Morphological variability of Agave landraces for artisanal spirit production in Jalisco, Mexico: regional patterns and conservation perspectives

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Abstract

Jalisco, located in western Mexico, is a key epicenter of agave spirit production driven by global demand. This demand has often led to the agro-industrial intensification of agave cultivation and the implementation of regulations, such as Designations of Origin, which unintentionally threaten plant genetic resource conservation and impact the livelihoods of traditional small-scale producers. The coastal and southern regions of Jalisco have a long tradition of producing artisanal spirits from plants belonging to the *Agave angustifolia* Haw. – *A. rhodacantha* Trel. complex. In these two regions, we evaluated 17 morphological traits in 385 agave plants, representing 24 traditional landraces, using multivariate methods. The high variability observed appears to be influenced by multiple factors at different scales, including taxonomic species, regional differences, and landrace distinctions. We delineated this complexity by identifying: 1) Landraces as the primary factor explaining morphological variation; 2) Five landrace groups with distinct morphological traits; 3) Five specific landraces—three in the coastal region and two in the south—as the most clearly delineated entities. Finally, we discuss the emerging patterns associated with each factor and highlight the significance of local agave production systems in generating and conserving agrobiodiversity.

INTRODUCTION

Mexico is the center of origin and diversification of *Agave* L. (Asparagaceae) with 160 native species (Villaseñor 2016), representing ca. 70% of the global diversity for the genus (García-Mendoza 2002). As many as 22 categories of use and around 40 specific uses have been reported for *Agave* spp., with food, fiber and beverage being the most widespread (Colunga-GarcíaMarín et al. 2017). Among these uses, alcoholic beverages—particularly distilled spirits—have massive economic importance and are associated with vast biocultural diversity, with 53 *Agave* species known to be used to produce distilled beverages in Mexico (Torres et al. 2015). Leading a boom of *Agave* spirits, tequila and mezcal have experienced a massive growth in production in the last decades (Tetreault et al. 2021). This has been possible due to both the expansion of cultivated area, and also as a result of a process of agro-industrial intensification, which has implied 1) the transition from diversified production systems to monocultures, 2) increased agrochemical usage, 3) shortened harvest cycles and 4) the increased focus on a few highly productive agave landraces (Bowen and Valenzuela-Zapata 2009; Torres-García et al. 2019; Valenzuela-Zapata and Nabhan 2003). The environmental costs of this economic success are apparent at different scales, ranging from deforestation, soil degradation, to the loss of traditional landraces and their resulting genetic erosion (Bowen and Valenzuela Zapata 2009; Tetreault et al. 2021).

The long-term interactions of diverse groups of people across Mexico with the biological diversity of *Agave*, over a large and heterogenous geography, has yield an even greater infraspecific diversity which is expressed in ample morphological variation, with multiple landraces typically recognized in each region (e.g. Cabrera-Toledo et al. 2022, 2024; Vázquez-Pérez et al. 2020). Morphological variation in *Agave* landraces has been widely studied in the context of their management and domestication; domestication syndromes have been classified into three types according to their main use (Colunga-

GarcíaMarín et al. 2017). Evidence suggest that species with ancient uses, such as food (Gentry 1982; Colunga-GarcíaMarín et al. 2007; Torres et al. 2015), fiber (Colunga-GarcíaMarín et al. 1996; Carrillo-Galván 2011) and fermented beverages (Figueredo-Urbina et al. 2021) show, in some species, clear domestication syndromes related with the traits selected for these uses (e.g. plant giantism, carbohydrate concentration, stem and peduncle inflorescence taste, leaves giantism, high amount of sap). Distilled beverages have a much more recent history, which may explain the absence of clear domestication syndromes associated with them (e.g. Vargas-Ponce et al. 2007).

Most research has focused on the morphological and genetic variation of industrially relevant *A. tequilana* F.A.C Weber var. Azul and *A. angustifolia* var. Espadín landraces (Gil-Vega et al. 2001, 2006; Rivera-Lugo et al. 2008; Rodríguez-Garay et al. 2009; Ruiz-Mondragón et al. 2022), both considered domesticated. However, for many other agave species/landraces, diversification and domestication is still in its early stages, occurring through ongoing diversification processes in peasant management systems, which have received limited attention (Vargas-Ponce et al. 2007, 2009; Cabrera et al. 2020, 2022). Understanding the role of peasant management in these processes—whether as drivers of morphological diversification (Vargas-Ponce et al. 2007, 2009) or as creators of domesticated forms (Figueredo-Urbina et al. 2021)—is a crucial first step in safeguarding them against the accelerated genetic erosion driven by the expanding agave agroindustry.

A. angustifolia was described by Gentry (1982) as “an extensive variable species”, acknowledging that the leaf size and armature (including terminal and marginal spines) of *A. rhodacantha* are also highly variable, with some forms being indistinguishable from *A. angustifolia*. Moreover, studies on the morphological and genetic variation of *Agave* landraces in southern Jalisco (Vargas-Ponce et al. 2007, 2009) and Oaxaca (Rivera-Lugo et al. 2018), suggests that these taxa should be treated as *A. angustifolia-A. rhodacantha* species complex. As multiple local landraces within this complex are managed to produce distilled spirits within and across regions in México (Torres-García et al. 2023), they serve as a relevant model to compare how morphological variation of managed agave landraces is structured across scales. For example, distinct morphological differences have been identified between wild gene pools and commercial crops, as well as taxonomic variations between *A. rhodacantha* and *A. angustifolia* across northern, central, and southern Mexico (Rivera-Lugo et al. 2018). Similarly, notable differences have been observed among wild populations, traditional farmer-managed cultivars, and commercial crops in central and southern Jalisco (Vargas-Ponce et al. 2007, 2009).

The state of Jalisco (Western Central Mexico) stands out for its *Agave* diversity and its tradition of agave spirit production including, tequila, mezcal, raicilla, tepe, and tuchi, among other distilled beverages (Bruman 2000; Valenzuela-Zapata and Gaytán 2012); being only tequila and raicilla within designation of origin (Diario oficial de la federación 1969, 2019 respectively). In particular, the north coast where *raicilla de la costa* is produced and south of Jalisco were *mezcal* is produced, are two regions with long tradition of production of artisanal spirits made with plants belonging to the *A. angustifolia - A. rhodacantha* species complex. Previous studies have morphologically and genetically characterized *Agave* landraces in the South confirming this species complex as the source of traditional landraces and

identifying three different morphological groups and two isolated landraces (Vargas-Ponce et al. 2007, 2009). Cabrera-Toledo et al. (2022) conducted a comprehensive analysis of the *Agave angustifolia–A. rhodacantha* complex in two regions of Jalisco known for their high cultivar diversity. Their findings reveal that the most frequently used cultivars in mezcal production in both regions are also the most genetically distinct from the rest. Additionally, they identified cases where some traditional farmer classifications aligned with genomic identities, as well as landraces that exhibited the closest genetic links to wild specimens. These insights provide valuable information about the genetic reservoirs from which the ancestors of modern mezcal cultivars originated. However, from a morphological perspective, this congruence could not be thoroughly analyzed due to the limited number of individuals representing each cultivar.

This study offers the most in-depth exploration to date of the morphological variation within the *Agave angustifolia – Agave rhodacantha* species complex, carried out in the context of traditional peasant management systems. By including a large number of individual plants and evaluating a broad set of morphological traits, our work captures the rich diversity shaped by generations of farmer selection and cultivation practices. Our sampling allows us to analyze the structure of morphological variation within species and locally defined landraces across different scales, including species, regions and landraces. In this context, we established the main research questions that guide our study: i) Are the determined species (i.e., *A. angustifolia* and *A. rhodacantha*), based on Gentry's typological criteria, morphologically distinct from each other? ii) Do landraces from the coastal and southern regions differ in terms of morphological features? iii) Are the landraces recognized by producers clearly defined, and which traits characterize each landrace? iv) Which of the aforementioned scales of variation (species, region, and landraces as defined by local farmers) better explains the morphological diversity observed in this complex?

By addressing these questions, we aim to uncover how morphological variation is structured by both natural and human history. Additionally, understanding the connections between two bioculturally significant regions—one of them (South) proposed as one of the primary sites where distillation first occurred (García Garza, 2021)—can provide deeper insights into the history of agave spirits. This, in turn, sheds new light on the legacy of Mestizo rural families, which is embedded in the morphological diversity of Jalisco's agave landraces and has important implications for their conservation and management.

MATERIAL AND METHODS

Study area and plant material

We assessed the morphological variation and geolocalized 24 agave landraces recognized by artisanal spirit producers in two regions of the state of Jalisco (Western Mexico) with longstanding tradition of agave spirit production. Our regions of study were the northern coast of Jalisco, mostly within the Cabo Corrientes municipality (hereafter the "Coast") where an agave spirit known as *raicilla de la costa* is

produced, and the southern region of Jalisco along its limits with the state of Colima, an area which includes five municipalities (hereafter the “South”), where *mezcal* is produced.

The Coast region, influenced by its proximity to the Pacific Ocean, has a subhumid tropical climate with a mean annual precipitation ca. 1,624 mm. Study sites here are located at elevations between 500 and 700 masl, natural vegetation is represented by semi-deciduous tropical forest and oak forest. Traditional raicilla production is concentrated in northern Cabo Corrientes, in small communities such as Chacala and El Refugio de Suchitlán. In contrast, the South region, has a hot semi-arid climate with a mean annual precipitation ca. 570 mm. Located in the Río Ayuquila-Armería basin, study sites range from 600 to 1000 m.a.s.l. in elevation. This area features thorn forests and deciduous tropical forests, especially in the lower valleys and canyons. While agroindustrial production of blue agave (*A. tequilana*) has extended throughout the region, traditional spirit production persists in the municipalities of Tolimán and Zapotitlán de Vadillo. In collaboration with local producers, we sampled most of the plant material in “mezcaleras”, local agave farming systems. Between 7 to 11 mature plants, each separated more than 10 m were recorded per producer property and landrace (population) to capture, as much as possible, the intra-varietal variability. A total of 385 records were included in the database (Table 1 and Figure 1), ca. half of them (200 records) were gathered during 2021 and 2022 in the Coast and the South. In the Coast, plants of 15 populations belonging to 8 different landraces were measured in Cabo Corrientes. In the South, plants of three different populations belonging to one landrace (Lineño) were recorded in Tolimán while plants of three populations of three local landraces were recorded in Zapotitlán de Vadillo. The second half of the data set (185 records) included samples analyzed in Vargas-Ponce et al. (2007); plants of 7 populations belonging to 7 different landraces from Zapotitlán de Vadillo, and from 7 populations of 7 landraces in Tolimán. In order to include some references of wild populations of both species, Vargas-Ponce et al. (2007) included one locality of *A.angustifolia* in Tuxcacuesco where plants grow in the dry forest, two populations in Tuxpan where plants do have some eventual management practices in the dry forest (Garabato, *A. angustifolia* and Sierrilla Verde Amarillento, *A.rhodacantha*) and the wild population of *A. angustifolia* located in Palo Alto, municipality of Tecolotlán. Additionally, one *Agave tequilana* var. Azul population was morphologically measured in Tonaya as a crop reference. Three plots were sampled twice, once in 2007 and the second in 2022 allowing to longitudinally explore change within properties after a 15 year-period. The taxonomic affinities for each population/landrace are proposed in Table 1, based on the expert opinions of botanic specialists García-Mendoza, A. and Carrillo-Reyes, P. (pers. comm), following Gentry’s typological criteria based on vegetative traits. Concisely, *sensu* Gentry (1982), both species have short stems (less than 1 m tall), but *A. rhodacantha* has longer (2 - 2.6 m) and broader (8-15 cm) linear leaves, green to faintly glaucous green, bigger teeth (4-8 mm long), closely spaced (1-3 cm apart) and, shorter spine (1-2.5 cm); meanwhile *A. angustifolia* has shorter (60 cm-1.20 m, although longer in cultivate) and narrower (3.5-10 cm) linear to lanceolate leaves, light green to glaucous gray, smaller teeth (2-5mm), and spine variable (1.5-3.5m) long.

Table 1. Number of individuals morphologically measured by population, landrace and municipality. The species taxonomy hypothesis and voucher herbaria number are also indicated. Number in super index

designates the same population. *Wild references of *A. angustifolia*; **Wild reference of *A.rhodacantha;*. The vouchers were deposited in IBUG (http://herbanwmex.net/portal/index.php)

Year	Region	Municipality	Local Landrace (abbreviation)	Species	Population Code	N	Voucher Number
2021-22	Coast	Cabo Corrientes	Amarillo (Ama)	*A. angustifolia*	PDSA	10	*Padilla del Muro et al. 243*
2021-22	Coast	Cabo Corrientes	Amarillo (Ama)	*A. angustifolia*	NJS	10	-
2021-22	Coast	Cabo Corrientes	Amarillo (Ama)	*A. angustifolia*	LJT	10	-
2021-22	Coast	Cabo Corrientes	Amarillo (Ama)	*A. angustifolia*	ARR	10	*Padilla del Muro et al. 215*
2021-22	Coast	Cabo Corrientes	Amarillo (Ama)	*A. angustifolia*	AJJ	10	-
2021-22	Coast	Cabo Corrientes	Amarillo (Ama)	*A. angustifolia*	LCS	10	*Padilla del Muro et al. 218*
2021-22	Coast	Cabo Corrientes	Cenicillo (Ceni)	*A. angustifolia*	DVDC	10	*Padilla del Muro et al. 236*
2021-22	Coast	Cabo Corrientes	Cenizo (Cen)	*A. rhodacantha*	TPT	10	-
2021-22	Coast	Cabo Corrientes	Cenizo (Cen)	*A. rhodacantha*	CJJ	7	*Padilla del Muro et al. 219*
2021-22	Coast	Cabo Corrientes	Chico Aguiar (ChiAgui)	*A. rhodacantha*	DVD	10	*Padilla del Muro et al. 235*
2021-22	Coast	Cabo Corrientes	Chico Aguiar (ChiAgui)	*A. rhodacantha*	CHJJ	10	*Carrillo Reyes et al. 9688*
2021-22	Coast	Cabo Corrientes	Criollo (Cri)	*A. angustifolia*	LMS	7	*Padilla del Muro et al. 246*
2021-22	Coast	Cabo Corrientes	Pencudo (Pen)	*A. rhodacantha*	DVDP	9	*Padilla del Muro et al. 238*
2021-22	Coast	Cabo Corrientes	Pencudo Verde (PenVer)	*A. rhodacantha*	PDS	10	*Padilla del Muro et al. 242*
2021-22	Coast	Cabo Corrientes	Verde (Ver)	*A. rhodacantha*	DVDV	10	*Padilla del Muro*

							et al. 237
2021-22	South	Tolimán	Lineño[1] (Lin)	*A. angustifolia*	LSJ	10	*Cabrera Toledo et al. 30*
2021-22	South	Tolimán	Lineño (Lin)	*A. angustifolia*	TTT	10	-
2021-22	South	Tolimán	Lineño (Lin)	*A. angustifolia*	JGLL	10	-
2021-22	South	Zapotitlán de Vadillo	Azul Telcruz[2] (AzuTel)	*A. rhodacantha*	ATLA	10	*Padilla del Muro et al. 273*
2021-22	South	Zapotitlán de Vadillo	Ixtero Amarillo[3] (IxAma)	*A. rhodacantha*	IALA	10	*Padilla del Muro et al. 271*
2021-22	South	Zapotitlán de Vadillo	Ixtero Verde (IxVer)	*A. rhodacantha*	IVLA	7	*Padilla del Muro et al. 269*
2007	Central	Tecolotlán	Palo Alto (PalAl)*	*A. angustifolia*	PA	9	-
2007	South	Tolimán	Cimarron Negro (CimNe)	*A. rhodacantha*	CN	10	-
2007	South	Tolimán	Hojudo (Ho)	*A. rhodacantha*	Ho	10	-
2007	South	Tolimán	Ixtero Amarillo (IxAma)	*A. rhodacantha*	IAc	10	-
2007	South	Tolimán	Lineño[1] (Lin)	*A. angustifolia*	Lc	11	-
2007	South	Tolimán	Mezcal Piña (MezPi)*	*A. angustifolia*	MP	11	-
2007	South	Tolimán	Soca (Soca)	*A. rhodacantha*	SO	10	-
2007	South	Tolimán	Verde Rápido (VerRa)	*A. angustifolia*	VR	10	-
2007	South	Tonaya	Agave Azul (Azul)	*tequilana*	AA	10	-
2007	South	Tuxcacuesco	Angustifolia (Angus)*	*A. angustifolia*	ATX	10	-
2007	South	Tuxpan	Garabato (Gar)*	*A. angustifolia*	G	10	-

2007	South	Tuxpan	Sierrilla Verde Amarillento** (SieVerAma)	*A. rhodacantha*	SVA	10	-
2007	South	Zapotitlán de Vadillo	Azul Telcruz[2] (AzuTel)	*A. rhodacantha*	TE	10	-
2007	South	Zapotitlán de Vadillo	Brocha (Bro)	*A. rhodacantha*	B	10	-
2007	South	Zapotitlán de Vadillo	Chancuellar (Chan)	*A. angustifolia*	CH	10	-
2007	South	Zapotitlán de Vadillo	Ixtero Amarillo[3] (IxAma)	*A. rhodacantha*	IA	10	-
2007	South	Zapotitlán de Vadillo	Lineño (Lin)	*A. angustifolia*	Lz	10	-
2007	South	Zapotitlán de Vadillo	Perempitz (Perem)	*A. angustifolia*	PE	7	-
2007	South	Zapotitlán de Vadillo	Prieto (Pri)	*A. rhodacantha*	P	7	-

Morphological data sampling

For each individual, as long as there were 7 to 11 mature plants per landrace and population, we recorded the following morphological traits (as measured in Vargas-Ponce et al. 2007, Cabrera et al. 2022): plant length (cm), leaf length (cm), maximum leaf width (cm), leaf width at middle (cm), terminal thorn length (cm), terminal thorn base width (cm), number of lateral teeth, distance between teeth (cm) and teeth length (cm). Munsell leaf colors were also recorded. This color system offers a descriptive and systematic approach to communicating color, using a code with three components: hue, value and chroma. Hue indicates the definition or “purity”, distinguishing one color from another. Value represents lightness or darkness, while chroma indicates intensity. The code was converted to xyY coordinates using the R packages *munsellinterpol* (Gama et al. 2022). As was mentioned earlier, producers recognized variations in leaves and spine shapes, as well as differences in the level of thorniness. To obtain some indicators of this thorniness, of the shape of the terminal spine and of the leaves, other five variables were calculated as combinations of the previous ones: teeth/leaf length, number of lateral teeth/leaf length; terminal thorn length/terminal thorn base width; leaf length/terminal thorn length, leaf length/leaf width at middle. Variables highly (>0.8) Pearson-correlated were removed for the following analysis.

Morphological diversity

First, a Principal Component Analysis (PCA) was conducted using population means and plotted using R packages *stats* (R core team 2022) and *factoextra* (Kassambara & Mundt, 2020). This analysis aims to

reduce the number of variables to a smaller set of components that explain most of the variance in the original variables.

Next, samples were organized by species, region, and landrace to perform a Discriminant Analysis of Principal Components (DAPC) to pre-defined groups (from 2 to 9) by the *k* mean algorithm thanks to the *adegenet* package (Jombart 2008, Jombart et al. 2010, Jombart and Ahmed 2011). DAPC is "a multivariate statistical approach in which variance in the sample is partitioned into a between-group and within- group component, to maximize discrimination between groups. Data is first transformed using a principal component analysis (PCA) and subsequently clusters are identified using discriminant analysis (DA)" (Grünwald et al. nd). The analysis provides membership probabilities for each sample based on the retained discriminant functions that were plotted using *clumpack* (Kopeman et al. 2015).

Finally, the mean and coefficient of variation (CV) were calculated for each retained variable in each of the populations. Mean values were normalized and shown together with the CV in a heatmap using *superheat* R package (Barter & Yu 2017), excluding the color coordinates.

Contribution of the different scales to variation

To determine which factor had the greatest influence in explaining the observed morphological diversity, a sequential Permanova was performed using the following model: Species * Region + Region/Local Landrace. In this model, species and regions were considered independent while the landrace was nested within the region factor, as each region comprises different landraces. This analysis was conducted using the Bray-Curtis distance matrix implemented in the *vegan* R package (Oksanen 2022). Specifically, samples collected in the wild and *A. tequilana* were excluded to refine the analysis.

Morphological diversity index

To identify the landraces that preserve greater morphological variation, the morphological diversity index (MDI) was calculated using both Shannon and Simpson indices. First, the data was filtered to select the four most important variables according to the PCA for each landrace: Terminal Thorn Base Width, the first coordinate of the color (xyY_x) (+), Plant Length (-) and Leaf Width at Middle (-). Next, ten classes were created by variable and the data was transformed into relative frequencies. Using the R package *vegan* (Oksanen et al. 2022), Shannon diversity was calculated for each population and variable, followed by the calculation of a mean value per population. Finally, the results were plotted by region and landrace using the R package *ggplot2* (Wickham 2016).

RESULTS

Morphological variability by species and regions

Among the 17 morphological variables used, only one [maximum leaf width (cm)] was removed due to high collinearity. The first two principal components of the PCA, constructed with population mean values and shown by species and region, are plotted in Figure 2. These components explained 24.2% and

20.4% of the variance, respectively. The variables contributing most to the first principal component were Terminal Thorn Base Width (+) and the first coordinate of the color (xyY_x) (+), while the second principal component was primarily influenced be Plant Length (-) and Leaf Width at Middle (-). The x-coordinate (xyY_x) defines the color's position along the red-to-green axis (hue). When analyzing the distribution of landrace populations by species, we observe that *ca.* eleven landraces, along with the wild population of *A. rhodacantha* (Sie.Ver.AmaSVA), are grouped near the center of these two principal components. This indicates an admixed phenotypic group of both species. However, a trend exists toward a *A. rhodacantha* phenotypic group, particularly in the positive zone of the first principal component. This trend is primarily driven by one population of Ixtero Amarillo, which exhibits a wider base of the terminal spine and higher xyY_x color coordinate values, meaning a shift towards toward a green-yellow hue. Additionally, it falls into the negative zone of the second principal component, indicating the longer plants with wider leaves in the middle section. Conversely, a trend toward an *A. angustifolia* phenotypic group is situated in the upper left zone, including the two wild populations of *A. angustifolia* (code as PalA.PA, Angus.ATX), mezcal piña (MezPi.MP) landrace from the south and, some Amarillo populations from the coast. This group exhibits a shorter base of the terminal spine and lower values of the xyY_x color coordinate values, indicating a shift toward a green-blue hue. Additionally, it falls into the positive zone of the second principal component, corresponding to shorter plants and narrower leaves at the middle section. Finally, *A. tequilana* is positioned near the *A. angustifolia* phenotypic group but further to the left, indicating a stronger green-blue color definition and slightly taller plants. When considering regional differences, we observe that *ca.* seven landraces from both regions overlap. However, the southern region is displaced towards the positive zone of the first component, while the coastal region is towards the negative zone. This suggests differences in terminal spine width (narrower in the coastal region) and the plants color (more green-yellow in the south). Both regions feature tall plants, but in the south, there are more landraces with shorter plants.

Morphological variability by landrace

The membership probabilities for each cluster (K2-K9) provided by the DAPC analysis are shown in Figure 3. When analyzing a small number of clusters (K2 and K3), the results do not show congruence or resolution at any variation scale, as samples from different species, *A. angustifolia*, *A. rhodacantha*, are not separated into distinct clusters, not even *A. tequilana.* According to the method manual, “caution should be taken when interpreting group memberships of a DAPC, since there are risks of overfitting the discriminant functions. Group memberships can be used as indicators of how clear-cut genetic clusters are; this is most useful for groups defined by an external criterion, i.e. defined biologically, as opposed to identified by k-means” (Jombart and Collins 2015). Thus, *A. tequilana* var. Azul, the only population included as representative of *A. tequilana* and with evidently scarce morphological variability (Fig. 4, see coefficient of variation), was useful as a reference for a group with high membership values. It is assigned separated in a different cluster at k5 and, at k6 is almost completely differentiated from the rest. Therefore, we consider k6 to be the most suitable number of clusters for evaluating the hypothetical landraces. Among eight landraces recognized by the coastal producers, Amarillo, Cenicillo, Criollo,

Cenizo, Chico Aguiar, Pencudo, Pencudo Verde, and Verde, the DAPC analysis grouped them in only three clusters, represented by the colors orange, blue and purple.

Similarly, in the south, the DAPC analysis identified fewer clusters with the highest membership probabilities, reducing the number of landraces perceived by producers. It is important to note that landraces in the south region were recorded at two different points in time: nine were measured in 2007, another different landrace (Ixtero Verde) was added in 2022, and three more (Lineño, Ixtero Amarillo, and Azul Telcruz), were measured in both years. Based on this dataset, eight landraces and one wild-managed population exhibited high membership probabilities linked to three groups: light pink, green and previously mentioned purple.

Following the subsequent DAPC analysis, we considered additional traits (Figure 4) that complemented the first two principal components identified in the PCA, refining the group definitions in K6. Then, based on six traits (though in some cases, seven may be useful), we can better describe these groups as following:

Blue cluster

This cluster included Cenizo and Chico Aguiar, landraces with mixed traits of *A. angustifolia* and *A. rhodacantha*. These are large plants with medium-short, wide leaves that have numerous medium-sized teeth and terminal spines with narrow bases. Most populations exhibit small variances in these traits, and the color is predominantly green-blue.

Purple cluster

Pencudo, Pencudo Verde, and two populations of Ixtero Amarillo (both measured in 2007) belong to this group. These plants are also large with wide leaves, but unlike those in the blue cluster, they have longer leaves, and a medium-low number of medium-sized teeth. They also have the widest terminal spines at the base, and their leaves are green-yellow. This group is the morphologically closest to *A. rhodacantha*.

Orange cluster

This cluster consists of medium-sized plants with shorter and narrower leaves compared to the previous groups. Their terminal spines are narrower at the base compared to those in the purple cluster, and they have a medium-low number of medium-sized teeth. These plants generally exhibit a green-blue coloration. This group is composed by Amarillo, Verde, and Soca.

Green cluster

The green cluster is similar to the orange cluster but exhibits more defined traits morphologically closer to *A. angustifolia*. All our wild reference populations including those collected in Palo Alto and Tuxcacuesco as well as Mezcal Piña in Tolimán, are included in this group. Only one landrace, Peremptiz

showed similar traits. These are the shortest plants among the studied groups, with the shortest leaves, a low number of short teeth, narrow terminal spines, and a reduced variance in all these traits.

Light pink cluster

These are plants of medium to short stature. They have few medium-sized teeth and terminal spines that are medium to narrow at the base. Their coloration ranges from green-blue and green-yellow. Azul Telcruz, Brocha, Ixtero Verde and some populations of Lineño (measured in 2007) belong to this group.

Burgundy cluster (*A. tequilana* var. Azul)

The only population of *A. tequilana* var. Azul included in this analysis (Fig. 3, burgundy color) is characterized by medium-sized plants and leaves of moderate length and width. These plants have numerous but very short teeth, narrow terminal spines at the base, and an extreme green-blue coloration. Among all landraces, they exhibited the lowest variance in the measured traits.

Contribution of different scales to variation

PERMANOVA results are presented in Table 2. All scales of variation were significant (Pr(>F)=0.001). Variation between regions accounted for 13%, variation due to species for 14%, and the interaction between regions and species 1.8%. The most substantial effect was observed in the variation among landraces nested within regions, which accounted for 35%. The unexplained variance (residuals) by any of these scales of variation represented 36% of the total variation. Different agave species can exhibit inherent morphological differences due to their genetic characteristics and phenotypic plasticity. Regional influences on morphology can stem from environmental factors such as climate, soil, elevation, and local cultivation practices. Landraces within each region explain most of the morphological variability, likely due to selection and management practices. Although landraces reproduce clonally, producers selectively cultivate specific clones that have demonstrated adaptation to local conditions and desirable characteristics. Furthermore, agricultural practices vary among different mezcaleras and producers within each region, further contributing to the observed variability (residuals).

Table 2. PERMANOVA analysis results with the variation explained by different factors. The analysis tested the significance of variation between regions, species, their interaction, and landraces nested within regions. The table includes degrees of freedom (DF), sum of squares (SumOfSqs), R-squared values (R^2), F-values, and p-values (Pr(>F)).

	DF	SumOfSqs	R^2	F	Pr(>F)
Region	1	0.49	0.126	115.6	0.001***
Species	1	0.55	0.142	129.5	0.001***
Region x Species	1	0.07	0.018	16.2	0.001***
Region / Local Landrace	19	1.37	0.35	16.9	0.001***
Residual	333	1.42	0.36		
Total	255	3.9	1		

Morphological diversity index

Mean MDI by population, landrace and region, based on the four variables that contributed the most to the PCA, are shown in Figure 5. In the Coast region, the highest values are observed in the Cenizo, Cenicillo, Chico Aguiar, and Amarillo populations, while the lowest values are found in Pencudo Verde. In the South region, the highest values are recorded for Ixtero Amarillo, Garabato, Perempitz, and Sierrilla Verde Amarillento, whereas the lowest values belonging to Cimarron Negro and Hojudo. Overall, the analysis reveals substantial intravarietal variability in diversity within and among landraces. Both the Coast and South regions exhibit similar mean values of diversity (0.669 and 0.662, respectively); however, the South region displays a broader range of diversity values. This suggests greater phenotypic variability within landraces in the South compared to the Coast. Additionally, comparisons across species highlight that *A. tequilana* var. Azul exhibits relatively lower diversity (0.342, not shown in Fig. 5) compared to *A. rhodacantha* and *A. angustifolia*. Although *A. rhodacantha* and *A. angustifolia* show similar mean values (0.66 and 0.67, respectively), *A. rhodacantha* has a much broader range of diversity across its local landraces.

DISCUSSION

This study confirms that agave plants belonging to mezcaleras from the north coast and south Jalisco conform a morphologically broad and overlapping complex. However, we delineated this complexity through the distinction of groups that present enough morphological evidence to be distinguished as dissimilar (Fig. 6), regardless of the established label (species, region, or landrace). Furthermore, it is relevant that 17 morphological traits explain this diverse landscape better than thousands of SNP did in Cabrera-Toledo et al. (2022). This suggests that phenotypic diversification may be occurring more rapidly than genetic diversification (Arenas et al. 2025). The recent origin and diversification of the *Agave* genus (Good-Avila et al. 2006) could explain its weak reproductive barriers, which may facilitate hybridization (Eguiarte et al. 2021). This, in turn, could promote genomic cohesion within the genus, depending on the extent of gene flow and selection acting on hybrids (The Marie Curie SPECIATION Network 2012; Herron and Freeman 2014).

Species and regions

The recognition of landraces within *A. angustifolia* and *A. rhodacantha* evaluated in this work helped to delimit the morphological gradient typical of species complexes in a management context. Since this is not a taxonomic study, we cannot strictly conclude systematic species delimitations. However, assessing phenotypic affinities of a species contributes to a better understanding of the biological context in which landraces are managed. It is estimated that the genus *Agave* may have appeared 7.8-10 million years ago, with high rates of speciation occurring around 6-8 and 2.5-3 million years ago (Good-Avila et al. 2006). The relatively recent emergence of the genus has made it difficult for phylogenetic studies using traditional techniques to resolve the relationships between its constituent species published to date given the hybridization susceptibility in this genus (Eguiarte et al. 2021). One of the latest works published (Jimenez-Barron et al. 2020), based on the ITS nuclear sequence, divided the genus into large groups with high support. However, the relationships between species within each group were not fully resolved. Despite this, interestingly, the sole representatives of *A. angustifolia* and *A. rhodacantha* were placed in different groups. In this work, recognizing specific taxonomic units in the sampled morphotypes was not possible, mainly due to the lack of reproductive characteristics that are typically key to identifying species (Rivera-Lugo et al. 2018; Figueredo-Urbina et al. 2017). Also, it has been proposed a hybrid genetic pool as a possible source of plants for the mezcaleras in south Jalisco (Vargas-Ponce et al. 2007, 2009). In this case, we established a taxonomic assumption for each landrace based primarily on vegetative traits, some of the characteristics recognized by the farmers. Our results suggest that only a few landraces are the most congruent with the typological concept of *A. rhodacantha* or *A. angustifolia* defined by Gentry (1982). Of 14 landraces assigned to *A. rhodacantha*, the ones with more morphological affinities were one population of Ixtero Amarillo (South, IxAmaIA, Fig.2), Pencudo, and Pencudo Verde (Coast). These three landraces represent the largest plants combined with three traits, i.e., being biased to green-yellow colors, with the widest leaves and base terminal thorns. This is congruent with the fact that at least one of these landraces, Ixtero Amarillo, is also one of the most genomically divergent, though with some populations more genomically similar to wild individuals (Cabrera-Toledo et al. 2022).

The most morphologically contrasting group of plants—those more similar to *A. angustifolia,* which, notably, includes the wild references of this species—consists of Mezcal Piña, Peremptiz (South), some populations of Amarillo (Coast), Palo Alto (a wild reference from central Jalisco), and one wild population in the south (Tuxcacuesco). All these populations (landrace and wild) were the ones measured by Vargas-Ponce et al. (2007) and have also leaves with low numbers of teeth (and short), as Rivera-Lugo et al. (2018) reported for the types of *A. angustifolia* located in Oaxaca. These landraces are not mentioned by producers nowadays, except for Amarillo, the most important landrace on the coast because it sustains more than 90% of raicilla production in the north coast region.

The studied landraces correlate in part with their respective geographic regions of origin. This is likely attributed to shared abiotic and biotic conditions of the territories added to similar selection processes and implemented agroecological conditions settled by producers within each region when establishing

local landraces (Casas et al. 2016; Jiménez-Rojas et al. 2019). A tendency of forms related within each area can be identified. Though we found a similar number of landraces in both regions with large sized plants with broad leaves, on the Coast, these plants tend to have terminal thorn base widths smaller than in the South and with colors biased to green-blue. Almost all landraces on the Coast tend to have these forms; only Amarillo is morphologically like *A. angustifolia,* which shows narrower leaves and shorter plants on average.

Landraces

Our results showed that even when most of the landraces named by the farmers involve an overlapping morphological scape, we found that landraces on the Coast showed a better morphological delimitation, though not wholly congruent with the farmer's perception. Mezcaleras as agricultural systems have been managed in both regions for at least four human generations, around 140 years (Sierra-Huelsz submitted; Vargas-Ponce, 2007); however, the time that individual landraces have been propagated vegetatively is variable. We interpret that the ones that are identified by most of the farmers have more time being propagated by different producers. On the Coast, this is the case of Amarillo, Verde (orange cluster), Cenizo, and Chico Aguiar (blue cluster). In the South, the more common landraces these days are Lineño (light pink cluster), Soca, and Cimarron (admixed of orange-light pink clusters), and historically, Ixtero Amarillo (purple cluster), Ixtero Verde, Azul Telcruz (purple-light pink) and Cimarron (blue orange). However, they all showed individual plants with admixed forms in at least one population, indicating that they were probably 1) propagated from more than one genetic source; 2) obtained by a recent seed provenance, which is not usual practice but we cannot discard it; or, 3) presented more phenotypic plasticity, which is an essential source of variation in processes of domestication in vegetatively propagated crops (Denham et al. 2020). Farmers mentioned that it is difficult to know how many genetically different plants they have because they take the offshoots from several plant mothers and mix them before their establishment in the mezcalera. The probability that any of the above options happens is higher for those landraces more frequently propagated in both regions (Amarillo in the Coast, Lineño, and Ixtero Amarillo in the South). This could explain why, for these landraces, we found some populations admixed while others were morphologically identical. However, in general terms, we saw more homogeneous forms on the Coast than in the South, possibly reflecting different histories of establishment and current management of these landraces.

A plausible hypothesis is that some coastal landraces may have originated in the South. Diverse evidence suggests connections between these two regions. Agave spirit production in both regions share interesting biocultural characteristics, notably agave cultivation in diversified agroforestry systems (Sierra-Huelsz et al. submitted; Torres-García et al. 2019), and processing practices including the same agave cooking and distillation techniques (Valenzuela-Zapata et al. 2008). Additionally, certain landraces share names across regions (e.g., Amarillo and Ixtero Amarillo, Verde and Ixtero Verde, or Verde Rápido), while others—despite having different names—exhibit morphological similarities, such as Ixtero Amarillo on the coast resembling Pencudo in the South, or Verde on the coast resembling Hojudo in the South. Although morphological analysis alone does not provide conclusive evidence of interregional exchanges,

farmers' testimonies and previous genomic analyses offer valuable insights. The precise origins of the mother plants that gave rise to the landraces we see today remain unclear. In both regions, farmers identified at least three main sources: (1) plants sourced from local wild populations, (2) plants shared among mezcal producers within the same region, and (3) in the case of the Coast region, farmers reported that most landraces are not native with the exception of the wild "Cerreño" (Sierra-Huelsz et al., submitted), a term referring to plants brought from the hills—though we were unable to measure these due to a lack of mature specimens. A previous genomic analysis of individual plants from four coastal landraces (Amarillo, Verde, Cenizo, and Chico Aguiar) revealed that while they are distinct from one another, they are genetically very similar to wild materials from South Jalisco (specifically in the municipalities of Zapotitlán de Vadillo, Tolimán, and Sayula; Cabrera-Toledo et al. 2022). Notably, Amarillo emerged as the most genetically distinct landrace of both regions. This finding aligns not only with farmers' perceptions of these landraces as separate entities but also with the possibility that they are not truly local, as farmers suggest. Nevertheless, further analysis, including samples from plants growing in the coastal hills, is necessary to confirm this hypothesis.

The measured variables account for less than half of the overall morphological variation evaluated by PCA in these landraces. This aligns with the findings of Figueredo-Urbina et al. (2021) for *A. americana, A. salmiana* and *A. mapisaga* but contrasts with those of Vargas-Ponce et al. (2007) for landraces from southern Jalisco (the same included in this study), which found that 66% of the variation was explained by the first two discriminant functions. It also differs from the study on *A. maximiliana* in west mountains of Jalisco, where 70% of the variation was explained (Cabrera-Toledo et al. 2020). Additionally, research on *A. inaequidens*, *A. hookery,* and *A. cupreata* in Michoacan (Figueredo-Urbina et al. 2017), as well as on the *A. angustifolia* complex in Oaxaca (Rivera-Lugo et al. 2018), included reproductive traits and found almost 90% of the variation was explained by the first two discriminant functions. Explaining this result is complex, but considering certain factors can help clarify the outcome. First, we evaluated landraces at different stages of domestication. A significant portion of the data analyzed in this study comes from Vargas-Ponce et al. (2007), who reported that at least 10 of the 14 landraces they studied have been recognized in the region—based on the collective memory of current producers—for at least 150 years (e.g., Ixtero Amarillo, Ixtero Verde, Soca, Cenizo). To enable a comparative analysis between these southern landraces and those from the coast, we measured the same variables. However, it is highly likely that our samples include landraces at different stages of domestication. Second, a more rigorous ethnobotanical approach might have revealed greater congruence with morphological evidence, ensuring that only landraces strictly recognized by producers according to consensual criteria were included (Labeyrie et al. 2019). It is important to clarify that all not morphological traits evaluated in this study are necessarily targets of selection. While plant size arguably is, most morphological traits function as identification markers that may be correlated with other characteristics of interest to farmers, such as contribution to spirit taste, sugar content, pest resistance, offshoot production, maturation time (Colunga-GarcíaMarín et al. 2017; Sierra-Huelsz et al. submitted). Whether or not these traits are intentionally selected ultimately shape the different management strategies that farmers may adopt now or in the future.

Farmers in both regions name their local landraces based on their landrace traits (e.g., Amarillo, Pencudo; i.e. yellow, long leaf), places of origin (e.g., Chancuellar, Azul Telcruz; i.e. name of towns in the region), uses (e.g., Ixtero, i.e. fiber producing), human names (Chico Aguiar) or allusion of wild provenance (e.g., Cimarron, Cerreño, Barranqueño; i.e. wild, from the mountain, from the gorge).

Identifying and naming local landraces allow traditional agave producers to manage agave on an individual plant basis, even when cultivated together, as each landrace has specific ecological characteristics and distinct use qualities. When diverse landraces are managed collectively, they are often treated as uneven-aged systems, with plants harvested individually as they each approach sexual maturity.

In contrast, agro-industrial agave production relies on monocultures designed to maximize yield and economic efficiency. In these systems, agave plants are managed as a uniform unit, established synchronously and harvested collectively when the average sugar concentration target is reached.

Traditional agave production systems tend to be diverse in terms of the landraces managed, which are often associated with varied uses and selection criteria. These systems also promote heterogeneity in plant ages and microenvironmental conditions, fostering greater agrobiodiversity and diverse morphological expressions (Colunga-García-Marín and Zizumbo-Villarreal 2007). In contrast, agro-industrial monocultures aim to create homogeneous growing conditions for a genetically uniform stock, resulting in a morphologically uniform expression. While one system is rooted in diversity and continually enhances it, the other is homogeneous and leads to progressively deteriorating genetically and culturally eroded production systems. We do not interpret the evaluated traits as part of some domestication syndrome, but this does not exclude an ongoing domestication process (cf. Parra et al. 2006). The fact that variation among landraces contributed more than other factors—such as species, regions, or species-region interactions—suggests that farmers' perception of landraces, despite being underrepresented, was useful in significantly explaining the landscape morphology of this complex. Moreover, it indicates that this perception has played a role in guiding the diversification process. In these terms, a broader conception of domestication is proper in the interpretation of our results, one that offers multiple proxies for documenting the process of coevolution between crops and humans (Clement et al. 2021; Denham et al. 2020), a conception that includes other criteria of interactions that do not pre-supposes a conscious and systematic selection that is the objective of modern farming domestication (Clement et al. 2021; Rindos 1984).

Morphological diversity index

Given that, in our study region, producers primarily propagate agave through vegetative offshoots, we expected a lower degree of morphological variation in landraces that have been present in the mezcals for extended periods or are more frequently found, assuming the same genetics among most of the plants of each landrace. The hypothesis that vegetative propagation leads to decreased crop diversity has already been demonstrated in other traditional agave management systems for distilled beverages production (Figueredo-Urbina et al. 2021). However, our results revealed a significantly different pattern.

On the one hand, some of the landraces that showed the highest morphological variability, Garabato and Sierrilla Verde Amarillento, aligned with the hypothesis, as they are wild populations with low levels of management. On the other hand, several of the most frequent or traditionally known landraces in both regions, Cenizo, and Amarillo on the coast, and Ixtero Amarillo in the South, also exhibited high MDI values. In fact, these landraces displayed the higher MDI reported for a vegetatively propagated *Agave* species used inspirits production (MDI=0.481 *A. hookeri,* Figueredo-Urbina et al. 2017). Their MDI values were even higher than other *Agave* species that reproduce sexually in natural populations or those propagated by peasants from seed (MDI = 0.413 *A. inaequidens*; 0.489 *A. cupreata*, Figueredo-Urbina et al. 2017; MDI = 0.628 *A. maximiliana*, Cabrera-Toledo et al. 2020). These findings suggest that while vegetative propagation is the predominant strategy in the mezcaleras, the high phenotypic plasticity of these landraces provides multiple options for selection.

Some of this variation may arise because landrace data often comes from multiple populations, meaning that producers may conserve different genotypes while referring to them with the same name. Morphological variation can result from both genetic differences and environmental influences, via epigenetic factors (Meyer 2015). In terms of genetic variation, differences may have been introduced within populations if multiple mother plants contributed to the lineage or if occasional sexual reproduction occurred within the mezcalera. Small-scale agave producers of both regions are only beginning to adopt seed propagation, making it premature to speak of intentionally directed genetic variability. However, there have been cases where some plants flowered before their inflorescence was cut, and they were just “left” allowing them to further develop and reproduce. In terms of environmental influences, differences in microenvironmental conditions and plant management could contribute to the observed morphological variation. Unlike monocultures, agroforestry systems are not as uniform, meaning that individual plants may have varying access to resources such as light, water, and soil. Notably, blue agave (*A. tequilana*), which is reproduced vegetatively in a monoculture system, showed one of the lowest levels of morphological variability. It would be valuable to compare the obtained values of wild and management populations as done by Cabrera-Toledo et al. (2020) in their study of *A. maximiliana* across different management intensities in forest systems. Their findings suggest that this *in situ* management helps to conserve morphological variation, as no significant differences were observed between wild and managed populations.

CONCLUSIONS

The local agave landraces cultivated and managed in the state of Jalisco to produce raicilla on the coast, and mezcal in the south exhibit high morphological variability, particularly when compared to that of blue agave, the only landrace used for tequila production. This variability appears to be influenced by multiple factors depending on the scale of variation, including taxonomic species and the region where they are managed. The observed patterns of morphological variability suggest that an agrobiodiversity conservation strategy for agaves in Jalisco should be highly adaptive and region-specific. Such strategy should promote genetic diversity conservation, support local management practices, and promote diversified production practices to prevent homogenization and ensure long-term sustainability. The

landraces most closely resembling the typological concept of *A. rhodacantha* were Ixtero Amarillo (South), Pencudo, and Pencudo Verde (Coast); while for *A. angustifolia* were Amarillo (Coast), Mezcal Piña y Peremptiz (South). Although regional morphological tendencies are weak, they are significant: landraces from the South tend to exhibit green-yellow coloration and broad wide terminal spines at the base, while those from the Coast are more biased to green-blue coloration and narrow terminal spines at the base.

Finally, farmers' perception of landraces as distinct entities plays a crucial role in shaping the morphological landscape of this species complex and guiding its diversification. However, future studies, should consider that the morphological traits evaluated here may not fully align with farmers' criteria for identifying landraces nor their selection targets. While landraces are identified based on phenotype, agave landraces are selected primarily for demographic, functional, and utilitarian traits—most notably precocity, sugar concentration, and reproductive capacity—which may not necessarily correlate morphological features. Therefore, studies on morphological diversity would benefit from being complemented by ethnobotanical research specifically designed to: 1) assess the extent to which producers agree (or disagree) in assigning individual plants to specific landraces; 2) document and analyze how farmers' selection targets relate to morphology.

All in all, maintaining diversified management systems and complex selection criteria that characterize small-scale agave producers for artisanal spirit production holds immense biocultural, environmental, and social value. The importance of actively conserving these processes is significant, not only from a biocultural perspective, as the beverages derived from them play a crucial role in shaping local cultural identities, but also from an ecological standpoint, as they are hotspots of agrobiodiversity and compatible with landscape conservation strategies. Additionally, they can represent a socially just livelihood that respects the lifeways of local producers.

Declarations

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Author Contribution

N.L. drafted the work and carried out the statistical analysis; O.V.P. and P.C.R. made substantial contributions to the conception of the work and the acquisition and interpretation of data, also partially organized fieldwork ; J.A.S.H. revised the work critically for important intellectual content and refined the writing style; D.C.T.conceived and designed the study, partially organized fieldwork, made substantial contributions to the conception of the work and the acquisition, analysis and interpretation of data.

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Data Availability

Raw data are available from the corresponding author on reasonable request.

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Figures

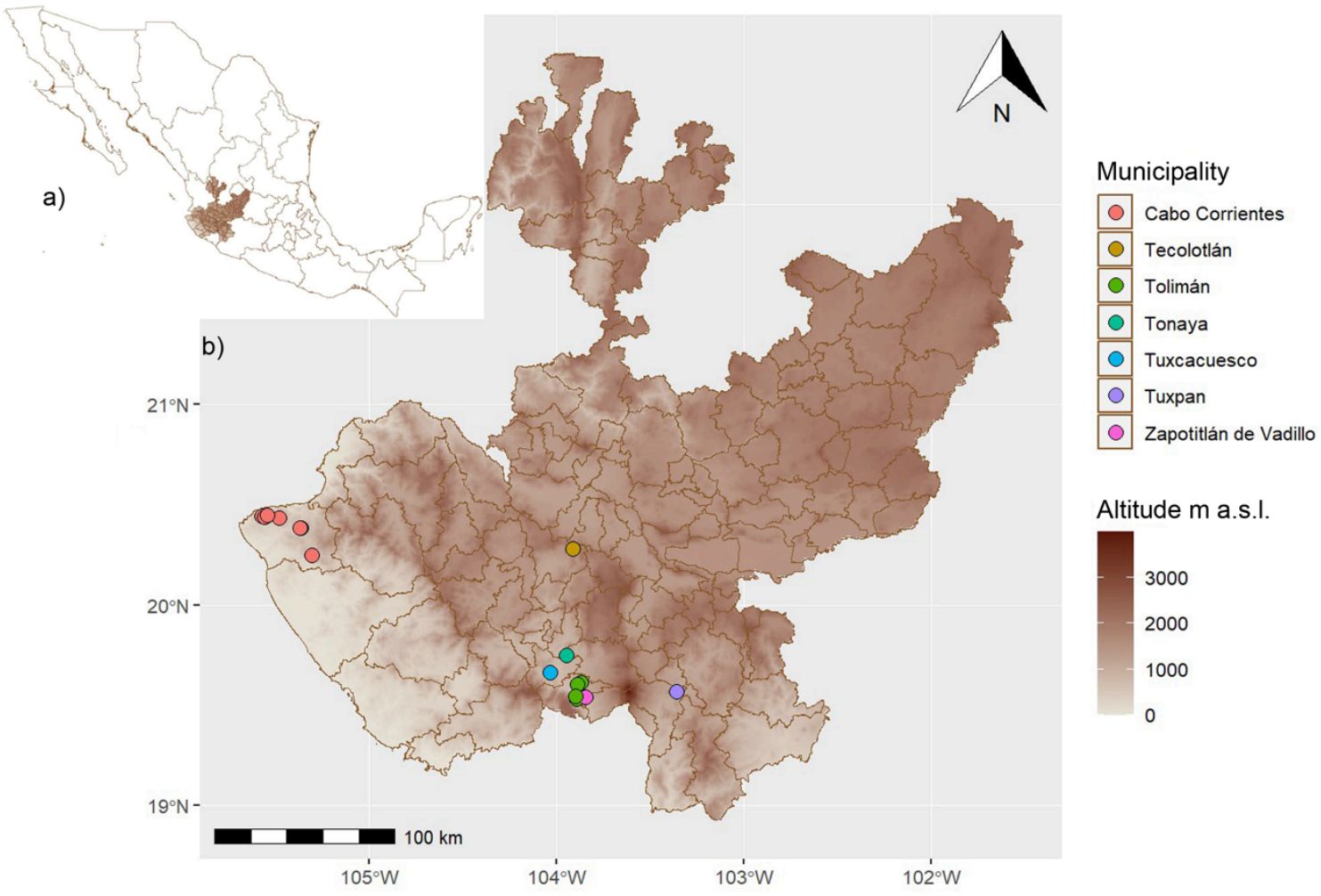

Figure 1

Geographic localization of the Jalisco state (b) in Mexico (a) and all the *Agave* populations sampled and listed in table 1

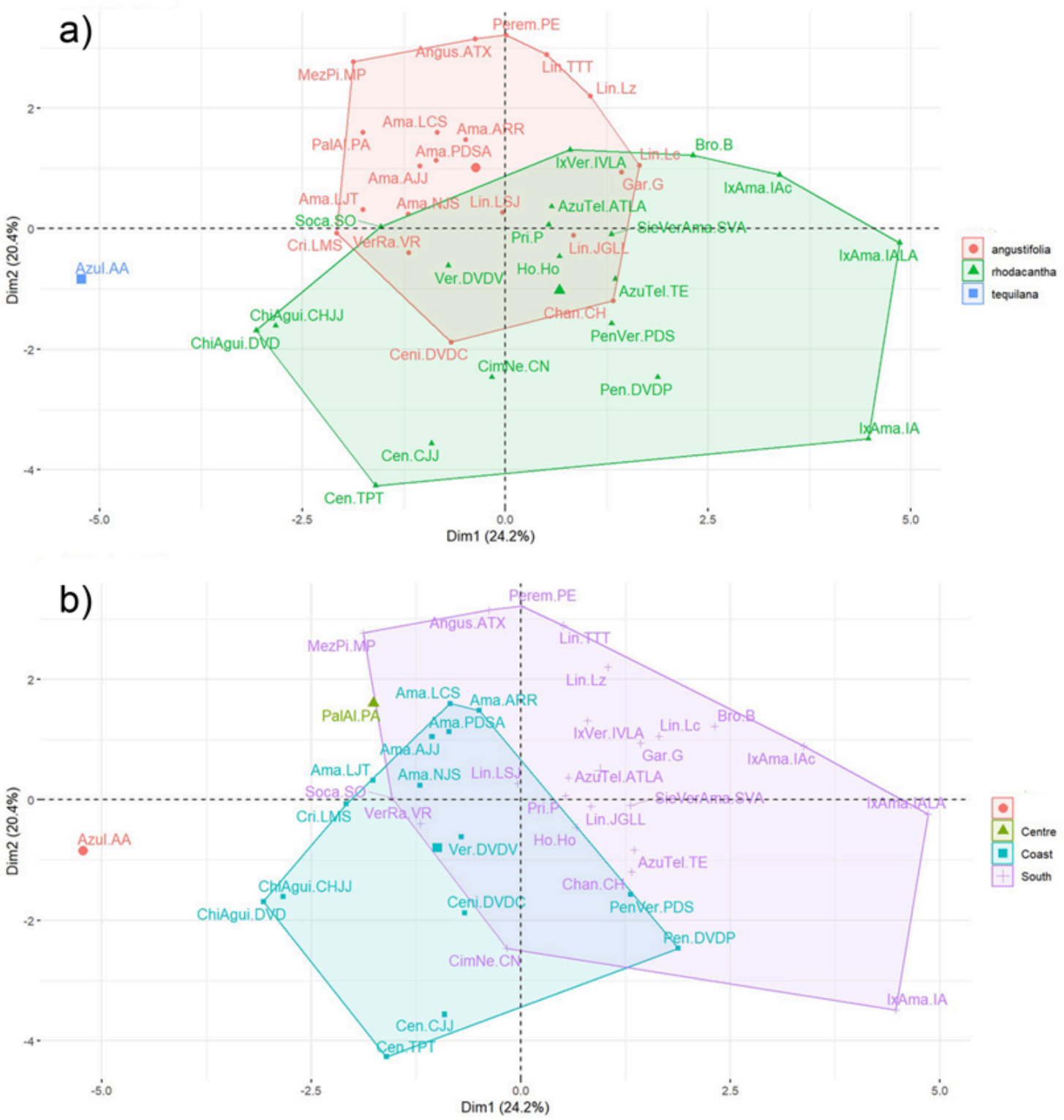

Figure 2

Landraces plotted in a two-dimensional space resulting from the PCA analysis. Figure 2a displays color-coded groupings by species, while Figure 2b shows groupings by region. Labels consist of abbreviated landrace names followed by the population code (e.g., 'Ama.AJJ' refers to the landrace 'Amarillo' from population AJJ, located in the municipality of Cabo Corrientes; see Table 1)

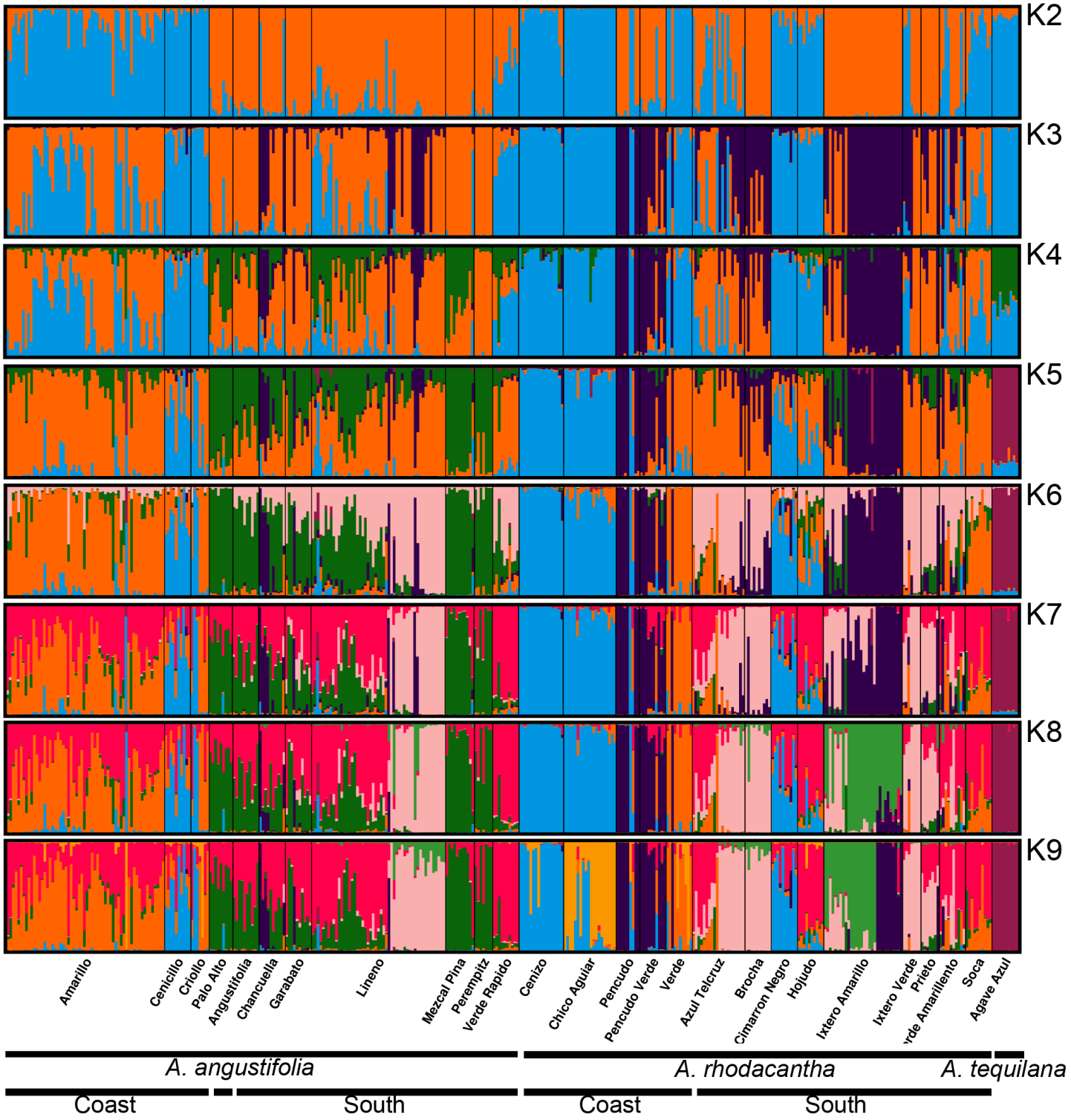

Figure 3

Membership probabilities of each of the 385 agave samples analyzed were provided by the k-means algorithm and the DAPC analysis when k2 to k7 groups were assumed. Each accession is represented by a vertical bar, and its length indicates the probability of belonging to each cluster. Accessions are ordered according to Species, Region and Landraces. *Palo alto region was recordered by centre

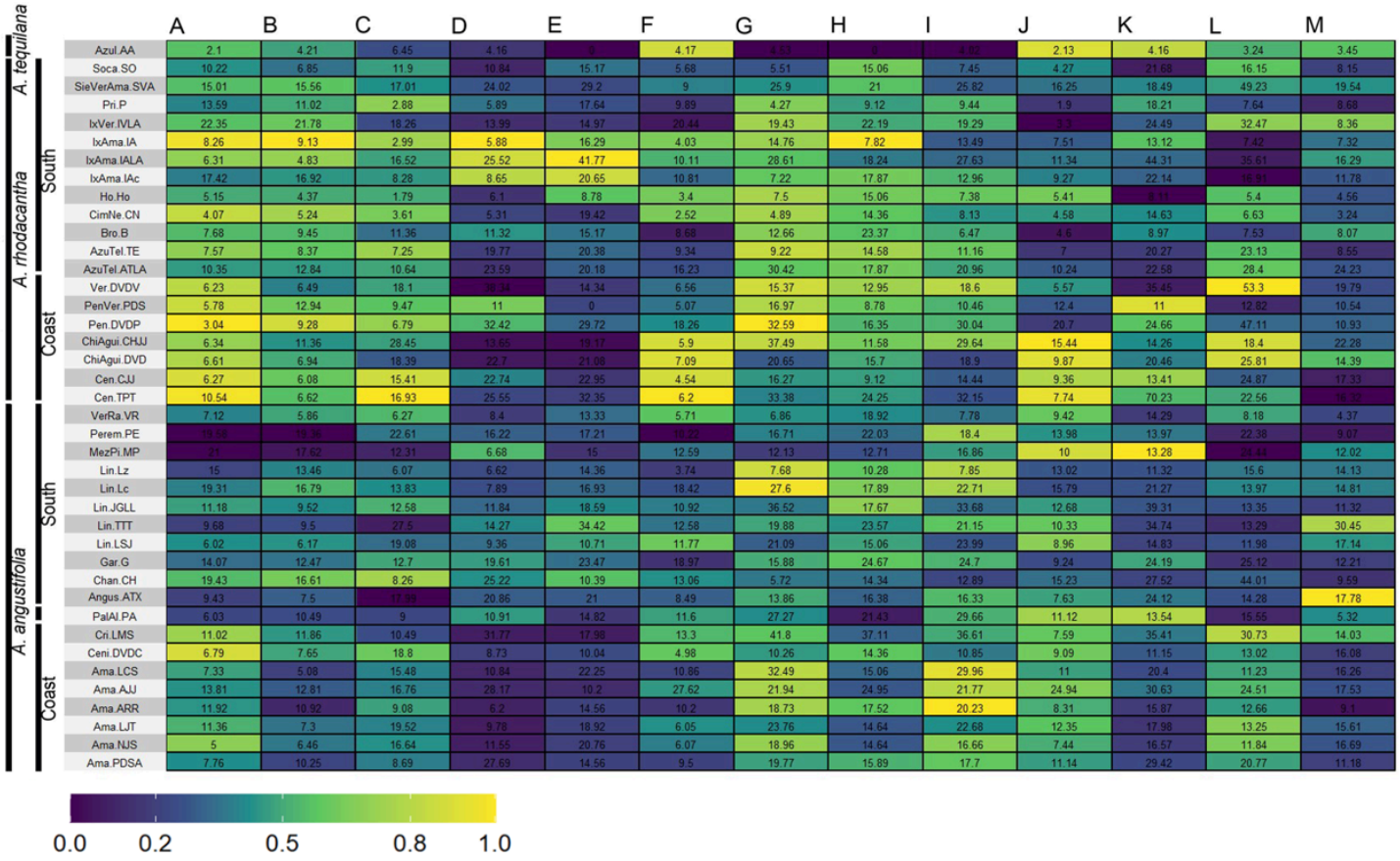

Figure 4

Normalized mean (color) and coefficient of variation (number) values for each retained variable ordered by column in the following order: A: plant length (cm), B: leaf length (cm), C: leaf width at middle (cm), D: terminal thorn length (cm), E: terminal thorn base width (cm), F: number of lateral teeth, G: distance between teeth (cm), H: teeth length (cm), I: distance between teeth/leaf length, J: number of lateral teeth/leaf length, K: terminal thorn length/terminal thorn base width, L: leaf length/terminal thorn length and, M: leaf length/leaf width at middle

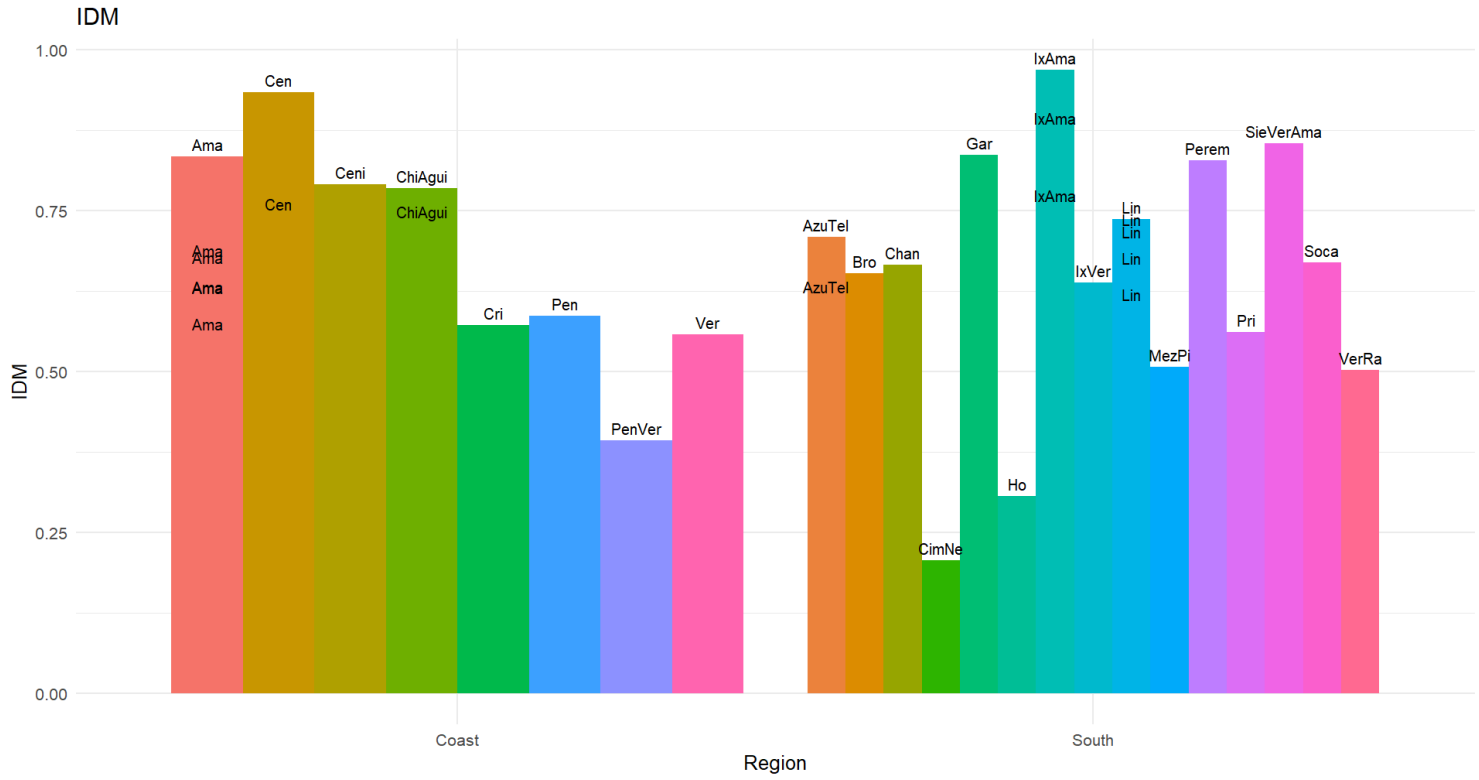

Figure 5

Mean MDI values for each local landrace and plot (population) are shown by region. Same landraces are displayed in the same bars

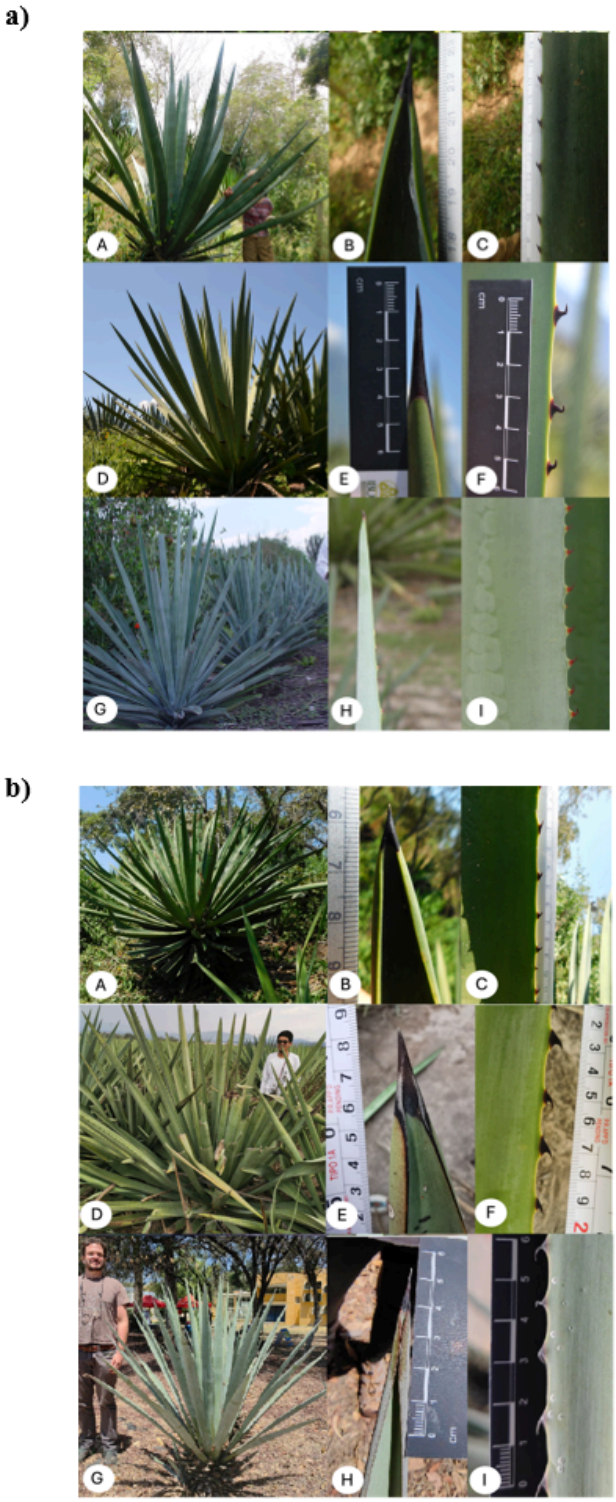

Figure 6

Agave landraces used for artisanal spirit production. The photos show representative plants from each cluster identified in the DAPC analysis described above. **a)** *A.* aff. *rhodacantha* Cenizo (A-C), blue cluster; *A.* aff.*rhodacantha* Ixtero Amarillo (D-F), purple cluster; *A.* aff.*angustifolia* Mezcal piña (G-I), green cluster. **b)** *A.* aff.*angustifolia* Amarillo (A-C), orange cluster; *A.* aff. *angustifolia*Lineño (D-F), light pink cluster; *A. tequilana* Azul (G-I), burgundy cluster