selective pressure from farmers who seek to obtain the most efficient raw materials to produce spirits. This domestication process, which has led to the modern commercial varieties, is consistent with the theory of consecutive periods of selection and domestication (Colunga-GarcíaMarín and Zizumbo-Villarreal 2007) carried out initially in western Mexico, the proposed site of origin of the germplasm. The objective of the primary selection process was probably to identify optimal genotypes for the production of fibers or as a food source. The hypothesis of a western origin for "Espadín" is supported by the genetic analysis performed in this study that shows a close relationship between *A. tequilana* and *A. angustifolia* "Espadín." In addition, Palomino et al. (2003) reported triploidy in the *A. tequilana* variety "Bermejo," which is morphologically similar to "Espadín" in that the plants are taller, have a larger rosette diameter and are more robust. A genetic analysis of these two varieties should be carried out to clarify whether they are the same taxon.

The second hypothesis for the origin of "Espadín" is based on historical and anthropological records suggesting the district of Tlacolula in the region of the Central Valleys of Oaxaca as the site of domestication of *Agave angustifolia*, where it was used for intensive production of mescal in the 1940s as described by Benítez (1980) and Urrutia (1986). In this scenario (in common with the first hypothesis), the distinctive morphological features of "Espadín," such as its large size, polyploidy and low percentage of mature seeds, could also have been generated and maintained by artificial selection (Colunga-GarcíaMarín et al. 1996; Levin 2002). However, the time of domestication would have been very short and due to the constant and significant differences between wild populations of *A. angustifolia* and the cultivated variety known as "Espadín," and its similarity to *A. tequilana*, the first hypothesis seems more likely.

The genetic diversity of the populations is consistent with other studies addressing the level of management and selection of optimal germplasm. *Agave tequilana* "Azul" and *A. angustifolia* "Sonora" are propagated by planting suckers and show low levels of genetic variation (Gil-Vega et al. 2006; Aguilar-Meléndez et al. 2009; Vargas-Ponce et al. 2009). Interestingly, *A. rodacantha* classified as under incipient domestication also shows a low level of diversity. This result is in contrast with the report of Vargas-Ponce et al. (2009) which found that populations maintained under traditional cultivation systems show levels of diversity similar to wild populations and may indicate that *A. rodacantha* has actually reached a higher level of domestication than was originally considered. On the other hand, *A. angustifolia* "Espadin" classified as cultivated, shows a level of diversity similar to the wild populations based on Nei's index, when diversity is calculated across all genotypes. However, this may be influenced by the polyploid nature of *A. angustifolia* "Espadin" since diversity within the "Espadin" population is low when individual genotypes are compared as can be observed in the phenogram.

Zizumbo-Villarreal and Colunga-GarcíaMarín (2007) have presented strong evidence that the process of distillation was introduced from the western coast of Mexico probably via the Philippines and spread throughout the country following the trade routes to the north, south and center. Colunga-GarcíaMarín and Zizumbo-Villarreal (2007) have also reported the presence of genotypes of the *A. angustifolia* complex close to the region where distillation was first introduced. Needham et al. (1980) and Needham and Lu (1985) have also suggested that the culture known as Capacha that thrived in the years 1500–1000 BC, what is now known as the state of Colima, employed vessels such as gourd pots which could have been used to obtain a distilled product. Zizumbo-Villarreal et al. (2009) present an experiment in which they used replicas of these modified gourd pots and an *Agave*-based fermented product to produce ethanol-containing distillates. This indicates that it is possible that distillation was carried out in Mesoamerica before the Spanish conquest.

It is very probable that desirable genotypes were transported along with distillation technology, foreign or domestic, and this may have had an impact on the genotypes exploited in different regions. In some cases the *Agave* populations already domesticated for the production of hard fibers and food, undoubtedly suffered a consequent event of domestication and management to produce raw material for distillation of spirits. Since vegetative propagation is almost exclusively employed for agave cultivation, specific clones could have been introduced, propagated and distributed extensively in a relatively short period of time (Zizumbo-Villarreal and Colunga-GarcíaMarín 2007).

Conclusions

Morphological and molecular evidence shows that the domesticated and semi-domesticated populations of the agave genotypes used to produce spirits are distinct from their wild relatives and show a lower level of diversity. This can be attributed to the selection and vegetative propagation of germplasm with desirable traits. Although *Agave tequilana*, *A. rhodacantha* and *A. angustifolia* "Espadín" can be distinguished both morphologically and genetically, the results also confirm a close relationship between them and support previous hypotheses that these genotypes belong within the *A. angustifolia* complex. The samples of *A. angustifolia* "Sonora," although in a separate subgroup, are also found within in the domesticated group.

As Gentry (1982) and other authors have previously indicated, the unraveling of the taxonomy of *A. angustifolia* is challenging and the exploitation of this species for commercial purposes has made this goal even more difficult. Although a more extensive analysis of *A. angustifolia* germplasm ranging from Oaxaca to Sonora is necessary in order to confirm our findings, based on these results we suggest that the *A. angustifolia* genotypes from Sonora should be recognized as a distinct variety under the name *A. angustifolia* var. *pacifica,* which was the name given by Trelease (1920) to these populations. Because of their close relationship with *A. tequilana*, and the results from this and other studies, the domesticated plants called "Espadín" could be considered as a cultivar of *A. tequilana*. Although the primary focus of domestication of *Agave* species by ancient populations was the production of fiber and food, our data suggest that the main factor leading to the degree of domestication of *A. angustifolia* observed today in the Oaxaca valley region was the secondary period of selection and management when the technology to produce spirits (developed either locally or imported from overseas) was introduced from the Pacific coast of Mexico.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Information on Electronic Supplementary Material

Online resource 1 Table showing the ploidy levels of the taxa analyzed.

Online resource 2 Figure showing the relative DNA content in two of the six populations analyzed (**a**) *Zea mays,* (**b**) *Agave angustifolia* var. *angustifolia* (diploid) and (**c**) *A. angustifolia* "Espadín" (triploid).

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