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Article

Harvest Season and Morphological Variation of Canistel (*Pouteria campechiana*) Fruits and Leaves Collected in Different Zones of Mexico

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Abstract: Canistel (*Pouteria campechiana* (Kunth) Baehni) (yellow sapote, canistel). Originally from Mexico, the fruit is edible, and the tree is used as an ornamental and medicinal plant. Descriptive studies were carried out with the objective of morphologically characterizing the fruits and leaves of trees located in different regions of Mexico to select outstanding specimens and propose their massive reproduction. The trees were selected in three zones. In zone 2 from flower anthesis to harvest 11 months, fruits with 3 seeds, greater weight (180 to 330 g) and quantity of pulp (198.88 g), subglobose shape, total soluble solids 33 %. The fruits of zone 1 at harvest 8 months and zone 3 took 9 months to be harvested, in the rest of the characteristics evaluated were exceeded by the fruits of zone 2. To characterize the canistel (*P. campechiana*) leaf and identify accessions or cultivars, the three main variables, according to the principal component analysis were: leaf area, perimeter, and minor diameter. The three zones where canistel fruits and leaves were characterized are important and can be recommended for propagation in zones with favorable climatic conditions for fruiting in tropical and subtropical regions of Mexico.

Keywords: yellow sapote; tree selection; viviparity; leaf characterization; early seed germination

1. Introduction

Due to climate change, it is no longer necessary to focus research in obtaining seeds with high productive potential, to satisfy a changing and dynamic market at national and international level; the theme should be directed to produce species with high nutritional and medicinal potential; even more if the center of origin is Mexico, such is the case of *Pouteria campechiana* (Kunth) Baehni (synonymy: *Lucuma salicifolia* Kunth, *Lucuma campechiana* Kunth, *Lucuma nervosa* A. DC, *Achras lucuma*); commonly known as egg fruit tree, canistel, drunken sapote and yellow sapote [1].

There are reports that canistel presents compounds in the fruit such as polyphenols with antioxidant activity [2]; in seeds and leaves it contains flavonoids (myricetin, quercetin); phenolic compounds, terpenes, among other compounds; ethanolic extracts of canistel seed and leaves are used in alternative medicine, recommended as anti-inflammatory and gastrointestinal disorders by the phenolic compounds (gallate acid)[3]; antioxidant by the flavonoid compounds (myricetin and quercetin (myricetin and quercetin) [4, 5]. The bioactive compounds reported for this species may not be the same or differ in concentration, such is the case of phenols reported by Ma *et al.* [6] and Kong *et al.* [7]; these differences may be attributed to the environment in which they develop, season of the year, soil, and management [8].

These references justify the study of canistel in Mexico, where there are specimens in backyard cultivation in the Morelos state; located in the municipalities of Yautepec and Jiutepec; also, in the

Emiliano Zapata Experimental field (thirty trees) (south side of the municipality of Yautepec), the latter is the seed from subsidiary 1 of Jiutepec [9]; because of the canistel is a tree reported ornamental, medicinal and fruitful interest, it is important to describe samples of these trees from these three localities to implement propagation by seed.

The objective was to characterize fruits, seeds, leaves and harvest time of canistel trees located in different areas of Mexico. This information was fundamental, since the data obtained led to recommend this fruit tree in other areas with similar climatic conditions; The initial assignments were to plant trees in public spaces, gardens, educational centers and/or in crops associated with other fruit trees.

2. Materials and Methods

2.1. Study zones

Canistel collection regions were geolocated, as well as the climate description; zone 1 and zone 2, each with a tree under backyard cultivation; zone 3, corresponds to seed subsidiary 1 of zone 2.

2.2. Planting material: growing conditions and selection

Once the trees to be sampled were located with constant walks and monitoring (fruiting), fruits and leaves were harvested. The maturity criterion for fruit harvest was semi-ripe [10]; Leaves were collected ripe and of homogeneous color without phytosanitary problems; both leaves and fruits were transported in a cooler to the laboratory and placed on a table, so that they would lose the heat of the field [11].

After receiving the material, the fruits and leaves were cleaned with wet flannel. The cleaned, sanitized, and dried leaves were placed between two thin transparent glasses for 6 h so that they would not lose their shape.

2.3. Fruit characterization

The canistel fruits were numbered (20 fruits harvested at random), weighed on a digital balance (MediaDeta DS-5); the polar diameter (from the base to the distal part or protuberance of the fruit), the equatorial diameter (the widest part of the fruit) (digital caliper brand: Titan model: 55674 of 15.24 cm. China). Then, both pulp and seeds were weighed, and the number of seeds with premature germination was recorded. Total soluble solids (TSS) which indicate sucrose content and includes carbohydrates, organic acids, proteins, fats, and minerals of the fruit (28 to 62 % Fisher (Japan) refractometer) [12].

2.4. Morphological leaf characterization

Images of five mature leaves from the adaxial and abaxial face (Figure 1A) were captured in RGB under a digital camera (FUJIFILM FinePIX S8600, f=4.5 - 162 1:2 9-6.9 lens, Japan) and connected to a personal computer and stored in JPG format at 1322 × 3128 pixels. These images were converted to a grayscale format with ImageJ 1.43u software (NIH, USA), to set the correct scale (Analyze > Set Scale > adjust the actual distance) and the Threshold tool (from 5 ± 1 to 79) was applied to segment the image (Image>Threshold>Apply), with the segmented image the dimensions were evaluated in mm, with after selection of the variables to be measured. These measurements were performed with the Analyze>Measure tools. The results of the variables assessed for leaf characterization were arranged in a data matrix for processing and analysis. The selected variables are indicated in figure one (Figure 1A–C):

- Area: The number of pixels within a shape bounded by a perimeter, also called leaf area.
- Perimeter: The number of pixels at the boundary of the object, in this case the leaf.
- Major diameter: corresponds to the length of the leaf.
- Minor diameter: is the width of the leaf.

- Circularity: It is a measure of the ratio of the area and perimeter, which excludes local irregularities this value is equal to 1 for a circular object and less than 1 for an object that departs from circularity.
- Solidity: It is the density of pixels, it indicates how many pixels are joined without leaving blank spaces, an object with higher solidity has no blank spaces, and an object with low solidity has blank spaces, like gaps.
- The angle of the leaves was evaluated by manually segmenting and marking the main and secondary veins of each leaf on the abaxial face as follows: Freehand line> Draw> Measure.

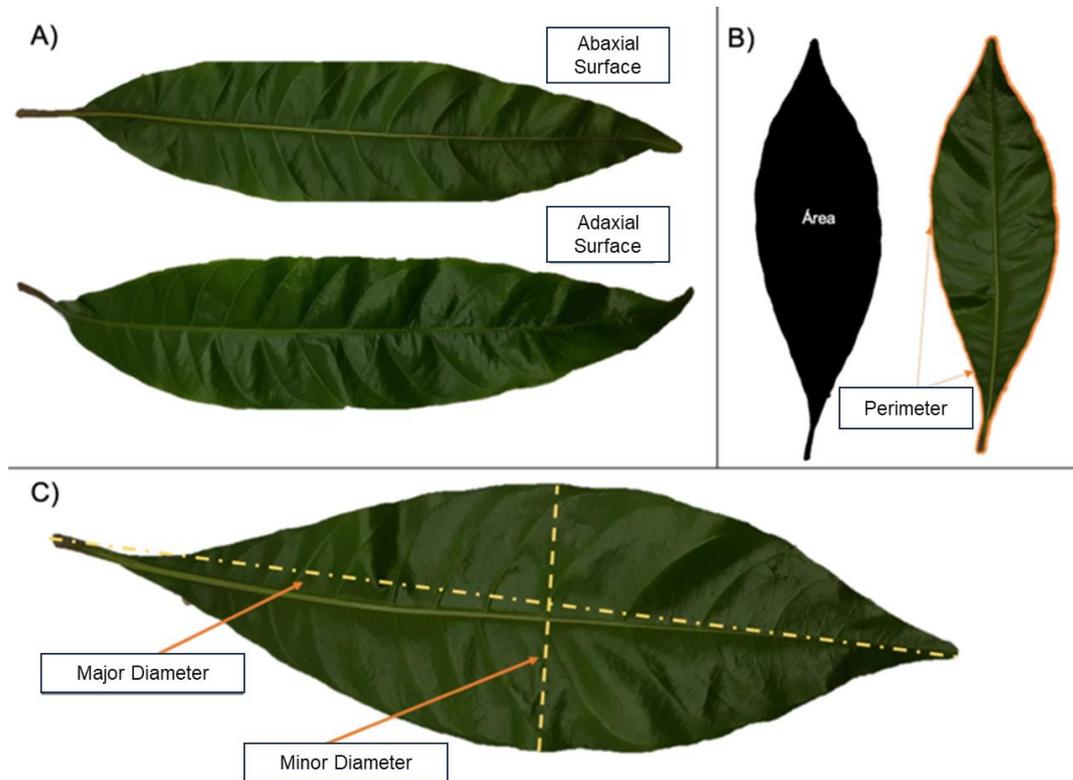


Figure 1. Canistel tree leaf with the evaluated variables description.

2.5. Statistical analysis

Statistical assessment of the fruits was performed using the mean \pm standard error of the mean (SEM), statistical significance by one-way analysis of variance (ANDEVA). Values $p < 0.05$ were considered significant. With the results obtained from the climate, fruit and leaf characteristics, the differences and similarities of the trees located in the three zones were statistically defined. The data obtained were processed by one-way analysis of variance (ANDEVA) with $p < 0.05$ and the application of Bonferroni's multiple comparisons test [13]. Values were analyzed with SPSS Statistics software version 20. Principal component analysis was performed with Minitab 17 software for leaf data only.

3. Results

3.1. Study zones

The characteristics of the three canistel leaf and fruit harvest zones were analyzed. Table 1 shows the climatic characteristics of each study zone to relate this information to the morphological variation of fruits and leaves (Figure 2A,B).

Table 1. Characteristics of the three collection areas of canistel (*Pouteria campechiana*) fruits and leaves considered as a phylogenetic resource in Mexico.

| Descriptive characteristics | Study Zones | | |
|-----------------------------|--|---|---|
| | Zone 1 | Zone 2 | Zone 3 |
| Geo location | 18.87595 LN-99.077464 LW | 18.884546 LN-99.17745 LW | 18.824988 LN- 99.096042 LW |
| Locality | Yautepec de Zaragoza | Jiutepec | Yautepec de Zaragoza |
| Neighborhood | Otilio Montaña | Centro de Jiutepec | San Isidro |
| m.a.s.l. ¹ | 1210 | 1350 | 1059 |
| Weather | Semi-humid sub-humid (66% of the year), the warmest of the temperate climates. Warm subhumid (34%), with summer rains, the driest of the subhumid climates, low oscillation. | Semi-warm and sub-humid (28 %), the coolest of the warm ones. Warm sub-humid with summer rainfall (72 %), the highest percentage of the year. | Warm sub-humid (100%), dry (low deciduous forest). |
| Precipitation | Summer, the intermediate of the sub-humid (rainfall concentrated in summer). Months without rain from November to April | The average annual rainfall is 1,021 mm and the rainy season is from June to October, with 890 mm. | Summer rainfall. Percentage of winter rainfall less than five. |
| Temperature | Hot summer (34 °C ± 4), extreme (at night temperature drops 18 °C ± 3); Ganges-type temperature march (with the highest temperature in May, before raining and the summer solstice; Ganges-type, due to the similarity of the climate in the Ganges River delta region in India. | Isothermal (the average thermal variation of the year does not exceed 3 °C), Ganges type temperature march. During the year it varies from 11°C to 32°C. The average 21.2°C; the maximum average variation is 31.4°C. The absolute maximum is 39.8 °C. The hottest months are April and May, and the coldest months are December and January. | Extreme hot summer, hot day (35 °C ± 3) in the evening temperature drops (20 °C ± 3); the rest of the year behaves similarly. |
| Harvest season (Months) | November- December | May- July | March- April |
| Early seed germination (%) | 0.2 | 30 | 0.1 |

¹m.a.s.l.: meters above sea level. [14].

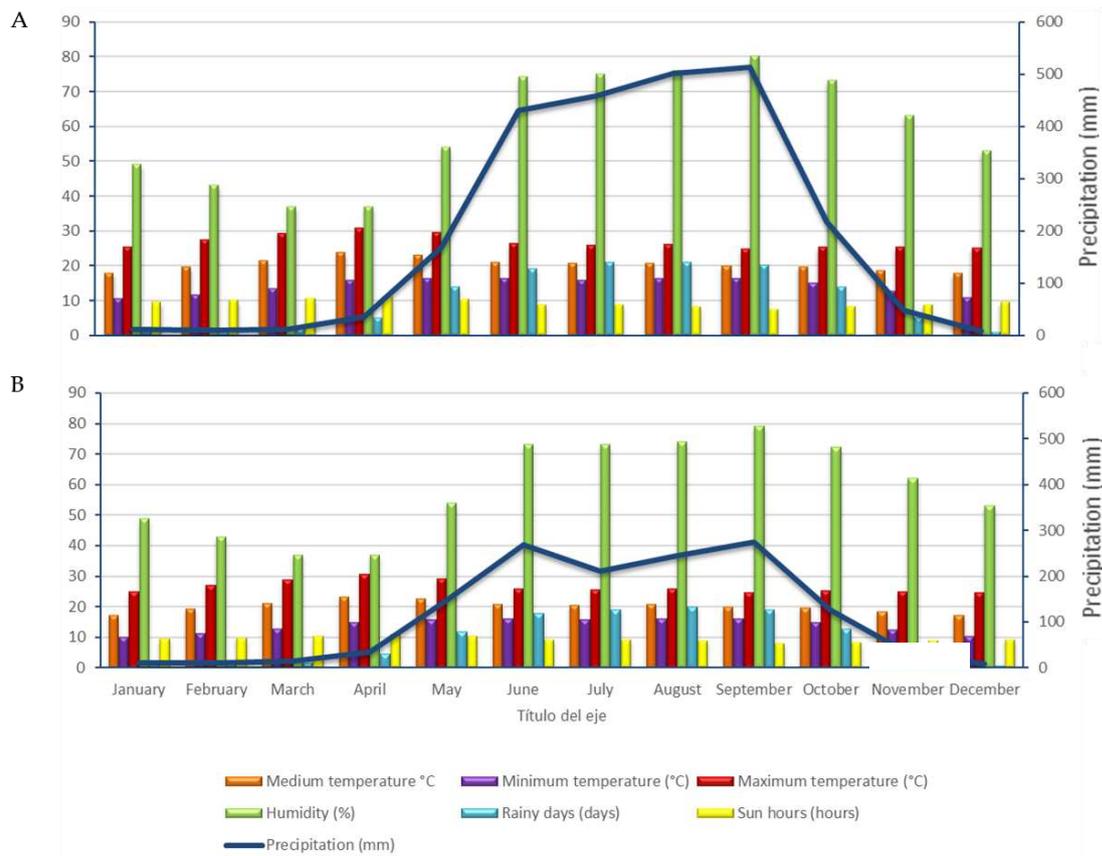


Figure 2. Climatic table with historical climate data in the collection zones of canistel (*Pouteria campechiana*) (A) Zone 1 and 3, (B) Zone 2.

3.2. Fruit characterization

The fruits of zone 2, presented greater weight (180 to 330 g), sub globose shape and quantity of pulp (198.88 g), 3 seeds with an average weight of 19.71 g. In addition, they showed significant statistical differences in polar and equatorial diameter and total soluble solids with respect to zones 1 and 3 (Table 2).

Table 2. Morphological and chemical characteristics of canistel (*Pouteria campechiana*) fruits collected in three areas of Mexico.

| Parameters | Zone 1 | | Zone 2 | | Zone 3 | |
|--------------------------------|---------------------|--------------------|----------------------|--------------------|---------------------|--------------------|
| | Mean | Standard deviation | Mean | Standard deviation | Mean | Standard deviation |
| Fruit weight (g) | 146.24 ^b | 25.11 | 261.94 ^a | 76.78 | 160.18 ^b | 51.64 |
| Fruit polar diameter (cm) | 9.49 ^b | 1.16 | 11.03 ^a | 0.84 | 9.07 ^b | 0.97 |
| Fruit equatorial diameter (cm) | 5.49 ^b | 0.46 | 7.29 ^a | 0.96 | 6.03 ^b | 0.93 |
| Pulp diameter (g) | 122.76 ^b | 27.62 | 198.88 ^a | 54.39 | 101.26 ^b | 35.87 |
| Number of seeds | 1.12 ^b | 0.33 | 2.59 ^a | 0.87 | 1.29 ^b | 0.47 |
| Seed weight (g) | 10.12 ^b | 2.26 | 19.71 ^a | 5.68 | 19.78 ^a | 3.29 |
| Seed polar diameter (cm) | 4.29 ^a | 0.45 | 4.49 ^a | 0.26 | 5.50 ^b | 0.57 |
| Seed equatorial diameter (cm) | 2.08 ^a | 0.28 | 2.24 ^{a, b} | 0.17 | 2.40 ^b | 0.22 |
| Total soluble solids (SST) | 27.47 ^b | 2.79 | 33.18 ^a | 0.39 | 26.12 ^b | 1.22 |

Values in the same row and sub table that do not share the same subscript are significantly different at $p < 0.05$ in the test for bilateral equality of column means. The tests assume equal variances ¹⁴Using the Bonferroni correction.

In canistel, from anthesis (Figure 2) to fruit harvest in zone 2 was 11 months, in zone 1 8 months and in zone 3 9 months; the morphological variation of the fruits can be seen in Figure 2, which shows the fruits collected in the 3 zones.

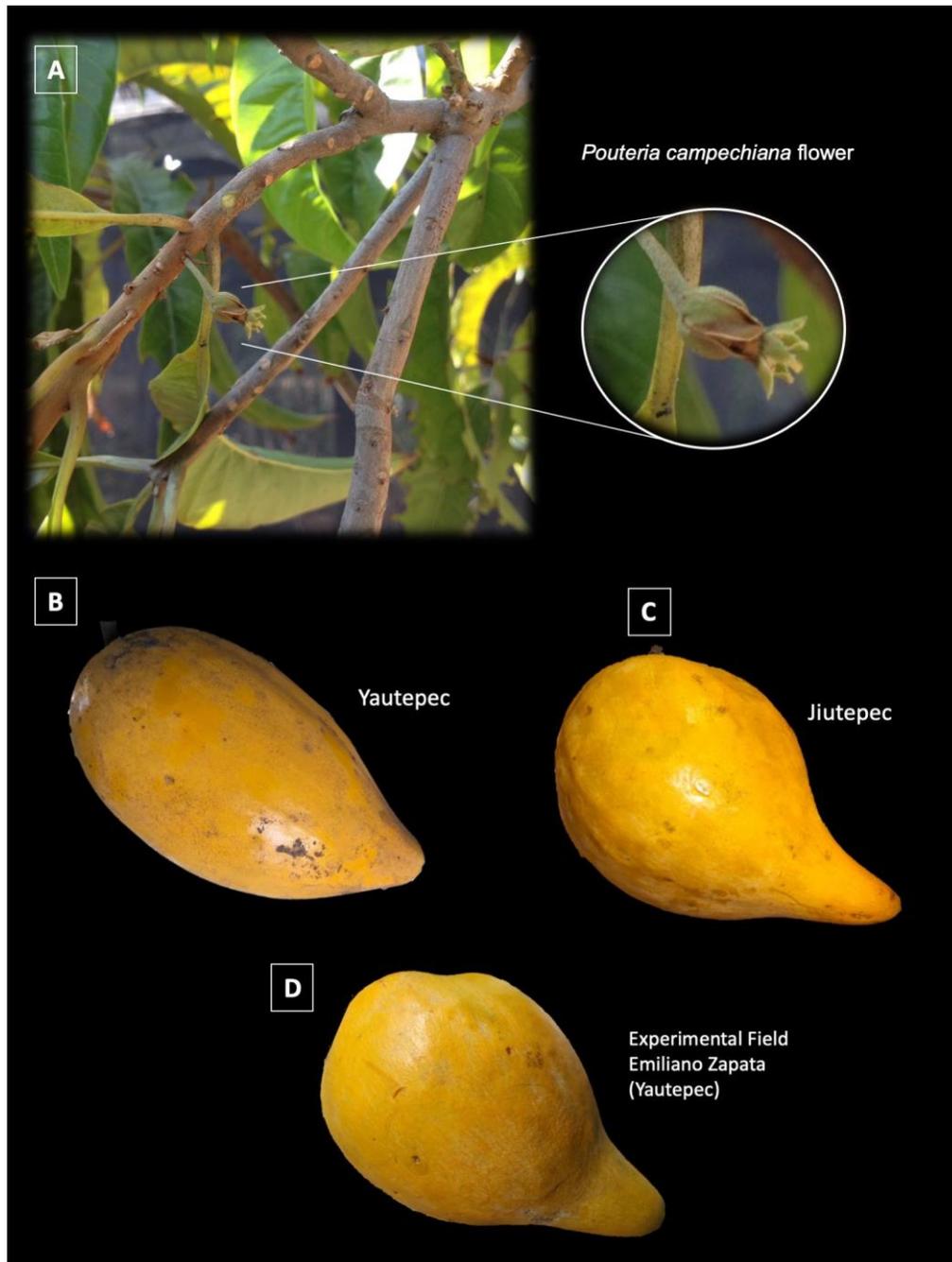


Figure 3. (A) Canistel (*Pouteria campechiana*) flower at anthesis of and fruits of the three zones, (B) zone 1, (C) zone 2 and (D) zone 3.

3.3. Leaf morphological characterization

The leaf area of the 3 zones was statistically different, with zone 3 having the largest leaf area. Leaf perimeter in the three zones showed different values, zone 3 with a greater perimeter than zone 2 and zone 1 respectively; major diameter (length) with a significant difference in zone 2, zones 1 and

3 are statistically equal; the minor diameter in the three zones was different. The circularity index for the three zones was different and for the soundness index only zone 3 showed a difference. The angle variable was different only in zone 3 (Table 3).

Table 3. Morphological characteristics of leaves collected in three zones of Mexico.

| Parameters | LEAVES | | |
|----------------|--------------------|--------------------|--------------------|
| | Zone 1 | Zone 2 | Zone 3 |
| | Mean | Mean | Mean |
| Area | 52.52 ^b | 48.95 ^a | 63.54 ^c |
| Perimeter | 44.79 ^b | 46.73 ^a | 48.46 ^c |
| Major diameter | 16.14 ^b | 16.52 ^a | 15.99 ^b |
| Minor diameter | 4.10 ^b | 3.77 ^a | 5.07 ^c |
| Circularity | 0.33 ^b | 0.28 ^a | 0.34 ^c |
| Solidity | 0.92 ^a | 0.92 ^a | 0.90 ^b |
| Angle | 48.46 ^a | 45.42 ^a | 56.67 ^b |

Note: Values in the same row and subtable that do not share the same subscript are significantly different at $p < 0.05$ in the test for bilateral equality of column means. Tests assume equal variances¹¹ Using Bonferroni correction.

3.4. Correlation analysis

In the correlation analysis of the morphological variables assessed in the canistel leaves, a very high correlation was found for the variables area and minor diameter, with a high correlation between perimeter and major diameter, as well as minor diameter and circularity. The variables area and circularity, area and perimeter were moderately correlated (Table 4).

Table 4. Pearson correlation between the variables measured in the canistel leaves of the 3 zones.

| | Area | Perimeter | Major diameter | Minor diameter | Circularity | Solidity |
|-----------------------|------------------------------|-------------------|-------------------------------|------------------------------|------------------------------|-------------------------------|
| Perimeter | 0.643 0.000** | | | | | |
| Major diameter | 0.515 0.000** | 0.760 0.000** | | | | |
| Minor diameter | 0.911 0.000** | 0.399 0.000** | 0.120 0.017* | | | |
| Circularity | 0.552 0.000** | -0.279 0.000** | -0.171 0.001** | 0.700 0.000** | | |
| Solidity | -0.291 0.000** | -0.466 0.000** | -0.076 0.129 ^{NS} | -0.324 0.000** | 0.162 0.001** | |
| Angle | 0.277 0.000 ^{NS} | 0.018 0.724** | -0.070 0.163** | 0.356 0.000 ^{NS} | 0.331 0.000 ^{NS} | -0.124 0.013 ^{NS} |

Not significant^{NS}, and significant at a $p \leq 0.05^*$ and $p \leq 0.001^{**}$.

3.5. Principal component analysis

In the principal component analysis, the eigenvalues indicated that the first principal component contributes 43.7 % of the total variability, the second component 28.1 %, and the third component

14.5 %; this shows that the combination of the first three components describes the 86.4 % variability of the data (Table 5).

Table 5. Eigenvalues, absolute and cumulative percentage of variance of the principal components of the canistel leaves analysis.

| Principal component | Eigenvalue | Variance (%) | Cumulative Variance (%) |
|---------------------|------------|--------------|-------------------------|
| 1 | 3.0615 | 43.7 | 43.7 |
| 2 | 1.9676 | 28.1 | 71.8 |
| 3 | 1.0175 | 14.5 | 86.4 |
| 4 | 0.7358 | 10.5 | 96.9 |
| 5 | 0.2118 | 3 | 99.9 |
| 6 | 0.0045 | 0.1 | 100 |
| 7 | 0.0013 | 0 | 100 |

In relation to the eigenvectors, the variable that contributed most to the first component was the area with 0.559, then was the smallest diameter with 0.507; in the second principal component were the variables perimeter (0.476) and circularity (-0.588); finally, in the third component were the variables solidity with -0.765 and largest diameter with -0.434 (Table 6).

Table 6. Eigenvectors of the first three principal components from the analysis of the variables in the canistel leaves.

| Variable | Principal Components | | |
|----------------|----------------------|---------------|---------------|
| | 1 | 2 | 3 |
| Area | 0.559 | -0.056 | -0.174 |
| Perimeter | 0.408 | 0.476 | 0.006 |
| Major Diameter | 0.300 | 0.454 | -0.434 |
| Minor Diameter | 0.507 | -0.270 | 0.029 |
| Circularity | 0.256 | -0.588 | -0.243 |
| Solidity | -0.254 | -0.216 | -0.765 |
| Angle | 0.212 | -0.313 | 0.370 |

The two-dimensional diagram shows the projection of the variables on the first 2 principal components. The variables projected on the plane, match the results obtained, where area and minor diameter contribute most to the variation of the data in the first component; in the second component they were perimeter and major diameter (Figure 4).

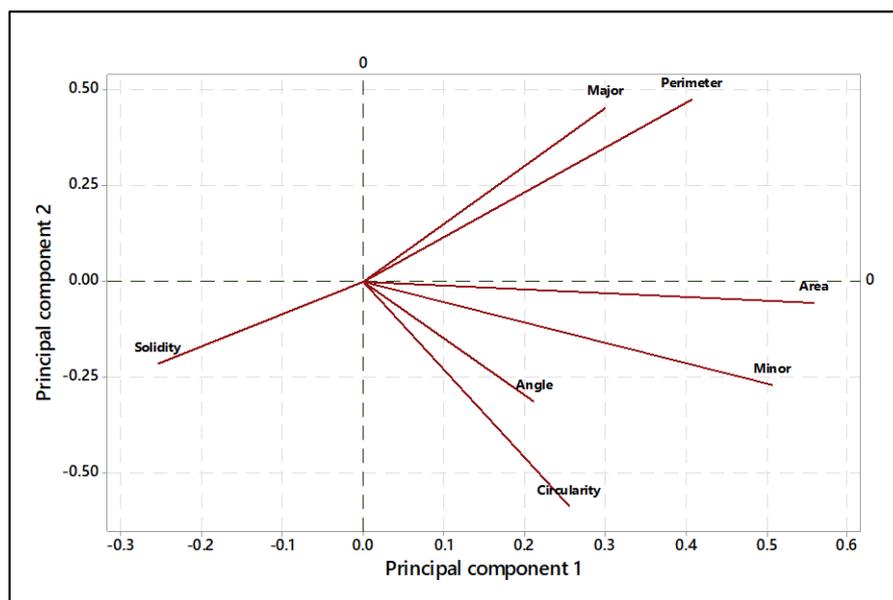


Figure 4. Two-dimensional diagram for the variables measured on the leaves, showing the projection of the zones on the first two principal components.

4. Discussion

The altitude above sea level (m.a.s.l.) and climate showed differences in the three collection zones (Table 1). Zone 2, had the highest altitude (1350 m), followed by zone 1 (1210 m) and 3 (1059 m). Pennington and Sarukhán [15], reported that canistel has been found at sea level and up to 1400 m in tropical and subtropical climates, this indicates that they are within altitude conditions. In general, altitude has an influence on the time of fruit development and ripening; this was reflected in the time of harvest; the fact that fruit development takes longer does not mean that the quality decreases [16] (Table 1).

Zone 2 is isothermal; this makes it different from the other two zones; 72% of the year it has a warm climate, the average annual temperature is higher than 22 °C; it is sub-humid most of the year. In the three zones, April and May are the months in which high temperatures predominate and precipitation is in summer; the heat wave is presented in the three zones, more markedly in zone 1, the community of this zone calls it Verano de Santiago (days when it does not rain), [17]

The climatic conditions of zone 2, could be related to the number of seeds, those of this zone presented on average 3 seeds and a high percentage of viviparity (30 %). In studies such as *Atapattu et al.* [18] (2014) reports weight of 175 g (Sri Lanka, average temperature of 29 °C); *Kong et al.* [7] reports weight of 118.09 ± 35.48 g (Malaysia, average temperature of 28 °C); The fruits of zone 2, exceed these weights, but not those of zone 1 and 3.

The fruits of zone 2, take longer to reach the semi-mature stage, and the climate is more stable, during the development period, the amount of precipitation is lower; as Dussi [16] mentions that the fruits may take more or less time to develop, this does not demerit the quality, apparently, they accumulated more sugars.

Multiple factors could contribute to premature seed germination, Cota-Sánchez *et al.* [19] mentioned that it is possible for the local environment and the age of the tree. Baskin and Baskin [16] mentions to these factors adds genotype; early germination in angiosperms can occur in fruit seeds in the same species but grown in different environments [20].

Among the factors also studied is temperature, Penfield, and MacGregor [21] report that high temperature is detected by the plant and transmitted to the fruit developing through signaling. Canistel fruits from zone 2, were harvested during the months of May to July (Table 1); These were the highest temperature months. This study suggests that the temperature factor had an influence on early seed germination [22].

The accumulation of proteins and starch also favors premature germination, as demonstrated by Borisjuk *et al.* [23] in broad bean (*Vicia faba*); in canistel in green state the humidity is 33.9 %, the pulp has 16 °Bx, and starch 38.9 % which could favor premature germination. Farnsworth [24] writes that to understand the physiology it is necessary to relate the ecology and evolution of the species. Colunga-García-Marín and Zizumbo-Villarreal [25], catalog the canistel as a non-domesticated fruit tree.

On the other hand, with the data obtained from the leaves, 84.6 % of the variability represented by the first three principal components is sufficient to explain the variability that exists among the leaves of the three study zones with the selected variables. According to these data, leaf area, minor diameter and perimeter are the variables that differentiate the leaves, of which the area measures the size, the perimeter the shape and the minor diameter the width or amplitude of the leaf, which is also positively related to the area. There are different factors by which morphological changes occur in plants that, although belonging to the species, there is variability mainly in shape and size, one of these factors is temperature [26], in this case, although the canistel crops are relatively close, the climatic conditions, especially temperature, directly influences the development of the plant and fruiting.

By its nature, the production of fruit trees is a source of permanent employment, income, other activities, or products derived from them, so that the cultivation of fruit species is, since its inception, an activity of great economic and social importance within the agricultural industry.

5. Conclusions

The canistel fruits and leaves studied morphologically in three zones of Mexico, it was found that the tree located at the highest altitude above sea level (zone 2) presented the highest number of seeds and high early germination; they took more months to become semi-ripe; the main attributes were the high percentage of flesh and the percentage of total soluble solids. The tree from zone 3 (subsidiary 1 from zone 2) behaved differently from those harvested in zone 1 and zone 2.

The leaves of the tree are elongated in shape and the angle formed by the primary ribs with respect to the secondary ribs are narrow (webbed appearance), the leaf area is smaller and consequently the perimeter is smaller. In terms of solidity, it means that the perimeter is smooth with very little undulation.

To characterize the canistel (*P. campechiana*) leaf with these three main variables (leaf area, perimeter, and minor diameter) it is possible to identify accessions or cultivars.

Author Contributions: Conceptualization, SEL and KMGV; methodology, SEL and KMGV; software, KMGV and JFPB; validation, SEL, TRG and JFPB; formal analysis, KMGV; investigation, SEL, KMGV, SLEA and JGCC; resources, SEL; data curation, writing—original draft preparation, SEL and KMGV; writing—review and editing, SEL, JFPB, TRG and KMGV; supervision, SEL; project administration, SEL; funding acquisition, SEL. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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