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Foliar Morphometric Study of *Jatropha curcas* L. (Euphorbiaceae)

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Abstract: *Jatropha curcas* L., is popularly known as physic nut, is native to Brazil and it belongs to the Euphorbiaceae family, whose seed is extracted an oil that might be used in the production of biodiesel. It is one of the only oil varieties that do not directly compete with food agriculture. Despite the great potential of *Jatropha*, several factors must be addressed for full domestication of the species. In this sense, studies aimed at establishing methodologies and morphoanatomical parameters which allow to detect whether or not differences between populations are essential. The objective of this study make a foliar morphometric analysis of three populations of *J. curcas* L. from the farm located in the Municipality of Piracuruca, Piauí, Carnauba. Geometric morphometry of anatomical landmarks was used. The study was conducted from March 2013 to June 2014. 30 leaves of each population were collected and selected eight anatomical landmarks. The principal component analysis showed that the first three principal components were significant using the "broken stick" model, comprising 60.5% of the total variance. MANOVA only showed significant difference between two of the three pairs of populations, when performed without the alignment of Procrustes. There are no significant differences between populations.

Keywords: Leaf Characters, Morphometry, Physic Nut

1. Introduction

The interest in renewable sources of energy has grown due to both economical and environmental high costs of production. *Jatropha curcas L.,* Euphorbiaceae, is an oily plant that has been highlighted as an alternative for the production of biofuels [1]. The adoption of pinhão-manso as a potential culture to fulfill the National Program of Biodiesel Production (PNPB) occurs mainly due to the potential of oil yield; the fact that it is a non-edible species; and also due to its handling, which is compatible to the profile of family agriculture. The oily plant has being implanted in several areas in Brazil [2].

Jatropha curcas L. is being considered an agricultural option for the Brazilian semiarid conditions because it is a native species which demands insolation and is strongly resistant to draught [3]. Pinhão-manso seeds are rich in oil and have all the necessary qualities to be transformed into diesel oil. The seed presents good conservation after harvesting, besides its smooth and easy cultivation, and it may become a great producer of raw material as an optional source of fuel [4]. Leaves are deciduous, alternate and subopposite, with a spiral phyllotaxis, and 105° away from the preceding one. 3-5-lobed, base cordate. New leaves present a dark red coloration due to the presence of anthocyanins. Old leaves of pinhão-manso are green, sparse and glossy, large and alternate, palmate with three to five lobes and petiolated, with whitish and salient veins in the lower face. Mature leaves have a green to yellow coloration and they soon get brown when totally dried [1]. The shape of the leave may have slight variations according to origin access of the plants [5].

Despite the potentialities of pinhão-manso, several factors may be solved for its complete domestication; for example, the knowledge of dimension and width of the available genetic variability is essential for the advancement and sustainability of the genetic gain process by selection in the improvement [6]. Not much is known about this plant and even some agronomic aspects should be better investigated, as it has a high genetic variability, with preferably entomophilic pollination, and may have possible high alogamy [7]. Behavioral aspects of the plant should be clarified in field or laboratory level, so that pinhão-manso may have full agricultural use [8]. Studies that use characters to investigate the diversity of organisms are important as long as they enable selecting those characters that better discriminate the accesses and discharge others that slightly contribute to the discrimination of genotypes of a certain species [9]. Although reseach on genetic diversity is also essential, there is a need to perform morphometric analyses and studies involving shapes and differences among populations, creating references for such comparisons, such as the physiological characterization of the plant and the evolutionary inference [10]. The potentialities of *Jatropha curcas* L. make it an interesting species for studies on morphologic changes.

The goal of this research was to check possible morphometric changes in *J. curcas* L. leaves from three populations in the municipality of Piracuruca-PI (Figure 1). Quantitative characters of leaves, i.e., anatomical markers, size and shape of the leaf, were used for this study. Our initial presupposition was that, since the populations were from different origins, there might probably be variations in the shape of the leaf, such as shown in research developed by [5].

2. Materials and Methods

Figure 1. *Location map of the municipality of Piracuruca-PI, where the samplings of Jatropha curcas L. were done. Source: Wikipedia.*

The collection of botanical material was performed at Fazenda Carnaúba, located in the municipality of Piracuruca, Piauí. Latitude: 03° 55’41”S; Longitude; 41° 42’ 33” W. At a 60 meters altitude. According to the Köppen classification, the climate of that area is classified as Aw- tropical with dry season. Three populations of *Jatropha curcas* L. were collected in three plots of Carnaúba farm, such as: EMBRAPA, Ecodiesel and Rio Pardo. Thirty plants out of each one of the three populations, and then three leaves of each plant were randomly sampled totaling 90 leaves. The selected leaves were pin fixed in a clear background with its abaxial surface (more prominent vein patterns) presented and individually photographed with a digital camera GE X500 vertically placed.

The selection of eight anatomical markers was based on the shape of the leaf and in the foliar venation on its border (Figure 2). The best based markers as homology are 1 and 2 respectively, representing the junction of the petiolate to the blade and the apex of the leaf.

Figure 2. *Marking and description of eight homologous points in Jatropha curcas L. leaf through the program TpsDig (Rohlf 1999).*

The number of markers was the same in all specimens. The images were imported to the program TpsDig [12], which was used for the digitalization of the eight anatomical markers, and for the capture of the coordinates of the anatomical markers producing a total of 16 coordinates for each leaf; each pair (x_i, y_i) represented one marker [13]. Those data were stored by TpsDig in a text file in “TPS” format. Each image was reduced up to approximately 100KB to enable the easy use of the program TpsDig2, and the coordinates of each leaf were stored in a “TPS” text file and imported directly into PAST software version 2.7 [14], which was used to perform all the analyses. Procrustes analysis [15] was used, and all analyses used this matrix of standardized coordinates as a starting point.

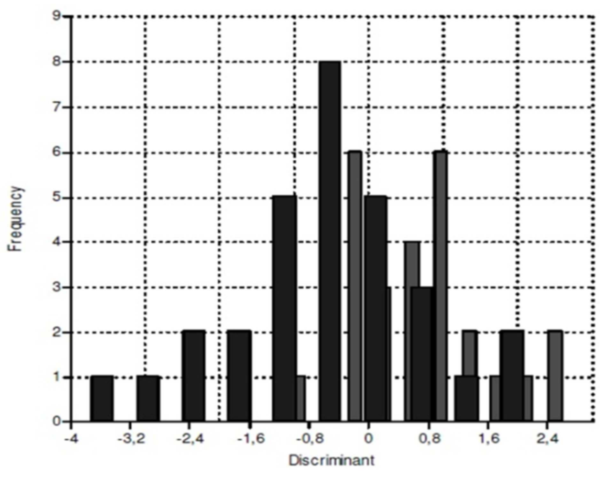
The PAST program was used to perform the Principal Components Analysis (PCA) of the linear data matrix. The individuals were treated as if they belonged to the same population, which allowed the study of the global variation of characters and the identification of the most important gradients in the variability of foliar morphology. Through the results obtained, the highest variation gradients were identified in the following data: the Principal Components. Principal Components Analysis (PCA) of the matrix of all the individuals was employed to study the global variation of anatomical markers settings and to identify the most important gradients in the variability of foliar morphology. Those gradients, the Principal Components (PCs), were visualized through “Thin Plate Spline” reconstruction of deformations corresponding to the minimum, medium and maximum values of each one of the two first PCs.

Discriminant Function Analysis (DA) was employed to compare the shape of the leaves. The implementation of this technique in PAST visualizes the separation or superposition of two groups through a frequency graphic as the three populations in contrasting colors. The Hotelling’s T^2 test was used to estimate the significance of the difference among populations.

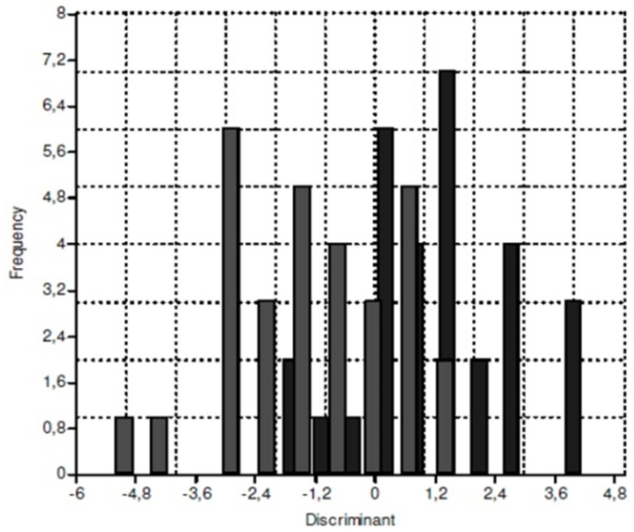
Canonical Variates Analysis (CVA) was used to compare the populations; the implementation in PAST enables the visualization of mean shapes of each population through a reconstruction of deformations through “Thin Plate Spline”. CVA was also used to compare variations in foliar shape of the three populations. MANOVA was used to compare the similarities among pairs of populations, using the option *Tranform>Procrustes* 2D/3D among populations.

3. Results and Discussion

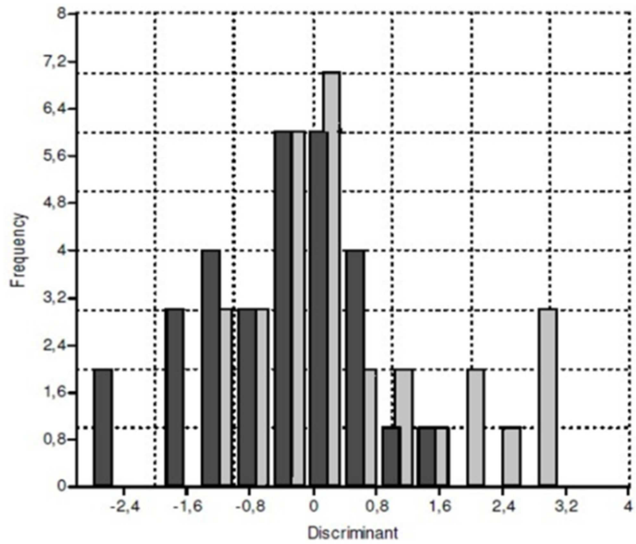
The results obtained through the plotting (Figure 3) show an overlapping in PC1 and PC2 among the populations, neither having a differentiation nor a grouping among the populations. The eigenvalues (*PCA scree plot*) showed that eight Principal Components were necessary to express 95% total variance; the four first PCs only accounted for 87.83% showing that there was not a significant variation among the three populations. The result of the Discriminant Analysis did not show a significant difference among population means. For the populations Ecodiesel and EMBRAPA [Hotteling’s T^2: p (same)=0.06983], only 68.33% of individuals were correctly classified, which was evident in the frequency plotting. For the populations EMBRAPA and Rio Pardo, [Hotteling’s T^2: p(same)=0.7424], only 62.07% of individuals were correctly classified. And for populations Ecodiesel and Rio Pardo, [Hotteling’s T^2: p(same)=0.08121], 70% of individuals were correctly classified. Accounting for a mean of 66.6% of individuals correctly classified among populations. The other individuals grouped in populations different from the population of origin, according to this analysis.



A)



B)



C)

Figure 3. *Discriminant Analysis (DA) of Jatropha curcas L. leaves (in A: Ecodiesel, B: EMBRAPA and C: Rio Pardo).*

The Multivariate Analysis of Variance (MANOVA) did not result in significant differences among populations when it was done with Procrustes 2D/3D alignment. Ecodiesel and EMBRAPA (Wilks lambda =0.755, df1=16, df2= 43, F=0.8719, p(same)= 0.6029). Populations EMBRAPA and Rio Pardo (Wilks lambda =0.7649, df1=16, df2= 43, F=0.8262, p(same)= 0.6504). And Ecodiesel and Rio Pardo (Wilks lambda =0.7267df1=16, df2= 43, F=1,01 p(same)= 0.4647). However, when the same test was performed without the Procrustes 2D/3D alignment there was a significant difference between the pairs of populations Ecodiesel/EMBRAPA and EMBRAPA/Rio Pardo. It could be noticed that the Rio Pardo population was the most differentiated from the others because with or without Procrustes 2D/3D it did not present any significant differences when compared to the populations EMBRAPA and Ecodiesel.

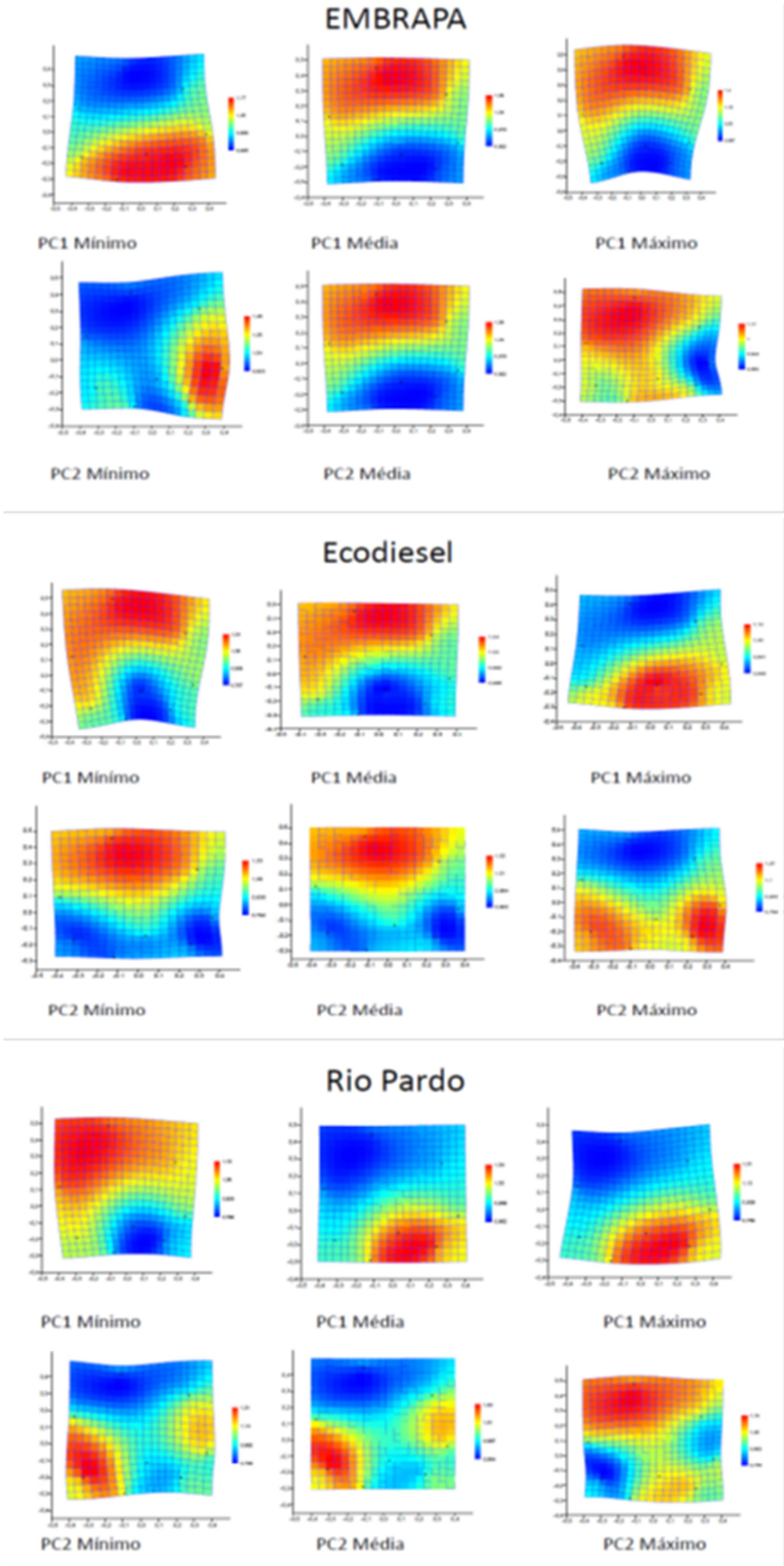


Figure 4. *Principal Components Analysis (PCA), pinhão-manso leaves (Jatropha curcas L.). EMBRAPA, Ecodiesel and Rio Pardo. Deformations represented by the first two Principal Components. A background progressively to red expresses expansion; to blue, it expresses narrowing in the deformations. The terms minimum, medium and maximum indicate deformation score values respectively along the axis of the canonical variates. Computed in PAST vers. 2.17 (Hammer et al. 2001).*

The main aim of the Principal Components Analysis in this study was to indicate which types of variation in the shape of the leaf were inside the total set of 90 leaves. Each principal component represents a shape variable and, therefore, it expresses a type of variation. PCA tries to express the variance and the correlation of original characters by changing them into a lower number of new variables (the principal components) that encompass more than 95% total variance and covariance of the sampling [13]. The main deformations along the first two Principal Components were visualized (Figure 4). They are extreme shapes along the two axes that separate more the means of the populations regarding their foliar shape. And highlight that the extreme shapes correspond to low values (left) and high levels (right). As for the symmetry of Ecodiesel foliar deformation, PC1 expressed asymmetric deformations while PC2 presented symmetry in relation to the main axis of the leaf. In PC1 the score decrease is followed by a contraction in the base of the leaf and a longitudinal expansion in the upper half of the leaf. In PC2 the score decrease causes decrease in the right and left lower lobes and apex expansion. Score increase results in the opposite. In EMBRAPA, concerning symmetry, the opposite of Ecodiesel happened when compared to PCs 1 and 2. Rio Pardo presented symmetry in PC1 and a great symmetry in PC2. PC1 with score decrease in base contraction and apex expansion. With score increase in PC2, however, expansion happened in both the apex and the base. By comparing PC2 of Ecodiesel and Rio Pardo, it was possible to observe that they are opposite. Comparing EMBRAPA and Rio Pardo, PC1 has opposite behaviors with score increase or decrease. In relation to PC2, it could be noticed an arrangement similar to the reflection of a mirror, which highlighted its significant separation that was also shown in MANOVA analysis.

It is reasonable to infer that symmetric PCs of greater importance, such as Rio Pardo's PC1, EMBRAPA's PC2 and Ecodiesel's PC1 are more influenced by genetic factors than by asymmetric factors, bearing in mind research such as [17] that demonstrated greater correlation between symmetric form and genetic factors variables in leaves and petals of growing varieties of soybean, citrus and primrose. Those studies showed that the asymmetric variation tends to express more environmental factors. When associated to MANOVA, it was possible to infer about the foliar variation of the sampled populations, which highlighted the distant from the Rio Pardo population in relation to the others. However, those comparisons revealed that there is a difference between the shapes of the leaves among populations, but it was only significant in statistical parameters. Gradients of morphologic variation established in this study may serve as a basis for a higher scale study, comparing more distant and more isolated populations.

4. Conclusion

The expected result based on previous studies would be a difference in the shape of the leaf among populations, since they are from different origins; however, the study did not show any significant difference between the pairs of the populations Ecodiesel-EMBRAPA and Ecodiesel–Rio Pardo. Based on MANOVA analyses there are significant differences between the pair EMBRAPA/Rio Pardo. More detailed studies need to be done for a higher understanding of foliar variations in pinhão-manso, such as the genetic ones, because different growings may be genetically different, although significant differences were not detected in the shape of the leaves with such analysis.

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