

Potential of Plants from the Genus *Agave* as Bioenergy Crops

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Abstract *Agave* is a succulent genus within the monocot family Agavaceae. The plants have a large rosette of thick fleshy leaves, each ending generally in a sharp point, and are native to arid and semi-arid regions from the southern USA to northern South America. The most important commercial species is *Agave tequilana* grown for production of tequila. Several cultivated species of *Agave* such as *Agave sisilana* and *Agave salmiana* can perform well in areas where rainfall is insufficient for the cultivation of many C₃ and C₄ crops. The key feature of the crassulacean acid metabolism photosynthetic pathway used by agaves is the stomata opening and CO₂ uptake during the night, thus allowing less water to be lost by transpiration. Alcoholic beverages, sweeteners, fibers, and some specialty chemicals are currently the main products coming from agave plants. The recovered information related to productivity, biofuel processability, by-products, etc. suggests that some *Agave* species have a real potential to compete economically with other bioenergy crops. But more than compete, it could complement the list of bioenergy crops due to its capacity to grow with very little rainfall and/or inputs and still reach good amount of biomass, so unused semi-arid land could be productive. Although *Agave* has great potential to be

developed as a bioenergy crop, more laboratory and field research are needed.

Keywords *Agave* · Bioenergy crop · Biofuel · Biofuel feedstock · Crassulacean acid metabolism

Abbreviations

°C	Degrees Celsius
CAM	Crassulacean acid metabolism
EPI	Environmental productivity index
EST	Expressed sequence tag
ha	Hectare
Mg	Metric tons
mm	Millimeters
PEP	Phosphoenolpyruvate
PEPCase	Phosphoenolpyruvate carboxylase
Rubisco	Ribulose biphosphate carboxylase oxygenase
TRS	Total reducing sugars
WSC	Water-soluble carbohydrates
WUE	Water-use efficiency

Introduction

Biofuels, Sustainability, and Water Usage

Until recently, productivity, processability, and agronomic inputs have been viewed as the most important parameters to consider during the selection and development of new biomass feedstocks for biofuel production. However, issues such as water usage, sustainability, greenhouse-gas emissions, biodiversity, competition with food supply, and general impacts on society are now recognized as equally important for consideration [49]. As example, the three Bioenergy Research Centers funded by the US Department

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of Energy—The Bioenergy Science Center, the Joint Bioenergy Institute, and the Great Lakes Bioenergy Research Center—have considered some of these issues like sustainability and greenhouse-gas emissions, although their central mission is the development of technology to generate economically competitive liquid biofuels from plant biomass [3, 10, 13, 43, 47, 52].

The optimal use of water to grow a selected feedstock is of critical importance because water scarcity, more than any other factor, determines whether land is suitable for growing food crops. Thus, growing plants with high water-use efficiency on land that is too dry to grow food crops is a potentially powerful strategy for producing biomass feedstocks in large amounts while minimizing competition with the food supply. Additionally, making productive use of semi-arid land can have positive effects on poor rural areas.

The water-use efficiency (WUE) value (grams CO₂ fixed/kilogram water transpired) varies markedly among plants with different types of photosynthetic metabolism. C₃ plants typically have WUE values of 1–3; C₄ plants, between 2 and 5; whereas crassulacean acid metabolism (CAM) plants have values between 10 and 40. Therefore, CAM plants can be cultivated in arid or semi-arid land normally unsuitable for the cultivation of most C₃ and C₄ crops [35]. It is exceedingly unlikely that a C₃ or C₄ plant could be developed, with or without genetic modification, with water-use efficiency approaching that of CAM plants. Moreover, CAM plants are native to essentially every state in the USA except Alaska, although they are prominent parts of ecosystems only in the Southwest.

In spite of this potential, CAM plants have received much less systematic study or development as energy crops relative to inherently less water-efficient plants such as corn (maize), sugarcane, switchgrass, *Miscanthus*, poplar, sugar beets, *Jatropha*, soy, and canola.

Among CAM plants, species of *Agave* have started to attract increasing attention as energy crops [48]. *Agave* is a succulent genus within the monocot family Agavaceae. The plants have a large rosette of thick fleshy leaves, each ending generally in a sharp point, and are indigenous to both arid and semi-arid regions from the southern USA to northern South America.

The genus *Agave* traditionally includes about 166 species; however, the genus is paraphyletic to the genera *Manfreda*, *Polianthes*, and *Prochnyanthes*. The entire clade of 208 species has been termed *Agave sensu lato* [18]. The most important commercial species are *Agave tequilana* grown for production of tequila; *Agave angustifolia*, *Agave salmiana*, *Agave americana*, and several other species that are grown commercially in Mexico for the production of mescal (a distilled beverage similar to tequila); *Agave sisalana* that has been cultivated in the Caribbean, Brazil,

India, many Pacific islands, Australia, and parts of Africa for fiber production; and *Agave fourcroydes* and *Agave lechuguilla* which are the species of choice for fiber production in Mexico. The saponins tigogenin and hecogenin are extracted from the waste residues of *A. sisalana* and *A. americana* fibers and are important raw materials in the synthesis of steroid hormone [8, 24, 35].

This article reviews the somewhat diffuse information on the genus *Agave* that appears important for the consideration of these species as biomass feedstocks. It can be concluded that some *Agave* species have a real potential to be bioenergy crops, although this potential requires further validation through both laboratory and field research.

Water-Use Efficiency and Crassulacean Acid Metabolism

About 7% of all plant species possess CAM [58], many of which represent the predominant plant biomass in arid, semi-arid, or marginal regions of the world. Normally, a CAM plant has approximately 33% of the water requirement of a C₄ plant and approximately 16% of the water requirement of a C₃ plant to produce the same amount of biomass.

Thanks to CAM, several cultivated species of *Agave* can reach good productivities in areas where rainfall is insufficient for the cultivation of many C₃ and C₄ crops. For example, the productivity of *A. salmiana* under only 32 cm of annual rainfall was 10 Mg ha⁻¹ year⁻¹ [38]. CAM permits the net uptake of CO₂ at night end, thereby dramatically improving water-use efficiency for carbon assimilation in plants growing in arid habitats [2].

Stomata (the microscopic pores in leaves) open to allow CO₂ to enter to carry out photosynthesis. This opening leads to the loss of water vapor (transpiration). C₃ and C₄ plants open their stomata during the day when the temperatures are higher, the sun is brighter, and the loss of water by transpiration is high. The key feature of the CAM photosynthetic pathway used by agaves is the opening of stomata and CO₂ uptake during the night, thus allowing less water to be lost by transpiration [35]. During the daytime, CAM plants tend to close their stomata, so any CO₂ fixed during this period must come from within the plants.

Phosphoenolpyruvate carboxylase (PEPCase) is the enzyme employed by C₄ and CAM plants for the capture of atmospheric and respiratory CO₂ in mesophyll cells, but whereas in C₄ plants the four-carbon acid products are transported to the bundle-sheath cells where they are fixed in photosynthetic products by the ribulose biphosphate carboxylase oxygenase (Rubisco), in CAM plants, the whole process occurs in mesophyll cells, but at different times. At night when the stomata are open, the PEPCase generates a four-carbon acid product (such as oxaloacetate)

which is converted to malate in the cytosol by the malate dehydrogenase. Malate is transported and sequestered in the vacuole as malic acid due to the high concentration of H^+ . Since CO_2 uptake and malate accumulation continue during the night, malate can reach concentrations as high as 200 mM in the vacuole by dawn. During the day this malate is exported to the cytosol where it is decarboxylated. Malate decarboxylation can occur by several routes and enzymes depending on the CAM species [11, 12, 20]. It is not yet clear how malate decarboxylation occurs in *Agave*, but the products could be pyruvate or phosphoenolpyruvate (PEP) and in both cases CO_2 . This CO_2 is now available to Rubisco in the chloroplast for photosynthesis in the light, and the pyruvate can be converted to PEP and either be re-used by the PEPCase or else used for the biosynthesis of storage products which in *Agave* are fructans and soluble sugars (Fig. 1). The stomata remain closed during the daytime, avoiding the escape of the internally released CO_2 and at the same time preventing the loss of water by transpiration [1, 2, 35, 51].

Agave Productivity

The best productivities registered for *Agave* species are 38 and 42 $Mg\ ha^{-1}\ year^{-1}$ for *Agave mapisaga* and *A. salmiana*, respectively, growing close to Mexico City [36] (Table 1), but higher yields have been suggested for *A. americana* growing in Australia or *A. tequilana* growing in

Jalisco, Mexico (Arturo Velez, personal communication; Fig. 2).

Documented results in *A. salmiana* or *A. mapisaga* suggest that these species can perform extraordinarily when comparing their highest productivities with those of other dedicated bioenergy crops (Table 1). *Agave* can have higher productivity than switchgrass and poplar, similar to the highest values for *Miscanthus* (Table 1). Sugarcane and sorghum appear to show higher yields than the highest yields for *Agave*. However, these comparisons should be interpreted with care, since neither the rainfall value for the *Agave*, nor the inputs for the sugarcane or sorghum studies, were specified.

There are reports where the yearly productivity of *Agave* has been much lower, for example *A. tequilana*, 25 $Mg\ ha^{-1}$ [40]; *A. fourcroydes*, 15 $Mg\ ha^{-1}$ [32], or *Agave deserti*, 7 $Mg\ ha^{-1}$ [37]. This large range in reported productivities is likely due to the different environmental conditions [9], with the productivities in the lowest range being due to growth under unfavorable conditions. Some *Agave* species are more productive than others under unfavorable conditions, for example the yearly productivity of *A. lechuguilla* with 427 mm of rainfall was 4 $Mg\ ha^{-1}$ [39] while that of *A. salmiana* with only 320 mm was 10 $Mg\ ha^{-1}$ [38].

An Environmental Productivity Index (EPI) was developed as a quantitative tool to predict productivity on a wide geographical and under different environmental conditions, to help evaluate the agronomical potential of *Agave* [33, 35]. A correlation exists between EPI and the number of leaves unfolding for several *Agave* species; this easy measurement can be used to predict biomass yields [17].

Using relationships between carbon assimilation and night temperature [25], theoretical CO_2 assimilation data were calculated for *A. tequilana* at four specific geographical locations in Mexico. The estimated results ranged from 39 to 42 $Mg\ CO_2\ ha^{-1}\ year^{-1}$ (21–23 $Mg\ biomass\ ha^{-1}\ year^{-1}$) [9], which are close to the actual productivity of 25 $Mg\ ha^{-1}\ year^{-1}$ [40]. However, the actual productivities of other *Agave* species on these four locations were far from the calculated range, suggesting that the night temperature and carbon assimilation relationship is not the same in different *Agave* species [9]. Therefore, one area for potential development of *Agave* as a bioenergy crop could be creating and/or using existing models to predict CO_2 assimilation and hence biomass yields of important *Agave* species under optimal and sub-optimal conditions.

Highlighting the potential of *Agave* is the fact that, as CAM plants, agaves have higher WUE values than any C_4 or C_3 crops. For example, the productivity for *A. salmiana* of 10 $Mg\ ha^{-1}\ year^{-1}$ with only 32 cm of annual rainfall [38] is remarkable. About three times more rainfall is

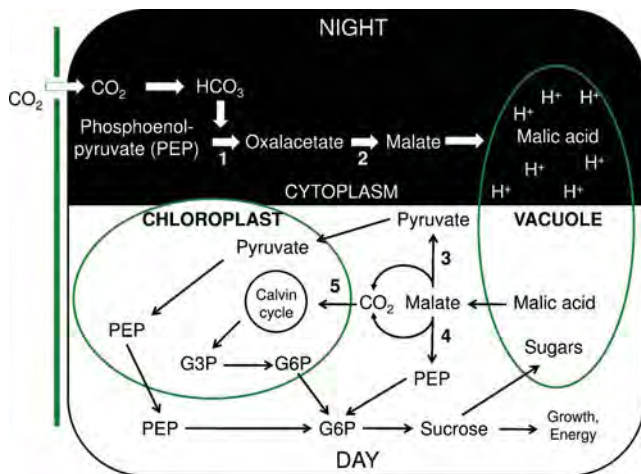


Fig. 1 The CAM pathway in a mesophyll cell. Black and white areas represent the cell during the night or day. The green line on the left of the diagram represents the leaf epidermis, with the gap representing a stomatal pore. The most important enzymes acting during night time are: (1) phosphoenolpyruvate carboxylase (PEPC) and (2) malate dehydrogenase. During the day, decarboxylation of malate occurs, but it is not totally clear whether this occurs via pyruvate by the NADP-malic enzyme or NAD-malic enzyme (3) or via PEP by PEP carboxykinase (4) in *Agave*. Both steps would generate CO_2 which would be available to Rubisco (5) in the chloroplast for photosynthesis

Table 1 Comparison of typical agronomic traits relating to above-ground dry biomass productivity and approximate rainfall required for *Agave* and several designated bioenergy crops

Crop	Tolerance to drought	Productivity (Mg ha ⁻¹ year ⁻¹)	Rainfall (cm yr ⁻¹)	Location	Reference
Agave	High				
<i>Agave</i> spp.		10 to 34	30 to 80	ns	[48]
<i>Agave salmiana</i>		42	ns	Mexico, Mexico	[36]
<i>Agave salmiana</i>		10	32	San Luis Potosi, Mexico	[38]
<i>Agave maysiaga</i>		38	ns	Mexico, Mexico	[36]
<i>Agave deserti</i>		7	43	Sonora Desert, CA, USA	[37]
<i>Agave fourcroydes</i>		15	100	Yucatan, Mexico	[32]
<i>Agave tequilana</i>		25	108	Jalisco, Mexico	[40]
<i>Panicum virgatum</i> (switchgrass)	Good				
Var. Alamo		23 ^a	80 to 160	AL	[31]
Alamo and Kanolow		5.2 to 11.1	50 to 70	NE, SD, ND	[44]
Var. Alamo		11 to 20	60 to 130	TX, AR, and LA	[4]
Other energy crops					
<i>Zea mays</i>	Low		50 to 80	ns	[48]
Grain		7			
Stover		3			
<i>Populus</i> spp.	Moderate	5 to 11	70 to 105	ns	[48]
<i>Miscanthus giganteus</i>	Low	15–40	75 to 120	ns	[48]
<i>Saccharum officinarum</i>	Moderate	50–67	ns	Guyana; Hawaii, USA; Queensland, Australia	[34]
<i>Sorghum bicolor</i>	Good	47	ns	California, USA	[34]

ns not specified

^aThis value is a 10 -year average. The year with maximal yield had 34.6 Mg ha⁻¹ year⁻¹ with a rainfall of about 130 cm

needed to get similar amounts of biomass with poplar (Table 1). Any bioenergy crop under similar amounts of rainfall would be very far from reaching the above-mentioned productivity of *A. salmiana*, and many of them would even struggle to survive.



Fig. 2 Photograph taken at an experimental plantation of *A. americana* in Australia. The biggest agave individuals weighed 1.2 Mg (Arturo Velez, personal communication)

One of the strongest arguments to support further studies of *Agave* as a potential energy crop, therefore, is its ability to produce very good amounts of biomass with very little rainfall and/or input. This could enable us to make productive use of the currently unproductive semi-arid land which constitutes approximately 18% of the terrestrial surface.

Agave Processability

Cellulose and hemicelluloses are the main components in the cell wall that can be hydrolyzed to simple sugars for further fermentation to produce ethanol or other liquid biofuels. The resistance of cell walls to breakdown to sugars is called recalcitrance and is the major limitation to converting lignocellulosic biomass to biofuel. Lignin, the other major component of cell walls, has a negative effect on hydrolysis of polysaccharides and is believed to be a major, if not the major, cause of recalcitrance. Transgenic plants with reduced lignin levels have clearly improved sugar release [6, 16, 22].

The relative compositions of lignin, cellulose, and hemicelluloses in *Agave* look positive for processability in comparison with other crops (Table 2); the cellulose content is higher while the lignin content is in the low range.

Table 2 Comparison of biomass feedstock composition

Fiber source	Cellulose	Hemicellulose	Lignin	Solubles	Reference
Agaves					
<i>A. tequilana</i>	65	5.3	16	12.5	[21]
<i>A. fourcroydes</i>	77.6	5–7	13.1	3.6	[54]
<i>A. lechuguilla</i>	79.8	3–6	15.3	2–4	[54]
<i>A. sisalana</i>	77.3–84.4	6.9–10.3	7.4–11.4	nd	[29]
Corn stover					
Study 1	44	30	26	nd	[50]
Study 2	39.4	33.1	14.9	8.9	[7]
Switchgrass					
Study 1	44.9	31.4	12	nd	[26]
Study 2	41	ND	30	nd	[53]
Study 3	ND	ND	20–25	nd	[45]
<i>Miscanthus</i>					
Study 1	41	nd	27	nd	[53]
Study 2	41.9	26.6	13.3	15	[27]
Other crops					
Eucalyptus	49.4	21.2	18.2	nd	[21]
Poplar	48.2	30.7	17.7	nd	[23]
Wheat straw	34.9	22.5	21.3	11.9	[26]
Sugarcane	48.6	31.1	19.1	nd	[42]

nd not determined

As seen in Table 2, there is considerable variation in biomass composition between different studies using the same crop. For example, the lignin content of switchgrass varies from 12.3% to 30% between studies, and in *Miscanthus*, this variation is from 13.3% to 27%. This variation could be related to environmental and physiological conditions (for example stress conditions or drought) as well as variation in data analysis. These comparisons are therefore difficult to make because of the lack of environmental information referenced above, and because other components or special structural features of the cell wall could influence processability, we conclude that further sugar release studies using the same methodology are needed for multiple *Agave* species as well as other bioenergy crops grown under standardized conditions.

Other important carbohydrate reserves in *Agave* that can be used for ethanol production are the water-soluble carbohydrates (WSC), also called non-structural carbohydrates, which are released after thermal treatment. The WSC in mature *A. tequilana* heads can be as high as 90% of the dry matter [28, 55]. Most of the sugar released after the treatment comes from fructans; polymers were composed mainly of fructose units, the hydrolysis of which results in release of fructose (80–86%) and glucose (10–15%). Fructans are the principal WSC in *Agave* species and represent more than 60% of total soluble carbohydrates. Fructan content in heads of several *Agave* species ranges from 35% to 70% of dry matter [28]. *Agave* leaves also

contain non-structural sugars, but in much lower levels and decreasing from the base to the tip. The total reducing sugars in *A. tequilana* leaves range from 9.4% (base) to 3.3% (tip) of fresh weight [21]. Two varieties of *Agave fourcroydes*, a well-known fiber-producing plant, were used as a proof of concept to produce ethanol from its reducing sugars using a native yeast strain *Kluyveromyces marxianus* (selected for its rapid fermentation and high temperature tolerance). Nineteen kilograms of *Agave* heads was required to produce 1 l of 40% ethanol. Although reserve carbohydrates of easy access like fructans are used for more valuable products such as *Agave* syrup and/or alcoholic beverages such as mescal, in a situation of overproduction, these reserves could be used for biofuel production thus avoiding the recalcitrance of lignocelluloses.

A. tequilana has been selected for many years to produce a higher content of sugars, and agronomic practices have been developed to further increase sugar content since this varies depending on age and time of harvest [30]. *Agave* heads have much higher sugar content than leaves, and the sugar content in leaves decreases from bottom to the top [28, 55]. The tequila industry therefore uses the heads, while the leaves are left on the soil [9]. A bioethanol plant processing 400 Mg per day of *A. tequilana* heads (the size of Tequila Sauza Company) can produce 61 million l year⁻¹ of 100% ethanol without considering the cellulosic parts of *Agave* leaves and head bagasse which could increase production to about 110 million l year⁻¹ of ethanol. To

support this production, it would be necessary to harvest 6,083 ha annually [9]. An economic analysis of the production of ethanol under current tequila industry practices using *Agave* heads indicates a very high cost. Conversion of leaves and head bagasse biomass is probably essential to allow *Agave* to compete as an economically viable biomass feedstock [41].

Agave Uses and Potential By-products

Alcoholic beverages, sweeteners, fibers, and some speciality chemicals are currently the main products coming from agave plants (Table 3).

Beverages

Among the most common products of agave are alcoholic beverages, tequila (from *A. tequilana*) and mescal (mainly from *A. angustifolia*) [35]. Another product is the nectar or syrup, consisting of non-structural carbohydrates and used as a sweetener. Recently, this has appeared internationally in chain grocery stores [57].

Fibers

These are the vascular bundles that carry water from the soil. They have been used for bindings, nets, sacks, twines, and ropes, etc. (Fig. 3). The preferred species for fiber production have been *A. lechuguilla*, *A. fourcroydes*, and *A. sisalana*. The agave fiber industry once consumed over 1 million ha of land, but this has now been reduced by about 90% due to the growth of the synthetic fiber industry [30, 35].

Chemicals

The steroidal saponins tigogenin and hecogenin, extracted from the waste residues after production of sisal fibers from

A. sisalana and *A. americana*, are important raw materials in the synthesis of steroid hormones. They are used as starting materials in the production of corticosteroids (cortisone, cortisol, prednisolone, prednisone, dexamethasone, betamethasone, triamcinolone, etc.). They have cholesterol-lowering, anti-tumor, and anti-inflammation activities [24, 56]. Other saponins identified within the *Agave* genus include manogenin, yucagenin, agavogenin, sarsasapogenin, texogenin, esmilagenin, gitogenin, clorogenin, diosgenin, gentogenin, and ruizgenin. *A. lechuguilla* leaves contain between 1% and 2% of the dry matter as steroidal saponins [19]. These could serve as valuable co-products from *Agave* species cultivated primarily as bioenergy crops.

Availability of Molecular Biology Tools in *Agave*

A number of traits could be improved to increase the value of *Agave* as a bioenergy crop. Among these, freezing tolerance is particularly important. Most of the *Agave* species (or at least the commercial ones) have poor cold tolerance. *A. tequilana*, *A. angustifolia*, *A. salmiana*, *A. sisalana*, and *A. fourcroydes* are among the most sensitive to cold, having frost tolerance to between -2°C to -4°C [14], such that their sustained annual cultivation is not possible outdoors in most of the US territory. There are some other *Agave* species with better frost tolerance and good potential for productivity. For example, *A. americana* and *Agave weberi* have frost tolerance down to -8°C and -11°C , respectively [14], although this means that their cultivation would still be limited to the southern regions of the USA. The *Agave* species with the highest frost tolerance is *Agave utahensis* (-23°C), but its potential productivity is low. Therefore, improving cold tolerance would be an important trait to increase the land area where *Agave* can be cultivated. Other traits to manipulate are those related to bioenergy production such as improving fructan content, decreasing recalcitrance by

Table 3 Current uses of *Agave*

Use	Species	Production	Area cultivated (ha)	Reference
Beverages				
Tequila	<i>A. tequilana</i>	>300 million l	80,000	[35]
Mezcal	<i>A. salmiana</i> , <i>A. mapsiaga</i> , and eight others	20 million l	30,000	[35]
Pulque/aguamiel	<i>A. salmiana</i> , <i>A. mapsiaga</i> , and eight others	200 million l	20,000	[35]
Fiber	<i>A. sisalana</i> and <i>A. americana</i>	ND	100,000	[33]
Chemicals				
Tigogenin and hecogenin (saponins)	<i>A. sisalana</i> and <i>americana</i>	ND	By-product of the fiber industry	[24, 56]

Pulque is a fermented non-distilled beverage. "Aguamiel" (honey water) is sap collected from certain agaves. Tigogenin and hecogenin are important raw materials in the synthesis of steroid hormones

ND No data available



Fig. 3 Mostly artisanal products from *Agave* fibers (ixtle) now remain in the market

manipulating cell wall polymers [6, 16, 22], or enhancing specific valuable by-product levels.

Genetic improvement of *Agave* spp. by conventional breeding is difficult due to the long period the plants take to reach maturity, 7–8 years in *A. tequilana* and even longer in some other species like *A. salmiana* [5]. Genetic transformation could potentially be used to overcome this limitation. Several species of *Agave* have been micropropagated in vitro, but there is currently only one report on successful genetic transformation of *Agave* (*A. salmiana*), using both *Agrobacterium tumefaciens* and particle bombardment [15]. The most effective method of transformation was the co-cultivation of explants with *Agrobacterium* containing a binary vector. The *uidA* gene (β -glucuronidase) was used as a reporter, and the transformation efficiency was 2.7% [15].

The molecular biology tools available for *Agave* are at present very limited. A search of the NCBI GenBank using “Agave” as search word currently returns practically nothing in nucleotide or expressed sequence tag (EST) sequences. Thus, generating extensive EST or genomic sequence data would be another area of potential research for the development of *Agave* as potential bioenergy crop. Two research institutes in Mexico have recently been producing general transcriptome data for *A. tequilana* [46]. Although the data are not yet publicly available, the authors mention that they will be released soon. They give a brief description of the results obtained and examples of transcriptome data mining for genes with potential bioenergy application. For example, related to fructan accumulation, they found 33 sequences encoding fructosyltransferases or invertases. For cell wall-related cellulases, they identified 3 exoglucanases, 22 endoglucanases,

and 32 β -endoglucanases. In the case of lignin biosynthesis, they found sequences for all ten enzymes involved in monolignol biosynthesis. The agave transcriptome database could be available for interested researchers by contacting the authors [46].

Conclusions

Literature data related to productivity and processability (for biofuel purposes) indicate that some species of *Agave* would be able to compete economically with other bioenergy crops. However, the characteristic that makes certain *Agave* species outshine other bioenergy crops is their capacity to grow with very little rainfall and/or inputs and still reach a good amount of biomass [38]. Unused semi-arid land with a rainfall of 450 mm or less is plentiful worldwide and is ready to use, forests do not have to be cleared, and cropland does not need to be taken. Neither switchgrass nor *Miscanthus* would be productive in such arid environments, and most other bioenergy crops would likely fail to survive.

Considering that increases in food production will be needed for the coming generations, one of the most important issues to attend related to sustainability is water scarcity. Therefore, crops that require minimal amounts of water and no irrigation, and do not compete for food, like *Agave*, should be preferred for biofuels. Related to greenhouse-gas emissions, the input required for *Agave* cultivation is minimum, so we predict that the carbon balance would be very positive. Moreover, many of the semi-arid regions are poor or undeveloped, so making this

land productive can bring some wealth to the indigenous population.

It is possible to envisage an economically competitive *Agave* biofuel industry if it is used as the most adequate species, cultivated under optimal agronomical practices for bioenergy, with concurrent production of valuable by-products. However, more laboratory research and field trials under standard conditions are needed, using the most productive species (like *A. salmiana*, *A. mapisaga*, *A. americana*, and *A. tequilana*) in order to obtain truly comparative data on characteristics such as productivity, agronomic practices, cold tolerance, and water requirement. Further research on biomass conversion and bio-process engineering is also critical to improve the efficiency of biofuel and co-product production.

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