Agave biotechnology: an overview

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REVIEW ARTICLE

Agave biotechnology: an overview

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Abstract

Agaves are plants of importance both in Mexican culture and economy and in other Latin-American countries. Mexico is reported to be the place of *Agave* origin, where today, scientists are looking for different industrial applications without compromising its sustainability and preserving the environment. To make it possible, a deep knowledge of all aspects involved in production process, agro-ecological management and plant biochemistry and physiology is required. *Agave* biotechnology research has been focusing on bio-fuels, beverages, foods, fibers, saponins among others. In this review, we present the advances and challenges of *Agave* biotechnology.

Keywords

Agave, applications, biofuels, beverages, fibers, foods

History

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Introduction

Agave is a source of nutrients, for both humans and animals. These plants grow in different regions of the country and have capacity of adaptation to several environmental conditions (Pinos-Rodríguez et al., 2008). In Mexico, *Agave* has a high economic importance because of its many industrial applications such as beverages, food, biofuels, fructans and natural fibers.

Agave is being used in Mexico with different purposes such as food, building, pharmacy, religion, textile and decoration. The *Agave* species with the highest economical importance in this country is *Agave tequilana* Weber blue variety, commonly known as *Agave* azul (Paz et al., 2011). Although, there are over 200 species of the *Agave* plant (Figure 1), the most economically important plant type of this genus is named ''maguey''. This word may have a relationship with the Caribean term ''taino''. Although it is known that they were named after the Spanish that conquested America (Aguirre et al., 2001). In the Azteca cosmogony, the Maguey goddess was named ''Diosa Mayahuel''.

Agave plants are composed of two main parts, large leaves with spines, where it is possible to extract sisal type fibers; and the second part named ''píña'', which is cooked to obtain juices and produce many beverages, including tequila, the principal Mexican alcohol beverage that is consumed worldwide (Whitney et al., 2002).

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Agave farming is performed for various purposes: to produce thicker fibers which could be utilized during paper and fertilizers production (Narváez-Zapata & Sánchez-Teyer, 2009) or alcoholic beverages. There are established specific agronomical practices for each kind of final product and biomass production. Surprisingly, some species of *Agave* yield large biomass volume in semi desert zones, with life cycles from 5 to 6 years (Somerville et al., 2004).

The Mexican Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA), reported that in 2009, the Mexican states with the highest production of *Agave* were Jalisco with 6.59×10^5 tons by year, followed by Oaxaca with 3.06×10^5 and Nayarit with 1.18×10^5 tons (Paz et al., 2011). This plant has been reported to be grown in African countries such as Kenya and Tanzania, as well as Mexico and USA in North America and there is recent interest in Australia to produce *Agave* crops (Davis et al., 2011, Holtum et al., 2011). *Agave* ''pencas'' have little use as the cover of some Mexican dishes such as ''barbacoa'' and ''mixiote'', and are considered as a barely exploited residue. At present, there is an industrial interest to use the whole plant, for some of the uses mentioned above. Employment of extensive and profitable agricultural and food residues that apparently had no added value is actually the main raw materials for the biotechnological industries (Goldbeck et al., 2013).

During natural sources utilization, one should always take into account plant conservation programs and environment impacts (sustainability). These requirements are based on three aspects, one already mentioned, sustainability, without forgetting, the social and economic aspects, involving biological and humanistic areas. The other ones determine the application of correct action for problem resolution by humans. Sustainability as an environmental component

should increase knowledge to understand the environment – *Agave* species interactions. These aspects have a main objective to maintain population and ecosystems capacity to assimilate waste, regenerate renewable natural resources and available energy from these materials (Baena Gonzales, 2005). *Agave* plays an important role in diverse areas and are used from food to ornamental decoration. Table 1 shows a summary of some *Agave* uses (García Herrera et al., 2010).

Figure 1. *Agave atrovirens* picture taken from the Saltillo, Coahuila vicinity.

Taxonomy

Agave was sacred to the Aztec population of prehispanic Mexico and in náhuatl was named *Metl.* Charles Linneo used *Agave* which is greek for *noble* and *admirable* in latin and that name was given due to the ability of these plants to can grow predominantly or exclusively on dry environments, although it can be found on other ecosystems (López & Mancilla-Margalli, 2007).

Agave belongs to the *Asparagales* order, within the *Agavaceae* family, with more than 200 species and 47 intra-specific categories. Mexico has been reported to have 75% of the *Agave* species. Narváez-Zapata & Sánchez-Teyer (2009) reported that at least 135 endemic species of *Agave* in Mexico. There are other reports that classify the *Agave* into 12 sections with 82 species, 21 subspecies and 23 varieties, these being 197 taxa as a whole. Studies-based angiosperm phylogenetic relationships, age estimation and diversification rates indicate that the order of *Asparagales* is traced back 120 millions of years (Magallón & Castillo, 2009). Commonly, *Yucca* and *Aloe* are mistaken for being of the *Agave* genus (Bogler et al., 2005), and the family *Xanthorrhoeceae* has been split from the order of the *Asparagales* and that family begat *Aloe* which is different from *Agave*. Yucca shares the same subfamily of *Agavoidea* (formerly *Agavaceae*) with

Table 1. Principal uses of socioeconomic and agroecological importance of the *Agave*.

Uses	Product	Part of the plant
Food	Sugar	Stem (piña)
	Stews	Flowers and fruit
	Candy	
	Barbecue wrapping	Flower scape (Quiote)
	Mixiotes	Leaves
	White worms	Cogollo cuticle
	Red worms (Chinicuiles)	Leaves
	Pulque bread	Stem (piña)
	Tortillas	Flower perianth + nixtamal
Beverages	Aguamiel, honey, atole de aguamiel, Pulque, Mezcal, Tequila, Sotol, Bacanora, Vinagre, Jarabe	Stem (piña)
Agricola	Living fence	Entire plant
	Avoid erosion by being soil former	Entire plant
	Leader plant of ecosystems	Entire plant
	Compost (fertilizer)	Leaves compost
Forage	Bovine, caprine, porcine	Leaves, flower scape, flowers, inflorescence, bagasse.
Building	Fences, shackes, pens	Floral escape
	Tiles to cover roof	Leaves
	Recollection canals for rain water	Leaves
	Compound materials: thermoplastic or thermophilic resins + fibers	Fiber residues
Fibers	Cordage, straw and basketry	Fiber leaves, roots
	Brushes, cleaning brushes with a soap included, polishing pads, woven and clothes	Fiber leaves
Medicinal	Useful to blows, internal injuries, no movement limbs, scurvy prevention	Leaves
	Anti-inflammatory, useful in cases of anemia	Honey and Pulque
Ornamental	Accessories like rings and earrings	Seed
	Christmas decoration	Whole plant
	Floral arches	Fiber leaves
	Gardens, streets and ridges	Whole plant
Domestic	Soap and detergent to dishes and clothes Shampoo	Leaves, stems and roots
	Pots and water container	Stem (piña)
	Lid of containers	Leaves and stems
	Chopsticks to extract edible worms	Terminal thorn
	Needle with thread	Terminal thorn plus thread leave
Others	Chemical and pharmaceutical industry, drugs and steroidal products (saponins)	Leaves, roots, stems and seeds
	Cellulase products for paper	Leaves (pulp and fiber residues)
	Ethanol production, cellulose and glucosides	Leaves (pulp and fiber residues, bagasse, juice)

Source: Centro de Propagación de *Agave* del Estado de Guanajuato.

Agave (Bogler et al., 2005). According to Good-Ávila et al. (2006), *Agave* and *Yucca* differs in their reproductive ecology, they are monocarpic and polycarpic respectively and *Agave* is a younger genus approximately 7 to 10 million years and yet it harbors much more species than *Yucca*, which is a genus of a 100 million years and less species. Apart from *Yucca* and *Agave*, Davis et al. (2011), reports the most influential *Agave* species as most productive are: *A. utahensis*, *A. vilmoriniana*, *A. deserti*, *A. fourcroydes*, *A. sisilana*, *A. lechuguilla*, *A. salmiana*, *A. tequilana* and *A. mapisaga*. Most of these species are used to produce fibers, rope and soap; however, *A. tequilana* is used to produce tequila and *A. salmiana* and *A. mapisaga* to produce pulque (Alfaro-Rojas et al., 2007).

Distribution

Agave plants are perennial. Their metabolism, physiological and morphological characteristics allow them to survive under extreme conditions. They are found in valleys, plains, hills and stony hillsides, including mountain places with high altitude. *Agave* plants grow in soil with neutral pH or slightly alkaline. The crassulacean acid metabolism (CAM) is used for the *Agave* genus. These plants decrease total water consumption and transpiration, because CO_2 absorption occurs during the night. They assimilate sugar by photosynthesis during the day (Black & Osmond, 2005). During the night, they open their stomata to liberate less amounts of water as is normal in the day. In this way, *Agave* species have a low demand for nutrients and efficient use of water, which is six times higher than some C3 species like wheat that sequester the carbon by a photosynthetic cycle that involving intermediaries which contain three atoms of carbon (Paz et al., 2011).

Until 2010 in Mexico, the number of *Agave* species was controversial. Some authors have mentioned that there are 166 recognized species, some 200 and other 273 different *Agave* species in the American continent. Some *Agave* species grow in specific area and others are widely distributed. For example, the Agave, from which mezcal is obtained, is commonly found in most Mexican states, except in Tabasco and the Yucatán Peninsula (Illsey-Granich et al., 2004).

Physiology

Agave plants are very well adapted to several habitats, including arid environments, and can grow individually or as populations. *Agave* can grow to 1.8 m tall, a succulent rosetta is formed by the leaves and it can weigh up to 250 kg in the fresh state. The stem is thick and fibrous and a flower emerges as the stem grows along with the leaf bases and acts as an energy source storage in the form of carbohydrates. The roots penetrate approximately 30 cm into the ground. When an *Agave* growth cycle nears its end, the flower appears and the life span range is from 8 to 20 years (Martínez-Salvador et al., 2005).

This plant is propagated by seeds, and vegetative stems and propagules from the inflorescence, depending on the species. Gómez-Pompa (1963) mentioned that in this genus, sexual reproduction is limited or absent, although it could occur but the seeds have only 33% germination. Commonly, *Agave* plants are cultivated in an asexual form with vegetative stems, derived from the rhizome, which are emitted from the mother plant after the first year of plantation. When plants are small, they could be maintained in a nursery for 12 months or more until its definitive plantation.

There are some attempts for genetic improvement of these plants with methods such as *in vitro* reproduction, to achieve better characteristics like precocity, faster growth, leaves without spines, more and better fiber, high weight yield, resistance to drought, diseases or alkalinity, more reducing sugars content, etc. (García-Herrera et al., 2010).

Traditional uses of the *Agave* plant

Beverages type depends on the *Agave* specie; ''Mezcal'' is primarily obtained from *A. angustifolia, A. potatorum,* and *A. salmiana;* ''Bacanora'' from *A. angustifolia, A. potatorum* and *A. pacifica;* ''Pulque'' from *A. salmiana* and ''Tequila'' from *A. tequilana.* On the other hand, some *Agave* beverages like ''natural Aguamiel'' and/or juices are obtained from fresh or cooked ''piñas'' can be utilized as sources for polysaccharides, fructose syrup, *Agave* fructans extractions, Maillard components and biofuels production (Narváez-Zapata & Sánchez-Teyer, 2009).

The most consumed national alcohol beverage in Mexico is ''Tequila'', which is a distillated beverage obtained through fermentation of sugars of *Agave tequilana Weber* var. azul juices (López-Alvarez et al., 2012). Tequila has a long and rich history in México and the USA. The name comes from the náhuatl ''tequitl'' and ''tlan'' words, which mean work and place, respectively. So it could be said that Tequila means ''place where work is carried out'' (Cedeño & Álvarez-Jacobs, 1995). ''Tequila reposado'' is obtained after a mature process from three to 12 months of resting in wood barrels, when the maturation time is from one to 5 years, it is named ''Tequila añejo'' (Lappe-Oliveras et al., 2008).

Agave plants are ready to be harvested (cut) for beverages production when they are 8-10 years-old. At this time, farmers remove the inflorescence, with the objective to concentrate sugars into the stem, and avoid sugar consumption by the ''quiote''. Diverse patents have been obtained to accelerate the maturation process of cooked stems. Normally, the leaves are removed and only *Agave* stems are used for tequila production (Orendain & Rodriguez Flores, 2007). An efficiency of 70% in sugar transformation is observed during fermentation, but on some occasions, this may be due to less yeast stress (Arrizon et al., 2010). Tequila can be produced only in certain areas for it has ''denominación de origin'', similar to the term used in the European Union ''protected designation of origin''. This includes 181 counties belonging to five Mexican states, such as, 125 from Jalisco, eight from Nayarit, seven from Guanajuato, 30 from Michoacán and 11 from Tamaulipas (Consejo Mexicano Regulador del Tequila, 2011). The species from which this beverage must be obtained is *Agave tequilana Weber* var. azul, this species also has ''denominación de origin'' to be cultivated only in the States mentioned before. It must have certain relevant characteristics such as a high concentration of inulin (20–24%), small fiber content and certain compounds which will give the specific flavor that characterize the *Agave tequilana* (Bautista et al., 2001).

The traditional production process for tequila involves conical holes dug underground. The duration of cooking depends on the oven type, for example 48 h in masonry ovens, while in autoclaves 12 h (Carrillo, 2007) is adopted. During cooking several biochemical reactions are involved, for example, sugars and fructo-oligosaccharides are hydrolyzed, because these cannot be solubilized in water, and in consequence, they are not fermented (Pessoni et al., 1999). When the cooking process is over, the material is ready for juice extraction. After that, *Agave* pulp is obtained using a milling process, where sugars (12%) are obtained and lately are separated from stem residues, collected and transported to the next production step (Guzmán, 1997). When fermentation ends, distillation is undertaken in copper or stainless tanks. This process is conducted twice, where the first one is known as destroying; when the vinazas are eliminated from water, ''heads'' and ''lines''. The product obtained is named ordinary tequila with 25–30°GL. The second distillation is termed rectification and has the objective to concentrate ethanol and make separate the heads and lines, having at the end 45 to 50°GL (Rodriguez, 2001). The final content must be from 35–45°GL (NOM-006-SCFI-2005). Depending on the process after distillation, the liquor is named white tequila, youth or old gold, rested and old extra (Buenrostro et al., 2010).

The second most common beverage produced from *Agave* is ''Mezcal'', which similar to Tequila, has ''denominación de origin'' (Martínez-Aguilar & Pena-Alvarez, 2009). Production of ''Mezcal'' starts with the harvest of heads ''piñas'' with an approximate age of 8 to 12 years. In this case, maguey was previously (1 or 2 years) castrated (elimination of inflorescence before its development), this allows the ''piñas'' to gain weight and mature rapidly. It should be mentioned, that there are no patents on the Mezcal process, in contrast with the numerous patents for ''Tequila'' (Narváez-Zapata & Sánchez-Teyer, 2009). The original process includes certain steps like cooking, fermentation and distillation. However, it depends on the mezcal producer, this gives a large variation and there is lack of quality standards in some cases. Each piña weighs between 350–400 kg. Cooking is performed in an underground oven, with a conical shaped oven formed with rocks and firewood and heat is maintained for 3 days. Reserve polysaccharides like inulin, are hydrolyzed to release fermentable monosaccharides, which later would be converted by alcohol fermenting microorganisms. In some cases, an acid hydrolysis pretreatment is performed, but this implies separation of electrolytes, which could be toxic in subsequent steps. The previously cooked sample is milled, and fermented for 1 to 2 weeks. When fermentation ends, fermented broth is placed in an ''alambique'', and then the broth is heated, vaporized and condensed slowly through a condenser to obtain a crystalline liquid. This process is repeated for a second time, the product obtained is called Mezcal (García-Soto et al., 2005).

There is also a very common beverage in some Mexican states, named ''Pulque''. This beverage has a viscous texture, milky and foamy with 4 to 9°G.L. It takes *Agave* plants 12 years to produce this beverage. Plant stored sugar emits a single flower stalk that could be until 20 feet in height, but in plants for Pulque production, the inflorescence must be cut, leaving a concave surface 12-18 inches in diameter. Now the maguey sap, commonly known as Aguamiel, is fermented naturally to produce Pulque, this process can be performed inside the plant or in a ''Tinacal''. Aguamiel is collected twice a day. Between gatherings many insects are trapped which are used as food. Many plants can produce this beverage for about 6 months, before death. Previous to fermentation, mature seed pulque is added in the containers to start the process (Buenrostro et al., 2010). Native microorganisms like yeast, lactic bacteria, and some ethanol and exopolysaccharides producers participate during fermentation. These microorganisms convert part of the sugars present in the ''Aguamiel'' (Baena Gonzales, 2005). Aguamiel fermentation is carried out from 12 to 48 hours at 25 °C; an important quality issue is contamination with inhibitory substances to the mesophilic microorganisms. In the last decade, aguamiel has been used for ethanol and polysaccharides production, like β-glucans and dextrans, and when they are present, viscosity increases, producing a non Newtonian fluid (Cervantes & Pedroza, 2007).

''Pulque'' production is actually in decline; however, some Mexican agencies have initiated projects and visualizing the beverage and plant as an alternative source to produce ethanol. *Agave* does not compete for soil and water with food commodities, because its requirements for soil moisture and nutrients are very low. In addition, its adaptation to dry and semi-dry environments is very high; these plants are good and effective alternative sources for diverse products other than ''Pulque''. In fact, these plants are found forming part of sustainable systems of production, because they are used as live barriers, fences and protective/formers of terraces in erosion control and yeast production for other fermentations (Paz et al., 2011). In 2002, worldwide were planted more than 500 000 ha of Agave. The main use for these plants is fibers, foliage production, and alcohol production such as ''tequila'', (Borland et al., 2009).

Henequen (*Agave fourcroydes Lem.*) is a plant, originated from *A. angustifolia*. Successful cultivation of this plant has been achieved in the Yucatan Peninsula, and was first domesticated by the pre-Hispanic Mayas. This plant has been used for obtaining fibers, preserving the morphologic variation because of its geographic distribution; however henequen fiber demand has declined drastically because of the availability of synthetic fibers, this has resulted in abandonment of plantations leading to a declined growth of an associated cottage industry. Henequen ''piña'' is used for production of an alcoholic beverage that has been patented-IMPI 219235 (Rendón-Salcido et al, 2009). Because of its large biomass production and less demand for nutrients, this plant is a promising source of biofuels with a high energetic value which may generate very little greenhouse gases emission (Paz et al., 2011).

A diverse species of insects that infest *Agave* plants, are edible. These insects are consumed in different stages of their biological cycle, in various regions. In the arid and semi-arid zones these insects are collectively called ''magueyeras'' which among other uses, serve as substrate for edible insects production. Escamoles (*Liometopum apiculatum M.*) are ant larvae. The escamolera ants are found in maguey roots.

They require little water and feed on leaves, seeds, flowers, fruits, insects and dead animals.

White worms (*Acentrocneme hesperiaris*) have a special flavor that is greatly valued in Mexico, North America and Europe, this insect can be identified in the plant because they produce a coloration in the leaves like wilting. This insect can be extracted with a needle and is useful as food and feed.

Red worms (*Hypopta agavis B.*) cause critical damages to *Agave* plants. The larvae stage is found inside the *Agave* stems, but these worms can cause plant death. Worms are recollected by cutting the plant base (García-Herrera et al., 2010).

Agave as a source of prebiotics and bioactive compounds

Many species of *Agave* are distributed in Mexico and some other countries. This plant has been used as the cure of many bacterial diseases and oxidative stress (Ahumada-Santos et al., 2013). Additionally, some other activities such as immunomodulatory (Chen et al., 2009), antifungal (Verastegui et al., 2008), anti-inflammatory (Da Silva et al., 2002), antiparasitic (Orestes et al., 2008) and antihypertensive activities (Duncan et al., 1999) have been demonstrated.

Within the secondary metabolites, we find as part of the *Agave* plant, many compounds such as triterpens, steroids, tannins, volatile coumarins, flavonoids, alkaloids, free anthracenic derivatives, cardiotonics and reducing sugars are also present in *Agave* plants. *A. ornithobroma* is one of the species with more diversity of phytochemicals (Ahumada-Santos et al., 2013). As well as the compounds mentioned above, tannins, flavonoids and steroidal saponins have bioactive activity (Hostettman & Marston, 1995). Tannins and flavonoids have the attribution of possessing antioxidant, antitumoral and antimicrobial activity. Antibacterial activity has been demonstrated for some organic extracts from species of *Agave* against *Streptococcus* group A-4, *Salmonella enterica typhi, Shigella dysenteriae, Escherichia coli* 25922*, Pseudomonas aeruginosa* 27853*, Enterococcus faecalis* 29212*, Staphylococcus aureus* 3*, Escherichia coli* A011 and *Staphylococcus aureus* 29213*;* with remarkable special action from *A. tequilana.* The release of antioxidant activity is evident for species such as *A. rzedowskiana* among others (Ahumada-Santos et al., 2013).

Water soluble carbohydrates (WSC) are reported to be present in about 28.3 g/100 g (fresh weight), and 86.7 g/100 g (dry weight). It is important to note that the nature of *A. tequilana Weber* fructans are more complex than the one for chicoria, which have been previously studied by many researchers (Waleckx et al., 2008).

Hydroxymethyl furfural (HMF) is found in about 50 mg/kg, but when the *Agave* is utilized in tequila production, as for example the *A. tequilana weber*, the concentration could increase until 3000 mg/Kg at the end of cooking. This effect shows us the generation of Maillard compounds, as well as dehydration of carbohydrates (Waleckx et al., 2008).

The cooking honey extracted from the cooking process of this beverage (Tequila) could contain more than 4000 ppm of HMF (Lopez et al., 2003), which contribute to the importance in the flavor and aroma of the final product (Lamas-Robles et al., 2004).

This compound also has inhibitory properties against yeasts such as *Saccharomyces cerevisiae*, which play an important role in the fermentation of tequila (Waleckx et al., 2008).

Saponins are compounds that can be found in different plant species, the main characteristic is its skeleton descends from the thirty carbon precursor oxidosqualene, to this compound are engaged residues type glycosyl (Figure 2; Vincken et al., 2007). Saponins are glycosylated terpenoids, esteroids or alchaloids that have applications such as: antifungal, antibacterial, anti-cancer, adjuvants and anti-hemolytic activity, amongst other properties (Güclü-Üstündag & Mazza, 2007).

The agavaceae family is recognized as an important source of sapogenins with steroidal nature and primarily for saponins, which is the principle in steroidal hormones in laboratory synthesis (Eskander et al., 2010). Based on *Agave*

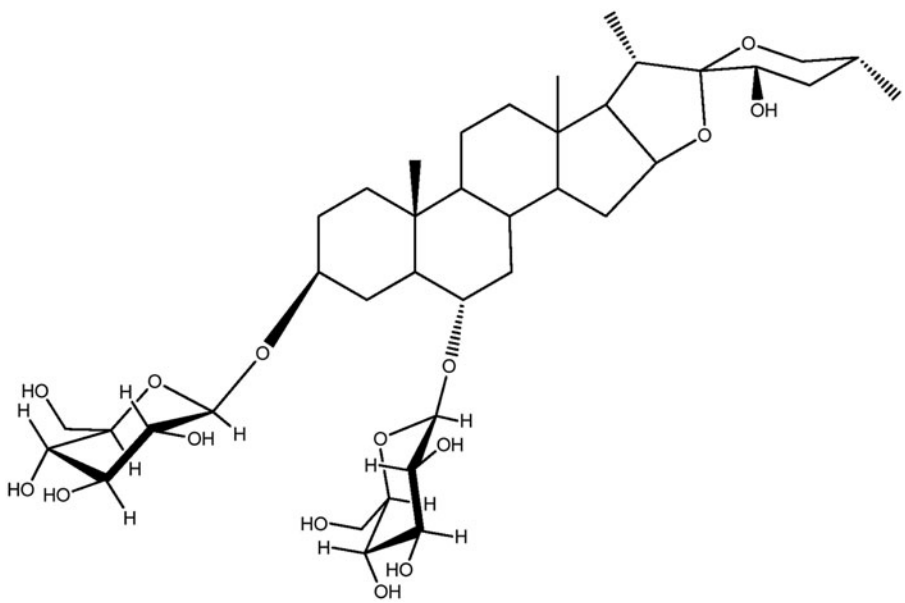

Figure 2. Saponin structure (Eskander et al., 2010).

applications during drug synthesis, many species have been studied, with the objective of identifying steroidal saponins (Eskander et al., 2010).

Some patents have been granted regarding the extraction and use of saponins from *Agave* fibers for control of microorganisms infecting the digestive system of some animals (McNeff & McNeff, 2008). Eskander et al. (2010) identified four different types of saponins in *Agave macroacantha* leaves some of these saponins were previously isolated from other *Agave* types. Yokosuka & Mimaki (2009) reported the isolation of 15 steroidal saponins, five of them were spirostanol and three more furostanol. Most processes for saponins, include extractions with methanol where chromatography is employed to elucidate the presence of saponins. In addition, the structure is determined by spectroscopic analysis. Saponins isolated from *Agave* were tested for their cytotoxic activity against HL-60 cells, and four of these compounds showed a moderate cytotoxicity in comparison to the etoposide control (Yokosuka & Mimaki, 2009). Saponins have been isolated by diverse scientists from different *Agave* species like *sisalana* and *uthahenesis*.

Natural fibers have been applied during reinforcement of materials (Bessadok et al., 2009). Sisal *(Agave sisalana)* and henequen *(A. fourcroydes)* fibers are the more widely obtained from *Agave*, mainly as structural construction material of light weight (Narváez-Zapata & Sánchez-Teyer, 2009). The increased awareness on recycling of fibers, has conducted to propose plants like *Agave* to this goal (Elenga et al., 2009). *Agave* fibers have certain advantages to the synthetic fibres, for example, lower density and cost, besides they are biodegradable and can be recycled (Kestur et al., 2013).

All *Agave* plant parts have commercial applications. For example, discarded leaves from the stems are used for extraction of a resin, which improve fiber quality. It is used for furniture. In addition, it is used for some industrial articles such as construction blocks and paper (Narváez-Zapata & Sánchez-Teyer, 2009).

Diverse species of higher plants, monocotyledons, dicotyledons and green algae present structures belonging to oligo or polysaccharides of fructose, named fructans and other ones known as fructooligosacharides (FOS). Fructans act like reserve carbohydrates in the CAM metabolism and as a protector of osmosis in dry conditions. The place where they can be found and where they are synthesized is in the stems (Arrizon et al., 2010). They vary in molecular structure and weight. In mature plants these material may be present in 13 to 17% (w/w) of fresh weight (Avila-Fernández et al., 2011).

In nature, we can find different types of fructans, they are classified by linkage of fructose units and by glucose location in the arrangement. The five groups are: inulins, neoseries inulins, levans, neoseries levans, and the mixed fructans (Figure 3; Waleckx et al., 2008). Inulins present one linear β-(2-1)- fructosyl chain; Neoseries inulins are composed of two linear β-(2-1)-linked fructosyl chains, bounded to a fructosyl residue of sucrose, and the other one bounded to a glucosyl residue; Levans have β-(2-6)-linked fructosyl chain bounded to a fructosyl residue of sucrose; Neoseries levans have two linear β-(2-6)- linked fructosyl chains, one bounded to fructosyl residue of the sucrose, and the other one to the glucosyl residue. Mixed fructans have two different linkages (β(2-1)) and (β-(2-6)) between fructose, when two types of linkages are in the same fructose, they are ramified and contain probably the glucose intern, which is typical of this group (Benkeblia, 2013).

These fructans are distributed in a colloidal form in diverse species of *Agave* (Arrizon et al., 2010). *Agave* genus presents a high content of oligomers which may form fructans, which are degraded by enzymatic or thermal action. The fructans belonging to the *A. tequilana* Weber var. azul are in the levan group, and they have received the name of agavins (Figure 4; Muñoz-Gutierrez et al., 2009). These kinds of fructans have been used for Tequila production, dietary products and some systems of drug delivery (Arrizon et al., 2010).

The *Agave* fructans have a unique feature. The molecules of fructose have β(2-1) linkages and also present from 3 to 29 degree polymerization (DP) with β(2-6) linkages, and, as a consequence, are classified as mixed fructans and neoseries fructans (Lopez & Mancilla-Margalli, 2007).

Agave fructans can be converted to free sugars, primarily fructose, after fermentation. One of the potential uses of these fructans is tequila production, which exists only in one report of the influence of the *Agave* age in the fermentation process (Pinal et al., 2009). However, Arrizon et al. (2010) says that there exists a significant increase in the sugar content and the morphology in different plants of *A. tequilana* in diverse ages. The fructan level reaches 3.07 and 4.46-fold for plants of *A. tequilana* after 4 and 6 years. The same behavior could be seen for the sugar content 2 to 4 years after the ''hijuelos'' plantation.

Along with inulin and fructooligosaccharides (FOS) are the two main ingredients in functional foods with prebiotic properties. FOS can be obtained by enzymatic activity via fungal fructosyltransferases or by the action of an endo-inulinase from chicory inulin. These enzymes are known also as inulo-oligosaccharidases or oligofructases (Crittenden & Playne, 1996). By their complexity, non-inulin FOS derived from *Agave* fructans exhibit best properties with those of commercial derivatives (Avila-Fernandez et al., 2011).

Inulin is extracted from roots of diverse plant species like chicory, sugar beet and yucon because they have high sugar content. The industrial process for inulin extraction is well known and recently has been used for inulin extraction from *Agave* stems, but it has been necessary to adapt this procedure because characteristics of raw material (Agave) in order to obtain the best results and reduce costs (Salazar, 2013).

The production of fructans is influenced by various factors like growth region, nutrients of the planted place, climatic changes, seasonal time, and water level. Besides, it is also influenced by the species of Agave; A. *tequilana*, A. *potatorum*, *Agave cantala*, A. *fourcroydes* and A. *angustifolia* have differences in the glucose chains, in the same way to the linkages (Arrizon et al., 2010).

Diverse patents have been obtained for fructans extractions from *Agave* raw materials. (Roman et al, 2008). Other patents have been presented on sweetener fractions containing inulin from plants sap, or for nutritional improvement of foods using a special blend of fructans, which are easy and difficult to be fermented. In fact, there is also a patent to obtain fructans from large chains, and also to produce inulin by an interaction with sucrose (Meuser et al., 2009).

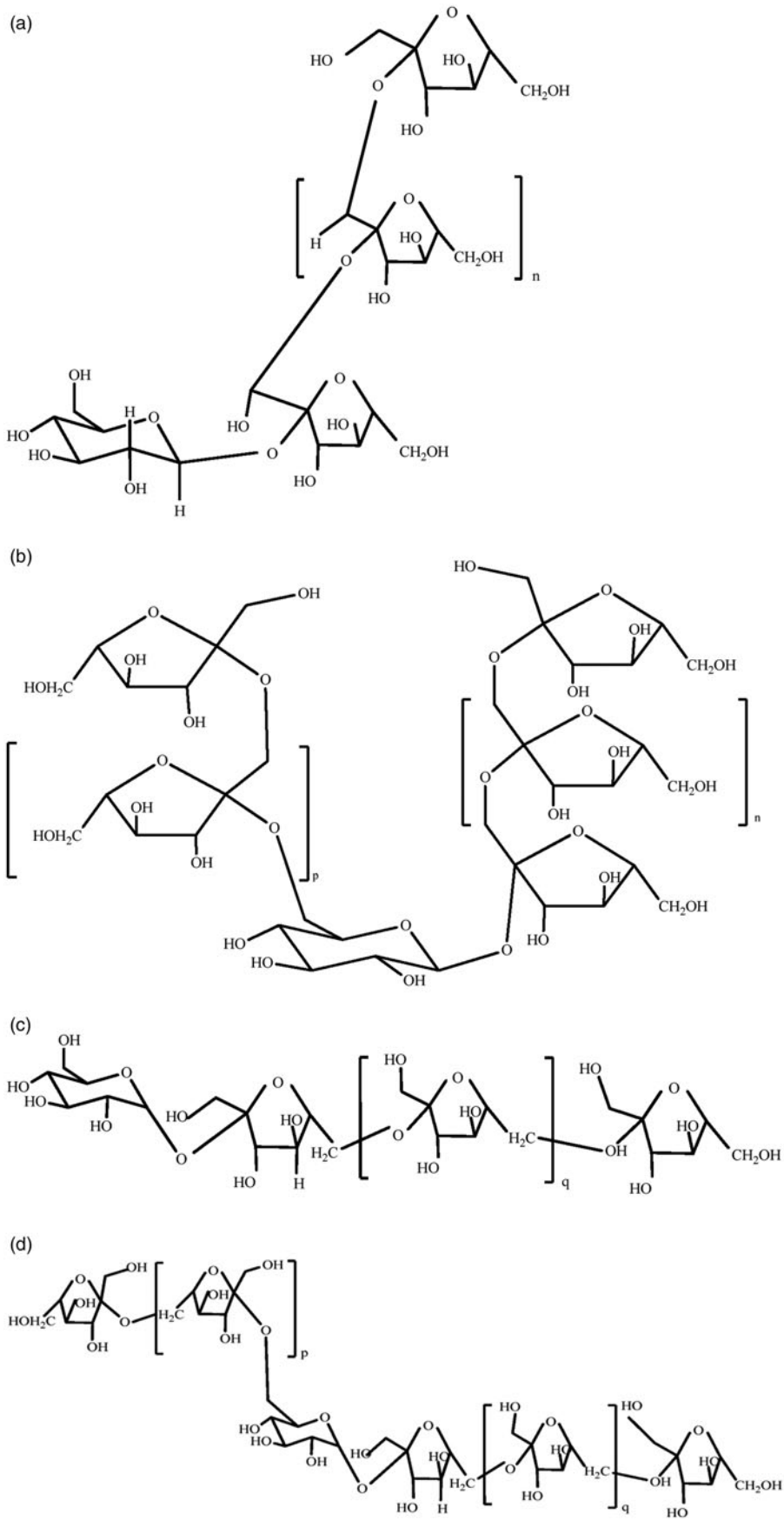

Figure 3. Fructan groups: (a) Inulins; (b) Neoseries inulins; (c) Levans; (d) Neoseries levans.

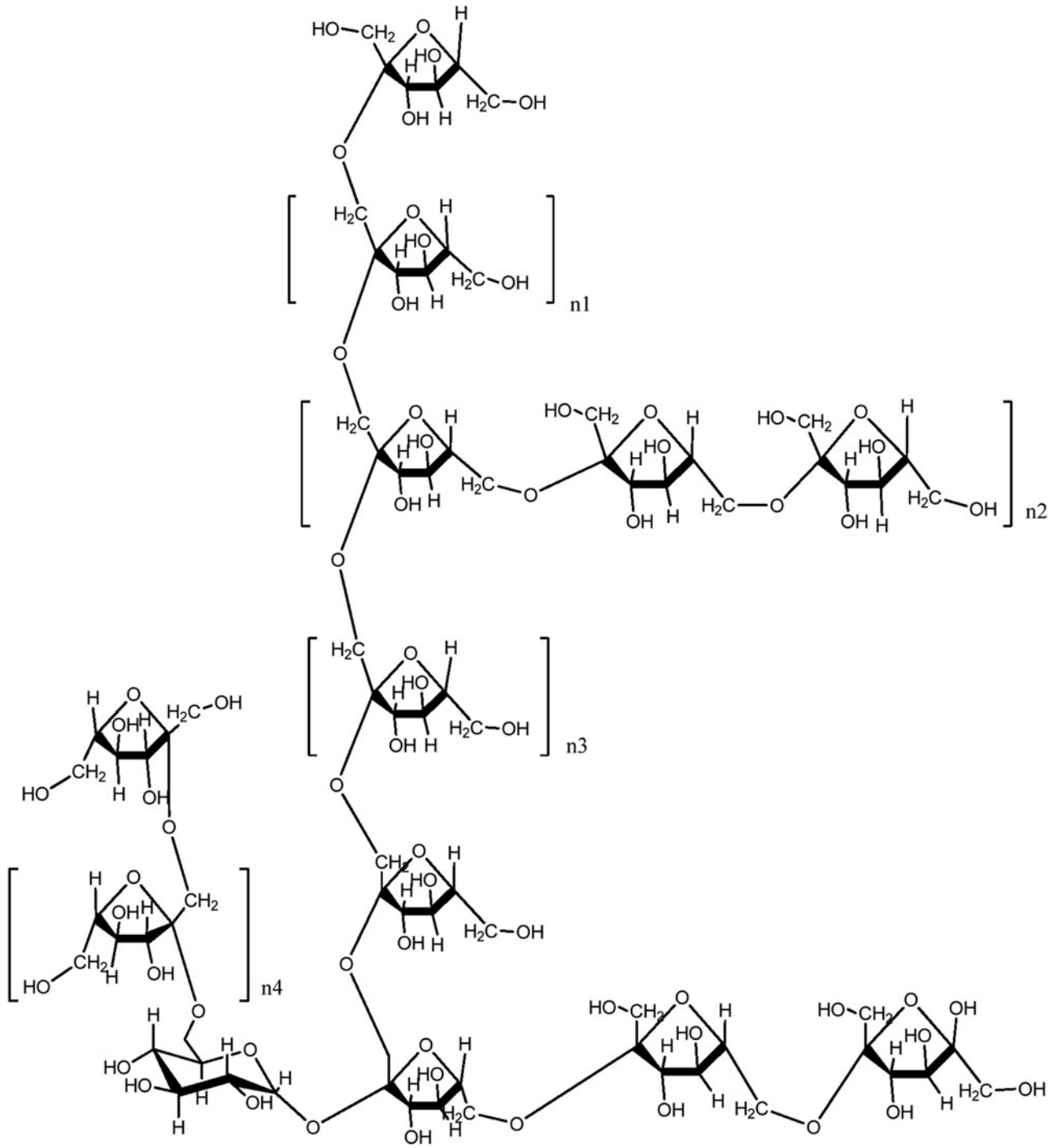

Figure 4. Gutiérrez Structure of the polysaccharide Agavin found in *Agave* species (Lopez & Mancilla-Margalli, 2007; Muñoz-Gutiérrez et al., 2009).

Applied and potential biotechnological uses of *Agave*

Biotechnological uses of *Agave* includes the use of biological or biochemical aspects native to the plant, or bioprocesses that take place on the plant or parts of it.

Agave as energy crop

An important characteristic of *Agave* plants is the high accumulation of soluble non-structural carbohydrates, which could make them a very important raw material for ethanol production (Sukumaran et al., 2009). For their morphological and biochemical characteristics, *Agave* does not compete with cereals and vegetables used as food, so it could be a sustainable raw material for biofuels production (Bansal et al., 2012). Crassulacean Acid Metabolism (CAM), present in *Agave* plants has not been studied profoundly as a potential raw material for biofuels (Borland et al., 2009). Actually, there is not much knowledge on these plants in large scale production for this kind of energy (Conde-Mejía et al., 2012). This type of metabolism operates in such a way that during the night it stores high amounts of malate which is formed from CO_2. In daytime, malate is additionally used in the photosynthesis.

This metabolism occurs among plants that grow in arid environments where the rain is scarce (Black & Osmond, 2005). The advantage for CAM plants, in this case *Agave*, is that water is more efficiently used because the stomata in leaves are closed at night, which decreases water loss. Regarding biofuels, this type of metabolism allows *Agave* to accumulate mono and polysaccharides that functions as energy reserve (Davis et al., 2011). These carbohydrates can be processed to become fermentable sugars by ethanologenic microorganisms to produce ethanol (Caspeta et al., 2014). *Agave* plants are able to accumulate high amounts of sugars, not hard to degrade compared to cellulose, thus making it an alternative for energy crop.

Another alternative in using *Agave* as biofuel source is the use of the cellulosic residues from alcoholic beverages production or the leaves. *Agave* plants have an important lignocellulosic content, primarily in the leaves, which is a potential source of diverse materials, and different procedures have been developed for obtaining these materials (Kuo & Lee, 2009) among them, products used in pharmacy, chemistry and biomaterials have been reported (Ruiz et al., 2011). Pre-treatments are very important for cellulose de-polymerization process (Gupta et al., 2011) where many procedures have been employed (Dadi et al., 2006). Within the procedures used for obtaining these products (Nlewem & Trash, 2010) are: ammonia fiber explosion (Ko et al., 2009), radio frequency (RF) dielectric heating, microwave radiation (Hu et al., 2008), electric pulse field (Kumar et al., 2009), hot compressed water (Sheng et al., 2010), calcium hydroxide (Chang et al., 1997), sodium hydroxide (Barcelos et al., 2012) and acid pre-treatments (Sluiter et al., 2013). Although, some of these procedures do not apply in every lignocellulosic source, different types of pre-treatments are needed for a given plant, in order to increase yield of the lignocellulosic material (Narra et al., 2012). Pre-treatments promote further enzymatic saccharification of cellulose by using cellulases. Pretreatment makes cellulose more susceptible to be hydrolyzed (Medina-Morales et al., 2011b). The most used approach is by biotechnological means, which is the enzyme addition to pretreated cellulosic material for further fermentation. The mechanism for cellulose degradation consists in the presence of three enzymatic activities: endo-glucanase, exo-glucanase and β- glucosidase. The first one hydrolyze the inner chain of cellulose polymer exposing reductive and no reductive ends of the linear polymer of glucose, while the second ones attack these terminations to liberate cellobiose and cellooligosaccharides (Bansal et al., 2011). The β- glucosidases will degrade cellobiose units to liberate finally the desired product, glucose (Yah et al., 2010). Glucose can then be fermented by ethanologenic microorganisms to produce ethanol. There are reported studies where fibers from *Agave atrovirens* leaves were enzymatically hydrolyzed where glucose obtained were up to approximately 0.28 g of glucose per gram of *Agave* leaf dry fiber (Medina et al., 2011a). In a more recent report, a study where residues of *Agave* were hydrolyzed by dilute acid and enzymatic hydrolysis and were able to accumulate 0.51 g/g of fermentable sugars for potential ethanol conversion (Caspeta et al., 2014).

Cellulase production

As previously stated, cellulases are fundamental to achieve biofuel production from cellulose. Although difficult to degrade, there are microorganisms that can convert lignocellulosic material, by enzymatic action of cellulose (Liao et al., 2011). In nature, there exist many microorganisms capable of degrade lignocellulosic material, and as consequence involved in cellulase production, the list contains aerobic and anaerobic bacteria (Chung-Yi et al., 2009), soft rot fungi, white rot (Lo et al., 2010) and anaerobic fungi (Dashtban et al., 2009). These enzymes are produced by microorganisms like filamentous fungi, actinomycetes and in aerobic bacteria. The more used are filamentous fungi like *Trichoderma, Penicillium, Fusarium, Humicola, Phanerochaete*, etc, have proved their capacity to produce this type of enzymes (de Siqueira et al., 2010).

Trichoderma is one of the microorganisms with more relevance, because it produces high yields of cellulases (Liu & Yu, 2012). This fungus has the capacity to degrade lignocellulosic material, it has the ability of promoting mycelial growth that permit the fungus move nitrogen and iron to a lignocellulosic substrate with very low nutrients that in fact form part of its carbon source (Taqhizadeh & Zabihollah, 2008). Cellulases, produced by *Trichoderma,* are mainly endo and exoglucanases, and the amount of β-glucosidase is low, or has little enzymatic activity (Zhou et al., 2008). Production of β-glucosidase from other microorganisms (Kumar et al., 2008) such as *Aspergillus* spp. (Gao et al., 2008) is recommended as an alternative to generate an effective cellulolytic enzymatic complex.

The use of solid state culture for production of cellulases is becoming a relevant fermentation system (Zhu et al., 2010). This process is performed culturing microorganisms on a solid substrate but without free water that could be dispersed in the medium but with enough moisture for fungal biochemical reactions (Orzua et al., 2009). SSC has been used for biopesticides, biosurfactants, aromatic products and biofuels production (Singhania et al., 2009). In this process, *Agave* fibers from leaf or stem bagasse could be used as carbon source for fungi such as *Trichoderma* to produce cellulases under this kind of culture system. Medina et al. (2011a), reports cellulose content of approximately 65% in leaf fibers of *Agave salmiana* and *Agave atrovirens*. Hernández-Salas et al. (2009), mentioned that bagasse from *Agave tequilana* has 43% cellulose. These residues can be used for biotechnological purposes such as fungal enzyme production. According to our search on the subject, there is no published work on fungal growth on *Agave* fibers in solid state culture, but our research group has unpublished results on this matter.

Inulinase production

Agave is an important source of fructans such as inulin and biotechnological aspects of it has been studied (Ávila-Fernández et al., 2011). In the food industry, inulinases are fructanases used to produce fructose syrups from inulin (Astolfi et al., 2011). The biotechnological aspects of *Agave* inulin and *Agave* itself include the enzyme production from the polysaccharide and microbial isolation from the *Agave*

plant with enhanced capacity to produce inulinase (Cruz-Guerrero et al., 2006). This enzyme catalyzes the depolymerization of inulin to fructose units and a smaller amount of glucose that is present in the polysaccharide. There are microorganisms that are reported as inulinase producers such as filamentous fungi isolated from *Agave* fields (Huitron et al., 2008), or yeast from *Agave* sap (Cruz-Guerrero et al., 2006). Inulinolytic enzymes from *Aspergillus niger* have been used to enhance hydrolysis from *Agave tequilana* juice, and increase ethanol production in subsequent fermentation processes. In raw *A. tequilana* juice the use of inulinases from *Aspergillus niger* CH-A-2010 helped increase ethanol yield to 97.5% compared to a lower yield of 83% obtained after acid hydrolysis on the juice (Huitrón et al., 2013). This strain was previously reported as being isolated from *Agave* fields and inulinase producing after submerged culture on *Agave* residues (Huitrón et al., 2008). Strains of *Kluyveromyces marxianus* and *Kluyveromyces lactis* were isolated from *Agave* sap and from fermented sap, or pulque and *K. marxianus* produced high levels of inulinase. These were isolated from a medium with sucrose and fructans, they were very effective compared to *K. lactis*, probably due to the fact that this strain was recovered from pulque and it contains ethanol and lower levels of inductors (Cruz-Guerrero et al., 2006). Flores et al. (2013) reported strains of *K. marxianus* in a process of simultaneous saccharification and fermentation, were *K. marxianus* DV4 was capable to produce 200 U/L of inulinase activity and 1200 U/L on *Agave tequilana* fructans. These are a few examples of microorganisms associated with *Agave* that produce this kind of enzyme, but there are many more from bacteria, fungi and yeast that are able to do so (Chi et al., 2009).

Omics studies on *Agave* genus

Different molecular biology tools have been applied to understand *Agave* physiology, genetic diversity, feasibility for genetic transformation and study of its pathogens. There are recent reports about use of omics science on *Agave* physiology. Martínez-Hernández et al. (2010) developed cDNA libraries of expressed genes which were used for physiological studies on *Agave tequilana.* These authors reported sequences and analysis of expression of four genes with importance on *Agave* physiology: *rbcS* which codify for the small RuBisCO subunit, the gene codifying for malic enzyme depending of NADP+; *1-ST* fructosiltransferase gene; and a gene codifying for abiotic stress LEA type. Delgado-Sandoval et al. (2012) using transcriptomic analysis identified seven candidates MADS genes which may be involved in flower induction regulation in *Agave tequilana.* On the other hand, there are reports on the effect of tissue culture on epigenetic mechanisms, De-la-Peña et al. (2012) reported that DNA methylation and histone modifications were affected under micropropagation of *Agave fourcroydes* and *A. angustifolia*. DNA methylation was two-fold in *A. fourcroydes* in comparison to *A. angustifolia* independent of the *in vitro* system used. In addition, the same authors mentioned that *Agave* cloned plants with higher DNA methylation during micropropagation were better adapted to *ex vitro* conditions and *A. fourcroydes* and *A. angustifolia* displayed differential expression of the KNOX1 gene, which is epigenetically regulated. In addition, there are studies on transcriptome mining of *Agave tequilana* and its relation to bioenergy applications (Simpson et al., 2011).

Genetic diversity in wild and commercial *Agave* populations has been accessed using different molecular markers. Use of the *Agave* species (tequila, mescal, bacanora and fiber) and propagation types have been also considered. *A. tequilana* Weber var. Azul is the only variety permitted for tequila production. Gil-Vega et al. (2001) accessed genetic variation in commercial populations of *A. tequilana* var. Azul using randomly amplified polymorphic DNA (RAPD) markers. In addition, ''Chato'' and ''Siguin'' varieties were also studied. *A. tequilana* showed one of the lowest levels of polymorphism detected in crops; this is attributed to a single conserved genotype because it is asexual propagation. Siguin variety was more closely related to *A. tequilana* var. Azul than Chato var. Later, using more informative markers, the same group of investigation found that AFLP (amplification fragment length polymorphism) analysis of nine *A. tequilana* varieties reported significant level of diversity among samples obtained from the same plantation and from different plantations (Gil-Vega et al., 2006). The effect of the type of propagation on polymorphism and methylation patterns in *Agave tequilana* Weber var. ''Azul'' was accessed by Díaz-Martínez et al. (2012). These authors used AFLP analysis and found low levels of variation between mother plants and offsets. However, a higher level of polymorphism was observed between mother plant and bulbils. Plant families obtained from commercial plantations showed lower levels of variation in comparison to that cropped as ornamentals. In addition, these authors studied epigenetic variation by analyzing changes in methylation patterns where offsets and bulbils had an overall decrease in methylation, while *in vitro* cultured plants presented methylation patterns specific to each generation which may suggest a correlation between methylation, expression of transposable element associated genes and somaclonal variation. Somaclonal variation has been also reported in *Agave tequilana* using other type of molecular markers. Torres-Moran et al. (2010) used inverse ISTR (sequence-tagged repeat) to determine genetic fidelity of plants obtained from field and propagated from rhizomes and of those propagated by *in vitro* somatic embryogenesis and proliferation of axillary buds, and found genomic changes not only in plants cultured *in vitro* but also evident in those propagated by rhizomes. Genetic differences among *Agave* species may be accesses more efficiently using new molecular marker methodologies and identify the tequila agaves as a distinct phylogenetic group. Bousios et al. (2007) isolated different Ty1-copia retrotransposon terminal sequences from *A. tequilana* and developed sequence-specific amplification polymorphism (SSAP), these molecular markers showed high levels of retrotransposon polymorphism throughout *Agave* varieties and species.

On the other hand, mezcal can be produced from different *Agave* species and at different Mexican states. By this reason, genetic diversity has been accessed using different *Agave* species which are used for mezcal production. Vargas-Arreola (2009) studied the intra and interpopulation genetic

variability using two *Agave durangensis* populations and one *Agave asperrima*, genetic variability was analyzed using ISTR. This kind of marker was useful to differentiate both species and to detect differences between the two A. *durangensis* populations. A high genetic variability was observed for A. *durangensis* which suggests that this taxon is in an active process of specialization or it includes various taxonomic entries. Genetic diversity in wild *Agave* populations may be generated by asexual propagation. Infante et al. (2006) compared the banding patterns of AFLPs and ISTRs between the mother plant and rhizome-derived daughter plants, and they observed that is possible to generate genetic variability during asexual reproduction. Recently, Aguirre-Dugua & Eguiarte (2013) assessed the degree of genetic erosion of five exploited populations of each *Agave cupreata and Agave potatorum* using ISSRs (Inter Simple Sequence Repeats) finding high levels of variation and moderate to high differentiation which suggests that these species are not genetically eroded. At least 200 *Agave* species have been reported in Mexico, some of them may be at risk because of over-exploitation. Eguiarte et al. (2013) described studies on genetic variability of 22 *Agave* species using different genetic markers. At the genus level, a high level of genetic variation, and low genetic differentiation was found. These authors remark that at least three *Agave* species are at risk, and should be subject to special conservation genetic efforts, especially *A. victoriae-reginae, A. angustifolia,* and *A. tequilana*.

Sanchez-Teyer et al. (2009) measured the genetic variability within and between natural A. *angustifolia* populations using AFLP's, finding some degree of genetic variation between mother plants and their vegetative produced rhizomes. Although, total expected heterozygosity was high and gene flow was intermediate, other genetic parameters like Nm, FST and GD indicated that it is urgent to take measures in order to preserve the genetic diversity of A. *angustifolia*.

Henequen (*Agave fourcroydes* Lem), is a plant propagated asexually, with economic importance because of its fiber. Analysis using AFLP, showed differences in AFLP patterns in a natural population. Analysis of five different plantations exhibited differences at the population level, which suggest that genetic variability is generated and transmitted to descendants through asexual reproduction (Infante et al., 2003). Later the same research group found that ISTR analysis was more informative than AFLP. In addition, ISTR had a better capacity for quantifying genetic diversity in *A. fourcroydes* Lem through different genetic parameters like: average number of alleles per locus, expected heterozygosis, effective number of alleles per locus, and total number of effective alleles (Demey et al., 2004). *Agave* cytogenetics has been also benefited with the use of omics sciences. Robert et al. (2008) studied different *Agave* species with different ploidy levels and found a proportional increases in chromosome number, genome size, and genetic markers with increasing ploidy. Thus, they reported a complete additivity in genome structure with increasing ploidy. In addition, they used the telomeric sequences (TTAGGG)*n* as a genetic marker, locating this marker in the *Agave* telomere sequences.

Genetic transformation

There are few reports about *Agave* genetic transformation, mainly using *Agrobacterium*-based transformation and bioballistics methods. Flores-Benitez et al. (2007) mentioned the genetic transformation of *A. salmiana* using two methodologies *Agrobacterium tumefaciens* and particle bombardment, in both cases, it was necessary to optimize the shoot regeneration and rooting condition using leaves and embryogenic calli. In addition, the *uidA* (β-glucuronidase) and the *nptII* genes were used as reporter and selectable markers respectively. After the transformation and regeneration condition were optimized, it was possible to generate transgenic plants being the *Agrobacterium* method, the most effective for genetic transformation. Other reports mentioned a different perspective of *Agave* genetic transformation, where *A. salmiana* plants were transformed using *Agrobacterium rhizogenes,* so in this case transformed roots were obtained, which later were efficient colonized by *Glomus intraradices.* The most efficient transformation was obtained using stem cells (Rodríguez- Hernández et al., 2007).

Study of *Agave* pathogens

Genomics have been used to study *Agave* pathogens. Avila-Miranda (2012) studied genetic variation of twenty-five *Fusarium oxysporum* stains which were isolated from *Agave* plants with and without foliar wilt. *Fusarium* genetic diversity was accessed by sequencing of ITS (internal transcribed spaces), DNA Box-PCR molecular markers and vegetative compatibility groups. ITS sequencing showed that 15 out of 25 strains had a point mutation, which makes them different to that sequences reported for pathogenic *Fusarium* strains. Dissimilarity analysis based on BOX-PCR patterns showed that twelve strains grouped in a cluster and nine strains were grouped in other different cluster. Sixteen strains were grouped in two different vegetative compatibility groups and nine did not show vegetative compatibility and only two *Fusarium* strains were pathogenic to *Agave* plants.

Conclusions

There are multiple biotechnological applications using *Agave* plants, but these depend on the level of knowledge regarding this plant. In Mexico, there exist various processes that need improvement and only using scientific procedures can these improvements be achieved. Still in areas where these plants are scarce or are not useful because of bad management, these plants are a source of jobs for many, not only in the *Agave* production places, but also other places in many countries. For this reason, it is necessary to establish techniques that improve the benefits obtained from this plant for different processes using biotechnology.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article. We thank the Mexican National Council for Science and Technology (CONACYT) for the support to this investigation, and to the reviewers for helpful comments.

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