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Morphological Characterization of *Agave tequilana* Weber Variety *azul* (Asparagaceae) under Cultivation in the Municipality of Llera, Tamaulipas, Mexico

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Abstract

The blue agave (*Agave tequilana* Weber variety *azul*) is a species that is widely used to produce tequila. For this reason, in the last decade, large extensions of terrain have been established for its cultivation. However, much of this land has been abandoned in a short time because at the beginning it was not known that at least six years were needed before the agave could be harvested. However, when the growers wanted to reintegrate some of these crops into the productive process, their exact age and the cultural activities needed for harvesting the agave were unknown because information regarding its vegetative development relative to its age was lacking. The hypothesis of this work was: If the morphological properties reflect the development state of the population individuals, then the properties height, basal area, rosette diameter, the north, south, east and west leaf, leaves number and foliar verticil will help to characterize the age categories in *Agave tequilana* Weber variety *azul* population under cultivated conditions. To this end, six plots with plants of different ages (one to six years) were selected in the municipality of Llera, Tamaulipas, Mexico. In each plot, 60 individuals were selected at random, and their morphological properties (height, basal area, rosette diameter, northern leaf, southern leaf, eastern leaf, western leaf; number of leaves and leaf whorl) were measured. With these data, a principal component analysis was performed to determine which of these properties explains the most variation of the data. The results indicate that height and basal area are the properties that are most closely associated with plant age. It is also for the first time demonstrated that harvest time can be reduced by one or two years, which favors producers economically because it could generate savings in production costs.

Keywords

Morphological Properties, Vegetative Development, Principal Components, Blue Agave

1. Introduction

Agaves (genus *Agave*, family Asparagaceae) are a group of plants representative of Mexico that are highly valued both ecologically and economically. The genus was first described by Carl Linnaeus in 1753 (Ciaramello & De Paiva, 1975; García-Mendoza, 2007). They are part of the floristic composition of a wide variety of vegetation types, among which are included conifer forests located in mountain systems above 3500 m altitude and coastal desert scrub characteristic of arid and semiarid areas of the Baja California Peninsula at 0 m above sea level (Solís-Aguilar et al., 2001; Bautista-Justo et al., 2001).

These plants have been used for 8000 - 10,000 years. Today, they are used as a source of food, traditional medicine, biofuel (bioethanol), shelter, ornament, raw material for natural fiber articles and, in greater proportion, for alcoholic beverages. These many uses motivated the indigenous and mestizo peoples of Mexico to represent agaves as mythological gods, to which our ancestors conferred great cultural importance that continues today (Alanís-Flores & González-Álvarez, 2011; Pérez-Hernández et al., 2016).

One of the gods was Ometochtli, god of pulque, an alcoholic drink made of fermented aguamiel juice of the agave, particularly of the species *Agave americana* L., *A. atrovirens* Karwinsky ex Salm-Dyck, *A. ferox* K. Koch, *A. hookeri* Jacobi, *A. mapisaga* Trel., *A. marmorata* Roezl, *A. salmiana* Otto ex Salm-Dyck, *A. scaposa* Gentry and *A. seemanniana* Jacobi (Gentry, 1982; Álvarez-Duarte et al., 2018). Mezcal is another agave-based beverage made from mainly the species *Agave angustiofolia* L., *Agave asperrima* Jacobi, *Agave potatorum* Zucc and *Agave salmiana* Otto ex Salm-Dyck. Later, tequila emerged, obtained only from the species *Agave tequilana* Weber variety *azul*. Mezcal and tequila are obtained by distilling the extract of the cooked stems (heads) of the agave (Gentry, 1982; Eguiarte, 1995; Acosta-Navarrete et al., 2007; Alanís-Flores & González-Álvarez, 2011).

Over time, tequila became more important economically, and it was necessary to plant monocrops of *Agave tequilana* Weber variety *azul* obtained by asexual propagation, eliminating genetic variability and impacting the reproductive biology of the interacting species that depend on it, such as bats (genus *Leptonycteris*) that have been recorded as important pollinators of this and a large number of plant species (Castillo-Hernández & Treviño-Carreón, 2009; Trejo et al.,

2015) and whose number have been diminishing (Trejo et al., 2017). It has been observed that this species of agave does not reproduce sexually because when they reach physiological maturity (four to six years old) and begin the reproductive stage, the developing bloom stalk is cut (*desquiote*) to prevent the plant from using the energy accumulated during the growth period and extend the time that *jima* (extraction of the core, or *piña*, for processing) can take place without altering its commercial value. However, some authors indicate that it is highly important to maintain the structure and dynamics of populations of the species for their conservation (Vázquez et al., 2011). Over exploitation of this resource has put some of these species at risk and is currently found in the Norma Oficial Mexicana NOM-059-SEMARNAT, 2010 and/or in the international red list (UICN).

In Mexico, an estimated 170,000 ha are used for cultivation of agave (Álvarez-Sánchez et al., 2010). However, this area is distributed in the states of Guanajuato (seven municipalities), Nayarit (eight municipalities) Tamaulipas (11 municipalities), Michoacán (30 municipalities) and Jalisco (126 municipalities). Jalisco produces 98.3% of the national total. These states possess the Denomination of Origin of Tequila (DOT) granted by the Tequila Regulating Council, based on the Norma Oficial Mexicana NOM-006-SCFI, 2012, Bebidas Alcohólicas-Tequila-Especificaciones.

Cultivation of the agaves generates income for the people involved in the production chain and, in some cases, it is the daily sustenance for entire families (Magallán-Hernández & Hernández-Sandoval, 2000; García-Herrera et al., 2010; Treviño-Carreón et al., 2011).

Crops are affected by environmental properties, agronomic management, and chronological and genetic heterogeneity of the plantations, which are affected by diseases caused by fungi, such as *Cercospora agavicola* known as grey spot or leaf smut (Ayala et al., 2005) or by bacteria such as *Pectobacterium carotovorum* (Jones) Waldee that causes rot of the apical meristem and necrosis. Agaves are also attacked by insect pests such as *Scyphophorus acupunctatus* Gyllenhal, the *Agave* weevil, one of the most aggressive pests (Rubio, 2007).

Some blue agave crops have been abandoned because of the long growing period. When a grower wants to recover these plantations, they do not know the exact age and so planning the *jima* is impossible (Gerritsen et al., 2010). Therefore, this study aimed to determine the relationship that exists between *Agave tequilana* Weber variety *azul* plant age and a series of morphological properties that could be used to estimate age and re-introduce the agaves into the production system. The results can increase knowledge of the resource and its use would enable growers to recover the crop, generate employment, recover investment in the production chain and improve rural living conditions (Martínez-Palacios et al., 2015). In the municipality of Llera, Tamaulipas, Mexico, the stem (piña or head) is extracted at 7 years of age. After this time, the plant begins to use the stored sugars to produce the bloom stalk (*quiote*). The general objective of this study was to characterize, morphologically, the development stages of *Agave tequilana* Weber variety *azul* from plantation to harvest and to determine the morphological properties that estimate plant age.

2. Materials and Methods

2.1. Study Area

As study plots, we used certified *Agave tequilana* Weber variety *azul* cultivated in the municipality of Llera, Tamaulipas, Mexico, which has the Denomination of Origin of Tequila (DOT). These plantations included all the vegetative development stages of the plants. We then selected six plots that had plants of different ages distributed randomly (Table 1), from transplant to harvest considering a one-year separation between plants, that is, each plant represented an age that was one year older than the one before. In each plot, 60 individuals dispersed in four rows in the center of the plot to eliminate the edge effect were selected and measured for nine morphological properties (Figure 1).

2.2. Evaluation of Morphological Properties

Based on observations of *Agave tequilana* plants, we selected morphological properties that reflect their development; some of these are related to vegetative organs plant, such as the leaf and stem (Figure 2).

1) Height, using a tape metric, it was measured vertically from the plant base (neck) to the meristem apex (heart) where the terminal spine.

2) Basal area, for this the stem basal diameter was obtained by measuring the stem base and then the basal area is obtained with the circle area formula.

3) Rosette diameter, obtained by measuring the distance between the apexis of distal and horizontal leaves, considering that blue agave leaves form a half sphere.

4) to 7) Leaf length, obtained by measuring the distance between the leaf base and the apex of its terminal spine. Four leaves were selected, each in a cardinal point: north (property 4), south (property 5), east (property 6) and west (property 7), with the aim of detecting whether there is difference in length related to cardinal position.

Table 1. Location of *A. tequilana* Weber variety *azul* plots in four ejidos of Llera municipality, Tamaulipas.

Plot (Age)	Latitude	Longitude	Municipality	Ejidos
Plot 1	23˚11'38,02"	98˚49'24,00"	Llera	Voz Campesina
Plot 2	23˚12'07,01"	98˚50'14,08"	Llera	Voz Campesina
Plot 3	23˚12'01,06"	98˚50'20,00"	Llera	Adolfo Ruíz Cortines
Plot 4	23˚11'01,04"	98˚47'21,06"	Llera	Mariano Escobedo
Plot 5	23˚11'38,03"	98˚47'16,06"	Llera	Ignacio Zaragoza
Plot 6	23˚12'28,03"	98˚40'21,00"	Llera	Voz Campesina

Figure 1. Color pictures showing the *Agave tequilana* Weber variety *azul* in the municipality of Llera, Tamaulipas, Mexico.

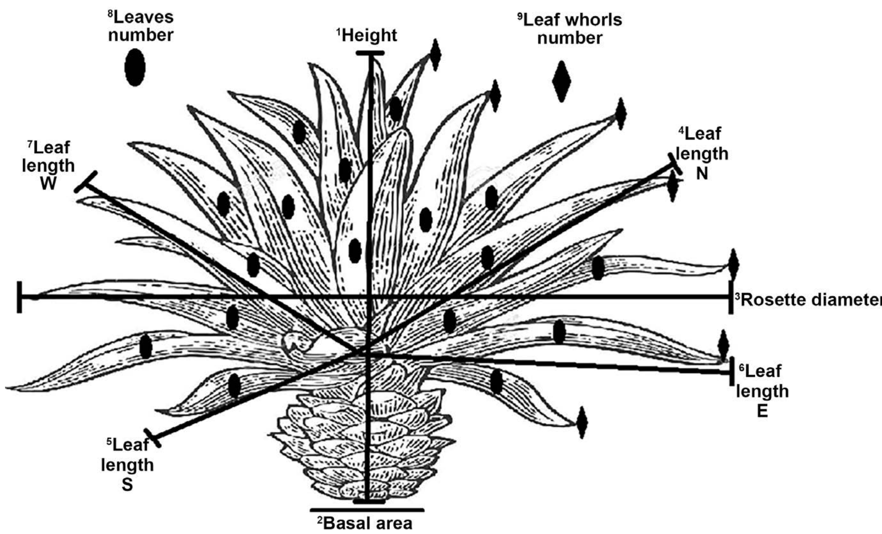

Figure 2. Measurement of morphological properties in blue agave in Llera, Tamaulipas, Mexico.

8) Leaves number, obtained by counting all leaves on each plant.

9) Leaf whorls number, which consists of a row of leaves formed around the stem (pineapple), this whorls are counted from the base of plant to the meristem (central cone). Leaves that are totally separated from the apical meristem were included and those leaves that die or were dying during the growing stage were excluded from the count.

2.3. Statistical Analysis

An analysis of variance (ANOVA) was performed with the software STATGRAPHICS PLUS 2.0 for Windows to determine significant statistical difference between length leaf north (property 4), south (property 5), east (property 6) and west (property 7), with respect to the age of the plants from the sampled plot, with a probability equal to or less than 0.05. There were significant differences, thus, the Kruskal-Wallis multiple range tests were used to contrast the means of the properties of each of the plots. STATA 12 was then used for principal components analysis to obtain the properties that best estimate age of the plants in each plot. A linear regression analysis of each of the properties resulting from the principal components analysis, relative to the age of the plants from each plot, was performed.

3. Results and Discussion

3.1. Analysis of Variance (ANOVA) of the Property Leaf Length

The results of the analysis of variance (ANOVA) used to comparison of means of this properties, determined that there are no significant statistical differences to cardinal point of leaf position, that is to say, the leaves have the same length, not change with respect to cardinal point (F = 379.36; df = 23; $P > 0.001$), but there were significant differences between leaf length and plot age. Therefore, we decided to group the values of the properties north leaf length, south leaf length, east leaf length and west leaf length into a single property called "leaf length".

3.2. Principal Components Analysis

The principal components analysis resulted in a total of two components, both correspond to the properties related to plant age of each plot. Component one is the property height, while component two is the property basal area. These two properties can be used for more precise age estimation of the plants of each *A. tequilana* Weber variety *azul* plot that has been abandoned (Figure 3).

3.3. Analysis of Variance (ANOVA) of the Property Height

The result of analysis of variance (ANOVA) of the age data, it indicates that there is a significant statistical difference among the means to the property height of each plot. The F relationship is equal to 1890.53 and belongs to the estimation between groups. For the estimation within the group, the *P* value of the F test is below 0.001, and therefore, there is a significant statistical difference between the mean height from one plot to another (F = 1890.53; df = 5; $P < 0.001$) with a confidence level of 95.0%. The Kruskal-Wallis multiple range test formed five groups, indicating that the means of the property height were different, relative to the age of the plants of each plot.

The results indicate that only the two- and three-year-old plots belong to the same group, while the rest remain as individuals, suggesting that plant development permanent when they reach a height of about one meter. However, this increases continuously in the following years.

3.4. Linear Regression Model Analysis of the Property Height

The regression model that had a coefficient of correlation close to one ($R^2 = 0.9409$) was the third-order polynomial trend line. The equation is the following:

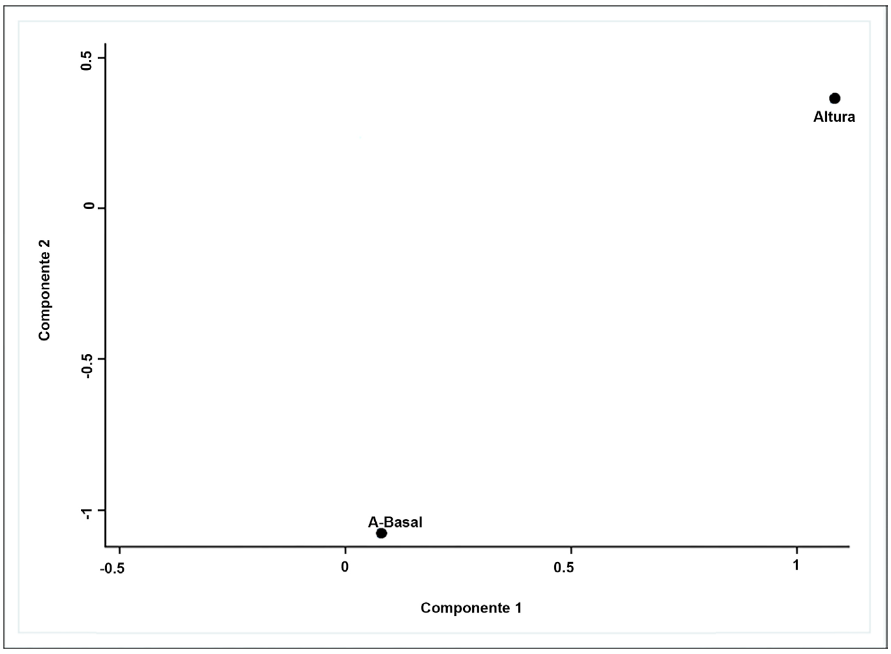

Figure 3. The two principal components resulting from the analysis of nine *A. tequilana* morphological properties.

$y = 1.2193 + 6.527 \times 10^{-2}A - 6.1637 \times 10^{-4}A^2 + 3.17 \times 10^{-6}A^3$, where y = Plots (age) and x = Height (cm) (Figure 4).

3.5. Analysis of Variance (ANOVA) of the Property Basal Area

The ANOVA of the age values indicates that there is a significant statistical difference between the mean age and basal area of the plants; the F relationship is equal to 461.15 and belongs to the estimation among groups. For the estimation within groups, the *P* value of the F test is less than 0.001. Therefore, there is a significant statistical difference between the mean basal area on the plot level with respect to the rest ($F = 461.15$; df = 5; $P < 0.001$) at a confidence level of 95.0%.

With the Kurskal-Wallis multiple range test, six groups were formed, indicating that the means of the basal area are different with respect to the age of plants of each plot.

The results indicate that only the six-year-old plot is smaller than the four and five-year-old plots, assuming that the plants are losing biomass. This suggests that plant development slower when they reach a basal area of 31.53 cm^2. However, this area decreases continuously in subsequent years because the plant is preparing to develop the inflorescence and thus consumes part of the stored sugars.

3.6. Linear Regression Model Analysis of the Property Basal Area

The regression model that had the coefficient of correlation close to one ($R^2 = 0.7953$) was the third-order polynomial trend line with the following equation:

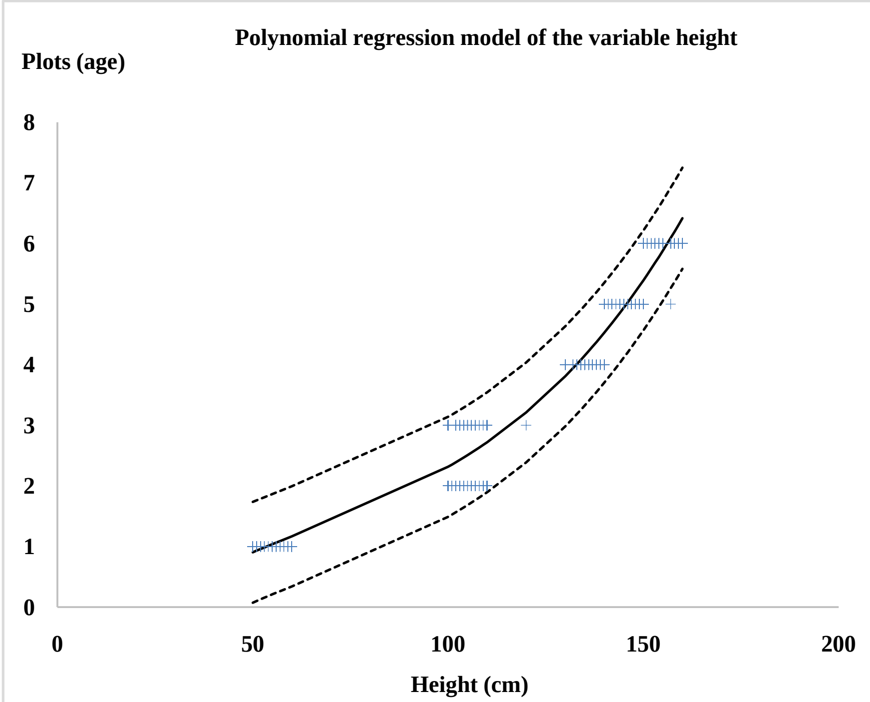

Figure 4. Polynomial regression model of the property height with respect to plot age.

$y = 3.5547^{-1} + 1.641 \times 10^{-2}AB^2 - 1.879 \times 10^{-5}AB^2 + 6.863405 \times 10^{-9}AB^3$, where y = Plot (age) and x = Basal area (cm^2) (**Figure 5**).

There are few accessible registered research on the topic of morphological properties of *Agave tequilana* Weber variety *azul* that permit comparison of the different stages of vegetative development of the plants from transplant to harvest. Nevertheless, Saldaña-Robles et al. (2012), Gutiérrez-Vaca et al. (2012), they evaluated the plant weight and found than some properties are related directly and proportionally to each other. Háuad et al. (2010), only describes the averages of plant height, leaf length and maximum width in *Agave tequilana* Weber variety *azul*.

In our study, we observed that two-year-old plants have an increase of 100% than one-year-old plants when they are transplanted to a new site under optimum conditions because competition among the suckers, or pups, and the mother plant is eliminated, permitting constant development (García-Mendoza, 2007). This growth is reflected in an increase in the diameter of the stem base, which is related to the basal cover area resulting in an increase in the total number of leaves. This, in turn, results in larger leaf area, a higher photosynthetic rate, and therefore, accelerated vegetative growth. Moreover, we observed an increase in the number of leaf whorls, while other morphological properties, such as rosette diameter, leaf length (north, south, east and west) and total plant height, reach their maximum values, and stop growth. At the same time, the plants stem increases in diameter because of the rapid absorption of nutrients provided by the substrate and the photosynthetic rate.

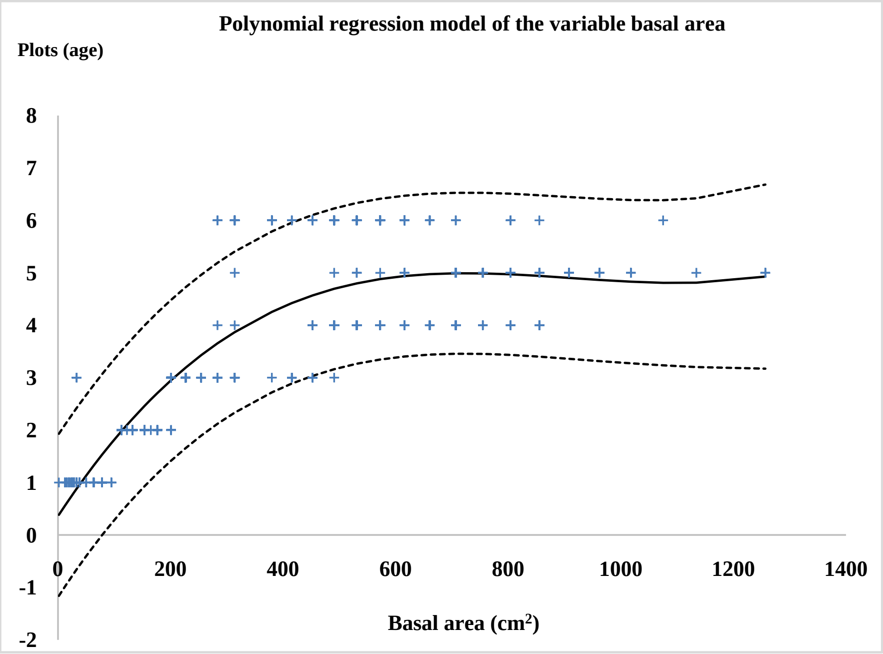

Figure 5. Polynomial regression model of the property basal area with respect to plot age.

Comparison in values morphological properties of three-year-old plants with those of two-year-old plants, shows that the properties basal area and total leaf number remain constant, while the height property is slower, permitting basal cover area to increase gradually. Comparison the values of the height property in the plot with four-year-old plants with the three-year-old plot, increased 100%, while the basal area property increased, leading to increased height and rosette diameter and a larger number of leaves and leaf whorls, because as the plant increases in biomass, it needs to absorb more resources from the soil to increase its reserves and prepare for flowering.

Comparing the results of five-year-old plants with those of the four-year-old plot, we observe that the property basal area increases gradually since the plant is preparing to produce the bloom stalk. Also, there is a direct and proportional relationship between basal area and number of leaves, which contribute to stem development. The values obtained from the plants of the six-year-old plot compared with those of the five-year-old plot, indicate that the plant permanent in terms of the properties basal area, rosette diameter and leaf whorls, while the number of leaves increases. Possibly due to these changes, the plant could begin to consume its stored resources, and thus, basal area does not increase. This could indicate that in the municipality of Llera, Tamaulipas, Mexico, the optimum age for harvesting the stems of this species begins at five years.

Comparing the values obtained of the plants of the six-year-old plot with those of the five-year-old plot, we observe that the plant properties basal area, rosette diameter and leaf whorl remain stable, while the number of leaves increases because stored plant resources are being consumed reducing the increase in basal area.

4. Conclusion

Abandonment of plantations of *Agave tequilana* Weber variety *azul* plantations leads to great economic loss for the producers. The principal components analysis identified the morphological properties that can be used to estimate the age of plants in abandoned plantations so that they can be restored. The properties that best represent the degree of total development of the plants are height and basal area, which conformed five and six groups based on the values of each of the properties. Moreover, the linear regression analysis resulted in a polynomial trend line of the property height, showing that this property is directly proportional to plant age, while the pattern of increase of property basal area does not continue in plants older than five years.

STATA 12 indicates that the plant can be harvested as of five years of age when, if *jima* is not carried out, it will begin to lose biomass.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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