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REVIEW

A new agenda for blue agave landraces: food, energy and tequila

ANA VALENZUELA

Universidad de Guadalajara, Guadalajara, Mexico

Abstract

Agave tequilana Weber (Rigidae, Agavaceae), blue agave, is a native Mexican plant that has been associated with tequila since the 17th century. The tequila industry has matured over time and now has a geographical indication (Denominación de Origen; DOT). The tequila industry has grown substantially in the last 15 years (19.82% annual increase between 1995 and 2008), resulting in an increase in agave production and associated residue (leaves) and bagasse that can be used for second-generation biofuels. At a time when the biofuel industry is undergoing unprecedented changes, with diversified demand and predictions of increased competitiveness, this paper presents a review of agave landraces that have been affected by tequila production but may be beneficial for a biofuel industry. Conventional botanical studies have revealed domestication syndromes in races related to blue agave ('azul listado', 'sigüín' and 'pata de mula') specifically for production of fructans in the plant core as would be expected in mezcal agaves (including those used for tequila). Some others, such as the 'moraleño' and 'bermejo' cultivars (Sisalanae) show domestication syndrome only in the fibers, while others, such as 'chato,' A. americana L. subtilis (Americanae) show domestication syndrome in fructans and fibers and 'zopilote,' A. rhodacantha (Rigidae) a relatively low domestication syndrome. No specimens of the cultivars named 'mano larga', 'mano anchaque' and 'cucharo' were found in the Tequila Region of Origin (Western Mexico). The genetic resources from landraces ignored by the tequila industry may be valuable for both ethanol production and conservation.

Keywords: Agave tequilana landraces, CAM plants bioethanol, tequila

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Introduction

Blue agave is not a basic food crop, and does not require irrigation, making this plant and its close relatives excellent candidates for biofuel research and development. Agave has been used in the production of distilled alcoholic beverages in Mexico since the 17th century. The most popular of these beverages are tequila and mezcal. Recently, agave plants have been proposed as a bioenergy feedstock to mitigate negative effects of climate change, and the first generation of bioethanol production from blue agave (*Agave tequilana* Weber *Rigidae*) in Jalisco (western Mexico) is beginning to emerge as a byproduct of tequila production. In 2008, the tequila industry produced 312.1 million liters and the bioethanol industry is predicting to have an annual

Correspondence: Ana Valenzuela, e-mail: ana.valenzuela@gmail.com

production potential of 110 million liters for domestic fuel (El Informador, 2010). Furthermore, the leaves of the plant and the bagasse have traditionally not been used (Mancilla-Margali & Lopez, 2006), making it a candidate for use as a fuel.

This review first introduces blue agave agriculture and tequila production, then addresses the need for the study and conservation of landraces and concludes with a description of a new agenda that includes biofuels applications and the reintroduction of genetic resources in blue agave. The aim of this review is to present opportunities in conservation genetics for increasing the breeding potential of blue agave for biofuel.

Blue agave (A. tequilana Weber Rigidae Agavaceae) and tequila production

A. tequilana plantations grow in Mexico's tropical zone with a 90% probability of annual rainfall over 600 mm

(Valenzuela-Zapata, 1992) and moist, well-drained soils. Most of the production is in rain-fed areas in western Mexico, specifically in the state of Jalisco (Valenzuela-Zapata, 1985). Blue agave farming today involves a high degree of mechanization to work the soil and apply inputs to establish and maintain plantations using chemical weed control, inorganic fertilizers, pest and infection prevention and control, pruning, sucker removal, removal of the flower stalk, and harvest (Valenzuela-Zapata, 2003).

The tequila-producing region is under Protected Geographical Status (*Denominación de Origen Tequila*; DOT, Secretaría de Economía, 2006a), a category recognized by the World Trade Organization as a geographical indication which 'identifies a product as originating from a particular territory of a World Trade Organization Member, or region or locality in that territory where a given quality, reputation or other characteristics of the product are essentially attributable to its geographical origin.' Tequila Protected Geographical Status covers an extensive area of three million hectares which is not defined by edaphic or climatic limits.

Between 1999 and 2003, blue agave cultivation expanded into areas formerly used for growing grains, such as corn (Martínez et al., 2003). The small tequila farm system has been abandoned, and in two periods during the last 15 years (1995-1998 and 2004-2010) the current tequila industry demand of 35 million plants per year was exceeded (El Informador, 2010). Rampant 'blue gold fever' or 'agavization' was fueled by the unprecedented boom in agave prices that took place between 1999 and 2003. However, blue agave prices fell again in 2007 (Fig. 1). Over the past 15 years, tequila industry growth has been driven more by a surplus of raw material than as a response to increased consumer demand (Valenzuela-Zapata & Macias, 2010). Given this, there has been a quest for a new agenda that includes first-generation biofuels.

In 2006, 90% of 400 million plants according to the Tequila Regulating Council have become concentrated in two regions in Jalisco: the Tequila Region of Origin (TRO) and Los Altos Region (LAR) (Macias & Valenzuela-Zapata, 2009). In both regions, tequila growers have previously experimented with intercropping agave with grains, pastureland, squash–corn–bean rotations, irrigated orchards, beans, peanuts and winter legumes, and cucumbers (Valenzuela-Zapata, 1985, 1994b, 1995, 1996, 1997, 2005).

The TRO is located northwest of Guadalajara and is known as the Lowlands. The climate in the TRO is warm subtropical, and the *municipio* where the most agave is grown (Amatitán) is at an elevation of 1310 m. The LAR is located northeast of Guadalajara and is known as the Highlands. In the LAR, the high plateau

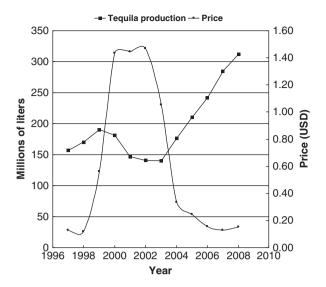


Fig. 1 Tequila production and real price per kilogram of agave standardized to 2007 Mexican pesos MXN and presented as US dollars USD (June 2010 USD/MXN exchange rate) 1997–2007. From Consejo Regulador del Tequila (CRT) data standardized by Valenzuela-Zapata and Macias.

of the *municipio* of Arandas is at 2000 m with a temperate subtropical climate. Blue agave plantations are considered to be most productive in temperate to semiwarm subtropical climates at elevations between 1600 and 2200 m (Pimienta-Barrios *et al.*, 2001). Thus, LAR is an optimal zone for agave cultivation, whereas TRO is suboptimal. This is likely the reason why efforts to extend agave cultivation towards the southern coast at elevations below 1000 m have not met with success (Ruiz *et al.*, 2002).

Agave plants prefer well-drained cambisols or volcanic litosols with medium-textured granular structure (Valenzuela-Zapata, 1994a). These soils are naturally poor in organic material and slightly acidic, like the majority of tropical soils (Hartemink et al., 1996). Calculation of the nutrient index in the TRO showed that nitrogen was one of the deficient nutrients for A. tequilana (Nobel, 1989). Soils in the LAR (Gobeille et al., 2006) and TRO (Valenzuela-Zapata, 1995) were found to have low exchange capacity and soil tilling may mineralize organic material, reducing nutrient retention capacity. Many more studies have been published on agave plantation weeds (López Muraira, 2008) and pests (Solís-Aguilar et al., 2001; Jiménez et al., 2004; Ayala-Escobar et al., 2005; Virgen Calleros, 2010) than on soil nutrients and management. It is in soil management where agroecological practices offer an opportunity for improvement of agave agriculture. For example, the leaf blight known as 'red ring' is often seen in soils with low fertility such as those in the LAR and TRO (Valenzuela-Zapata, 1994a; Salamanca Camacho, 2007). Studies conducted in the TRO show that between 1997 and 2003 large industrial agave plantations received more applications of herbicide (6-14) and insecticides and fungicides (1-8) than nutrients and soil treatments (two to five applications) (Valenzuela-Zapata, 2005).

Mezcal agaves have a long history of use as sweet food additives, and are today the basis for the production of fermented beverages or mezcals (Colunga-GarciaMarin & Zizumbo-Villarreal, 2007). As a mescal agave, the stem of blue agave is rich in fructans, also called agavins (~ 52% sugars; Mancilla-Margali & Lopez, 2006). Agave is harvested after the plant reaches maturity (5–8 years) just before flowering, to maintain the accumulated fructans. The leaves are removed and the stem and leaf base, collectively called the 'head' (cabeza) or 'piña,' is harvested. The carbohydrates in the head are then hydrolyzed into sugars using heat, and the extracted juice is then fermented.

Agaves have three natural reproductive mechanisms: seeds, offshoots from rhizomes and plantlets from bulbils. Blue agave is primarily propagated asexually using rhizome offshoots (Valenzuela-Zapata, 1994a), and more recently, in vitro culture methods due to the need for homogeneity and the difficulty of sexual propagation of semelparous plants. This has resulted, in part, in agave domestication syndromes, in which some characteristics have been selected for cultivation at the expense of reproductive capacity (Table 1), and very low genetic diversity.

Del Real Laborde (2010) reported that 2 million agave, 5.7% of the total plant harvest, were cloned between 2002 and 2010. Industry demand is predicted to continue to expand beyond 35 million plants per year (El Informador, 2010). While some view blue agave plagues and diseases as the most significant problems limiting production

Table 1 Domestication syndromes in cultivated agaves for sugars and fibers

Domestication syndromes
AS
AF
AS
AF
AS
AF
AS
AF

AS, agaves for sugars in cooked hearts 'mescal' (Hodgson, 1996, 1999; Hodgson & Slauson, 1995); AF, fibrous agaves (Colunga-GarciaMarín & May-Pat, 1997).

(Narvaez-Zapata & Sanchez-Teyer, 2009), they can also be viewed as a symptom of genetic erosion (Valenzuela-Zapata & Nabhan, 2004; Valenzuela-Zapata, 2005). These plant pathogens can spread when a crop is abandoned because of price instability, loss of soil fertility, inadequate nutrition and a surplus of mature plantations left to rot in the countryside. Hybrid vigor could be increased by developing landraces that are currently ignored in commercial agave production.

Searching the blue agave gene pool and their relatives

The growth in the tequila industry, which began in the 1970s, was based on expanded use of one landrace as its exclusive use became part of the obligatory tequila standards in a manner similar to industry and sales strategies set by varietal wine industries outside Europe (US, Australia and others). Like other crops in industrial agriculture, homogeneity of blue agave plantations is a desirable characteristic for the automation, mechanization and standardization of agricultural management of a raw material. With the development of homogenous commercially scaled agave production, there is the risk of losing the genetic diversity that was once inherent to the Mexican landscape.

Interviews of farmers and tequila industry employees were conducted in the 1980s in the area of Tequila, Mexico to collect data regarding agave landraces that were formerly described by Pérez, (1887): azul (blue agave), sigüín, pata de mula, moraleño, bermejo, chato, zopilote, mano larga, mano anchaque and cucharo. Synonyms, but not descriptions, were found for all the plants (Valenzuela-Zapata, 2003). Collections were made for conservation and future studies; herbarium voucher specimens were deposited at the Instituto Tecnológico *Tlajomulco* (ITT) in Jalisco and a collection of live plants and herbarium vouchers were deposited at the University of Guadalajara Botanical Garden (IBUG). Additional agave cultivars from traditional mezcal taverns were collected and planted at ITT botanical garden for later identification using Gentry (1982). Additional interviews were conducted from 2006 to 2009 with smallscale mezcal producers at six taverns in Cabo Corrientes, Mascota, Mazamitla, Tapalpa and two medium industries in Autlán and Tonaya municipios in the state of Jalisco.

To determine if the genetic diversity found in the old tequila landraces were still cultivated, anatomical and genetic characteristics of various agave landraces were compiled from the census data. The landraces are presented here as two groups: those belonging to the A. tequilana complex (the Tequila Agave Group; sigüín, pata de mula and azul listado, Table 2) and those not related to related to *A. tequilana* (*chato, zopilote, moraleño* and *bermejo*, Table 3) according to Gentry (1982).

Agave flowering stalks have key morphological differences that are necessary in order to differentiate the cultivars and clarify the relationship between the landraces (Figs 2 and 3). For example, a century ago, agave plants used for tequila production were briefly described by Trelease (1909, cited by Conzatti, 1981) using incomplete samples (with no flowers). Although three of the cultivars were registered by Gentry (1982) as synonyms of *A. tequilana*, Trelease described 'chato' as *A. subtilis, 'mano larga'* or 'chino bermejo' as *A. palmaris,* and 'pie de mula' or 'pata de mula' as *A. pes-mulae*. These nomenclatures were decided without considering reproduction strategies and flowering morphologies.

The length of life cycle, rosette size, rhizome number and sugar content of landraces are also distinguishing characteristics; these traits were consistently similar within groups and different between groups. Here, we are not including all complete descriptions made by Valenzuela (2003) for each landrace, just the most important differences. It is evident that landraces of the Tequila Agave Group have been selected for characteristics that are favorable for tequila production and cultivation (e.g. sugar content, short life

cycle, teeth and spines with little obstruction and higher rhizome number). This, combined with molecular genetic marker analysis by Bousios et al. (2007), and the evidence that all three Tequila Agave Group varieties can be crossbred, suggests that the Tequila Agave Group cultivars (including 'pata de mula') be added to the A. angustifolia ssp. tequilana complex. All the landraces in the Tequila Agave group are small to medium plants with diffuse umbels, 'azul' has the largest harvest index 'head', suggesting greater productivity than 'siguin' and 'pata de mula'. By redefining phylogenetic relationships among the groups in this way, opportunities emerge for new lines of research on conservation and genetic improvement of domesticated plants. We can also ascertain from the morphological data that A. rhodacantha var zopilote has a low domesticated syndrome. The landraces have rhizomes and bulbils and low capacity of sexual reproduction. All of them have a size reduced in flowering stage and faster floral dehiscence after anthesis is shown. In addition, A. rhodacantha has a very long life cycle, more persistent fruits and less rhizomes and rarely bulbils, so higher sexual reproduction capacity, more fertile seeds and leaves with strong teeth in the margins (vs. glaucous blue-green color with weak teeth) (personal observation).

Table 2 Characteristics for Agave angustifolia ssp. tequilana landraces

	Landraces of Agave angustifolia ssp. tequilana Tequila Agave Group				
Characteristics	Azul	Siguin	Pata de mula		
Cycle	Short	Short	Slightly shorter than that of 'azul'		
Rosette	1.7–2.0 m	1.7–1.9 m	1.0–1.2 m		
Stem	Ovoid, spherical	Spherical	Small spherical		
Leaves	90–140 leaves	80–95 leaves	60-80 leaves		
	Broad leaves basis	Broad leaves basis	Less broad leaves basis		
	Medium	Medium	Small		
	Linear-lanceolate glaucous blue	Linear-lanceolate glaucous greenish	linear bluish-green		
Flowers	68–79 mm	60–75 mm	40–50 mm		
	Bluish-green glaucous	Greenish	Greenish		
Floral axis	5–6 m length	3–5 m length	2–3 m length		
	Oval shape	Oval shape	Oval shape		
	20–28 branches	20–25 branches	10-20 branches		
	Diffuses umbels	Diffuses umbels	Diffuses umbels		
Rhizomes and bulbils	Numerous	Less than that of 'azul'	Less than that of 'azul'		
Sugars	High				
Fibers	Fibrous	Less Fibrous	Less fibrous		
	Leaves not easily peeled	Leaves not easily peeled	Leaves not easily peeled		
Ploidy	Diploid*	Atypical diploid *,†	Not reported		

Descriptions are based in Valenzuela-Zapata (2003) and personal observations.

Long floral tube, succulent and fragile.

^{*}Palomino et al. (2008).

[†]Largest genome size (Gil-Vega et al., 2006).

Table 3 Characteristics of Agaves not related to Tequila Agave Group landraces

	Agave landraces					
Characteristics	Moraleño§	Bermejo§	Chato¶	Zopilote		
Cycle‡	Medium	Long	Long	Longer		
Rosette	1.2–1.4 m	2.0–2.2 m	1.7–2.0 m	1.6–1.8 m		
Stem	Ovoid	Ovoid	Spherical-short	Ovoid		
Leaves	150–280 leaves Small lanceolate glaucous blue	120–160 leaves Long lanceolate greenish-blue	80–100 leaves Long spatulate glaucous bluish-green	100–120 leaves Medium linear green		
Flowers	68–79 mm Bluish-green glaucous shiny	60–75 mm Greenish	80–100 mm Green glaucous yellowish	50 mm Greenish		
Floral axis	4–8 m length Oblong narrow shape 25–35 branches Compacted umbels	6–8 m length Pyramidal ensiform shape 30–35 branches Big open umbels	6–10 m length Oval pyramidal shape 25–35 branches Compacted umbels	5–6 m length Oval shape 30–35 branches Dense umbels		
Rhizomes and bulbils	Few	Few	Few	Rare		
Sugars	Low Narrow leaves basis	Low Narrow leaves basis	High Broad leaves basis	Not reported Narrow leaves basis		
Fibers	Fibrous Fibers short and hard	Fibrous Fibers long, soft and shiny	Fibrous Fibers long and very hard	Less fibrous Irregular in length		
Ploidy	2n*	3n*	4n-5n* larger genome size†	Not reported		

^{*}Palomino et al. (2008).

§Sisalana group with leaves easily peeled, fetid flowers in 'bermejo'

We propose that the landraces 'moraleño' and 'bermejo' be moved to the species group Sisalanae and listed as cultigens (Gentry, 1982). These landraces were not found in natural conditions or in cultivation in western Mexico (Cházaro et al., 2007; Hernandez et al., 2007; Vázquez-García et al., 2007). 'Moraleño' and 'bermejo' panicles sizes and shapes are similar to those of A. desmettiana and A. sisalana, both in section Sisalanae (Gentry, 1982), and show characteristics such as fibrous leaves easily to decorticate, long stems and numerous leaves, indicative of domestication syndrome for fiber use. More than 200 short leaves are observed in 'moraleño' with asperous and hard fibers very different to 'bermejo' with 160 long leaves and soft fibers.

The proposed distinction between the Tequila Agave Group and the group of other agave landraces is also supported by the taxonomical grouping of species used for mezcal production in other regions of Jalisco. According to Gentry (1982) A. tequilana, belongs to the A. angustifolia complex. A. rhodacantha and A. angustifolia

complexes are found in the wild and in cultivation (Vargas-Ponce et al., 2007, 2009; Rodríguez-Garay et al., 2009), but these complexes are not the same in tequila agaves sensu Pérez (1887) nor lines identified by Valenzuela-Zapata (2003). Based on flower morphology, we hypothesize that A. rhodacantha 'zopilote' originated as a landrace from traditional mezcal production in Jalisco. The origin of the 'chato' landrace, a tetraploid-quintaploid variety (Palomino et al., 2008) with the largest genome size (Gil-Vega et al., 2006), is unknown. 'Chato' has linear-spatulate and rigid leaves similar to Rigidae section (Gentry, 1982) but the flower morphology, specifically a long oval pyramidal panicle, large yellow tepals, are similar to A. americana (century plant). Therefore, we propose that 'chato' be classified as A. americana ssp. subtilis as Trelease named it in 1909 (cited by Conzatti, 1981).

Domestication syndromes observed in tequila agaves support the fact that blue agave forms a phylogenetic group distinct from its near relatives (Valenzuela-Zapata,

[†]Gil-Vega et al. (2006).

[‡]All the landraces are longer in cycle than that in 'azul', descriptions are based in Valenzuela-Zapata (2003) and personal observations. Leaves collapse abruptly from the main stem during fruiting as in Agave sisalana.

[¶]A. americana ssp. subtilis.

^{||} *A. rhodacantha* leaves not easily peeled.

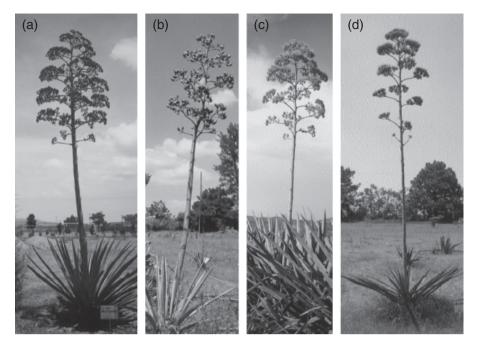


Fig. 2 Flowering plants of landraces of the Tequila Agave Group *Agave angustifolia* ssp *tequilana*: (a) *azul*, (b) *azul listado*, (c) *siguin* and (d) *pata de mula*. The panicles have an oval shape. *Siguin* and *pata de mula* have slender inflorescences and smaller rosettes than azul.

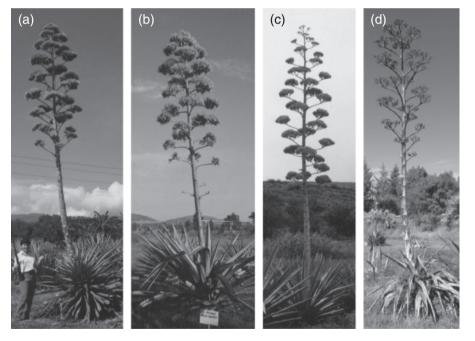


Fig. 3 Flowering plants of landraces cultivated in the Tequila region in the nineteenth century: (e) moraleño (cultigen Sisalanae), (f) Bermejo (cultigen Sisalanae), (g) Agave americana cv subtilis Americanae, (h) A. rhodacantha cv zopilote Rigidae.

2003; Bousios *et al.*, 2007). Through a review of the aforementioned interviews, data regarding agave and tequila production published in the last 10 years, and

data from field work tracing native genetic resources related to tequila agaves (Pérez, 1887; Valenzuela-Zapata, 1994b, 1997, 2003), it is clear that new markets and

growing raw material demands have affected agave landraces. One can therefore conclude that the expansion of blue agave in traditional mezcal regions has affected the germplasm diversity (Colunga-GarciaMarin & Zizumbo-Villarreal, 2007).

Despite the apparent decline in genetic diversity, there are opportunities for expanding the gene pool utilizing cultivars that were not previously considered for tequila production. Of course, reintroducing those mentioned above as an A. angustifolia ssp. tequilana complex. Numerous cultivars of A. angustifolia and A. rhodacantha, as well as wild populations of A. inaequidens Koch Crenatae, A. maximiliana Baker Crenatae and A. valenciana Cházaro & A. Vázquez are used for the production of traditional Jaliscan agave beverages. These cultivars are different from those used for tequila production now and in the 19th century (Valenzuela-Zapata et al., 2008).

New agenda and landrace conservation

After repeated episodes of blue agave oversupply, partly in response to skyrocketing prices (Fig. 1), an effort was made to utilize surplus agave by building up the inulin and fructose syrup industry (Coelho, 2007; Secretaría de Ecomonía, 2009), and more recently, the agave biofuel industry in Mexico. The manufacture of beverages called distillates or aguardientes (agave 'eaude-vie'), under Mexican Standard NOM-EM-012-SCFI-2006 (Secretaría de Economía, 2006b), is another industry emerging from the surplus agave, including that which is grown in regions of Mexico not protected by geographical status.

There is; however, a lack of reliable data on agave syrup and inulin production. Based on the information available from the tequila industry, including blue agave plant inventories held by the National Chamber of the Tequila Industry (http://www.tequileros.org.mx) and the tequila makers union and the Tequila Regulation Council (CRT) (http://www.crt.org.mx), blue agave production exceeds current market demands. Despite increasing diversification of products, there is an opportunity to use biomass from blue agave for

The tequila industry does not set obligatory quality standards for raw material. Instead, each brand sets its own criteria regarding quality standards. Some consider sugar and product quality; Bautista Justo et al. (2001) classify good quality as 25-30% total reducing sugar (%TRS) concentrations after hydrolysis. Others place more importance on the factors which influence fermentation and therefore quality of the final product (Pinal et al., 2009). In addition, harvest age (Arrizon et al., 2010), soil and climate conditions and harvest

season (Larqué-Saavedra et al., 2010) were also found to affect sugar content and quality.

The market for agave could be diversified by using agave harvest residue (e.g. leaves), and bagasse (the residue left after the juices have been extracted) for use as production (Davis et al., 2011). Demand for tequila 100% agave sugars has recently increased (19.82% annual increase between 1995 and 2008), and the resulting increase in residue and bagasse can be used for secondgeneration biofuel production (Chavez-Guerrero & Hinojosa, 2010; Valdez-Vazquez et al., 2010). If the demand for agave sugars for bioethanol production grows as predicted, then the quantity of agave residues is likely to double in the next few years. There is currently little use for agave bagasse although it has been considered for use as compost (Iñiguez-Covarrubias et al., 2010) animal feed and fiberboard production (Iñiguez-Covarrubias et al., 2001b). These residues can now be considered for use as a solid fuel or for ethanol production due to their high sugar content. Leaves constitute 32% of the total biomass of the agave plant and contain 13.1-16.1% TRS (Iñiguez-Covarrubias et al., 2001a). Bagasse represents 40% of the total wet weight of milled agave (Iñiguez-Covarrubias et al., 2001b) and contains 5-20% TRS (Alonso-Gutierrez, 2005).

Discussion

We find ourselves at a historic crossroads as blue agave demand changes. Increased demand from the three main agave industries (tequila, fructose syrup and bioethanol) and an unquantified inventory of blue agave can be expected to lead to new relationships between industry and agave producers. Use of tequila production residues would increase diversification in the agave industry, and add additional value. The entire blue agave plant could be mechanically harvested and used for biofuel production, using lignocellulosic materials and sugars without separation (Holtum et al., 2010).

The synthesis provided here reveals a need for conservation and reintroduction of the three blue agave landraces named here as Tequila Agave Group (Table 2) to conserve phytogenetic resources and to increase productivity. The blue agave interbreeding with their nearest relatives could offer heterosis and more genetic variability relative to the landrace used now, which is derived from high selection pressure. Genetic improvements to produce agave hybrids using interbreeding techniques developed in sisal in Africa (Lock, 1962) and Brazil (Macedo, 1999) have been used for some time. Some researchers have even investigated intravarietal (intralandrace) clones of A. tequilana to find high productivity profiles (Madrigal Lugo & Velázquez Loera, 2010).

Identifying plants in flower (Valenzuela-Zapata, 2003) and then using molecular markers from the blue agave gene pools (Bousios et al., 2007) ('azul listado', 'sigüín', and 'pata de mula') would help to select potential landraces that can increase the hybrid vigor of the blue agave variety. Results from other authors (e.g. Gil-Vega et al., 2006) RAPD molecular markers assume all landraces are varieties of A. tequilana, ignoring plant descriptions of Tequila Agave Group landraces, and are therefore limited for hybrid genetic improvement and germplasm analysis (Spooner & Lara-Cabrera, 2001). Incorporating landrace diversity in new agave crops will also serve conservation goals, and help mitigate the loss of intervarietal diversity (Gil-Vega et al., 2001). Genetic variability has been reduced because asexual reproduction dominates agave agro-ecosystems like 'henequen' (Abraham-Juarez et al., 2009; González et al., 2003; Infante et al., 2003), and variation is likely to be much greater if pollination of flowering stalks occurs.

Agave species domesticated principally for fibers ('bermejo', 'moraleño') are rich in cellulose and perhaps useful for biofuels and other products in the future. Landraces like 'chato' with two domestication syndromes for fibers and sugars (long leaves and hard fibers and big heads and broad leaves basis) could have multiple bioenergy uses. Thus, there are opportunities for new utilization of landraces of agave that might satisfy the emerging and diversified market for agave products.

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References

- Abraham-Juarez MJ, Ramírez-Malagón R, Gil-Vega KC et al. (2009) AFLP Analysis of genetic variability in three reproductive forms of Agave tequilana. Revista de Fitotecnia Mexicana, 32, 171–175.
- Alonso-Gutierrez MS (2005) Valorisation de la bagasse de l'agave tequilana W. cv azul: caractérisation, étude de la digestibilité et de la fermentation des sucres. Thesis Sciences des Agroressources, Institut National Polytechnique de Toulouse, Toulouse, France, 188 pp.

- Arrizon J, Morel S, Gschaedler A *et al.* (2010) Comparison of the water-soluble carbohydrate composition and fructan structures of *Agave tequilana* plants of different ages. *Food Chemistry*, **122**, 123–130.
- Ayala-Escobar V, Yanez-Morales MD, Braun U *et al.* (2005) *Cercospora agavicola* – a new foliar pathogen of *Agave tequilana* var. azul from Mexico. *Mycotaxon*, **93**, 115–121.
- Bautista Justo M, Garcia Oropeza L, Salcedo Hernandez R *et al.* (2001) Azucares en Agaves (*Agave tequilana* Weber) cultivados en el estado de Guanajuato. *Acta Universitaria*, **11**, 1–33.
- Bousios A, Saldana-Oyarzabal I, Valenzuela-Zapata AG *et al.* (2007) Isolation and characterization of Ty1-copia retrotransposon sequences in the blue agave (*Agave tequilana* Weber var. azul) and their development as SSAP markers for phylogenetic analysis. *Plant Science*, **172**, 291–298.
- Chavez-Guerrero L, Hinojosa M (2010) Bagasse from the mezcal industry as an alternative renewable energy produced in arid lands. Fuel, 89, 4049–4052.
- Cházaro BMJ, Vázquez-García JA, Valencia O (2007) Nuevas especies y nuevos registros de *Agave* en Jalisco, México. In: *Agaves del Occidente de México* (eds Vázquez-García JA, Cházaro BMJ, Hernández Vera G *et al.*), pp. 13–18. Universidad de Guadalajara, Guadalajara.
- Coelho A (2007) Eficiencia colectiva y upgrading en el cluster del tequila. Análisis Económico XXII, 49, 169–194.
- Colunga-GarciaMarín P, Larqué A, Eguiarte L et al. (2007) En lo ancestral hay futuro: del tequila, los mezcales y otros agaves. ISBN: 978-968-6532-18-0. CONABIO, México, 304 pp.
- Colunga-GarciaMarín P, May Pat F (1997) Morphological variation of henequen (*Agave fourcroydes, Agavaceae*) germplasm and its wild ancestor (*A. angustifolia*) under uniform growth conditions: diversity and domestication. *American Journal of Botany*, **84**, 1449–1465.
- Colunga-GarciaMarin P, Zizumbo-Villarreal D (2007) Tequila and other Agave spirits from west-central Mexico: current germplasm diversity, conservation and origin. *Biodiversity* and Conservation, 16, 1653–1667.
- Conzatti C (1981) Flora Taxonómica Mexicana, Vol. 2. CERETI, Guadalajara, pp. 93–118.
- Davis SC, Dohleman F, Long SP (2011) The global potential for *Agave* as a biofuel feedstock. *Global Change Biology Bioenergy*, **3**, 68–78.
- del Real Laborde I (2010) Agave propagation for commercialscale production. Agave and Bioenergy Workshop, May 12–13, 2010, Guadalajara, Mexico.
- El Informador (2010) *Producción de etanol complicará planeación estratégica del agave.* Periodico 17 de mayo de 2010, Guadalajara, México.
- Escobar-Guzmán RE, Zamudio Hernández F, Gil Vega K (2008) Seed production and gametophyte formation in *Agave tequila-* na and *Agave americana*. Botany, **86**, 1343–1353.
- Gentry HS (1982) Agaves of Continental North America. University of Arizona Press, Tucson, AZ.
- Gil-Vega K, Díaz C, Nava-Cedillo A et al. (2006) AFLP analysis of Agave tequilana varieties. Plant Science, 170, 904–909.
- Gil-Vega K, González Ch M, Martínezde la VO et al. (2001) Analysis of genetic diversity in Agave tequilana var. Azul using RAPD markers. Euphytica, 119, 335–341.

- Gobeille A, Yavitt J, Stalcup P et al. (2006) Effects of soil management practices on soil fertility measurements on Agave tequilana plantations in Western Central Mexico. Soil and Tillage Research, 87, 80-88.
- González G, Alemán S, Infante D (2003) Asexual genetic variability in Agave fourcroydes II: selection among individuals in a clonally propagated population. Plant Science, 165, 595-601.
- Hartemink AE, Osborne JF, Kips PA (1996) Soil fertility decline and fallow effects in ferralsols and acrisols of sisal plantations in Tanzania. Experimental Agriculture, 32, 173-184.
- Hernandez VG, Cházaro BMJ, Flores-Berrios EP (2007) Inventario, distribución y habitat del género Agave en Jalisco, México. In: Agaves del Occidente de México (eds Vázquez-García JA, Cházaro BMJ, Hernández Vera G et al.), pp. 6-12. Universidad de Guadalajara, Guadalajara.
- Hodgson WC (1996) Agaves and archaelogy: Agave delamateri adds to both stories. The Sonoran Quaterly, 50, 6-9.
- Hodgson WC (1999) Agaves and humans: the continuing story of a continuing connection. The Sonoran Quarterly Desert Botanical Garden Bulletin, 53, 6-8.
- Hodgson WC, Slauson L (1995) Agave delamateri (Agavaceae) and its role in the subsistence patterns of pre-Columbian cultures in Arizona. Haseltonia, 3, 130-140.
- Holtum JAM, Chambers D, Tan DKY (2011) Agave as a biofuel feedstock in Australia. Global Change Biology Bioenergy, 3, 58-67.
- Infante D, González G, Echeverría-Peraza L (2003) Asexual genetic variability in Agave fourcroydes. Plant Science, 164, 223-230.
- Iñiguez-Covarrubias G, Acosta N, Martinez L et al. (2010) Compostaje de bagazo de agave y vinazas tequileras. In: Composting Experiences in Industrial and Animal Residues (ed. Iñiguez-Covarrubias G), pp. 25-52. Universidad de Guadalajara, Guadalajara.
- Iñiguez-Covarrubias G, Díaz-Teres R, Sanjuan-Dueñas R (2001a) Utilization of by-products from the tequila industry. Part 2: potential value of Agave tequilana Weber azul leaves. Bioresource Technology, 77, 101-108.
- Iñiguez-Covarrubias G, Lange SE, Rowell RM (2001b) Utilization of by-products from the tequila industry title: part 1: agave bagasse as a raw material for animal feeding and fiberboard production. Bioresource Technology, 77, 25-32.
- Jiménez HI, Virgen-CG, Martinez de la VO et al. (2004) Identification and characterisation of bacteria causing soft-rot in Agave tequilana. European Journal of plant pathology, 110, 317-331.
- Larqué-Saavedra A, Rendon LA, Magdub A et al. (2010) Climate and age important factors for ethanol production by henequen. Agave and Bioenergy Workshop, May 12-13, 2010, Guadalajara, Mexico.
- Lock GW (1962) Sisal. Twenty five years, sisal research. Sisal Growers Association Longmans, Tanganyka.
- López Muraira IG (2008) Manual de Maleza del Cultivo del Agave tequilana en Jalisco, 1st edn. Instituto Tecnológico de Tlajomulco y Tequila Sauza, S.A. de C.V., Mexico.
- Macedo VR (1999) Melhoramento genético do sisal. In: O agronegocio do sisal no Brasil (eds da Silva Ferreira OR, de Macedo Beltrao NE), pp. 93-110. Embrapa, Campina Grande.
- Macias MA, Valenzuela Zapata A (2009) El tequila en tiempos de la mundialización. Comercio exterior, 59, 459-472.

- Madrigal Lugo R, Velázquez Loera A (2010) Agave feedstock for bioethanol, Agave and Bioenergy Workshop, May 12-13, 2010, Guadalajara, Mexico.
- Mancilla-Margali NA, Lopez MG (2006) Water-soluble carbohydrates and fructan structure patterns from Agave and Dasylirion species. Journal of Agriculture Food Chemistry, 54, 7832-7839.
- Martínez RLM, Iñiguez LI, Palomera GC et al. (2003) Impacto ambiental, social, y económico del cultivo del agave azul (Agave tequilana Weber) en el municipio de Tonaya, Jalisco. Working paper, University of Guadalajara-CUCSUR, Autlan, Mexico.
- Narváez-Zapata JA, Sánchez-Teyer LF (2009) Agaves as a raw material: recent technologies and applications. Recent Patents on Biotechnology, 3, 185-191.
- Palomino G, Martínez J, Méndez I (2008) Karyotype studies in cultivars of Agave tequilana Weber. Caryologia, 61, 144-153.
- Pérez L (1887) Estudio sobre el agave llamado mezcal. Boletín de la Sociedad Agrícola Mexicana, 11, 132-136.
- Pimienta-Barrios E, Robles-Murguia C, Nobel PS (2001) Net CO₂ uptake for Agave tequilana in a warm and a temperate environment. Biotropica, 33, 312-318.
- Pinal L, Cornejo E, Arellano M et al. (2009) Effect of Agave tequilana age, cultivation field location and yeast strain on tequila fermentation process. Journal of Industrial Microbiology and Biotechnology, 36, 655-661.
- Rodríguez-Garay B, Lomelí-Sención JA, Tapia-Campos E et al. (2009) Morphological and molecular diversity of Agave tequilana Weber var. Azul and Agave angustifolia Haw. var. Lineño. Industrial Crops and Products, 29, 220-228.
- Ruiz CJA, Pimienta-Barrios E, Zañudo-Hernández J (2002) Regiones térmicas optimas marginales para el cultivo de Agave tequilana en el estado de Jalisco. Agrociencia, 36, 41-53.
- Ruvalcaba-Ruiz D, Rodríguez-Garay B (2002) Aberrant meiotic behavior in Agave tequilana Weber var azul. Plant Biology, 2, 1–10.
- Salamanca Camacho M (2007) Cambios en la estrategia de manejo de maleza en el agave tequilero. In: Estado actual del manejo de plagas y enfermedades del agave tequilero: Memorias Simposio Prioridades Fitosanitarias Actuales en el cultivo de Agave tequilana W. var azul (eds Bernal JS, Orozco Hernandez A, del Real Laborde JI et al.), pp. 163-182. Colegio de Postgraduados, Mexico.
- Secretaría de Economía (2006a) Norma Oficial Mexicana NOM-006-SCFI-2005, Bebidas alcohólicas-Tequila-Especificaciones, Diario Oficial de la Federación, January 6.
- Secretaría de Economía (2006b) Norma Oficial Mexicana de Emergencia NOM-EM-012-SCFI-2006, Bebidas Alcoholicas-Bebidas Alcoholicas-Destilados de Agave-Especificaciones, Informacion Comercial, Etiquetado y Metodos de prueba, Diario Oficial de la Federación, November 30, 2006.
- Secretaría de Economía (2009) Norma Mexicana NMX-FF-110-SCFI-2008, Productos alimenticios – Jarabe de agave- Especificaciones y métodos de prueba. April 22, 2009. 28 pp, Mexico City.
- Solís-Aguilar JF, González-Hernández H, Leyva-Vázquez JL et al. (2001) Scyphophorus acupunctatus Gyllenhal, plaga del agave tequilero en Jalisco, México. Agrociencia, 35, 663-670.
- Spooner DM, Lara-Cabrera S (2001) Sistemática molecular y evolución de plantas cultivadas. In: Enfoques contemporáneos para el estudio de la biodiversidad (eds Hernández HM, García

- Aldrete AN, Álvarez F *et al.*), pp. 57–114. Instituto de Biología UNAM. Mexico.
- Valdez-Vazquez I, Acevedo-Benítez JA, Hernández-Santiago C (2010) Distribution and potential of bioenergy resources from agricultural activities in Mexico. *Renewable and Sustainable Energy Reviews*, 14, 1–7.
- Valenzuela-Zapata AG (1985) The tequila industry in Jalisco, Mexico. Desert Plants, 7, 65–70.
- Valenzuela-Zapata AG (1992) Fertilización en plantas jóvenes de agave tequilero, (Agave tequilana Weber, variedad azul). Master Sciences Thesis. Escuela de Graduados Universidad de Guadalajara.
- Valenzuela-Zapata AG (1994a) El agave tequilero su cultivo e industrialización. (ed. Agatha), Guadalajara, Mexico.
- Valenzuela-Zapata AG (1994b) Los agaves tequileros. Boletín Amaranto, 7, 1–8.
- Valenzuela-Zapata AG (1995) La agroindustria del agave tequilero Agave tequilana Weber Boletín de la Sociedad Botánica de México, 55, pp. 15–25.
- Valenzuela-Zapata AG (1996) Utilización, producción y propagación convencional de *Agave tequilana*, la agavácea de mayor superficie cultivada en el mundo. In: *Técnicas convencionales y biotecnológicas para la propagación de plantas de zonas áridas* (eds Izquierdo J, Palomino G), pp. 87–110. Regional Office of the FAO for Latin America and the Caribbean, Santiago, Chile.
- Valenzuela-Zapata AG (1997) El agave tequilero. Cultivo e industria. (ed. Litteris), Guadalajara, Mexico, 204 pp.
- Valenzuela-Zapata AG (2003) El agave tequilero. Cultivo e industria de México. (ed. Mundiprensa), México, 215 pp.

- Valenzuela-Zapata AG (2005) *Indicaciones geograficas y sustent-abilidad: el caso del tequila*. Doctoral thesis, Universidad Autonoma de Nuevo Leon, Monterrey, Mexico, 126 pp.
- Valenzuela-Zapata AG, Macias MA (2010) La Indicación geográfica Tequila: lecciones de la primera denominación de origen mexicana. Universidad de Guadalajara (in press).
- Valenzuela-Zapata AG, Nabhan GP (2004) *Tequila. A Natural and Cultural History.* University of Arizona Press, Tucson, AZ.
- Valenzuela-Zapata AG, Regalado PA, Mizoguchi M (2008) Influencia asiática en la producción de mezcal en la costa de Jalisco. El caso de la raicilla. *México y la Cuenca del Pacífico*, **11**, 81–116.
- Vargas-Ponce O, Zizumbo-Villarreal D, Colunga-GarciaMarin P (2007) *In situ* diversity and maintenance of traditional Agave landraces used in spirits production in west-central Mexico. *Economic Botanu*, **61**, 362–375.
- Vargas-Ponce O, Zizumbo-Villarreal D, Martinez-Castillo J et al. (2009) Diversity and structure of landraces of agave grown for spirits under traditional agriculture: a comparison with wild populations of *A. angustifolia* (Agavaceae) and commercial plantations of *A. tequilana. American Journal of Botany*, **96**, 448–457.
- Vázquez-García JA, Vargas-Rodríguez L, Cházaro BMJ (2007) Diversidad, endemismo y rareza de especies de agave en Jalisco, México. In: *Agaves del Occidente de México* (eds Vázquez-García JA, Cházaro BMJ, Hernández Vera G *et al.*), pp. 23–31. Universidad de Guadalajara, Guadalajara.
- Virgen Calleros G (2010) Pest and diseases in *Agave tequilana* Weber var azul. *Agave and Bioenergy Workshop*, May 12–13, 2010, Guadalajara, Mexico.