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Genetic Diversity Analysis of *Jatropha* Species from Costa Rica Using AFLP Markers

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Abstract

The genetic diversity from species of the genus *Jatropha* collected from Costa Rica was analyzed by AFLP (amplified fragments length polymorphism). The study consisted of 114 accessions from 15 populations of 4 different species: *J. curcas*, *J. costaricensis*, *J. gossypifolia* and *J. stevensii*. These were collected from different locations in Costa Rica. Three different primers were used, resulting in 428 loci, and they were classified in three categories: unique and double bands (UBD), rare bands (RB) and shared bands (SB). The UBD were excluded for a total of 339 polymorphic loci used for the UPGMA dendrogram and principal component analysis (PCA). The species that obtained the highest average of polymorphic loci was *J. curcas*, which obtained the highest percentage of polymorphic loci (80.24%), followed by *J. gossypifolia* (79.35%), *J. costaricensis* (78.76%), and finally *J. stevensii* (40.71%). The average for the polymorphic loci was of 69.76%. Thus, the phylogeny of the *Jatropha* species in Costa Rica was elucidated, showing *J. curcas* more related to *J. stevensii* and this one with *J. costaricensis*, and *J. gossypifolia* as the most distant member of the genus. For the best of our knowledge this is the first report of a genetic analysis of *J. costaricensis* and *J. stevensii*. The obtained molecular evidence showed high levels of polymorphisms in the present study compared with reports from Africa, India, and China. The molecular diversity estimated in our analysis, together with agronomical or morphological data, can be very useful for plant breeding programs, given the importance of *Jatropha* species in oil production. Interestingly, Central American *Jatropha* material can be used to increase the genetic base of *J. curcas* populations localized in Asia and Africa, where reduced genetic diversity has been reported.

Keywords

***Jatropha* spp., AFLP, Molecular Markers, Polymorphism, Genetic Diversity**

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1. Introduction

The genus *Jatropha* is morphologically diverse with 160 - 175 old and new world woody species comprising of trees, shrubs, rhizomatous subshrubs, tuberous perennial herbs, geophytes, and facultative annuals, which are distributed chiefly in the tropical and sub-tropical regions of America, Africa and India [1]. *J. costaricensis* is described from Guanacaste, Costa Rica [2], *J. stevensii* is described from collections in Dept. Boaco in Nicaragua [3], and *J. gossypifolia* L. is a major weed in Australia [4]. *Jatropha stevensii* represents yet another extension of the Mexican xerophytic element into Central America, and the second record for *Jatropha* subg. *curcas* [3]. Easy adaptation to different kinds of marginal lands, drought endurance, avoidance by animals, and its short time interval to give first yield make this plant species more attractive for cultivation [5]. The species can grow even in areas with extreme climate and soil conditions which could not be inhabited by most of the agriculturally important plant species that circumvent the food versus fuel resentment [6].

Assessment of genetic diversity using molecular markers is crucial for the efficient management and conservation of plant genetic resources in gene banks [7]. There are very few studies on the genetic diversity of *J. curcas* that involve a variety of populations and molecular marker technologies [7]-[13]. Two common features of the majority of these studies are the inclusion of only a narrow selection of genetic materials, typically within one or a few countries [7]-[9] [12] [14], and the fact that they have generally revealed low genetic diversity within countries [10] [15]-[18].

Most of the research was focused on *J. curcas* originating from India, and it showed low genetic diversity [8] [9] [14] [15] [19]. Ranade *et al.* [15] characterized 22 accessions from six regions using RAPD and amplification of minisatellite DNA (DAMD) profiles and separated the wild and non-wild accessions; they observed a low level of variation among accessions within a group [15]. The analysis of the genetic diversity among 28 accessions using RAPD and amplified fragment length polymorphism (AFLP) revealed low genetic diversity in *J. curcas* [19], and similar results have been reported by Basha and Sujatha [9] and Tatikonda *et al.* [8]. In another research done in India, Sudheer *et al.* [14], using microsatellite markers, also concluded a genetic disequilibrium of *J. curcas*. The narrow genetic base of *J. curcas* in India has been attributed to the small number of introduced plants and their vegetative propagation [7].

For Chinese samples of *J. curcas*, 58 accessions were assessed in a study based on simple sequence repeat (SSR) and AFLP analysis, it demonstrated low levels of variation [7]. Therefore, the genetic diversity of *J. curcas* in China may only represent part of the Indian population [7].

In this way, it is likely that there is a greater genetic diversity in *J. curcas* individuals from the center of origin; however, with the exception of research using germplasm from Guatemala [20] and Mexico [10] [13] [16], relatively less work has appeared from its center of origin, as well as Africa and other regions of the tropics where it occurs extensively. Although genetic diversity of *J. curcas* in its native Central America is still unknown, it is suggested that germplasm for genetic improvement programs should be introduced from its native range rather than from other introduced populations [7]. Therefore, a collaborative global *Jatropha* genetic diversity evaluation effort is immediately needed for a better use of this valuable species in breeding programs.

The present study surveys the molecular diversity in four wild species of *Jatropha* from Costa Rica: *J. costaricensis*, *J. curcas*, *J. gossypifolia* and *J. stevensii*, by using amplified fragment length polymorphism analysis (AFLP). AFLP is a rich information marker system due to its ability to generate a large number of polymorphic/informative loci simultaneously in a single lane with a single-primer combination, as compared to RAPDs, RFLPs and microsatellites [8]. With AFLP, which does not require any prior DNA sequence information, the main disadvantage of SSR markers is that they need to be isolated again from most species being examined for the first time, and the development of these markers is both time consuming and expensive [8].

2. Materials and Methods

2.1. Plant Materials

Samples of leaves of *Jatropha* spp. were collected from Diriá and Santa Rosa National Parks, off road and commercial availability, for a total of 114 samples (**Table 1**).

2.2. AFLP Analysis

The genomic DNA of *Jatropha sp*. samples were extracted using the NucleoSpin Plant II commercial system

Table 1. Details of the *Jatropha* spp. accessions used for the AFLP analysis collected in Costa Rica.

Species	N. of individuals	N. of populations	Site
J. costaricensis	7	1	Península Santa Elena, Area Conservación Guanacaste, La Cruz. Cantera La Marbolita.
	4	1	Península Santa Elena, Area Conservación Guanacaste, La Cruz.
J. curcas	22	1	Cerro La Flor, Playas del Coco, Carrillo, Guanacaste.
	3	1	Tárcoles, Puntarenas.
	16	1	Fila cenizosa, Parque Nacional Diriá, Santa Cruz, Guanacaste.
	12	1	Parque Nacional Diriá, Santa Cruz, Guanacaste.
J. gossypifolia	42	4	Liberia, Guanacaste.
		1	Nicoya, Guanacaste.
		1	La Cruz, Guanacaste.
		1	Carrillo, Guanacaste.
		1	Palmares, Alajuela.
J. stevensii	8	1	Parque Nacional Santa Rosa, La Cruz, Guanacaste.
Total	**114**	**15**	

(Macherey-Nagel). AFLP plant mapping kit, with ligation and pre-selective amplification core mix modules, (Life Technologies, USA) was used for AFLP fingerprinting according to the manufacturer's instructions. The restriction-ligation reactions were performed using genomic DNA, with EcoRI and MseI enzymes, EcoRI and MseI adaptors and T4 ligase at room temperature overnight. The restriction-ligation product was diluted 1:2. The DNA prepared by restriction-ligation was pre-amplified using EcoRI-A and MseI-C primers. The pre-amplified product was diluted 1:20 with sterile deionized water. The diluted pre-amplification products were amplified using selective fluorescent dye-labeled EcoRI primers with the following combination: E-AGC/M-CTA, E-ACT/M-CTA and E-AGG/M-CTA [8] [19]. Data collection of selective amplification products was carried out on a 3130xl genetic analyzer (Applied Biosystems, USA). The polymorphic loci were classified in 3 categories: unique and double bands (UBD), rare bands (RB) (present in less than 10% accessions) and shared loci (SB).

2.3. Data Analysis

The genetic diversity analysis (principal coordinates, AMOVA, banding pattern) were in GenAlEx with binary database of AFLP, both for species and for populations. The phylogenetic tree was made in the Infotstat software using the Jaccard index. The genetic distance matrix resulting from GenAlEx analysis was used in the SigmaPlot 12.0 software to generate 3D graphic. The GenAlEx program was used to obtain allele frequencies, heterozygosity estimated, values of "p" and "q" to calculate the statistics (PIC, EMR, MI, RP, DI) of the primers. Equations were programmed in excel and were run with data.

AFLP scoring was conducted using GeneMapper v4.1 with default parameter settings (Applied Biosystems, USA). Sample fingerprints were individually scored and statistically analyzed, assuming fragment size as a locus, they were considered as biallelic (present = 1, absent = 0) and made the binary matrix. Phylogenetic tree according to Jaccard and band pattern analysis were constructed using the binary matrix. The genetic distance was calculated as proposed by Huff *et al.* [21]:

$$D = n\left[1 - \frac{2n_{xy}}{2n}\right] \quad (1)$$

Equation (1), Here, $2n_{xy}$ = number of shared character states, n = total number of binary characters. When calculated across multiple loci for a given pair of samples, this is equivalent to the total of state differences among the two DNA profiles. The genetic distance matrix was subject of principal coordinates and AMOVA analysis using GenAlEx v6.5.1. Analysis of molecular variance (AMOVA) procedure follows the methods of Peakall and Smouse [22]:

$$\Phi_{PT} = \frac{V_{AP}}{\left(V_{AP} - V_{WP}\right)} \quad (2)$$

Equation (2), Where, V_{AP} = among-population variable, and V_{WP} = within-population variable.

Genotyping data obtained from the AFLP primer combinations was used for assessing the discriminatory power of AFLP primer combinations by evaluating three parameters [23]: 1) polymorphism information content (PIC), 2) marker index (MI), and 3) resolving power (RP).

The PIC value for each AFLP primer combination was calculated as proposed by Roldan-Ruiz *et al.* [24]:

$$\Phi_{\mathrm{PIC}_i} = 2 f_i \left(1 - f_i\right) \quad (3)$$

Equation (3), where $\mathrm{PIC_i}$ is the polymorphism information content of marker i, f_i the frequency of the marker fragments, which were present and $1 - f_i$ the frequency of marker fragments, which were absent. PIC was averaged over the fragments for each primer combination. The marker index was calculated as given in Varshney *et al.* [25]:

$$\Phi_{\mathrm{MI}} = \mathrm{PIC} \times \mathrm{EMR} \quad (4)$$

Equation (4), where EMR: "The effective multiplex ratio (E) is defined as the product of the total number of loci/fragments per primer (n) and the fraction of polymorphic loci/fragments (β) (E = n * β)."

Resolving power of each primer was calculated according to Prevost and Wilkinson [26]:

$$\mathrm{RP} = \sum l_b \quad (5)$$

Equation (5), where l_b represents fragment informativeness. The l_b can be represented into a 0 - 1 scale by the following formula:

$$l_b = 1 \times 2\left(\left|0.5 - p\right|\right) \quad (6)$$

Equation (6), where p is the proportion of the 48 accessions containing the fragment.

Diversity values were calculated for each locus as proposed by Russell *et al.* [27]:

$$DI = 1 - \sum Pi^2 \quad (7)$$

Equation (7), Pi is the phenotypic frequency for each AFLPs-primer combination.

3. Results

3.1. AFLP Profiling and Marker Polymorphism

A total of 3 combinations of AFLP primers were used in the study to generate AFLP profiles over 114 accessions of *Jatropha* species collected in the province of Guanacaste, Costa Rica. In the analysis of the AFLP profile, only the loci with high resolution were considered. A total of 428 loci were generated and distributed homogeneously within 3 primer combinations (**Table 2**). The polymorphic loci were classified in 3 categories: unique and double bands (UBD), rare bands (RB), and shared loci (SB) (**Table 3**). The first consist of loci present in one or two samples by specific primer combinations. The UBD loci were excluded and, for further analysis, a total of 339 loci (TL) were used (**Table 3**). By this analysis, *J. curcas* obtained the highest percentage of polymorphic loci (80.24%), followed by *J. gossypifolia* (79.35%), *J. costaricensis* (78.76%), and finally *J. stevensii* (40.71%). The average for the polymorphic loci was of 69.76%.

3.2. Discriminatory Power of AFLP Primer Combination

3.2.1. Polymorphism Information Content (PIC)

The PIC value for polymorphic fragments oscillated between 0.245 and 0.269, with an average of 0.257 per primer combination. With the purpose of distinguishing different primer combinations, the PIC value for all the generated fragments by primer combination were averaged to obtain an average PIC value, corresponding to each primer combination. The highest PIC value was observed for the E-ACT/M-CTA primer combination and the lowest for the E-AGG/M-CTA primer (**Table 2**).

Table 2. Marker attributes for AFLP combinations used.

Primer combinations	PIC[a]	EMR[b]	MI[c]	RP[d]	DI[e]
E-ACT/M-CTA	0.269	112	30.99	26.50	0.911
E-AGG/M-CTA	0.245	110	26.96	21.81	0.943
E-AGC/M-CTA	0.256	116	29.72	23.47	0.944
Average	0.257	113	29.22	23.93	0.933

[a]Polymorphism information content (PIC); [b]Effective multiplex ratio (EMR); [c]Marker index (MI). [d] Resolving power (RP); [e]Diversity Index (DI)

Table 3. Number of polymorphic bands in relation to primer combinations. TB = Total bands. PB = Polymorphic Bands. MB = Monomorphic Bands. UBD = Unique and Double Bands. RB = Rare Bands < 10% from samples. SB = Shared Bands > 70% from samples.

Primer combinations	TB	PB	MB	% Polymorphism	UBD	RB	SB
EcoRI-ACT X MseI-CTA	146	146	1	99.34	33	76	4
EcoRI-AGG X MseI-CTA	138	138	0	100	28	75	2
EcoRI-AGC X MseI-CTA	144	144	0	100	28	76	0

3.2.2. Marker Index (MI)

The MI value had a range between 26.96 and 30.99 with an average of 29.22 per primer combination. The highest MI value (30.99) was observed for the E-ACT/M-CTA primer and the lowest MI value (26.96) for the E-AGG/M-CTA primer (**Table 2**).

3.2.3. Resolving Power (RP)

The RP is a characteristic for each primer combination that indicates the discriminatory potential of the primer combination. The RP range was 21.81 to 26.50 with an average of 23.93 per primer combination. The highest value (26.50) was shown for the E-ACT/M-CTA primer and the lowest for the E-AGG/M-CTA primer (**Table 2**).

3.2.4. Diversity Index (DI)

The diversity index value for 339 polymorphic loci ranged between 0.911 and 0.944 with an average of 0.933 per AFLPs-primer combinations. Highest value (0.944) was scored with the primer pair for E-AGG/M-CTA and the lowest value (0.911) for the primer pair E-ACT/M-CTA (**Table 2**).

3.3. Genetic Relationships of *Jatropha* Populations

The results of the principal component analysis (PC) showed that samples grouped by species in four groups (**Figure 1**). By doing the same analysis by species, the distribution was confirmed and the variation increased, explained by 100% (**Figure 2**). The cluster analysis shows that the species *J. curcas* and *J. stevensii* are genetically close, and at the same time, these were close with *J. costaricensis*. Consequently, *J. gossypifolia* is the farthest compared to the others (**Figure 3**).

Analyzing the total band patterns by species, *J. curcas* presented the highest band quantity (273), followed by *J. gossypifolia* (271), then *J. costaricensis* (268), and finally *J. stevensii* (145). From the 281 bands of *J. costaricensis*, 242 have a frequency equal or higher than 5%, resulting in the species with the highest quantity. Furthermore, *J. curcas* with 238 bands, then *J. gossypifolia* with 214 bands, and *J. stevensii* with 145 bands. *J. costaricensis* was the species with the highest quantity of private bands (24), then *J. gossypifolia* (13 bands), *J. curcas* (5 bands), and *J. stevensii* (none) (data not showed). In relation with the average of the expected heterozygosity, *J. curcas* obtained the highest value (0.177), then *J. costaricensis* (0.161), *J. gossypifolia* (0.160), and finally *J. stevensii* (0.124) (**Figure 4**). The AMOVA analysis of the different species of *Jatropha* showed that genetic differences exist both between and within species (n = 114, PhiPT = 0.471, P < 0.01) (**Figure 5**). Subsequently, an analysis for genetic difference was performed for the *J. costaricensis* and *J. curcas* species. For *J. costaricensis*, I and II populations showed no genetic difference between them (n = 11, PhiPT = 0.01, valor P = 0.40), but together they presented differences with population III (n = 33, PhiPT = 0.47, value P < 0.01) (**Figure 6**).

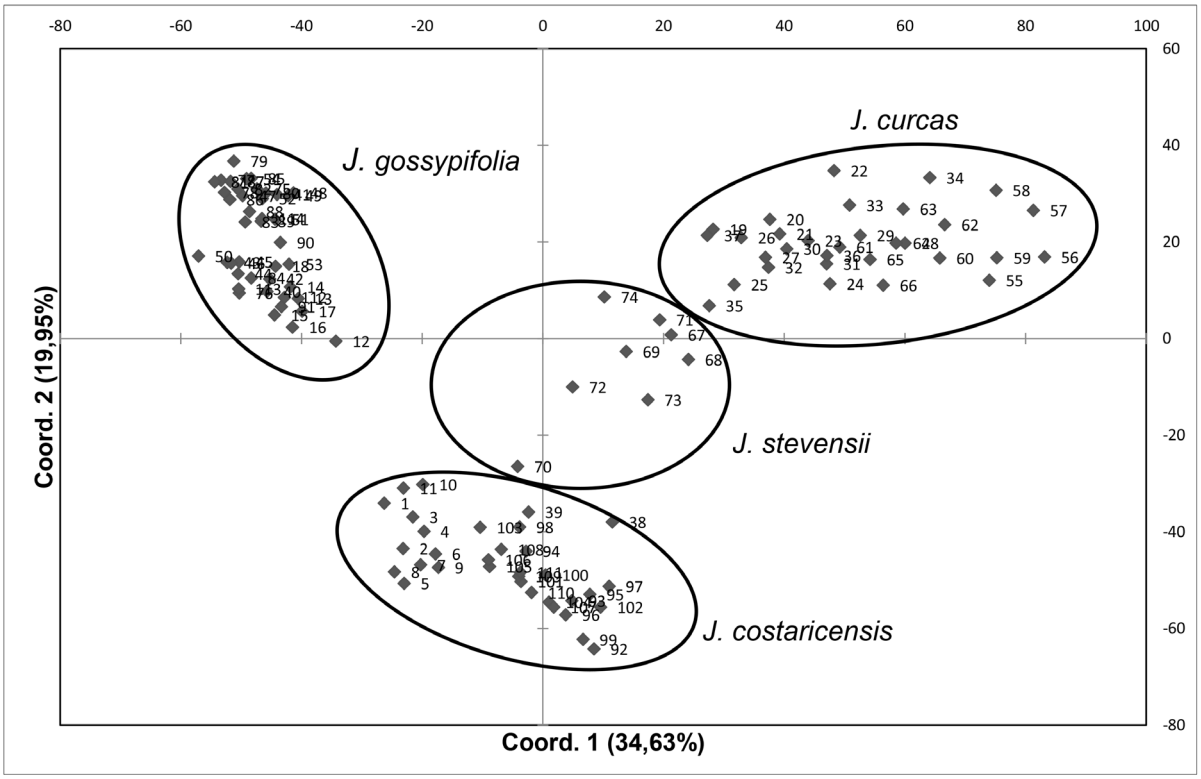

Figure 1. Genetic relations of 4 species of *Jatropha* based on the principal component analysis (PCA). Percentage of variation explained by the 2 axis: 53.82%.

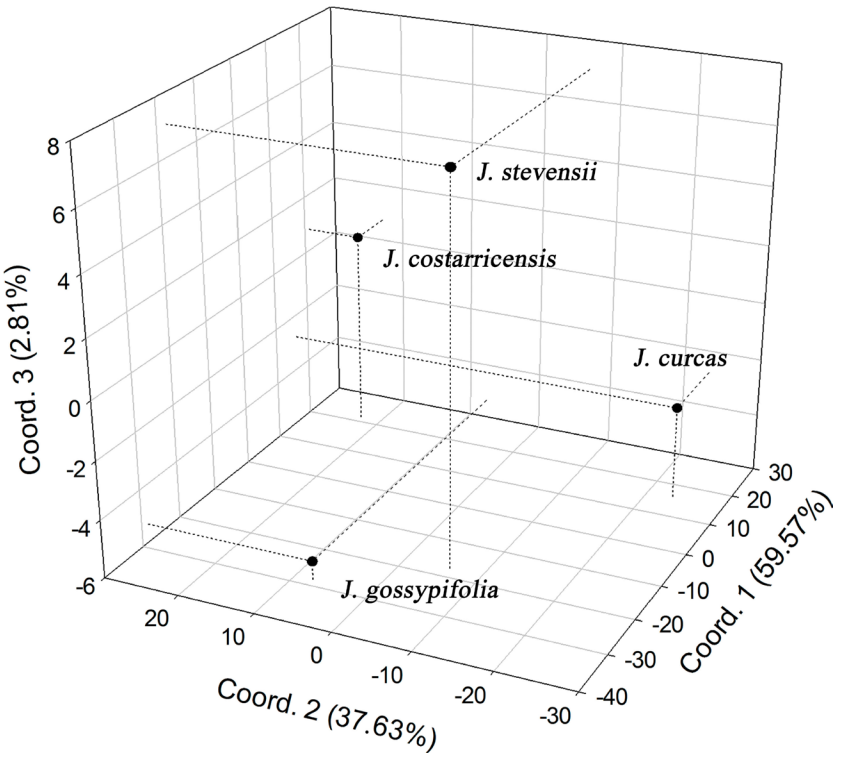

Figure 2. Principal coordinates per species. *J. curcas* n = 31, *J. costarricenses* n = 33, *J. gossypifolia* n = 42, *J. stevensii* n = 8. Percentage of variation explained by the 3 axis = 100%.

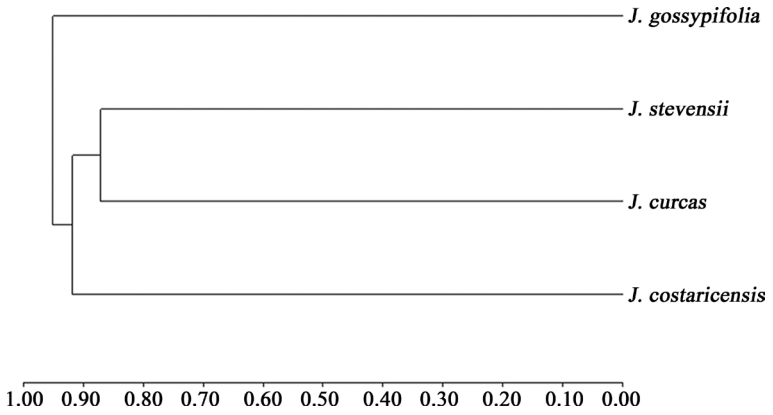

Figure 3. Phylogenetic tree for 4 species of *Jatropha* based in Jaccard coefficient similarity. cophenetic correlation = 99. *J. curcas* n = 31, *J. costarricenses* n = 33, *J. gossypifolia* n = 42, *J. stevensii* n = 8.

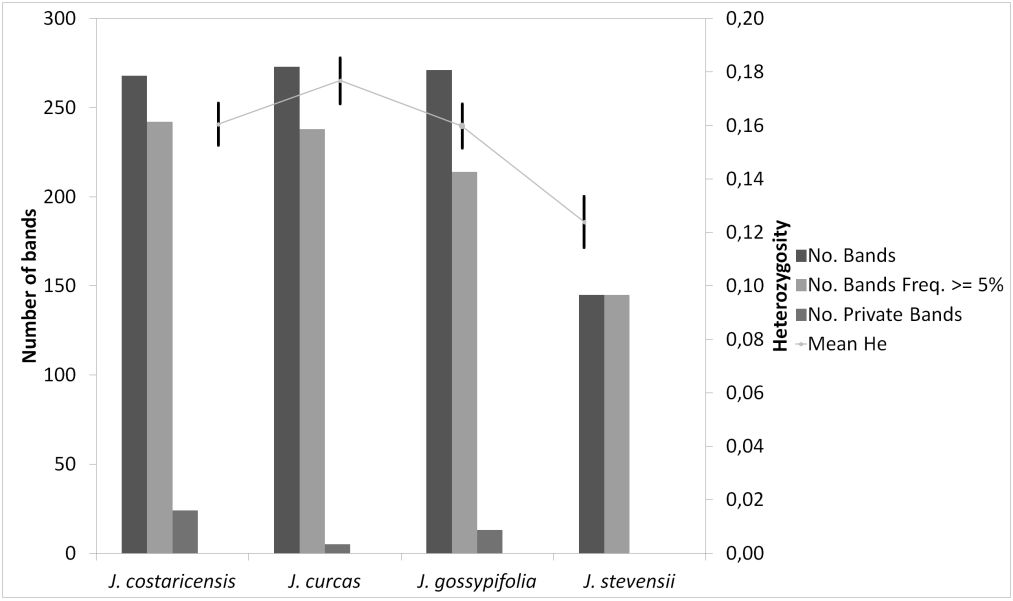

Figure 4. Banding pattern between species of *Jatropha*.

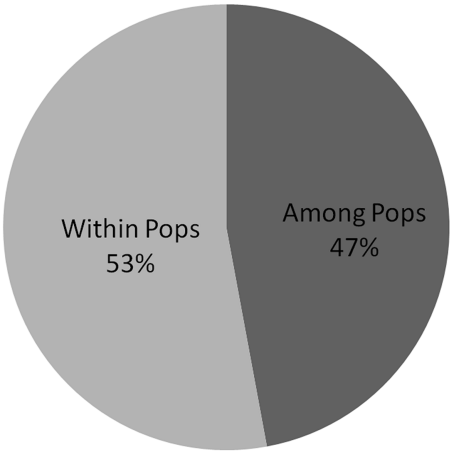

Figure 5. Percentage of molecular variation between and within *J. curcas*, *J. gossypifolia*, *J. costaricensis* and *J. stevensii*. Pops: populations.

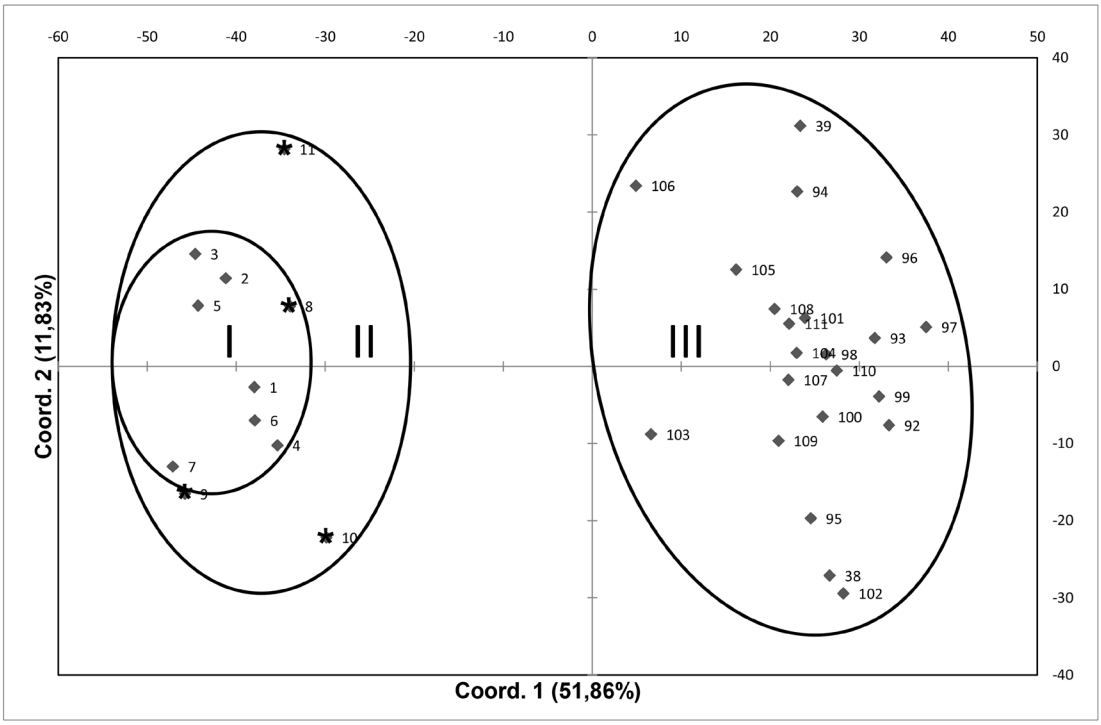

Figure 6. Principal coordinates between the different populations of *J. costaricensis*. Percentage of variation explained by the 2 axis = 63.69%. I = Península Santa Elena, Area Conservación Guanacaste, La Cruz, Guanacaste. Cantera La Marbolita. II = Península Santa Elena, Àrea de Conservación Guancaste, La Cruz, Guanacaste. Nearness of river Potrero Grande. Samples marked with an asterisk. III = Cerro La Flor, Playas del Coco, Carrillo, Guanacaste.

Unlike *J. costaricensis*, the 3 populations of *J curcas* are genetically different from each other (n = 31, PhiPT = 0.39, P < 0.01). Populations I and III had the greatest genetic difference in the study (n = 15 PhiPT = 0.54, P < 0.01). Although the populations II and III overlap in a region according to the principal coordinate analysis (**Figure 7**), these populations were genetically different (n = 28, PhiPT = 0.38, P value < 0.01). Similarly for the populations I and II, although their genetic difference is lower (n = 19, PhiPT = 0.24, P < 0.01).

4. Discussion

4.1. AFLP Profiling and Marker Polymorphism

It has been shown that the amplified fragment length polymorphism analysis (AFLP) is an efficient method for the molecular characterization and phylogenetic relation studies among the *Jatropha* species [14] [17] [19]. In this study, the level of polymorphism based on AFLP with 3 primer combinations was high and sufficient to distinguish the four species of *Jatropha* (*J. costaricensis*, *J. curcas*, *J. gossypifolia* and *J. stevensii*) collected in Costa Rica. This result agrees with other studies where AFLP technique allowed to estimate the genetic relationships of germplasm (*J. curcas*) and to detect a high level of polymorphism [13]. Unique, rare, shared, and double fragments detected by the 3 primer combination were identified. The primer combination named E-ACT/M-CTA was found to be more effective to detect a higher number of unique fragments and a good number of rare fragments. Therefore, these primer combinations are useful for the detection of informative fragments in the *Jatropha* germplasm. The availability of rare fragments present in different accessions, together with the genetic dissimilarity data, may be useful for the improvement of species through conventional methods as molecular improvement trials, like marker assisted selection (MAS) and the occurrence of shared fragments in accession. It is very useful to understand the relations and similarities between accessions [8].

4.2. Discriminatory Power of AFLP Primer Combination

A number of markers attributes like PIC, MI and RP have been used in different studies to evaluate the informa-

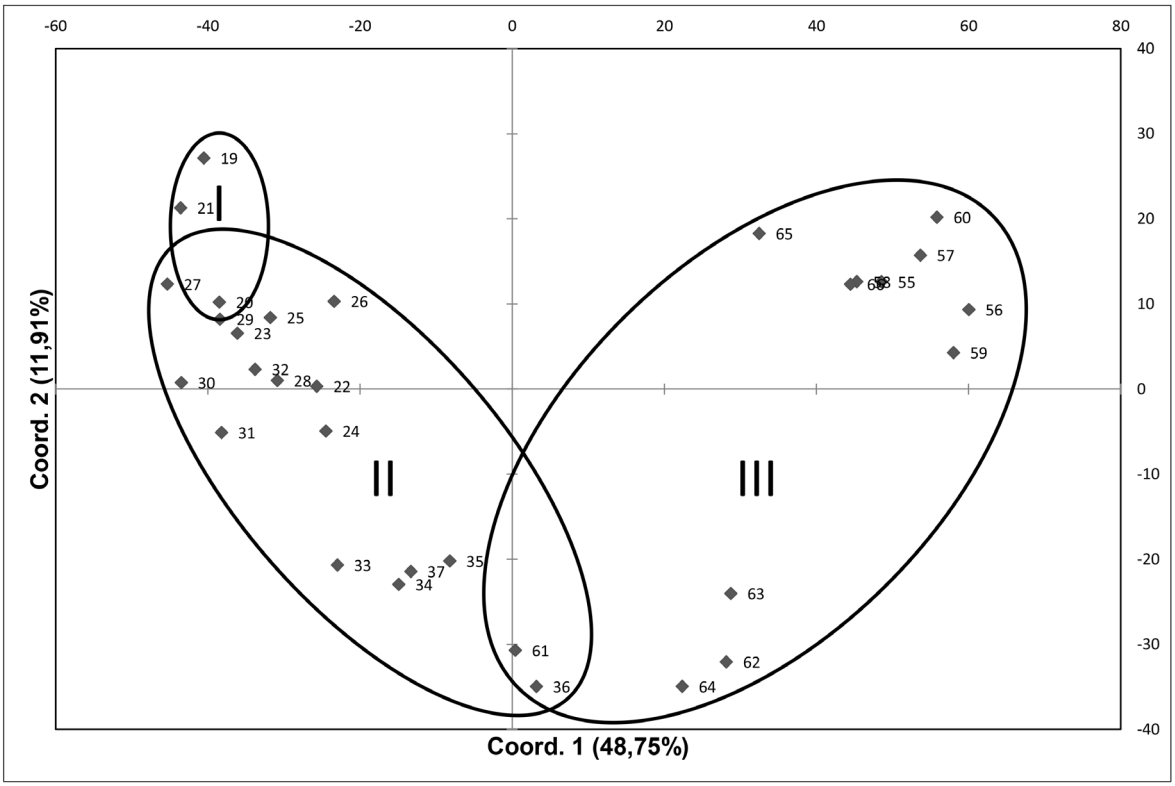

Figure 7. Principal coordinates between the different populations of *J. curcas*. Percentage of variation explained by the two axis = 58.78%. I = non toxic commercial variety. II = Fila cenizosa, Parque Nacional Diriá, Santa Cruz, Guanacaste. III = Parque Nacional Diriá, Santa Cruz, Guanacaste.

tive or discriminatory power in the primer combination in genetic diversity studies [8]. In this study, the 3 AFLP primer combinations showed an average PIC of 0.257. The maximum PIC value for bi-allelic markers as AFLP can be expected of 0.264. Based on the PIC value, the E-ACT/M-CTA primer combination is the recommended for the analysis of *Jatropha* germplasm in our study. The MI parameter confirmed in our study that the E-ACT/M-CTA combination is recommended to use it in the analysis of germplasm, which showed a value of 30.99. In this study, an MI value was obtained in the range of 26.96 - 30 (29.22 average) and a PIC value in the range of 0.245 - 0.269 (0.257 average), which are comparable to other studies. The primer combination used in our study showed RP values between 21.81 - 26.50 (23.93 average).

4.3. Genetic Relationships of *Jatropha* Populations

Also, it is important to optimize the design of the genetic improvement program to select genotypes and develop new populations [27]. In plant species, the genetic diversity tends to be higher near the geographical origin, which has been demonstrated in studies of *J. curcas* showing high genetic diversity in samples collected in Mexico (Veracruz, Puebla, Chiapas, Quintana Roo) [13] [16] [28]-[30]. This supports the hypothesis, based on morphological data and the natural distribution, indicating the Mesoamerican region (Mexico and Central America) as the possible center of origin of *J. curcas* [28]-[31]; although the average of the genetic diversity is limited, compared to the reported by other taxa from other endemic species.

Apart from *J. curcas*, the genetic diversity from other species of the genus considered native from Central America is still unknown. Therefore our research is pioneer in the study of the genetic diversity of 4 species of the genus *Jatropha* in the natural distribution area (Central America), and mainly of *J. costaricensis*. For the best of our knowledge this is the first report of a genetic analysis of *J. costaricensis* and *J. stevensii*. In the 114 accessions analyzed, high levels of polymorphisms were obtained compared with the reported from studies with materials from Africa, India and, China [13] [14] [17] [30] [32]. This pronounced genetic diversity indicates higher probabilities to improve the species by interspecific crossing, which has been successfully documented

previously (*J. curcas* × *J. integerrima*) [11] [19] [33]. The interspecific hybridization reveals the possibility of obtaining hybrids of *J. curcas* with other species of *Jatropha* [11].

Both PCA and UPGMA dendrogram divided the IV population in principal groups, coinciding by species. The dendogram shows high genetic similarity (Jaccard's similarity coefficient) between *J. curcas* and *J. stevensii*, and these two with *J. costaricensis*, and farthest with *J. gossypifolia*. This suggests that *J. curcas* with *J. stevensii* and *J. costaricensis* could have a common ancestor and more distant *J. gossypifolia*. This is consistent with the reports that point *J. curcas*, *J. integerrima*, *J. glandulifera*, and *J. gossypifolia* as native from America, and that they have been introduced to tropical countries in Africa and South East Asia (Indian, Indonesia, Malaysia and China) [30] [31] [34] [35].

However, our results differ from those found by Popluechai *et al*. [32], which report *J. gossypifolia* and *J. integérrima* closer to *J. curcas*. Also the same AFLP phylogram obtained by Sudheer *et al.* [19] showed a major cluster conformed by 5 species of *Jatropha* (*J. curcas*, *J. glandulifera*, *J. gossypifolia*, *J. integerrima*, and *J. tanjorensis*) and a minor cluster with *J. multifida* and *J. podagrica*, distributed in India. Basha and Sujatha [11], by combined data of RAPD and ISSR markers, grouped *J. curcas* with *J. gossypifolia* determining them as close relatives and as natural hybrid *J. tanjorensis*.

In another study using RAPD markers, all accessions of *J. curcas* were grouped in one group; a second group was formed of 6 species: *J. ramanadensis*, *J. gossypiifolia*, *J. podagrica*, *J. tanjorensis J. villosa* and *J. integérrima*, and finally a separate group of *J. glandulifera* [36]. *J. curcas* is considered the ancestral primitive species due to its morphological distinctive characteristics, where other species evolved from *J. curcas* and ancestral forms [37].

In our study, the principal component analysis (PCA) with only the accessions from *J. curcas* showed the formation of III principal groups, suggesting a broad genetic base from the germplasm. This germplasm base shows genotypes that could have few and probably unique genetic changes. This agrees with Pecina-Quintero *et al*. [13], where several accessions of *J. curcas* collected in three agro-climate regions of Chiapas, Mexico, showed high diversity. This shows nonrandom mating between groups and/or regions of accessions of *J. curcas*, which makes the selection of representative collections difficult. According to Pecina-Quintero *et al.* [13], these changes can be the result of the evolution of *J. curcas* to different environments or adaptation to different habitats, contrary to the study by He *et al.* [16], where AFLP analysis reported low genetic diversity from materials from Madagascar, Tanzania, and Surinam. Also, from four combinations of fluorescent primers, the genetic variation was characterized by 63 populations of *J. curcas* from 10 countries in Asia, Africa, and Mexico resulting in low genetic diversity between populations [17]. Nevertheless, the analyzed Mexican populations showed high values of genetic variation [16] [17]. In general, it has been demonstrating low genetic variation between accessions of *J. curcas* in Africa and Asia, and the reasons are not clearly defined [32]. However, Subramanyam *et al.* [24] reported high genetic diversity of *J. curcas* collected in 10 different eco-climate regions in India but by using RAPD markers. The variation of the genetic diversity within species is commonly related with the geographic range, reproduction mode, mating system, seed dispersal, and fertility [38].

Taking into account the local and global results [13] [16] [17] [32], we suggest that the high genetic variation detected between and within the populations of *Jatropha curcas* could be explained because of America being considered the range for the natural distribution of the species [31]. The introduction of this germplasm could be used to broaden the genetic base of *Jatropha* in countries like Africa and Asia (mainly in China and India), where the genetic diversity has been reported as low (35% - 50%), partly attributed to reduced genetic flow between populations, favored by the limited seed dispersal which occurs by gravity and anthropogenic dispersal (clonal reproduction) [10] [17] [32] [39]. It is agreed by several authors to use the germplasm from the range of natural distribution (Central America and Mexico) in genetic breeding programs for the species (mainly *J. curcas*) to broaden the genetic base [10] [17] [18] [32] [39], instead of germplasm from the same origins or introductions [7]. Knowledge of the genetic diversity of wild species *Jatropha* from Costa Rica will be useful for breeding programs and for the development of improved varieties. Understanding the genetic diversity, inter-relatedness, and differentiation of wild and introduced subpopulations of the species is a critical step towards selection and breeding of superior genotypes [17].

Conduct studies to determine whether the differentiation between populations of *J. costarricensis* is due to stochastic factors such as relief or wind direction, or if the distance between populations prevents pollen reach the nearest population, or flowering patterns differ between populations, or if there is an anthropomorphic factor, or the pollinators can't reach neighboring populations.

The number of samples obtained was due to factors such as difficult access to collection sites, the fact that the species is deciduous and scarcity of individuals in some species. It was not possible to analyze microsatellites because the genome of some of the species under study is unknown.

5. Conclusion

The AFLP primer combination AFLP E-ACT/M-CTA can be used in an efficient way for the differentiation of the four species, as it resulted in the highest number of polymorphic markers, which were specific for the species and populations of *J. curcas* and *J. costaricensis*. The identification of private bands per species can be used for screening samples of *Jatropha spp*. from unknown origin when comparing the unknown sample with the panel of private bands. The genetic analysis that elucidates the phylogeny of the *Jatropha* ssp. species states that *J. curcas* is more related to *J. stevensii*, those two with *J. costaricensis*, and *J. gossypifolia* as the farthest member of the genus. The populations of *J. curcas* are different between them. The identification collection and the maintenance of such genetically diverse wild germplasm from Costa Rica are of great importance in economic improvement of this species and for the selection of promising lines. Also the populations with very little or no human intervention may contribute more substantially towards the germplasm heterogeneity of *Jatropha costaricensis* for traits such as adaptability and oil yield.

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