Characterization of *Nopalea cochenillifera* clones using linear dimensions and multivariate analysis

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ABSTRACT. Forage cacti can contribute to increases in biomass yields in agricultural areas, improving the use efficiency of local natural resources. Forage cactus stands out as a forage alternative in semi-arid regions due to its high potential of phytomass production and energy value, large water reserve and easy propagation. The objective of this study was discriminate as morphometric characteristics of *Nopalea cochenillifera* cladodes in relation to Little and Giant Sweet clones. This research was conducted from March 2016 to July 2019. The design used was randomized blocks with five replicates using the forage cactus clones Little and Giant Sweet of *Nopalea cochenillifera* (L.) Salm-Dyck. The experimental unit was an area of 126.0 m² (12.6 x 10.0 m). Study sample is composed of 1018 cladodes (581 of Little Sweet clone) and (437 of Giant Sweet clone), randomly collected. Variables evaluated were the cladode length, width, thickness, area weight. T-tests, Pearson correlation coefficients, discriminant analysis and canonical variables analysis were used to evaluate, compare and discriminate the morphometric characteristics of forage cactus clones. The Giant Sweet clone presented the highest means for the variables length, width, area and weight. Fisher's discriminant function verified a 99.41% hit rate to differentiate groups of forage cactus clone. The hit rate for Little Sweet clone was of 98.97%, while for Giant Sweet clone was of 100.00%. With two canonical variables the explanation rate of the morphometric characteristics for the behavior of forage cactus clones is higher than 90%. Morphometric characteristics of cladodes can be used as parameters that help in the identification of *Nopalea cochenillifera* clones with high discriminatory power.

Keywords: little sweet clone; giant sweet clone; morphometric characteristics.

Introduction

Species adapted to arid and semi-arid environments, such as forage cactus (*Nopalea* spp. and *Opuntia* spp.), can contribute to increases in biomass yields in agricultural areas, improving the use efficiency of local natural resources (Diniz et al., 2017). The forage cactus stands out as a forage alternative in semiarid regions due to its high potential of phytomass production and energy value, richness of non-fibrous carbohydrates, ruminant acceptability, large water reserve and easy propagation (Pereira, Leite, Cavalcante, & Lucena, 2018).

One of the determining factors of biomass production is the absorption and use of solar radiation in the photosynthetic, as for forage cactus this function is conducted by cladodes (Lucena, Leite, Simões, Simões, & Almeida, 2018a). The forage cactus employs CAM (Crassulacean Acid Metabolism) and photosynthetic metabolism, with day closure of stomata and nocturnal carbon assimilation, thus providing a greater capacity to adapt to abiotic factors (Santos et al., 2016; Souza Filho, Ribeiro, Santos, & Macedo, 2016).

All these anatomical and morphophysiological adaptations acquired evolutionarily by forage cacti have contributed to the high agroecological success of this crop; high water use efficiency having resulted in their adaptation to environmental conditions that involve high atmospheric evaporative demand and reduced soil water content (Winter, Garcia, & Holtum, 2011; Hartzell, Bartlett, & Porporato, 2018). The yield of this crop is mainly influenced by light interception, which in turn is determined by morphological characteristics such as the cladode area (Pinheiro et al., 2014).

In recent years the quantitative study of the biology of anatomical form has also moved into morphometrics via the study of shape variation and its covariation with other variables (Adams, Rohlf, &

Slice, 2004). Several studies using linear dimensions in forage cactus have been established for species of the genus *Nopalea cochenillifera* as reported in studies with Little Sweet clone (Cunha et al., 2012; Silva et al., 2015; Gomes, Queiroz, Pereira, Costa, & Oliveira, 2016; Freire et al., 2018) and Giant Sweet clone (Padilha Junior, Donato, Silva, Donato, & Souza, 2016; Lucena, Pereira, & Leite, 2018b; Lucena et al., 2019a; Lucena, Leite, Simões, Izidro, & Simplício, 2019b), having generated high precision in estimative.

Although there is some information about the linear dimensions (length, width, thickness, area and weight) of the species *N. cochenillifera,* no studies have been reported in the literature relating some differences in morphometric characteristics of cladodes *Nopalea cochenillifera* in relation to Little and Giant Sweet clones. In order to find differences in the physiological structure of the forage cactus, the objective of this study was to discriminate the morphometric characteristics of *Nopalea cochenillifera* cladodes in relation to Little and Giant Sweet clones.

Material and methods

This research was conducted from March 2016, to July 2019, in the forage farming sector (Gefor) of the Federal Rural University of Pernambuco (UFRPE), Academic Unit of Serra Talhada (UAST), which was located in the following geographic coordinates (07° 57' 01" S e 38° 17' 53" E) at an elevation of 523 metres, Serra Talhada locality, northeast of Brazil. According to Koppen, the climate condition is a BSwh' with a rainy season during the summer, starting in November and ending in April. The average annual rainfall is 632.2 mm, the average annual air temperature is 26ºC, and the average air relative humidity is 60% (Leite, Lucena, Sá Júnior, & Cruz, 2017). Figure 1 shows the behaviour of maximum and minimum temperatures means and accumulated rainfall over the experimental period.

The soil used in the experiment was collected at a depth of 0-20 cm and classified as Typical Haplic Cambisol Ta Eutrophic, as described by *Empresa Brasileira de Pesquisa Agropecuária* (Embrapa, 2013). The soil sample was analysed by the soil fertility laboratory of the *Instituto Agronômico de Pernambuco* (IPA) and was characterized by the following chemical attributes: pH (water) = 6.80; P (extractor Mehlich I) = 40 mg dm^{-3}; K$^+$ = 0.45; Ca^{2+} = 5.50; Mg^{2+} = 1.60; and Al^{3+} = 0.0 cmol$_c$ dm^{-3}. The cleaning and preparation of the study area were performed manually in March 2016, after two cleanings being performed annually. Soon after, the cladodes of forage palms were planted. Organic fertilization was performed using 40 t ha^{-1} of bovine manure. The row spacing was 1.4 m, and the planting was performed using the card of deck system. In this system, a groove is made, and the cladodes are planted in a single file where one plant overlaps the other.

The design used was randomized blocks with five replicates using the clones Little and Giant Sweet of *Nopalea cochenillifera* (L.) Salm-Dyck. The experimental unit was an area of 126.0 m^2 (12.6 x 10.0 m), which consisted of 10 rows of cacti spaced in rows 1.40 apart, with four plants per linear meter corresponding to a planting density of 31746.03 plants per hectare. The rows and columns of the border were excluded. Cultural dealings were made where necessary. Two weeds were identified upon their emergence. The area was maintained in dry conditions throughout the crop cycle. Evaluations were performed 1200 days after planting (DAP).
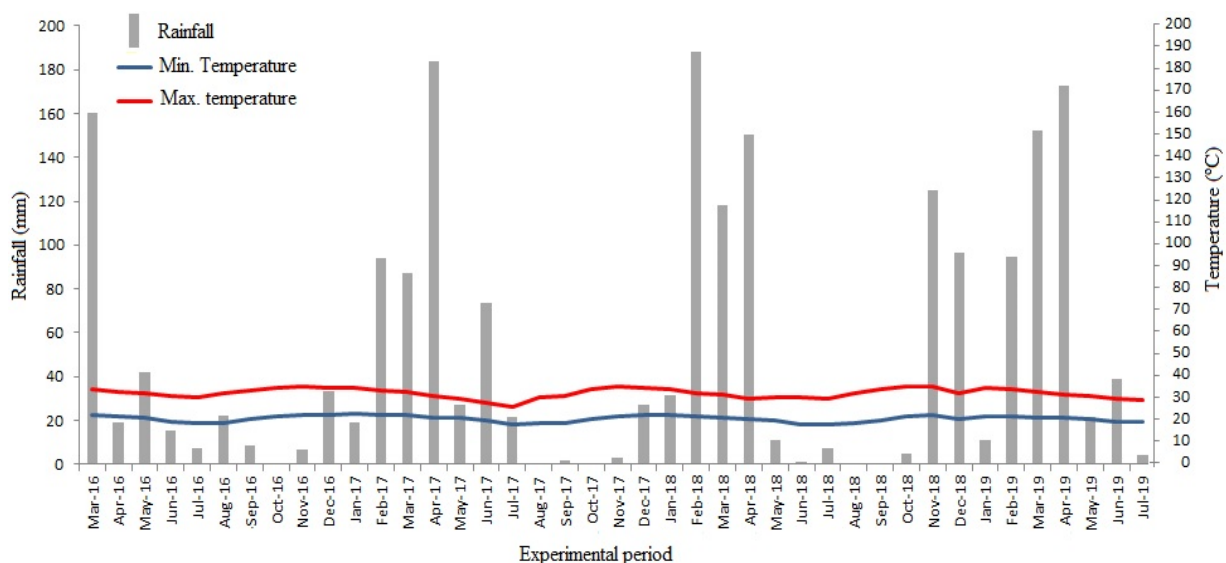

Figure 1. Distribution of rainfall and maximum and minimum temperature means of the experimental period.

To determine the cladode length (L; cm), width (W; cm), thickness (T; mm), area (ACR; cm^2) and weight (Wg; g), a randomized collection of 1018 cladodes (190 primary; 186 secondary and 205 tertiary of Little Sweet clone) and (167 primary; 127 secondary and 143 tertiary of Giant Sweet clone), free of damage, diseases or pests, was conducted according to methodologies established in the literature (Leite et al., 2017; Lucena
et al., 2018a; Lucena et al., 2019a). The cladodes were numbered from 1 to 1018 and then measured using a digital calliper; the length (L; cm), width (W; cm) and thickness (T; mm) of each cladode were also recorded. The regions of greatest width and length of each cladode were used to measure the two characteristics. The cladodes were weighed on a precision balance, and their values were recorded in grams (g).

Each cladode was spread over a millimetre graph paper, and the outline of the cladode was drawn following the methodology described by Lucena et al. (2019a). Using scissors, the area of the millimetre graph paper covered by the outline was cut and weighed on an electronic balance. From the same paper, a square of 10 x 10 cm was cut, equivalent to 100 cm^2, weighing 0.630 g. Thus, it was possible to calculate the proportional cladode area.

To evaluate the correlations between the variables, the Pearson correlation coefficient was used (Lucena, Leite, Cruz, & Sá Júnior, 2018c), which was denoted by Equation 1.

$$\rho = \frac{\sum_{i=1}^{n}(Y_i - \overline{Y})(X_i - \overline{X})}{\sqrt{\sum_{i=1}^{n}(Y_i - \overline{Y})^2}\sqrt{\sum_{i=1}^{n}(X_i - \overline{X})^2}} \qquad (1)$$

where:
Y_i and X_i are the i-th observations of the variables Y and X, while $\overline{Y}$ and $\overline{X}$ are the means of the variables Y and X, respectively. T-tests were used to assess the significance of the correlation between the variables. T-tests were used to compare the means of morphometric characteristics in relation to clones *Nopalea cochenillifera*.

Canonical variable analysis was used to verify the contribution of each morphometric characteristic as a function to different *Nopalea cochenillifera* clones. Canonical variables were defined according to Ferreira et al. (2003) and defined by Equation 2:

$$Y_i = a_{i1} X_1 + a_{i2} X_2 + \cdots + a_{ip} X_p \qquad (2)$$

where:
Y_i is the i-th canonical variable (i = 1, 2, ..., p); X_j is the j-th cladode morphometric characteristic (j = 1, 2, ..., p); a_{ij} is the weighting coefficient of the j-th cladode morphometric characteristic in the i-th canonical variable.

Canonical variables Y_i are linear combinations of the original variables and are deducted in descending order of importance, so the first canonical variable contains as much variation as possible in the original data, $VAR(Y_1) > VAR(Y_2) > \ldots > VAR(Y_p)$, where $VAR(Y_i)$ is called the variance of Y_i in the data set considered. Thus, it can be seen that the first canonical variables can explain most of the phenomenon variation. The canonical variables are obtained by solving the system of equations below Equation 3:

$$\det(\Sigma - \lambda_i I) = 0 \qquad (3)$$

where:
Σ is the covariance matrix, λ_i are the characteristic roots or eigenvalue of Σ and I is the identity matrix pxp.

The number of canonical variables was defined when the total variation was greater than 80%. The relative importance (Imp.) of each canonical variable is evaluated by the percentage of the total variation that it explains and is expressed as follows Equation 4:

$$\text{Imp.} Y_i = \frac{\lambda_i}{\sum_{i=1}^{p} \lambda_i} * 100 \qquad (4)$$

Discriminant analysis (Lucena et al., 2019b) was used to find functions of observed variables that explained the observed differences among clones of *Nopalea cochenillifera* and classify the cladodes by clones. Fisher's linear discriminant function is a linear combination of original characteristics which is characterized by producing maximum separation between two populations. Fisher's discriminant function is defined by Equation 5:

$$D(X) = [\mu_1 - \mu_2]'\Sigma^{-1}X \qquad\qquad (5)$$

where,

$X = [X_1, X_2, ..., X_p]$ is vector of the cladodes morphometric characteristics; μ_1 and μ_2 are the mean vectors of the morphometric characteristics of *Nopalea cochenillifera* clones and Σ is the covariance matrix of morphometric characteristics of *N. cochenillifera* clones.

The value of Fisher's discriminant function for a given set of morphometric characteristics of a respective cladode of *N. cochenillifera* is Equation 6:

$$D(x_0) = [\mu_1 - \mu_2]'\Sigma^{-1}x_0 \qquad\qquad (6)$$

The midpoint between the two mean vectors of the morphometric characteristics of N. cochenillifera clones is defined by Equation 7:

$$m = \frac{D(\mu_1) + D(\mu_2)}{2} \qquad\qquad (7)$$

The classification rule based on Fisher's discriminant function is allocate x_0 in the morphometric characteristics group of Little Sweet clone if $D(x_0) \geq m$ otherwise allocate x_0 in the morphometric characteristics group of Giant Sweet clone.

Results and discussion

Table 1 shows that there is some differences in morphometric variables among *Nopalea cochenillifera* clones. The Giant Sweet clone presented the highest means for the variables length, width, area and weight. This fact occurs due to the morphological structure itself *N. cochenillifera* genus, because the Giant Sweet clone cladodes presented, on average, higher values of length and width, and consequently higher area (cladode area is estimated using the product between length and width) and weight when compared to the Little Sweet clone cladodes. The thickness of the cladodes Little Sweet clone presented higher average than the cladodes Giant sweet clone, this may have occurred, due to the fact that the cladodes of the Little Sweet clone were smaller in length and width, tending to be thicker than cladodes of the Giant Sweet clone, owning to the fact that the forage cactus stores water to withstand long periods of drought in the cladodes.

Table 1. Comparison of cladode morphometric measurements as a function of *Nopalea cochenillifera* clones.

Morphometric variables	*Nopalea cochenillifera* (Mean ± SD)		p-value of T-test
	Little Sweet clone	Giant Sweet clone	
Length (cm)	18.77 ± 3.91b	22.77 ± 6.83a	< 0.0001
Width (cm)	7.82 ± 1.68b	10.65 ± 3.00a	< 0.0001
Thickness (mm)	14.11 ± 5.13a	11.28 ± 5.68b	< 0.0001
Area (cm^2)	135.04 ± 51.40b	235.45 ± 125.38a	< 0.0001
Weight (g)	124.63 ± 75.11b	201.31 ± 165.00a	< 0.0001

These results corroborate the findings of Freire et al. (2018); Gomes et al. (2016); Silva et al. (2015) and Cunha et al. (2012) in Little Sweet clone and Padilha Junior et al. (2016); Lucena et al. (2018b) and Lucena et al. (2019a) in Giant Sweet clone.

Figure 2a shows that all morphometric characteristics of the Little Sweet clone presented positive correlation. The highest correlations were between cladode area and length ($\rho = 0.88$), width ($\rho = 0.86$) and weight ($\rho = 0.82$). It was observed in Figure 2b that all morphometric characteristics of the Giant Sweet clone presented positive correlation. The highest correlations were between cladode area and length ($\rho = 0.92$), width ($\rho = 0.91$) and weight ($\rho = 0.82$).

Since the cladode area is estimated as a function of length and width, the bigger the cladode, the bigger its area will be, and consequently, the greater weight, as cladodes with smaller areas present lower weights, thus explaining the correlations mentioned.

Correlations between morphometric characteristics of forage cactus cladodes are known to be positively correlated, a fact that can be found in the reports of Cunha et al. (2012) observed a small positive correlation of cladode weight with length ($\rho = 0.59$), width ($\rho = 0.61$), thickness ($\rho = 0.73$) and area ($\rho = 0.65$) in *N. cochenillifera*

Little Sweet clone, Lucena et al. (2019a) in *N. cochenillifera* Giant Sweet clone (r > 0.95), Lucena et al. (2018a) in *Opuntia stricta* ($\rho = 0.90$), Reis, Gazarini, Fonseca, and Ribeiro (2018), ($\rho = 0.95$) in *Opuntia ficus-indica* correlating real cladode area with product of length by width and Guimarães, Donato, Azevedo, Aspiazú, and Silva Junior (2018) in *Opuntia ficus-indica* verified a positive correlation of weight with the cladode area of 0.86.
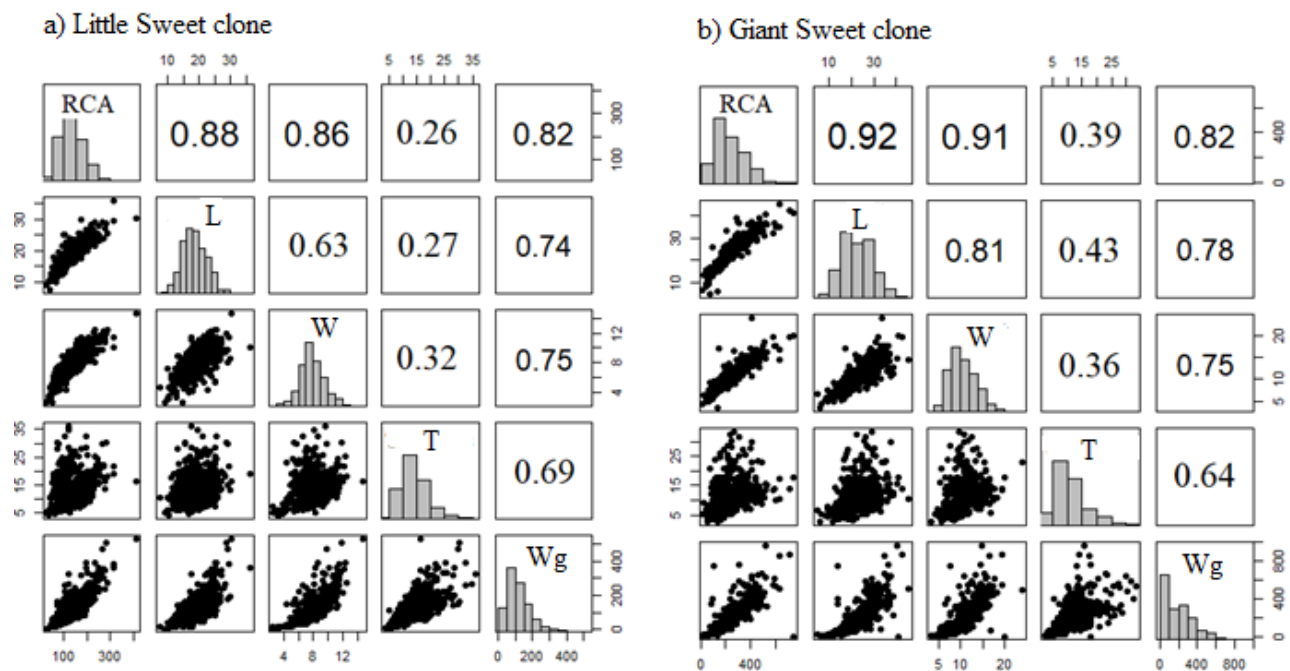

Figure 2. Correlation between morphometric measurements of *Nopalea cochenillifera* Little Sweet clone (a) and Giant Sweet clone (b).

Fisher's discriminant function was defined by Equation 8:

$$D(X) = -0.087L - 0.3W - 0.135T + 0.0056ACR + 0.002Wg \qquad (8)$$

Using the means of the morphometric variables of the Little Sweet clone cladodes (described in Table 1) the Fisher's discriminant function generated midpoint of D(X) = -4.88, while for Giant Sweet clone (means described in Table 1) the midpoint is given by D(X) = -4.98. Thus, to classify a cladode as Little Sweet the value of Fisher's discriminant function must be greater than or equal to (-4.93) = (-4.88-4.98)/2 otherwise cladode is classified as Giant Sweet clone.

Fisher's discriminant function can assist in the classification of *N. cochenillifera* cladode without the researcher's need to go to the field to observe which plant that cladode belongs to, only the measured cladode measurements should be at hand, thus saving time and cost.

Using Fisher's discriminant function verified a 99.41% $\left(\frac{575+437}{1018} * 100\right)$ hit rate to differentiate groups of *Nopalea cochenillifera* clone. The hit rate for Little Sweet clone was of 98.97% $\left(\frac{575}{581} * 100\right)$ while for Giant Sweet clone was of 100.00% $\left(\frac{437}{437} * 100\right)$ [Table 2].

The differentiation of the two clones of Nopalea cochenillifera with high precision when discriminant analysis is used can be explained due to the clones morphological structure itself, because the Giant Sweet clone cladodes are longer and wider, and consequently present a larger area and are heavier than the Little Sweet clone cladodes, and these characteristics enabled the high performance of clone discrimination.

Table 3 shows that with two canonical variables the explanation rate of the morphometric characteristics for the behavior of *Nopalea cochenillifera* clones is higher than 90%. Little Sweet clone with two canonical variables presented a contribution of 90.41%, while that Giant Sweet clone presented a contribution of 92.01%.

Table 4 shows that for Little and Giant Sweet clones the variables that most contributed to the explanation of the first canonical variable were the linear measurements (length, width, thickness and area), whereas for the second canonical variable the greatest contribution comes from the weight of the cladodes, contributing with 18.97% to Little Sweet clone characterization and 16.08% to Giant Sweet

clone. This cladode weight contribution rate for both clones can be explained due to the water storage that is performed by the cladodes.

Table 2. Classification of *Nopalea cochenillifera* clones by discriminant function.

Observed	Estimated		Total
	Little Sweet clone	Giant Sweet clone	
Little Sweet clone	575	6	581
Giant Sweet clone	0	437	437
Total	575	443	1018

Table 3. Importance of the canonical variables.

Canonical Variable	Little Sweet clone			Giant Sweet clone		
	Eigenvalues	Proportion of Variance	Cumulative Proportion	Eigenvalues	Proportion of Variance	Cumulative Proportion
CN1	3.5720	0.7144	0.7144	3.7965	0.7593	0.7593
CN2	0.9485	0.1897	0.9041	0.8040	0.1608	0.9201
CN3	0.3665	0.0733	0.9774	0.1830	0.0366	0.9567
CN4	0.0820	0.0164	0.9938	0.1735	0.0347	0.9914
CN5	0.0310	0.0062	1.0000	0.0430	0.0086	1.0000

Table 4. Contribution of morphometric characteristics for each canonical variable as a function of *Nopalea cochenillifera* clones.

Variables	Little Sweet clone		Giant Sweet clone	
	CN1	CN2	CN1	CN2
RCA	0.456	0.279	0.476	-0.189
L	0.460	0.188	0.464	-0.301
W	0.289	-0.852	0.309	0.873
T	0.498	0.315	0.490	-0.270
Wg	0.499	-0.248	0.471	0.196

Conclusion

Morphometric characteristics of cladodes can be used as parameters that help in the identification of *Nopalea cochenillifera* clones with high discriminatory power. Weight of cladodes is a very important measure in the characterization of clones of *N. cochenillifera*, it contributes with an explanation between 16 and 19% in the discrimination of clones. Fisher's discriminant function proved to be an important tool to differentiate the Little and Giant Sweet clones. Morphometric characteristics help us recognize physiological mechanisms of *Nopalea cochenillifera* clones.

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