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Sex expression and floral diversity in *Jatropha curcas*: A population study in its center of origin

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Sex expression and floral morphology studies are central to understand breeding behavior and to define the productive potential of plant genotypes. In particular, the new bioenergy crop *Jatropha curcas* L. has been classified as a monoecious species. Nonetheless, there is no information about its reproductive diversity in the Mesoamerican region, which is considered its center of origin and diversification. Thus, we determined sex expression and floral morphology in *J. curcas* populations from southern Mexico and Guatemala. Our results showed that most of *J. curcas* specimens had typical inflorescences separate sexes (monoecious), meanwhile the rest were atypical (gynoeceous, androeceous, andromonoecious, androgynomonoecious). The most important variables to group these populations, based on a discriminant analysis, were: male flower diameter, female petal length and male nectary length. From the southern Mexico "Guerrero" was the most diverse population and in Chiapas "Centro". On the other hand, a cluster analysis showed that the accessions from southern Mexico were grouped without showing any correlation with the geographical origin, while those accessions with atypical sexuality were grouped together. Additionally, a Mantel test showed a significant correlation between the distance matrix generated in this study and the genetic distance matrix (AFLP) previously reported for the same accessions. Our results contribute to design genetic improvement programs by using sexually and morphologically contrasting plants from the center of origin.

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Abstract

Sex expression and floral morphology studies are central to understand breeding behavior and to define the productive potential of plant genotypes. In particular, the new bioenergy crop *Jatropha curcas* L. has been classified as a monoecious species. Nonetheless, there is no information about its reproductive diversity in the Mesoamerican region, which is considered its center of origin and diversification. Thus, we determined sex expression and floral morphology in *J. curcas* populations from southern Mexico and Guatemala. Our results showed that most of *J. curcas* specimens had typical inflorescences separate sexes (monoecious), meanwhile the rest were atypical (gynoecious, androecious, andromonoecious, androgynomonoecious). The most important variables to group these populations, based on a discriminant analysis, were: male flower diameter, female petal length and male nectary length. From the southern Mexico "Guerrero" was the most diverse population and in Chiapas "Centro". On the other hand, a cluster analysis showed that the accessions from southern Mexico were grouped without showing any correlation with the geographical origin, while those accessions with atypical sexuality were grouped together. Additionally, a Mantel test showed a significant correlation between the distance matrix generated in this study and the genetic distance matrix (AFLP) previously reported for the same accessions. Our results contribute to design genetic improvement programs by using sexually and morphologically contrasting plants from the center of origin.

Keywords: breeding, Chiapas, flowering, Mexico, multivariate analysis

Introduction

Due to oil content of its seeds, *Jatropha curcas* is a plant that has recently attracted interest as a scientific model and as an agro-industrial crop. However, cumulative knowledge of this plant's biology and ecology is still limited, recent studies locate the Mesoamerican region as its center of origin and diversity.

Nonetheless, many studies about *J. curcas* have been performed with Asian and African accessions, where low genetic diversity is registered. Moreover, in the Mesoamerican region, particularly in Chiapas, Mexico there are few studies on genetic variation. Sánchez-Gutiérrez (2010) studied 147 accessions from five populations (Istmo, Frontera, Frailesca, Centro, and Soconusco) using AFLP markers; they found that the largest variation was within populations (94.2 %), while among populations the variation was 3.9 %, and the "Istmo" population was the most diverse. In other study, Pecina-Quintero *et al.* (2011) analyzed 88 accessions from seven regions in Chiapas by using AFLP markers; they found that one of the accessions (Tuxtla Chico) provided 100% of pistillate flowers (female) and showed the highest number of rare fragments. Thus, the use of molecular markers and morphological traits may be useful to differentiate populations. Heller (1996) described *J. curcas* morphological traits, and found size changes in canopy, stem, root, bark and leaves. These variations on morphological traits are being observed in Indian landraces, on seed allometry (Ginwal *et al.* 2005) and morpho-physiological variation (Saikia *et al.* 2009). The parameters evaluated by Saikia *et al.* (2009) were; plant height, stem girth, branches per plant and 100 seed weight. Nonetheless, there is a lack of information about inflorescences and flowers traits, which are considered highly conserved and could be used as estimators of *J. curcas* genetic diversity in Mesoamerica. In Chinese accessions (Wu *et al.* 2011), floral phenology was divided into twelve phases, where sexual differentiation in male and female flowers occurs in the seventh phase, besides that some plants possess mainly male and female

flowers. However, in the Mesoamerican region, considered as the center of origin there are no studies describing sex expression and floral traits. Therefore, this study aimed a) to describe *J. curcas* sex diversity; b) to assess the variability in Mesoamerican accessions with floral markers; and c) to analyze the relationship between floral traits and AFLP markers.

Materials and methods

Biological material and study site

We studied 103 *J. curcas* accessions (Table S1), collected in 33 sites in southern México (Ovando-Medina *et al.* 2011a). Three years old plants were grown in *Jathropa* Germplasm Bank of the Universidad Autónoma de Chiapas (Mexico) (14.4976 N, 92.4774 W and 58 meters above sea level; average annual temperature of 31°C, average annual humidity 80%, average of 2600 mm of rainfall and soil type andosol). From each accession, male flowers (n = 40) and female flowers (n = 20) were collected. Flowers were transported to the laboratory in polybags and stored at 4°C for up to 48 hours.

Classification of flowers

The number of inflorescences was determined every 28 days during a year. Flowers were classified as female, male and hermaphrodite; first the pedicel was removed from each of the flowers and then photomicrographs were taken by using a compound microscope (Zeiss AxioLab®) and / or stereoscope (Zeiss Stemi 2000 C®) equipped with a video camera (AxioCam MRc®) coupled to the Axio Vision © program.

92 *Floral traits*

93 For all the flowers we determined the number of sepals, petals and nectaries. For male and
94 hermaphrodite flowers the number of filaments, anthers and pollen count was determined. For
95 female and hermaphrodite flowers the number of ovules and the size of the pistil and ovary were
96 determined. We also measured the length and/or width and/or diameter and/or thickness of floral
97 character. The presence of trichomes was evaluated according to the following scale: glabrous,
98 moderately pubescent and abundantly pubescent flowers.

99

100 *Statistical Analysis*

101 In order to minimize differences observed in traits, our data were coded as shown in Table S2.
102 These data were processed by a multivariate discriminant analysis, where the most informative
103 characters were identified and populations were grouped. Then, a cluster analysis (Euclidean
104 distance and Ward grouping method) was performed to visualize the relationships among
105 populations. In both analyses two pathways were followed: one with all populations, and the
106 second one only with Chiapas populations (the most numerous). In addition, a Mantel correlation
107 test was performed between genetic distances (AFLP results with the same accessions, Sánchez-
108 Gutiérrez 2010) and Fisher's morphological distances generated in discriminant analysis.
109 Statistical analyses were performed with the XLStat© v 2014 and InfoStat© v 2014 software.

110 **Results and discussion**

111 *Flowering dynamics*

112 In our results, flowers were observed throughout the year only in four accessions (ARR-7,
113 CDCU-3, MAP-8 and PC-15). The rest blossomed from one to three times a year; 85.2 %

114 flowered from March to April, while in 59 % flowered during August and only 40.1 % of the
115 accessions flowered at the start of November (Figure S1).

116 As in all angiosperms, *J. curcas* flowering is the result of genetic variability, nutrients, phyto-
117 hormones and environmental conditions. Soil humidity may be involved in triggering flower
118 formation because we observed a clear relationship between the beginning of rainy season and *J.*
119 *curcas* flowering (Figure S2). Thus, the control of soil humidity could be key to induce
120 continuous blooming, as suggested by Sukarin *et al.* (1987). In Nicaragua flowering peak is
121 observed during April, May, June and August (Aker 2012) while in India from July to September
122 (Sukarin *et al.* 1987, Bhattacharya and Kumar 2005), which demonstrates that period, intensity
123 and frequency of rain, is influenced by the geographic location.

124 There are other factors that influence flowering, such as the genetic plasticity of the accession. In
125 this regard, the vegetative growth of *J. curcas* is sympodial and the inflorescence is terminal
126 (Halle *et al.* 1978), so that the number of inflorescences in the plant depends on the number of
127 mature branches (terminal sprouts) present at the time of sampling. Consistent with this model,
128 the inflorescences present at any time should be an even number, unless the development of the
129 branches is asynchronous. From our results, the genetic determinant of flowering in *J. curcas*
130 accessions used in this study seems a highly influential factor, since the number of inflorescences
131 was accession-specific.

132 *Sex of flowers in J. curcas accessions*

133 We found female, male and hermaphrodite flowers in the study accessions (Figure 1), and based
134 on proportion, plants were classified as gynoeceious, androeceious, andromonoecious,
135 androgynomonoecious (Table 1). Dehgan and Webster (1979) reported that *J. curcas* is known
136 as a monoecious plant (male and female flowers on the same inflorescence, also with

137 hermaphrodite ones), without specifying if this is a genotype-specific characteristic. In contrast,
138 it is also been reported plants with only female (Pecina-Quintero *et al.* 2011) or predominantly
139 male flowers (Wu *et al.* 2011).
140 We observed that most individuals were monoecious, but the presence of dioecism and complex
141 arrangements suggest that *J. curcas* sex is linked to a complex determinant (probably $Y_1 Y_2$).
142 Dioecism, in plants, is related to various ecological factors (Vamosi *et al.* 2009) and is observed
143 in several groups of unrelated plants. The appearance of dioecism has been reported in the genus
144 *Ribers* (Senters and Solis 2003) and has appeared at least twice in the genus *Silene* from a
145 gynodioecious ancestor (Desfeux *et al.* 1996). In particular, in the Siparunaceae family, dioecism
146 was originated from a monoecious ancestor (Renner and Won 2001). In monocots the dioecious
147 nature seems to have evolved from a transient gynodioecious state more than the monoecious
148 state (Weiblen *et al.*, 2000).
149 In contrast to animals, the sexual dimorphism appearance in angiosperms was not associated to
150 the evolution of sex chromosomes, with exception of a few species (Ruiz 2004). Heteromorphic
151 sex chromosomes are widely distributed in higher animals but in contrast to the study of the
152 origin and evolution of X and Y chromosomes in humans (Skaletsky *et al.* 2003), plants sex
153 chromosomes have been scarcely studied. It is estimated that animals sex chromosomes appeared
154 240 to 320 million years ago (Charlesworth 2002). Therefore, younger systems like dicots (Ruiz
155 2004) are interesting to prove this hypothesis. Although sexual determinism in plants and
156 animals is fundamentally different, the architecture of sex chromosomes is likely to be
157 comparable (Charlesworth 2002). It has been hypothesized that sex chromosomes evolved from
158 autosomes and have required at least two evolutionary events for the transition from
159 hermaphroditism to dioecism (Charlesworth 1991). Therefore, a male sterility mutation in

hermaphrodites (gynomonoecious) would give way to females, and then the males would appear from the suppression of female genes. However, in this study with *J. curcas* collected in its center of origin and diversity (Salvador Figueroa *et al.* 2015, Pamidimarri and Reddy 2014) we did not find gynomonoecious individuals, which could be possible by a) *J. curcas* gynoeceious plants originated by one mutational step from a hermaphrodite ancestor, or b) the gynodioecia emerged from androgynomonoecious populations (Figure 2). Therefore, it is necessary to perform a study involving more individuals to confirm the absence of gynomonoecious. In androgynomonoecious and andromonoecious individuals encountered, reminiscences could be found of sex chromosomes in *J. curcas*.

Variation of J. curcas floral characters

We observed casual and recurrent variations in *J. curcas* floral traits. The first type refers to the variability among inflorescences of the same plant, which may have the origin in the genotype-environment interaction (Heller 1996) or in epigenetic factors (Yi *et al.* 2010). Examples of occasional variation in male flowers are: number of petals (four or five), sepals (four, five or six), nectaries (four or five) and stamens (seven to ten). In female flowers occasional variation was found in the number of ovules (two, three or four). The second type of variation refers to features that were always present in the same plant, such as the amount of trichomes and the size of the characters. It is possible that the characters had recurrent variation, whether fixed or highly heritable (Ovando-Medina *et al.* 2011a).

Only the recurrent variation was used for studies of diversity on *J. curcas* populations from southern México.

Our results revealed that the typical male flowers whorls (Figure 1A) were composed of five petals, five sepals, five nectaries, ten stamens, ten anthers and pollen grains (27-44 μm diameter), and female flowers (Figure 1B); five petals, five sepals, five nectaries, one ovary, three ovules, one pistil. In hermaphrodite flowers (Figure 1C) there are five petals, five sepals, five nectaries, ten stamens, pollen grains ranging from 37-40 μm in diameter, one ovary, three ovules, one pistil. These data agree with those previously reported by other authors (Heller 1996, Toral *et al.* 2008, Wu *et al.* 2010).

Regarding flowers size, we found that male flowers are smaller (5-10 mm) than female (7-15 mm) and hermaphrodites (11-14 mm) ones; they also have a light green-yellow color, with most of flowers moderately pubescent. In contrast, Toral *et al.* (2008) mentioned that male and female flowers are small (6-8 mm), have a greenish-yellow color, with some pubescent flowers, and with versatile anthers and protruding sexual organs.

We also observed two hermaphrodite flower types; one with ten stamens around the ovary longer than the pistil and the second one with four to six stamens around the ovary shorter than the pistil. The first type could be considered as a strategy to be self-fertilized, because the pollen sac opens, and this can be used by the receptive stigma, without needing a dispersing agent (Ocampo-Velázquez *et al.* 2009). Pinilla *et al.* (2011) observed that *J. curcas* hermaphrodite flowers exhibit proterandry, since the development of the male and female phases first release pollen by manifesting as gynoecium growth in style and stigma, once produced senescence in the anthers, styles reach their ideal size when the papillae are already developed.

Discriminant analysis of floral morphological characters

204 For the first discriminant analysis we used all populations from Southern Mexico, and the most
 205 important characters were identified to form the five principal components and to group
 206 accessions by populations. Principal component 1 (F1) was the most important accounting for
 207 46.02% of the total variation (Table 2) and the most important variables for their contribution to
 208 F1 were: male flower diameter (MFD), female sepal length (FSL), female petal length (FPL),
 209 female flower diameter (FFD) and pistil thickness (PT). Principal component 2 (F2) accounted
 210 for 20.90 % of the variance (Table 2) and the variables that contributed most to this factor were:
 211 pistil thickness (PT), ovary length (OL), male nectary length (MNL) and female nectary length
 212 (FNL). The variables correlated to the main component 3 (F3) were ovule length (OL), quantity
 213 of trichomes in female flowers (TF), with 13.64% of the variance, whereas the main component
 214 4 and 5 only explained 5-8% of the total variation as shown in Table 2.

215 Many authors have performed studies on *J. curcas* morphological variation by using principal
 216 component analysis, e.g. Zapico *et al.* (2011) in the Philippines evaluated 21 quantitative
 217 morphological variables for 13 accessions of *J. curcas*, the five principal components accounted
 218 for 88.12% of the total variation, where the most important variables for the principal component
 219 1 (F1) were: plant height, number of leaves, percentage of seed germination and survival.
 220 Moreover, Vijayanand *et al.* (2009), studied 12 accessions of *J. curcas* to assess genetic diversity
 221 using 19 morphological characters, finding that the first three factors contributed 89.2% of the
 222 total observed variance, the variables that contributed most to the F1 were: stem diameter, length
 223 and width of the leaf, and plant growth, which contributed 35.7 % for F1. Machado (2011)
 224 conducted a study to characterize the morphological and productive variability of a collection of
 225 *J. curcas* in Cuba, taking into account 13 morphological characters, finding that plant height was
 226 the most variable indicator, followed by the thickness of stem and primary branches, number of

primary and secondary branches, accounting for 74.63% between the principal component 1 (F1) and principal component 2 (F2). However, there have been no previous investigations on floral variation found in *J. curcas* for possible comparison.

We observed that the grouping pattern of accessions by population, according to F1 and F2, accounted 66.92 % of the variance shown (Figure 3), where accessions belonging to Frontera and Oaxaca populations were grouped into F1, while accessions belonging to the Istmo and Centro populations in the F2. Guerrero's position in the lower left quadrant is due to the high percentage of variance shown by these accessions in the evaluated characters and their contribution to the F1 and F2. These results are probably due to existing plants in this population with hermaphrodite flowers.

The relationship between populations and centroids were plotted in Figure 4, in order to show how Centro and Istmo populations are closely related since Istmo is found within the centroid of Centro population. Also, this population is related to the populations of Oaxaca and Frailesca, which may be due to the geographical proximity between them. Meanwhile, populations of Guatemala, Frontera, Soconusco and Guerrero were separated from the rest and between themselves. It is remarkable how different are the Frontera and Soconusco populations, even though they are geographically closed, this may be due to the physical barrier represented by the Sierra Madre de Chiapas. Moreover, Guerrero was the most diverse population, because there are plants with hermaphrodite flowers in this population.

Ovando-Medina *et al.* (2011a), used the same accessions and studied the genetic diversity estimated with fatty acids of *J. curcas* seeds, they reported that the variation with respect to oil

content was 8.020% - 54.28%. In such a study, the principal component 1 (F1) and principal component 2 (F2) together explained 89.25% of the total variation. By a graph of centroids the relationship between the six populations was shown; the results were similar to the observed by grouping based on floral characters variation, as Guatemala was one of the most diverse populations in both studies, in addition to the close relationship in which the populations of the center and coast of Chiapas were grouped.

The second discriminant analysis exclusively studied accessions from Chiapas, because there is a greater extension of traditional cultivation of *J. curcas* in this state. The major characters were identified to form the five principal components and grouped the accessions by populations. F1 the most important explained 58.40% of the total variation (Table 3) and most important variables contributing to F1 were: diameter of the male flower (DMF), length of male sepal (LMS), male petal width (APM) and female petal length (LPF). Principal component 2 (F2) accounted for 20.92 % of the variance (Table 5) and the most important variables were: length of male nectary (LNM), width of the male anther (AAM), length of the ovule (LOvu) and the amount of trichomes on female flowers (TF). For F3 the most important variables were: the number of trichomes on male flowers (TM) and length of ovary (LO) accounting for a 16.81% variance, while the F4 contributed less variation than 4% of the total variance, as shown in Table 3. With respect to F1 and F2, the most important variables for all populations from Chiapas were the diameter of the male flower (DFM), female petal length (LPF), length of male nectary, male flower diameter (DFM), female petal length (LPF) and length of male nectary (LNM).

Pinilla *et al.* (2011), studied 246 accessions of *J. curcas* from Colombia through the study of 24 qualitative and quantitative morphological characters, finding that plant height, stem diameter, canopy projection, length and width of the eighth leaf, days flowering, weight and fruit length, length and width of the seed, explained a 93.62% of the variance for F1.

In Figure 5, the grouping pattern of accessions per population, according to F1 and F2, which together account for 79.31% of the variance, shows how accessions belonging to the population Soconusco is the only group around F1, while populations of Istmo and Centro are grouped at F2.

The relationships between the five populations may be visualized when the centroids (Figure 6) are plotted, in order to differentiate how a group containing the Istmo, Frailesca and Centro populations is formed; Frontera and Soconusco populations showed a marked differentiation between them and with respect to other populations. This shows that the Sierra Madre de Chiapas is a strong physical barrier between these populations and may be the main cause of diversification among populations. Centro was the most diverse population.

Cluster analysis of morphological characters of flowers

Two analyses of hierarchical ascendant classification (dendrogram or cluster analysis) were undertaken: one of them analyzed 103 accessions from southern México, finding a dendrogram with five groups as shown in Figure 7. The first group was the largest and contained 54 accessions from most populations, showing no correlation with geographical origin. The second group only gathered eight accessions, the majority from the population of Istmo. The third group

consists of 35 accessions, which belongs to Centro, Frontera, Soconusco, Guatemala and Veracruz populations. The fourth group consists of gynoecious plants i.e. that only produce female flowers. The last group was formed from two androecious accessions (plants), i.e. that only produce male flowers, and one andromonoecious plant, i.e. producing hermaphrodite and male flowers. There was no group according to geographical origin. The percentage of variation within groups was 59.43%, while between groups was 40.57%, which means that larger variance is within groups.

In the second analysis of hierarchical ascendant classification (dendrogram or cluster analysis) 90 accessions from the Chiapas state were studied and our results showed five groups (Figure 8). The first group was the second largest consisting of 38 accessions, also from all populations (Soconusco, Frontera, Istmo and Centro), but most were from Soconusco populations, while in the second group eight accessions were included, most from the Istmo population. The third group was the largest and consisted of 39 accessions of all populations. The fourth group was formed by two accessions; these plants were androecious, i.e. only produce male flowers. Finally, the last group consists of three gynoecious plants, i.e. accessions with only female flowers. Groups showed no correlation according to their geographical origin. The percentage of variation within groups was 57.55%, while between groups was 42.45%, meaning that the existing variance is greatest within groups.

The results coincide with those found by Sánchez-Gutiérrez (2010), who used the same accessions to study genetic variation in *J. curcas* in the state of Chiapas. The author found using cluster analysis, that the accessions are grouped without a geographical pattern.

318

319 A Mantel correlation test was performed between the distance matrix generated from genetic
320 data reported by Sánchez-Gutiérrez (2010) using the GenAlEx© version 6.3 program and the
321 matrix of distances based on morphological data, obtained by discriminant analysis. This
322 correlation test was performed by populations (5) and accessions (87). The results showed that
323 matrices generated by populations are not correlated ($p = 0.448$), while the matrices generated by
324 accessions did show correlation ($p = 0.001$). This means that the study of the diversity of *J.*
325 *curcas* using floral morphological markers reveals grouping patterns in accessions similar to
326 those obtained with AFLP molecular markers.

327

328 Although there are many studies of *J. curcas* diversity collected in different regions of the world
329 (for a review see Ovando-Medina *et al.* 2011b), no reports use floral markers as estimators of
330 variation, so we were unable to compare the findings of this study. A characterization study of
331 floral development in *J. curcas* was reported by Wu *et al.* (2011), who discussed in detail the
332 anatomy of flowers and inflorescences, but did not present data on variation between accessions.

333

334 The results of our study contributed to biodiversity *J. curcas* knowledge from the living fences in
335 southern México, which indicated, together with data from other research using phenotypic and
336 molecular markers, that the Mesoamerican region is a center of diversification for this species.

337

338 **Conclusions**

339 *J. curcas* floral traits in the Mesoamerican region are highly variable between accessions and
340 they are also informative, since they allow grouping at the population level. Flowering of *J.*

curcas occurs in three events (March to April, August and November), with the peak from March to April, corresponding to the onset of rains, where the greatest abundance of inflorescences unfolds, with 85.2% of the accessions in bloom. However, some accessions bloom all year (ARR-7, CDCU-3, MAP-8 and PC-15). This biofuel plant requires a rest period, which coincides with the dry season, then flowering is triggered with the onset of the rains, but is inhibited by an excess of humidity in the soil. Of the accessions, 93.2 % are monoecious, 1.9 % androecious, 2.9 % gynoeceous, 0.9 % andromonoecious and 1.9 % androgynomonoecious. The discriminant analysis revealed that the most important variables for their contribution to the variance were: male flower diameter, female petal length, male nectary length. Central and Istmo populations were closely related and Central shared floral characteristics with Oaxaca and Frailesca populations. For their part, populations from Guatemala, Frontera, Soconusco and Guerrero have fewer relationships with the other populations and with each other. Despite their geographical proximity, the Frontera and Soconusco populations are differentiated in their floral characters. The hierarchical ascendant classification analysis showed that *J. curcas* accessions from southern México were grouped without a geographic pattern, while accessions with atypical sexuality were grouped together; in this way, one group was formed by gynoeceous plants and another by androecious and andromonoecious accessions.

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- 443

Figure 1(on next page)

Types of *Jatropha curcas* flowers.

Differentiation of *Jatropha curcas* flowers according to sex, male flower (a), female flower (b), hermaphroditic flower (c).



C



B

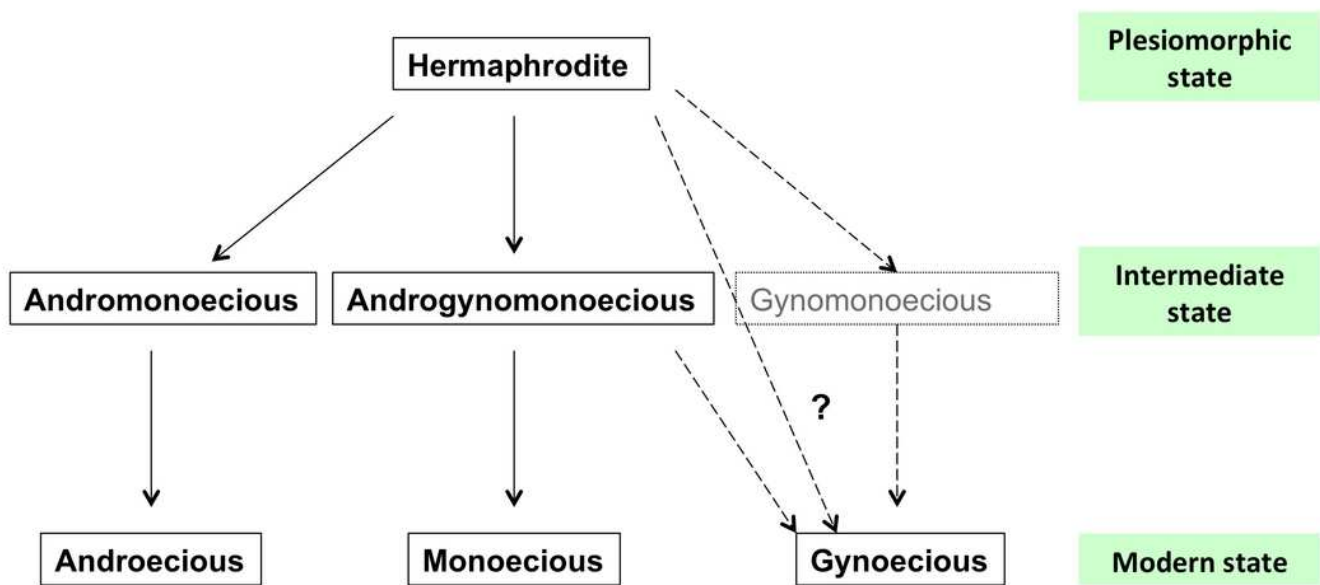


A

2

Sex expression in the biofuel plant *Jatropha curcas*.

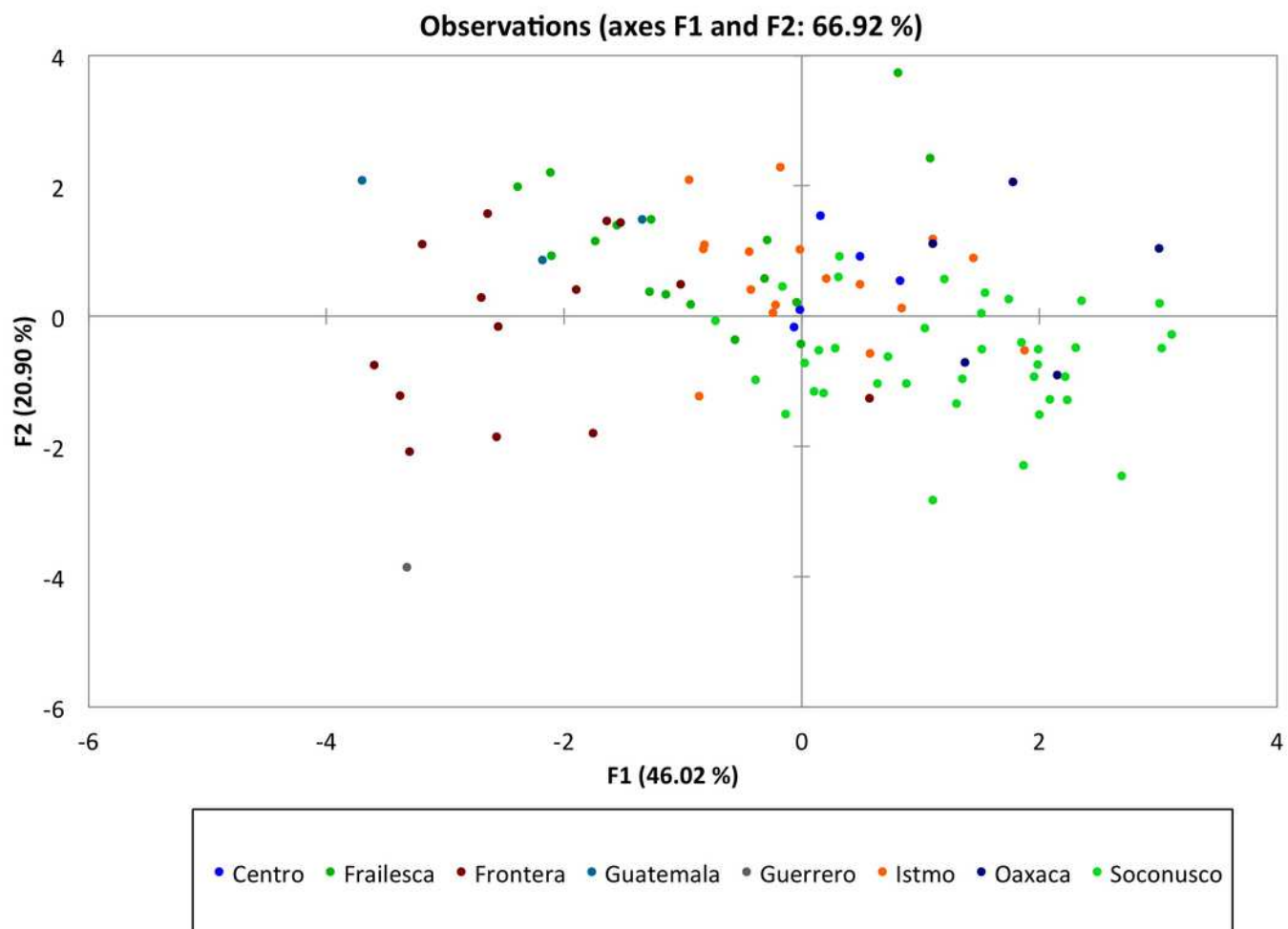
Phylogenetic hypothesis of the evolution of sexuality in the tropical tree *Jatropha curcas* L. (Euphorbiaceae).



3

Discriminant analysis of landraces of *Jatropha curcas* from Meso-America.

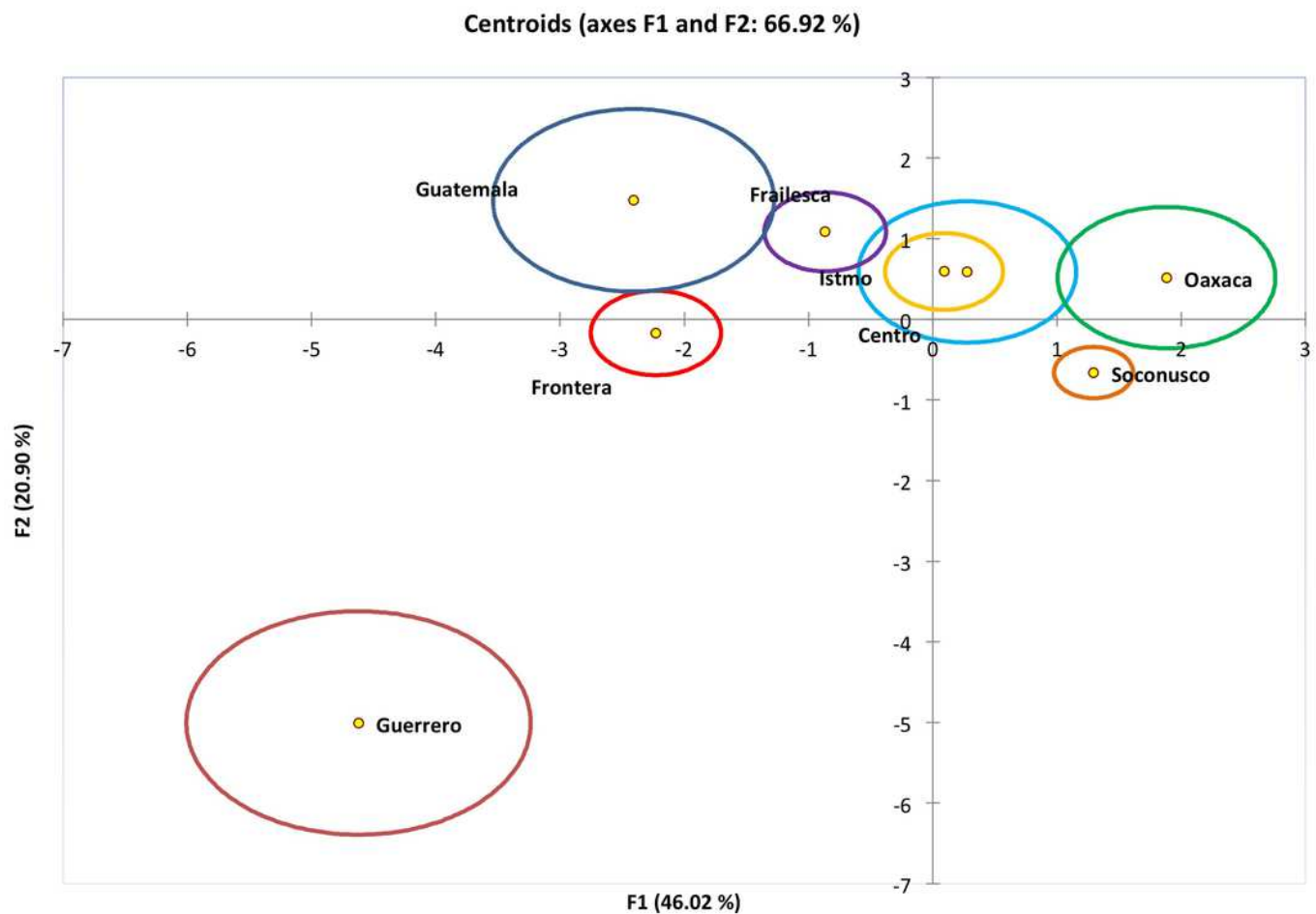
Groupings by populations of 103 accessions of *Jatropha curcas* L. of southern México, from the *Jatropha* Germplasm Bank of the Autonomous University of Chiapas.



4

Grouping of *Jatropha curcas* landraces based on multivariate analysis.

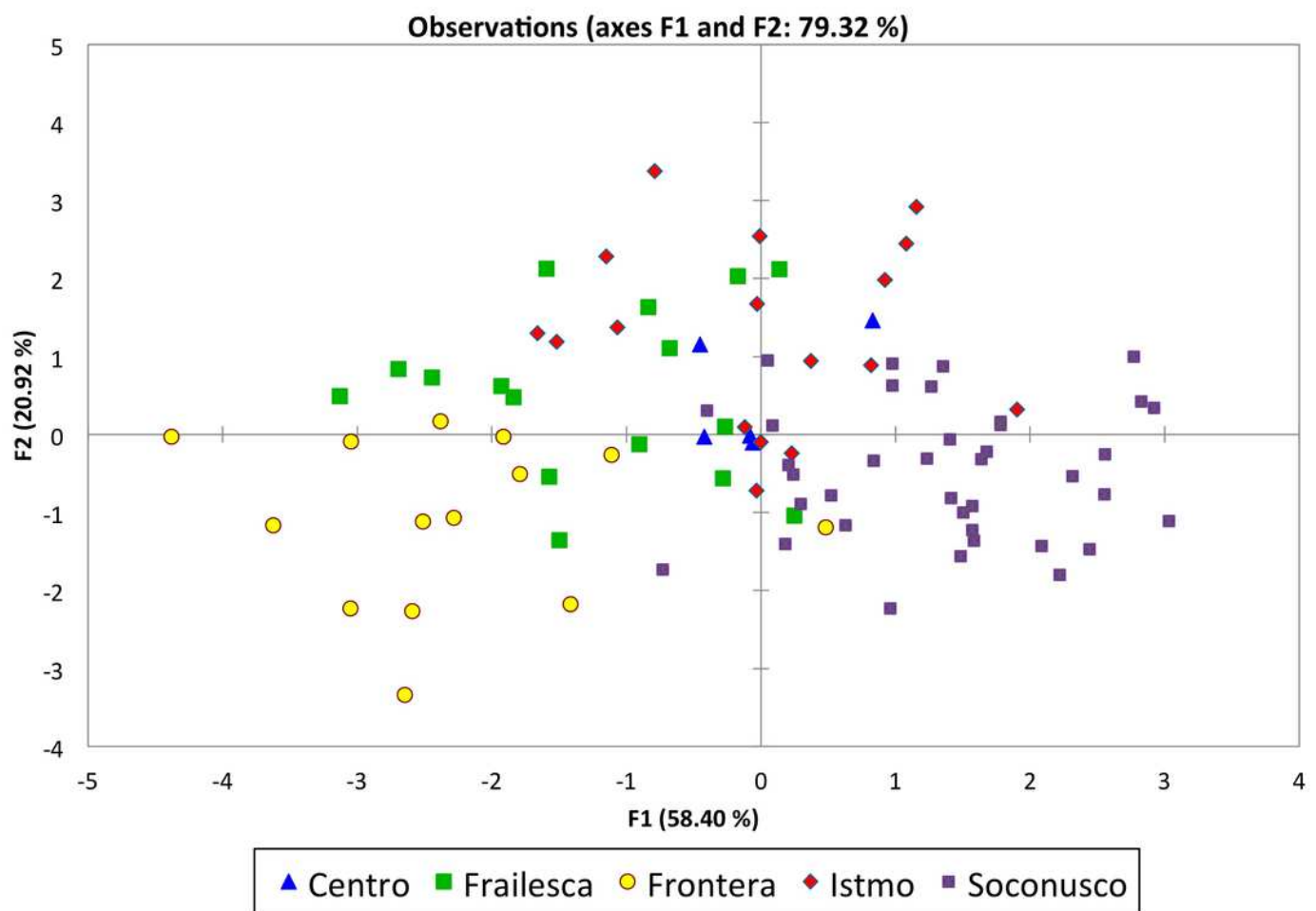
Graph of centroids of the eight populations of *Jatropha curcas* L. in southern México, from the *Jatropha* Germplasm Bank of the Autonomous University of Chiapas.



5

Discriminant analysis of *Jatropha curcas* landraces from Meso-America base on floral traits.

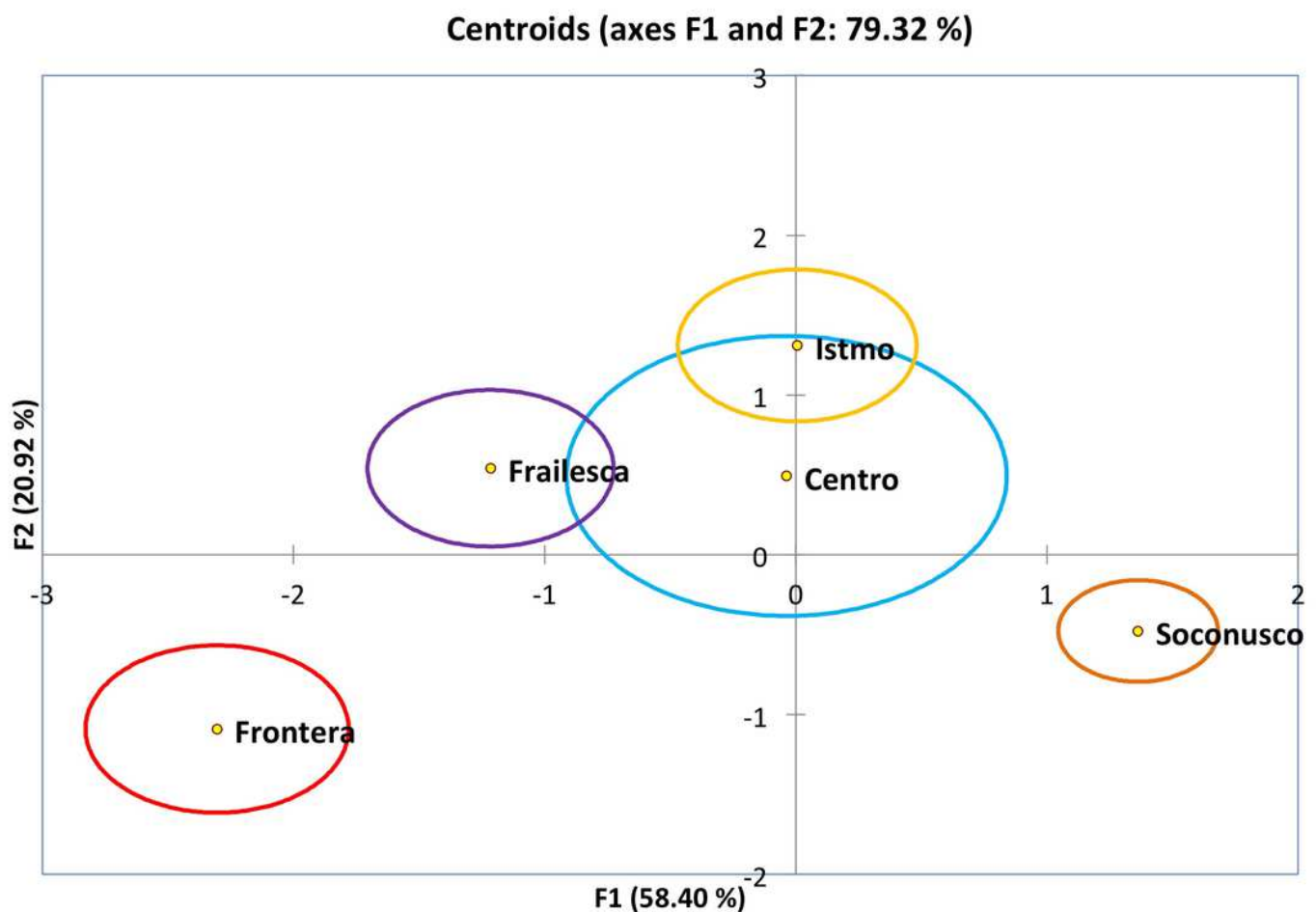
Grouping by populations of 90 accessiones of *Jatropha curcas* L. from Chiapas state, from the *Jatropha* Germplasm Bank of the Autonomous University of Chiapas.



6

Biplot of discriminant scores based on discriminant functions 1 and 2.

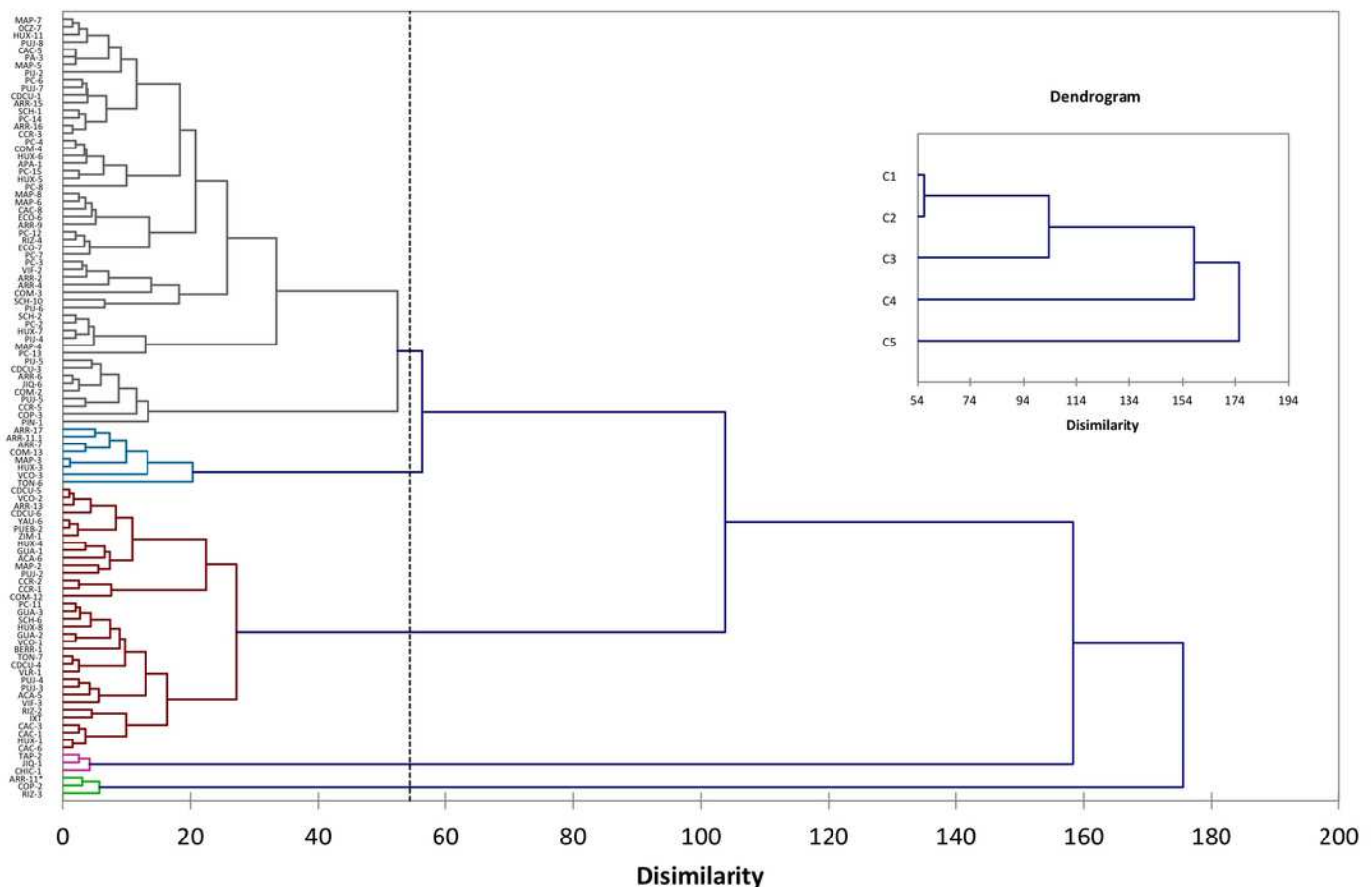
Chart of centroids of five populations of *Jatropha curcas* L. Chiapas state, from the *Jatropha* Germplasm Bank of the Autonomous University of Chiapas.



7

Clustering of *Jatropha curcas* accessions collected in Meso-America.

Dissimilarity dendrogram of 103 accessions of *Jatropha curcas* L. in southern México, from the *Jatropha* Germplasm Bank of the Autonomous University of Chiapas.



8

Clustering of *Jatropha curcas* accessions collected in the State of Chiapas, Mexico.

Dissimilarity dendrogram of 90 accessions of *Jatropha curcas* L. of Chiapas state, from the *Jatropha* Germplasm Bank of the Autonomous University of Chiapas.

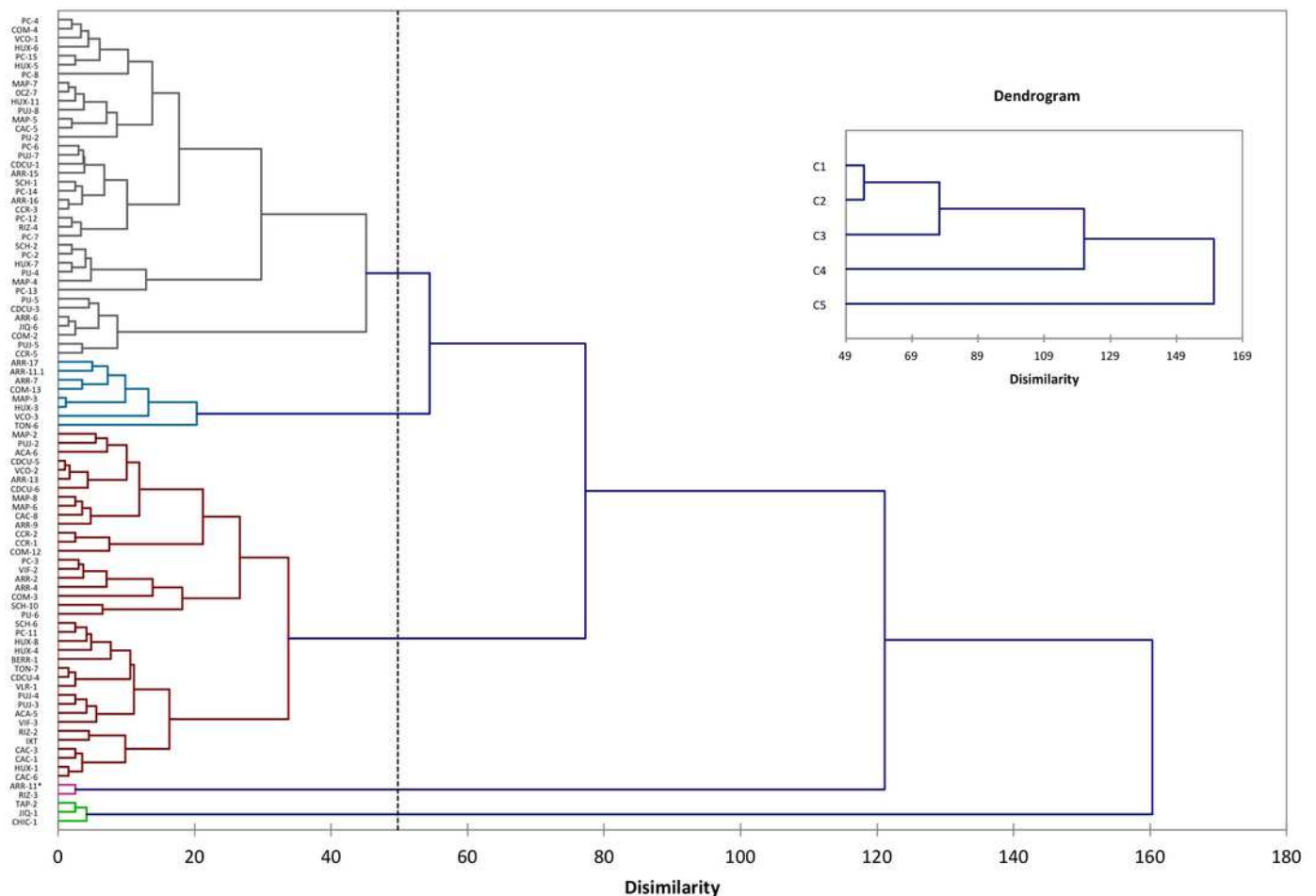


Table 1(on next page)

Sexuality of *Jatropha curcas* landraces collected in Meso-America.

Classification of 103 accessions of *Jatropha curcas* L. from the *Jatropha* Germplasm Bank of the Universidad Autónoma de Chiapas, based on their sexual descriptors.

Table 1. Classification of 103 accessions of *Jatropha curcas* L. from the Jatropha Germplasm Bank of the Universidad Autónoma de Chiapas, based on their sexual descriptors.

Type of plant	Accessions (n)	Description
Monoecious	95	Plants with male and females flowers in the same inflorescence
Dioecious		
Gynoeceious	3	Plants with only female flowers
Androeceious	2	Plants with only male flowers
Hermaphrodites		
Andromonoecious	1	Plants with male and hermaphrodite flowers
Androgynomonoecious	2	Plant that has mostly male flowers, with a few female and hermaphrodite flowers

Table 2 (on next page)

Multivariate analysis of flower traits of *Jatropha curcas* collected in Meso-America.

Eigenvalue of the five main components, based on floral characters of 103 accessions of *Jatropha curcas* L. in southern Mexico.

Table 2. Eigenvalue of the five main components, based on floral characters of 103 accessions of *Jatropha curcas* L. in southern Mexico.

Component	Total	Variance (%)	Cumulative Variance%
1	2.430	46.018	46.018
2	1.104	20.898	66.916
3	0.720	13.642	80.558
4	0.445	8.424	88.982
5	0.264	5.001	93.983

Table 3 (on next page)

Multivariate analysis of flower traits of *Jatropha curcas* from Meso-America.

Eigenvalue of the four principal components, based on floral characters of 90 accessions of *Jatropha curcas* L. in the State of Chiapas, Mexico.

Table 3. Eigenvalue of the four principal components, based on floral characters of 90 accessions of *Jatropha curcas* L. in the State of Chiapas, Mexico.

Component	Total	Variance (%)	Cumulative variance (%)
1	1.984	58.40	58.40
2	0.711	20.92	79.320
3	0.571	16.81	96.14
4	0.131	3.87	100