A peer-reviewed version of this preprint was published in PeerJ on 9 February 2017.

View the peer-reviewed version (peerj.com/articles/2931), which is the preferred citable publication unless you specifically need to cite this preprint.

Vásquez-Mayorga M, Fuchs EJ, Hernández EJ, Herrera F, Hernández J, Moreira I, Arnáez E, Barboza NM. 2017. Molecular characterization and genetic diversity of *Jatropha curcas* L. in Costa Rica. PeerJ 5:e2931 https://doi.org/10.7717/peerj.2931

Molecular characterization and genetic diversity of *Jatropha curcas* L. in Costa Rica

Marcela Vásquez-Mayorga [1], **Eric J Fuchs** [2], **Eduardo J Hernández** [1], **Franklin Herrera** [3], **Jesús Hernández** [4], **Ilenana Moreira** [5], **Elizabeth Arnáez** [6], **Natalia M Barboza** Corresp. [1, 7, 8]

[1] Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica, San Pedro, San José, Costa Rica
[2] Escuela de Biología, Universidad de Costa Rica, San Pedro, San José, Costa Rica
[3] Estación Experimental Fabio Baudrit Moreno, Universidad de Costa Rica, Alajuela, Costa Rica
[4] Ministerio de Agricultura y Ganadería, San José, Costa Rica
[5] Escuela de Biología, Instituto Tecnológico de Costa Rica, Cartago, Costa Rica
[6] Escuela de Biología, Instituto Tecnológico de Costa Rica, Cartago, Cartago, Costa Rica
[7] Escuela de Tecnología de Alimentos, Universidad de Costa Rica, San Pedro, San José, Costa Rica
[8] Centro Nacional en Ciencia y Tecnología de Alimentos, Universidad de Costa Rica, San Pedro, San José, Costa Rica

Corresponding Author: Natalia M Barboza
Email address: natalia.barboza@ucr.ac.cr

We estimated the genetic diversity of 50 *Jatropha curcas* samples from the Costa Rican germplasm bank using 18 EST-SSR, one G-SSR and nrDNA-ITS markers. We also evaluated the phylogenetic relationships among samples using nuclear ribosomal ITS markers. Non-toxicity was evaluated using G-SSRs and SCARs markers. A Neighbor-Joining (NJ) tree and a Maximum Likelihood (ML) tree were constructed using SSR markers and ITS sequences, respectively. Heterozygosity was moderate (He = 0.346), but considerable compared to worldwide values for *J. curcas*. The PIC (PIC = 0.274) and inbreeding coefficient (f = -0.102) were both low. Clustering was not related to the geographical origin of accessions. International accessions clustered independently of collection sites, suggesting a lack of genetic structure, probably due to the wide distribution of this crop and ample gene flow. Molecular markers identified only one non-toxic accession (JCCR-24) from Mexico. This work is part of a countrywide effort to characterize the genetic diversity of the *Jatropha curcas* germplasm bank in Costa Rica.

Molecular characterization and genetic diversity of *Jatropha curcas* L. in Costa Rica

Marcela Vásquez-Mayorga[1], Eric J. Fuchs[2], Eduardo J. Hernández[1], Franklin Herrera[3], Jesús Hernández[4], Ileana Moreira[5], Elizabeth Arnáez[5], Natalia Barboza[1,6,7]

1 Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica, San José, Costa Rica

2 Escuela de Biología, Universidad de Costa Rica, San José, Costa Rica

3 Estación Experimental Fabio Baudrit Moreno, Universidad de Costa Rica, Alajuela, Costa Rica

4 Ministerio de Agricultura y Ganadería, Costa Rica, San José, Costa Rica

5 Escuela de Biología, Instituto Tecnológico de Costa Rica, Cartago, Costa Rica

6 Escuela de Tecnología de Alimentos, Universidad de Costa Rica, San José, Costa Rica

7 Centro Nacional en Ciencia y Tecnología de Alimentos, Universidad de Costa Rica, San José, Costa Rica

Corresponding Author:

Natalia Barboza

Ciudad de la Investigación, San Pedro, San José, 11501-2060 Costa Rica

Email address: natalia.barboza@ucr.ac.cr

ABSTRACT

We estimated the genetic diversity of 50 *Jatropha curcas* samples from the Costa Rican germplasm bank using 18 EST-SSR, one G-SSR and nrDNA-ITS markers. We also evaluated the phylogenetic relationships among samples using nuclear ribosomal ITS markers. Non-toxicity was evaluated using G-SSRs and SCARs markers. A Neighbor-Joining (NJ) tree and a Maximum Likelihood (ML) tree were constructed using SSR markers and ITS sequences, respectively. Heterozygosity was moderate (He = 0.346), but considerable compared to worldwide values for *J. curcas*. The PIC (PIC = 0.274) and inbreeding coefficient (f = -0.102) were both low. Clustering was not related to the geographical origin of accessions. International accessions clustered independently of collection sites, suggesting a lack of genetic structure, probably due to the wide distribution of this crop and ample gene flow. Molecular markers identified only one non-toxic accession (JCCR-24) from Mexico. This work is part of a countrywide effort to characterize the genetic diversity of the *Jatropha curcas* germplasm bank in Costa Rica.

INTRODUCTION

The use of fossil fuels for energy production is being discouraged because of global warming and fluctuating market prices. This situation has motivated research on alternative fuel sources such as biodiesel from corn or palm oil (*To & Grafton, 2015*). *Jatropha curcas* is being explored as a new biofuel crop (*Islam et al., 2013*). It is planted on approximately 1.8 million ha in Indonesia, China, Brazil and Africa, and has the potential to become a biofuel crop in India and other tropical countries (*Carels, 2013*). *J. curcas,* a member of the Euphorbiaceae family, is native to America and has a pantropical distribution. It grows well under unfavorable climatic and soil conditions, making it an attractive biofuel crop. Average oil content per seed is 40-45 % (*Jongschaap et al., 2007*). Biofuel from this species is similar in quality to biofuels derived from conventional crops like canola, linseed and sunflower, and surpasses the quality of biofuels produced from soybean (*Basili & Fontini, 2012*).

The potential of *J. curcas* has not been fully exploited, mainly because of its variable and unpredictable oil yield that limits large-scale cultivation. Genetic improvement may alleviate this problem; however, characterization of the available germplasm is needed for breeding programs to be efficient (*King et al., 2015; Mastan et al., 2012*). Over the past decade, *J. curcas* germplasm has been genetically evaluated in India, China, Brazil, Mexico, Costa Rica and Central America (*Avendaño et al., 2015; Basha & Sujatha, 2007; China Plant BOL Group et al., 2011; Montes Osorio et al., 2014; Pecina-Quintero et al., 2014; Rosado et al., 2010; Wen et al., 2010*). Molecular markers such as RAPDs, ISSRs, AFLPs, genomic simple sequence repeats (G-SSR) and expressed sequence tags-SSR (EST-SSR) have all been used to assess the genetic diversity of *J. curcas* collections. These studies have revealed low levels of genetic variability in India and Brazil (*China Plant BOL Group et al., 2011; Rosado et al., 2010; Sun et al., 2008; Yadav et al., 2011*). Numerous

authors consider Mexico and Central America to be the center of origin and diversification (*Abdulla et al., 2009; Basha et al., 2009; Heller, 1996; Openshaw, 2000; Pamidimarri, Chattopadhyay & Reddy, 2008; Pamidimarri & Reddy, 2014; Pecina-Quintero et al., 2011; Tatikonda et al., 2009*), and high levels of genetic diversity in Guatemala (*Raposo et al., 2014*) and Mexico support this hypothesis (*Ambrosi et al., 2010; Ovando-Medina, Adriano-Anaya & Vásquez-Ovando, 2013; Ovando-Medina et al., 2011; Pamidimarri & Reddy, 2014; Pecina-Quintero et al., 2011*). However, these studies have consistently shown a lack of relationship between the geographic proximity of collection sites and the genetic similarity among accessions, because collection sites rarely represent the place of origin of accessions.

Another limitation to large-scale production of *J. curcas* is the possible toxicity of the seed. Both toxic and non-toxic genotypes of *J. curcas* are known (Insanu *et al.,* 2013). Varieties with a non-toxic seed cake would be more readily accepted by local farmers because sub-products of the oil extraction process could be utilized for animal feeding (*Makker & Becker, 2015*). Therefore, it is important to evaluate materials stored in germoplasm banks for non-toxicity. Non-toxic genotypes have been described in Mexican accessions (*Vera-Castillo et al., 2014*). As with variation in toxicity, genotypes with variable seed oil content and number of seeds may surface in other Latin American regions and contribute to *J. curcas* breeding programs all over the world.

In the past decade, SSR markers have proven useful for the analysis of genetic diversity. Simple sequence repeats that identify variability in transcribed genomic regions can be found in EST libraries. They may facilitate the identification of functional candidate genes, increase the efficiency of marker-assisted selection and serve as markers for comparative mapping (*Varshney, Graner & Sorrells, 2005*). Nuclear ribosomal-DNA internal transcribed spacers (nrDNA ITS) are sequence-based markers that have been used in phylogenetic studies and to assess genetic diversity

at the species level in a wide range of taxonomic groups (*Nieto-Feliner & Roselló, 2007*). ITS markers have been developed for *J. curcas* (*Pecina-Quintero et al., 2011*) and are believed to have greater discriminatory capacity than plastid *rbcL* and *matK* markers (*China Plant BOL Group et al., 2011*). SSR and ITS markers used together allow for a better understanding of the evolutionary history of undomesticated species such as *J. curcas*.

Prior to the current study, the genetic variability of the Costa Rican *J. curcas* germplasm bank had not been studied. The present study explores the molecular diversity of accessions using EST-SSR and G-SSR markers and evaluates the phylogenetic relationships between them using nuclear ribosomal ITS markers. Alleles associated with *J. curcas* toxicity were also evaluated with G-SSRs and sequence characterized amplified region (SCAR) markers.

MATERIALS & METHODS

DNA collection and extraction

The germplasm bank of *Jatropha curcas* in Costa Rica includes accessions from Honduras, Brazil, India, Mexico, El Salvador, Ecuador, Uganda, Colombia and South Africa. Costa Rican accessions include only spontaneously occurring individuals collected in the field and vegetatively propagated. The collection does not include material from commercial plantations (Fig. 1). International accessions originated from seeds collected from spontaneously occurring plants in each country. All accessions are maintained by vegetative propagation in the germplasm bank located at the Fabio Baudrit Experimental Station (10°00'10.3"N, 84°16'17.6"W) at Universidad de Costa Rica.

For genetic analysis, two young leaves were collected from each of 50 plants representing different accessions in the germplasm bank (Table 1). Vegetative material was frozen and later lyophilized

for DNA extraction. Nucleic acids were extracted using the *Möller et al.* (*1992*) protocol and quantified using a Nanodrop (Thermo Scientific).

SSR analysis

Eighteen EST-SSRs and one G-SSR marker (*Wen et al., 2010*) were used to analyze the 50 samples (Table 2). PCR was performed in a final volume of 25 µL with 1 X PCR buffer, 400 µM dNTPs, 0.4 µM of each primer, and 1 U Taq DNA polymerase (Thermo Scientific). Amplification conditions included an initial denaturation at 94 °C for 1 min, followed by 35 cycles of 94 °C for 1 min, 42-50 °C for 1 min (depending on the annealing temperature of each primer pair) and 72 °C for 1 min, with a final extension of 72 °C for 10 min. Results were visualized in 4-6 % polyacrylamide gels dyed with silver nitrate. To confirm the results, duplicates of 10 % of the samples were made. Acrylamide gels were scored manually in the GNU Image Manipulation Program (www.gimp.org) and a data matrix with band size data was created. The SSRs were scored according to amplicon size. The software Microchecker was used to test for null alleles and allelic dropout (*van Oosterhout et al., 2004*).

nrDNA-ITS region amplification and sequencing

ITS primers were used to elucidate phylogenetic relationships between accessions. The primer pair JCITS-1-F (5´-ACCTGCGGAAGGATCATTGTCGAAA-3´) and JCITS- 2-R (5´CCTGGGGTCGCGATGTGAGCGT 3´) was used (*Pamidimarri, Chattopadhyay & Reddy, 2008*) in a PCR reaction with a final volume of 25 µL and a final concentration of 1 X reaction buffer, 1.5 mM $MgCl_2$, 0.2 µM of each primer, 0.2 mM dNTPs, 1 U Taq DNA polymerase (Thermo Scientific) and 50 ng of DNA. The PCR program consisted of an initial denaturation step

of 94 °C for 1 min, followed by 30 cycles of 40 s at 94 °C, 65 °C for 1 min and 72 °C for 1 min. The final extension step was 5 min at 72 °C. The PCR products were sent to Macrogen® for sequencing. The obtained sequences were aligned and submitted to Genbank (www.ncbi.com).

Data analysis

Genetic diversity was quantified as the expected heterozygosity, the Polymorphism Information content (PIC) and the inbreeding coefficient (*f*). The PIC index describes the probability that two random accessions would have different alleles at a random locus (*Smith et al., 2004*). All estimates were calculated using the Powermarker 3.25 software (*Liu & Muse, 2005*). The validity of all estimates was assessed by means of 50 000 bootstraps.

To determine the genetic relation between accessions, we calculated the $\delta\mu^2$ genetic distance between accessions using the POPULATIONS software (http://bioinformatics.org/~tryphon/populations/). The $\delta\mu^2$ metric developed by *Goldstein et al.* (*1995*) is based on the stepwise mutation model and estimated distances are a linear function of divergence time; this distance is preferred for taxa that have diverged widely (*Goldstein et al., 1995*). We used the distance matrix to construct a Neighbor-Joining (NJ) tree using the default parameters in POPULATIONS. The standardized genetic distance matrix was also used to perform a Principal Coordinates Analysis (PCA) in GenAlEx 6.5 (*Peakall & Smouse, 2012*).

For phylogenetic analysis, ITS sequences for *J. curcas* samples from Mexico, India, Cape Verde, Spain, Africa and Madagascar were downloaded from GenBank (http://www.ncbi.nlm.nih.gov). Sequences from Costa Rican samples were edited with BioEdit software version 7.2.5 and aligned using the MAFFT algorithm in the GUIDANCE server. To compare the phylogenetic relationship of GenBank sequences with those from the Costa Rican *J. curcas* germplasm bank, we initially

used jModelTest 2.1.7 (*Santorum et al., 2014*) to define the optimum substitution model for all sequences and the GTR model (*Tavaré, 1986*) with uniform rates was chosen. A maximum likelihood (ML) tree was constructed with 3 000 bootstrap replications and expressed as the number of base substitutions per site using MEGA 6.0 (*Tamura et al., 2013*).

Toxicity evaluation

Plant toxicity was evaluated by the presence of alleles from three SSR markers associated with lack of toxicity (Table 3) (*Vischi, Raranciuc & Baldini, 2013*). PCR amplification was achieved in a final volume of 25 µL with a final concentration of 1 X reaction buffer, 1.5 mM of $MgCl_2$, 0.2 µM of each primer, 0.2 mM dNTPs, 1 U Taq DNA polymerase (Thermo Scientific) and 50 ng/µl DNA sample. The PCR program had an initial denaturation of 94 °C for 1 min, 30 cycles of 40 s at 94 °C, 65 °C for 1 min, 72 °C for 1 min and a final extension of 5 min at 72 °C. Results were visualized by genotyping with fluorescent dyes (FAM, VIC and PET) (*Vischi, Raranciuc & Baldini, 2013*) in a 3130 sequencer (Applied Biosystems). Two SCAR markers (ISPJ1 and ISPJ2) were also used to evaluate alleles for toxicity in all accessions following the protocol of *Basha & Sujatha* (*2007*). ISPJ1 amplifies a 543 bp fragment and is specific for toxic genotypes, while ISPJ2 is specific for non-toxic genotypes and amplifies a 1096 bp fragment. Results were visualized in a 2 % agarose gel run for one hour and dyed with GelRed (Biotium). Plants were scored as toxic or non-toxic based on the presence and size of amplicons.

RESULTS

Genetic diversity analysis

Genetic diversity was estimated using 18 SSR-ESTs and one G-SSR marker. Average heterozygosity (He) was 0.346 ± 0.062 (± SD). Polymorphism information contents (PIC) ranged from 0.042 to 0.677, with a mean PIC of 0.274 ± 0.165. We did not find evidence of inbreeding *f* = -0.102 ± 0.346 (Table 4).

The NJ tree did not show a clear clustering pattern (Fig. 2) and clusters did not reflect geographic proximity. Accessions from countries located in close proximity such as Colombia (JCCR-38) and Ecuador (JCCR-25) did not seem to be genetically close to each other. In contrast, samples from distant locations clustered together, for example, India (JCCR-INDIA) and Costa Rica (JCCR-14); South Africa (JCCR-47) and Honduras (JCCR-2); Brazil (JCCR-16) and Costa Rica (JCCR-MIR); and Mexico (JCCR-24) and Ecuador (JCCR-25). Within Costa Rica, there was no evidence of geographic structure. Samples collected from sites separated by more than 300 km grouped together (JCCR-20, JCCR-7).

Our PCA analysis produced comparable results. Genetic information accounted for 41.26 % of the observed variance; the first two components explained 19.17 % and 11.86 % of the total variance, respectively. We did not observe distinct groups of accessions in a biplot of the first two components (Fig. 3). Accessions from the same country did not group together, such as those from Mexico (JCCR-24 and JCCR-31) and India (JCCR-INDIA and JCCR-27 INDIA). Costa Rican accessions were scattered throughout the plot without any discernible pattern. These results were congruent with our NJ tree.

nrDNA-ITS sequence analysis

As with the NJ and PCA analysis, the observed patterns from the Maximum Likelihood (ML) tree did not reflect the geographic origin of the accessions (Fig. 4) and in most cases, the clades grouped in polytomies. International and Costa Rican accessions from this research were scattered

throughout the tree (JCC-38, JCCR-2, JCCRLEP, JCCR33-1, JCCR31, JCCR21, JCCR19, JCCR9, JCCR8, JCCR7, JCCR3), they grouped with sequences from Mexico and with individuals from Spain, Cape Verde, Africa and Madagascar (GenBank accession numbers EU700449, EU70055, EU70046, EU70045, respectively). It is important to mention that the observed clustering of germplasm independent of geographical origin could be an artifact of unequal sampling, as the majority of the samples used in this research were from Costa Rica and only one or two representatives from each of the other countries were included.

Toxicity evaluation

A single Mexican accession (JCCR-24) was identified as non-toxic by the SSR primer set (JCT-31, JCT-27 and JcSSR-26) and both SCAR primers ISPJ1 and ISPJ2. ISPJ1 also identified non-toxic genotypes in two other accessions: JCCR-32 and JCCR-43. Using ISPJ2, accessions JCCR-22 and JCCR25 were identified as non-toxic. These results need to be corroborated using *in vivo* assays.

DISCUSSION

Genetic diversity of *Jatropha curcas*

Our study is the first to provide genetic diversity estimates for Costa Rican *J. curcas* samples. Based on morphological and molecular evidence, different authors support the idea that Mexico and Central America may be the center of origin and diversification for *J. curcas* (*Pamidimarri & Reddy, 2014; Pecina-Quintero et al., 2014*). *Pecina-Quintero et al.* (*2014*) found high genetic diversity of *J. curcas* in Mexico. *Grativol et al.* (*2010*) analyzed 332 accessions from 12 locations in Brazil using ISSR primers and reported lower genetic diversity than that reported in Mexico. *Pamidimarri & Reddy* (*2014*) used RAPD and AFLPs to analyze the molecular diversity of 42

Indian accessions of *J. curcas* and found a mean percentage of polymorphism (PP) of 26.47. In the same study, the average PP of Mexican accessions was 33.18. The mean PP of the germplasm samples excluding the Indian accessions was 35.86, supporting the hypothesis that Indian germplasm is less diverse than germplasm in other regions of the world (*Colombo, Second & Charrier, 2000; Ram, Kumar & Bhatt, 2004*). *Montes et al*. (*2014*) also found higher genetic diversity in Mexican and Central American accessions compared to those from other parts of the world. Given the observed levels of genetic diversity, Costa Rica may be a secondary center of origin or diversification for this species (*Pamidimarri & Reddy 2014; Pecina-Quintero et al., 2014*). However, comparisons are limited since few studies have evaluated genetic diversity of *J. curcas* germplasm using EST-SSR markers. *Wen et al.* (*2010*) evaluated 45 accessions from Indonesia, Grenada, South America and two Chinese provinces, and found a mean genetic diversity of 0.3819. The most diverse locations were South America and Yunnan with H= 0.33 and H= 0.3473, respectively. In another study, 50 EST-SSR markers were used to evaluate 25 Indian accessions and an average He of 0.30 was found (*Yadav et al., 2011*). As in our study, accessions clustered independently of geographic origin. Our PIC estimates (PIC= 0.274 $\pm$ 0.165) were comparable to those obtained by *Yadav et al.* (*2011*) (PIC= 0.25 ± 0.16) and are considered moderately informative (*Botstein et al., 1980*).

In Costa Rica, *Jatropha curcas* is typically not cultivated commercially. Plants usually grow as hedgerows and are occasionally reproduced by farmers through cuttings. Our samples represent plants growing spontaneously in the field; no commercially grown material was included in the study. Therefore, our genetic diversity estimates represent the standing natural variation of this species. However, since EST-SSR markers were developed from expressed sequence tag libraries, they reside within genes and are subject to selection, which reduces unfavorable polymorphisms

(*Cova et al., 2012; Ellis & Burke 2007*). EST-SSR markers are less polymorphic than genomic SSRs (*Song et al., 2012*) and consequently, genetic diversity may have been underestimated in this study. *J. curcas* is a predominantly outcrossing species (tm= 0.683) (*Bressan et al., 2013*). As expected, we found no significant evidence of inbreeding (f = -0.102). High rates of gene flow should produce low levels of inbreeding, which would result in the low structure suggested by our NJ and ML clustering. Low inbreeding coefficients were also estimated in Mexico and South America (*Ambrosi et al., 2010*). Although inbreeding was negligible in the present study, in other parts of the world such as India and Brazil, lower genetic diversity has been attributed to increased selfing or a high paternity correlation due to the spread of introductions across the country through vegetative propagation, recent common ancestry, drift, and intensive selection of the currently cultivated materials since the time of introduction (*Basha & Sujatha, 2007; Bressan et al., 2013; Rosado et al., 2010*).

Phylogenetic analysis of *Jatropha curcas*

Several studies of *J. curcas* have shown that collection sites do not necessarily reflect the genetic origin of accessions (*Ambrosi et al., 2010; Maghuly et al., 2015*). Our NJ tree and PCA analysis (Fig.2 and Fig.3) showed no correlation between genetic similarity and geographic proximity. For example, the two Mexican accessions, JCCR-24 and JCCR-31, clustered in different putative groups. Also, the two Indian accessions (JCCR-27 and JCCR-INDIA) did not seem to be related. *J. curcas* is widely cultivated and plants are exchanged commonly. Accessions from the same country may come from diverse origins and thus may be placed in different clades. Our analysis suggests that collection sites may not necessarily represent local germplasm, but genetically distinct lineages from different geographic regions. Material exchanges between American,

African and Asian collections have occurred commonly over the last 200 years (*Heller, 1996*) and may have resulted in founder effects in Africa and Asia (*Henning, 2007; Lengkeek, 2007; Pamidimarri & Reddy 2014*). For example, according to *Pamidimarri & Reddy* (*2014*), Portuguese seafarers introduced accessions from Mexico and Central America to India through two dispersal routes: one brought *J. curcas* through Africa, Madagascar and finally to India, while the other passed through Spain on its way to India. These migration routes support our findings of a widely dispersed plant with little geographic structure.

The observed ML tree topology may be an artifact of the low level of genetic structure seen in our other analysis. Concurrently, low levels of inbreeding suggest considerable gene flow may be occurring in *J. curcas* in Costa Rica. Although our samples did not cluster similarly across analysis, a general lack of group structure was maintained throughout. The lack of consensus between clustering algorithms may be attributed to the nature of the different markers used. We analyzed multiple EST-SSR loci distributed throughout the genome (*Davies & Bermingham, 2002; Pamidimarri & Reddy, 2014*), and we are confident that we have accounted for a significant portion of the genetic variability in this species. Costa Rican diversity estimates may be improved by enriching the germplasm bank with more accessions from the Caribbean and southern parts of the country.

Toxicity evaluation of *J. curcas*

Our results show that only one of the accessions, Mexican JCCR-24, had all of the alleles that indicate non-toxicity. This accession was previously confirmed as non-toxic by *in vivo* evaluation (*Basha & Sujatha, 2007*). JCCR-24 could be used as a parental plant in a breeding program to obtain dual-purpose non-toxic plants, thereby increasing the attractiveness of *J. curcas* as a biofuel

plant (*King et al., 2013*). In other samples, only one or two toxic alleles were detected, depending on the primers used. This may have been due to variations present in Costa Rican genotypes. SSR markers are very polymorphic *(Powell et al., 1996)*. The primers used to detect non-toxicity were developed from Mexican, Asian and African accessions (*Basha & Sujatha, 2007; Phumichai et al., 2011*) and their ability to detect toxicity may differ for genotypes from other parts of the world. It is possible that other Costa Rican accessions have alleles that have not yet been identified as indicative of non-toxicity, and in this case, the non-toxic nature of the accessions would have been overlooked. *In vivo* evaluations are needed to confirm this hypothesis.

ACKNOWLEDGEMENTS

We thank the Estación Experimental Fabio Baudrit Moreno (EEFBM) of the Universidad de Costa Rica for the collection and maintenance of the germplasm bank.

ADDITIONAL INFORMATION

DNA Submission to GenBank

The following information was supplied regarding the submission of DNA sequences: GenBank accession no.**:** KU561375, KU561376, KU561377, KU561378, KU561379, KU561380, KU561381, KU561382, KU561383, KU561384, KU561385, KU561386, KU561387, KU561388, KU561389, KU561390, KU561391, KU561393, KU561394, KU561395, KU561396, KU561397, KU561398, KU561399, KU561400, KU561401, KU561402, KU561403, KU561404, KU561405, KU561406, KU561407, KU561408, KU561409, KU561410, KU561411, KU561412, KU561413, KU561414, KU561415, KU561416, KU561417, KU561418, KU561419, KU561420, KU561421, KU561422, KU561423, KU561424.

REFERENCES

Abdulla JM, Janagoudar BS, Biradar DP, Ravikumar RL, Koti RV, Patil SJ. 2009. Genetic diversity analysis of elite *Jatropha curcas* L. genotypes using randomly amplified polymorphic DNA markers. *Journal of Agricultural Science* 22:293-295.

Ambrosi DG, Galla G, Purelli M, Barbi T, Fabbri A, Lucretti S, Sharbel TF, Barcaccia G. 2010. DNA Markers and FCSS Analysis Shed Light on the Genetic Diversity and Reproductive Strategy of *Jatropha curcas* L. *Diversity* 2:810-836 DOI10.3390/d2050810.

Avendaño R, García Díaz E, Valdez-Melara M, Chaves Solano N, Mora Villalobos A, Aguilar Cascante F, Williamson Benavides B, Solís-Ramos L. 2015. Genetic Diversity Analysis of *Jatropha* Species from Costa Rica Using AFLP Markers. *American Journal of Plant Sciences* 6:2426-2438 DOI10.4236/ajps.2015.614245.

Basili M, Fontini F. 2012. Biofuel from *Jatropha curcas*: Environmental sustainability and option value. *Ecological Economics* 78:1-8 DOI10.1016/j.ecolecon.2012.03.010.

Basha SD, Sujatha M. 2007. Inter and intrapopulation variability of *Jatropha curcas* (L.) characterized by RAPD and ISSR markers and development of population-specific SCAR markers. *Euphytica* 156:375-386 DOI 10.1007/s10681-007-9387-5.

Basha SD, Francis G, Becker K, Makkar HPS, Sujatha M. 2009. A comparative study of biochemical traits and molecular markers for assessment of relationships between *Jatropha curcas* germplasm in China. *Biomass & Bioenergy* 7:1-12 DOI10.1016/j.plantsci.2009.03.008.

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *The American Journal of Human Genetics* 32:314-331.

Bressan EA, Sebbenn AM, Rodrigues Ferreira R, Lee TSG, Figueira A. 2013. *Jatropha curcas* L. (Euphorbiaceae) exhibits mixed mating system, high correlate mating and apomixis. *Tree Genetics & Genomes* 9:1089-197 DOI 10.1007/s11295-013-0623-y.

Carels N. 2013. Towards the domestication of *Jatropha*: The integration of science. In: Bahadur B, Sujatha M, Carels N (ed) *Jatropha* Challenges for a New Energy Crop: Volume II. Springer, New York, 263-299.

China Plant BOL Group, De-Zhu L, Lian-Ming G, Hong-Tao L, Hong W, Xue-Jun G, Jian-Quan L, Zhi-Duan C, Shi-Liang Z, Shi-Lin C, Jun-Bo Y, Cheng-Xin F, Chun-Xia Z, Hai-Fei Y, Ying-Jie Z, Yong-Shuai S, Si-Yun C, Lei Z, Kun W, Tuo Y, Guang-Wen D. 2011. Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *PNAS* 108:19641-19646 DOI 10.1073/pnas.1104551108.

Colombo C, Second G, Charrier A. 2000. Diversity within American cassava germplasm based on RAPD markers. *Genetics and Molecular Biology* 23:189–199.

Cova V, Perini D, Soglio V, Komjanc M, van de Weg E, Glessler C, Gianfranceschi L. 2012. Exploiting expressed sequence tag database for mapping markers associated with fruit development and fruit quality in apple. *Molecular Breeding* 29:699-715.

Davies N, Bermingham E. 2002. The historical biogeography of two Caribbean butterflies (Lepidoptera: heliconiidae) as inferred from genetic variation at multiple loci. *Evolution* 56:573–589.

Ellis JR, Burke JM. 2007. EST-SSRs as a resource for population genetic analysis. *Heredity* 99:125–132.

Goldstein DB, Ruiz Linares A, Cavalli Sforza LL, Feldman MW. 1995. An evaluation of genetic distances for use with microsatellite loci. *Genetics* 139:463-471.

Grativol C, da Fonseca Lira-Medeiros C, Hemerly AS, Ferreira PCG. 2010. High efficiency and reliability of inter-simple sequence repeats (ISSR) markers for evaluation of genetic diversity in Brazilian cultivated *Jatropha curcas* L. accessions. *Molecular Biology Reports* 38:4245-4256 DOI 10.1007/s11033-010-0547-7.

Heller J. 1996. Physic Nut *Jatropha curcas* L. *Promoting the Conservation and use of Underutilized and Neglected Crops.* Rome: IPGRI, 10–11.

Henning RK. 2007. *Jatropha curcas* L. In: van der Vossen HAM, Mkamilo GS, ed. Plant Resources of the Tropical Africa: Volume I. Vegetable oils PROTA Fondation, Wageningen, The Netherlands, 14:116–122.

Insanu M, Dimaki C, Wilkins R, Brooker J, van der Linde P, Kayser O. 2013. Rational use of *Jatropha curcas* L. in food and medicine: from toxicity problems to safe applications. Phytochemistry review 12:107–119.

Islam A, Taufiq-Yap YH, Chu CH, Ravindra P, Chan ES. 2013. Transesterification of palm oil using KF and NaNO3 catalysts supported on spherical millimetric γ-Al2O3. *Renewable Energy* 59:23–29.

Jongschaap REE, Corré WJ, Bindraban PS, Brandenburg, WA. 2007. Claims and Facts. In: *Jatropha curcas* L.: Global *Jatropha curcas* Evaluation, Breeding and propagation programme. Report 158 Plant Research International BV, Wageningen, The Netherlands, 42.

King AJ, Montes LR, Clarke JG, Affleck J, Li Y, Witsenboer H, van der Vossen E, van der Linde P, Tripathi Y, Tavares E, Shukla P, Rajasekaran T, van Loo EN, Graham IA. 2013. Linkage mapping in the oilseed crop *Jatropha curcas* L. reveals a locus controlling the

biosynthesis of phorbol esters which cause seed toxicity. *Plant Biotechnology J* 11:986–996 DOI 10.1111/pbi.12092.

King AJ, Montes LR, Clarke JG, Itzep J, Perez CA, Jongschaap RE, Visser RG, van Loo EN, Graham I. 2015. Identification of QTL markers contributing to plant growth, oil yield and fatty acid composition in the oilseed crop *Jatropha curcas* L. *Biotechnology for Biofuels* 8:1-17 DOI 10.1186/s13068-015-0326-8.

Lengkeek AG. 2007. The *Jatropha curcas* agroforestry strategy of Mali Biocarburant SA. In: Proceedings of the FACT Seminar on *Jatropha curcas* L. Agronomy and Genetics. Wageningen, The Netherlands, 597.

Liu K, Muse SV. 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21:228-2129 DOI 10.1093/bioinformatics/bti282.

Maghuly F, Jankowicz-Cieslak J, Parbinger S, Till BJ, Laimer M. 2015. Geographic origin is not supported by the genetic variability found in a large living collection of *Jatropha curcas* with accessions from three continents. *Biotechnology Journal* 10:536-551 DOI 10.1002/biot.201400196.

Makker HPD, Becker K. 2015. *Jatropha curcas*, a promising crop for the generation of biodisel and value-added coproducts. *European Journal of Lipid Science and Technology* 11:773-787 DOI 10.1002/ejlt.200800244.

Mastan S, Sudheer P, Rahman H, Ghosh A, Rathore M, Ravi Prakash C, Chikara J. 2012. Molecular characterization of intra-population variability of *Jatropha curcas* L. using DNA based molecular markers. *Molecular Biology Reports* 39:4383-4390 DOI 10.1007/s11033-011-1226-z.

Möller EM, Bahnweg G, Sandermann H, Geiger HH. 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Research* 22: 6115-6116**.**

Montes Osorio LR, Torres Salvador AF, Jongschaap REE, Azurdia C, Berduo J, Trindade LM, Visser RGF, Loo EN van. 2014. High level of molecular and phenotypic biodiversity in *Jatropha curcas* from Central America compared to Africa, Asia and South America. *BMC Plant Biology* 14:19 DOI10.1186/1471-2229-14-77.

Nieto-Feliner G, Roselló JA. 2007. Better the devil you know? Guideline for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. *Molecular Phylogenetics and Evolution* 44:911-919.

Openshaw K. 2000. A review of *Jatropha curcas*: an oil plant of unfulfilled promise. *Biomass & Bioenergy* 19:1-15 DOI 10.1016/S0961-9534(00)00019-2.

Ovando-Medina I, Espinosa GF, Nuñez FJ, Salvador FM. 2011. Genetic variation in Mexican *Jatropha curcas* L. estimated with seed oil fatty acids. *Journal of Oleo Science* 60:301-311.

Ovando-Medina I, Adriano-Anaya L, Vásquez-Ovando A. 2013. Genetic diversity of *Jatropha curcas* in southern Mexico. In: Bahadur B, Sujatha M, Carels N (ed) *Jatropha* Challenges for a New Energy Crop. Springer, New York, pp 263-299,

Pamidimarri SDV, Chattopadhyay B, Reddy MP. 2008. Genetic divergence and phylogenetic analysis of genus *Jatropha* based on nuclear ribosomal DNA ITS sequence. *Molecular Biology Reports* 36:1929-1935 DOI 10.1007/s11033-008-9401-6.

Pamidimarri SDV, Reddy MO. 2014. Phylogeography and molecular diversity analysis of *Jatropha curcas* L. and the dispersal route revealed by RAPD, AFLP and nrDNA-ITS analysis. *Molecular Biology Reports* 41:3225-34 DOI 10.1007/s11033-014-3185-7.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28:2537-2539.

Pecina-Quintero V, Amaya JL, Zamarripa A, Montes N, Núñez C, Solís J. 2011. Molecular characterization of *Jatropha curcas* L. genetic resources from Chiapas, México through AFLP markers. *Biomass & Bioenergy* 35:1897-1905 DOI 10.1016/j.biombioe.2011.01.027.

Pecina-Quintero V, Anaya-Lopez JL, Zamarripa-Colmenero A, Nunez-Colin CA. 2014. Genetic structure of *Jatropha curcas* L. in Mexico and probable center of origin. *Biomass & Bioenergy* 60:147–155 DOI 10.1016/j.biombioe.2013.11.005.

Phumichai C, Phumichai T, Kongsiri N, Wongkaew A, Sripichit P, Kaveeta R. 2011. Isolation of 55 microsatellite markers for *Jatropha curcas* and its closely related species. *Biologia Plantarum*

55:387-390.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* 2:225-238.

Ram J, Kumar A, Bhatt J. 2004. Plant diversity in six forest types of Uttaranchal, Central Himalaya, India. *Current Science* 86:975-978.

Raposo RS, Souza IGB, Veloso MEC, Kobayashi AK, Laviola BG, Diniz FM. 2014. Development of novel simple sequence repeat markers from a genomic sequence survey database and their application for diversity assessment in *Jatropha curcas* germplasm from Guatemala. *Genetics and molecular research* 13:6099-6106 DOI 10.4238/2014.August.7.25.

Rosado TB, Laviola BG, Faria DA, Pappas MR, Bhering LL, Quirino B, Grattapaglia D. 2010. Molecular markers reveal limited genetic diversity in a large germplasm collection of the

biofuel crop *Jatropha curcas* L. in Brazil. *Crop Science* 50:2372-2382 DOI 10.2135/cropsci2010.02.0112.

Santorum JM, Darriba D, Taboada GL, Posada D. 2014. *jmodeltest*.org: selection of nucleotide substitution models on the cloud. *Bioinformatics* 30:1310–1311 DOI 10.1093/bioinformatics/btu032.

Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Mitchell SE, Kresovich S, Ziegle J. 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. *Theoretical and Applied Genetics* 95:163–173.

Song YP, Jiang XB, Zhang M, Wang ZL, Bo WH, An XM, Zhang DQ, Zhang ZY. 2012. Differences of EST-SSR and genomic-SSR markers in assessing genetic diversity in poplar. *Forestry Studies in China* 14:1-7 DOI 10.1007/s11632-012-0106-5**.**

Sun QB, Li LF, Li Y, Wu GJ, Ge XJ. 2008. SSR and AFLP markers reveal low genetic diversity in the biofuel plant *Jatropha curcas* in China. *Crop Science* 48:1865-1871 DOI 10.2135/cropsci2008.02.0074.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30:2725-2729 DOI 10.1093/molbev/mst197.

Tatikonda L, Wani PS, Kannan S, Beerelli N, Sreedevi KT, Hoisington AD. 2009. AFLP based molecular characterization of an elite germplasm collection of *Jatropha curcas* L., biofuel plant. *Plant Science* 176:505-513.

Tavaré S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. In: American Mathematical Society. *Lectures on Mathematics in the Life Sciences* 17:57-86.

476 **To H, Grafton Q. 2015.** Oil prices, biofuels production and food security: past trends and future
477 challenges. *Food Security* 7:323-336 DOI 10.1007/s12571-015-0438-9

478 **van Oosterhout C, Hutchinson WF, Wills DP, Shipley P. 2004.** MICRO-CHECKER: software
479 for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*
480 4:535-538 DOI 10.1111/j.1471-8286.2004.00684.x.

481 **Varshney RK, Graner A, Sorrells ME. 2005.** Genic microsatellite markers in plants: features
482 and applications. *Trends in Biotechnology* 23:48-55 DOI 10.1016/j.tibtech.2004.11.005.

483 **Vera-Castillo YB, Cuevas JA, Valenzuela-Zapata AG, Urbano B, Gonzalez-Andres F. 2014.**
484 Biodiversity and indigenous management of the endangered non-toxic germplasm of Jatropha
485 curcas L. in the Totonacapan (Mexico), and the implications for its conservation. *Genetic*
486 *Resources and Crop Evolution* 61:1263-1278 DOI: 10.1007/s10722-014-0109-2.

487 **Vischi M, Raranciuc S, Baldini M. 2013.** Evaluation of genetic diversity between toxic and non
488 toxic *Jatropha curcas* L. accessions using a set of simple sequence repeat (SSR) markers. *African*
489 *Journal of Biotechnology* 12: 265-274 DOI 10.5897/AJB12.2656.

490 **Wen M, Wang H, Xia Z, Zou M, Lu C, Wang W. 2010.** Development of EST-SSR and genomic
491 SSR markers to assess genetic diversity in *Jatropha curcas* L. *BMC Research Notes* 3:4210 DOI
492 10.1186/1756-0500-3-42**.**

493 **Yadav HK, Ranjan A, Asif MH, Mantri S, Sawant SV, Tuli R. 2011.** EST-derived SSR markers
494 in *Jatropha curcas* L. development, characterization, polymorphism, and transferability across the
495 species/genera. *Tree Genetics & Genomes* 7:207-219 DOI: 10.1007/s11295-010-0326-6.

496

497

498

499

500

List of tables

List of figures

523 substitutions per site. Blue boxes show the sequences obtained from the current work in Costa
524 Rica. Other accessions were obtained from Genbank for comparison.

Figure 1

Map of Costa Rica

Map of Costa Rica with sites where *Jatropha curcas* accessions were collected. A) North Pacific Region. B) Central Pacific Region

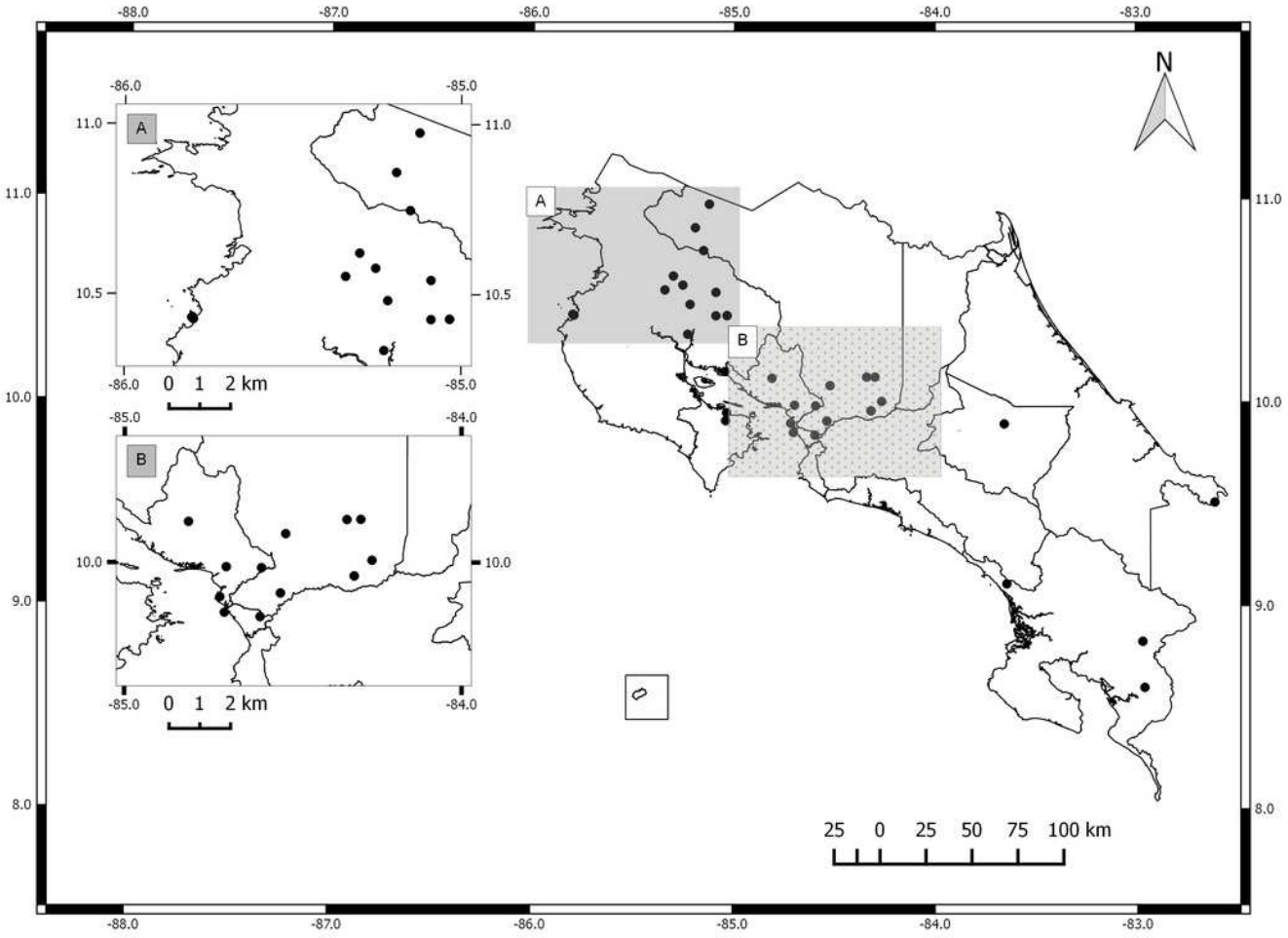

Figure 2

Neighbor-Joining (NJ) tree

Neighbor-Joining (NJ) tree based on a genetic distance matrix from 19 microsatellite data from 50 germplasm accessions of *J. curcas*.

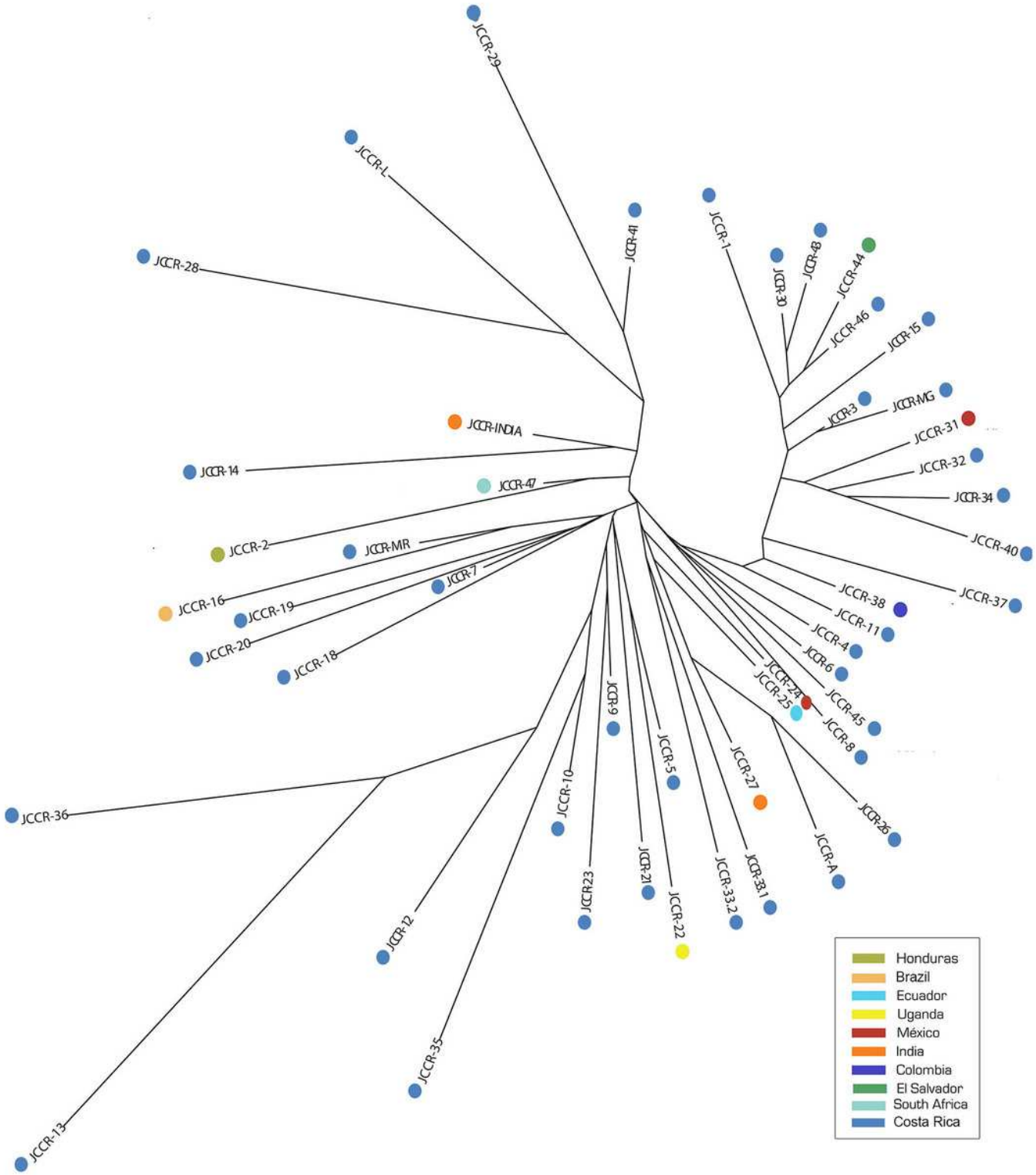

Figure 3

Principal coordinate analysis

Principal coordinate analysis created with GenAlEx based on the $\delta\mu^2$ genetic distance estimated in the Populations software with 19 microsatellites from 50 germplasm accessions of *J. curcas* from the Costa Rican germplasm bank evaluated in this study.

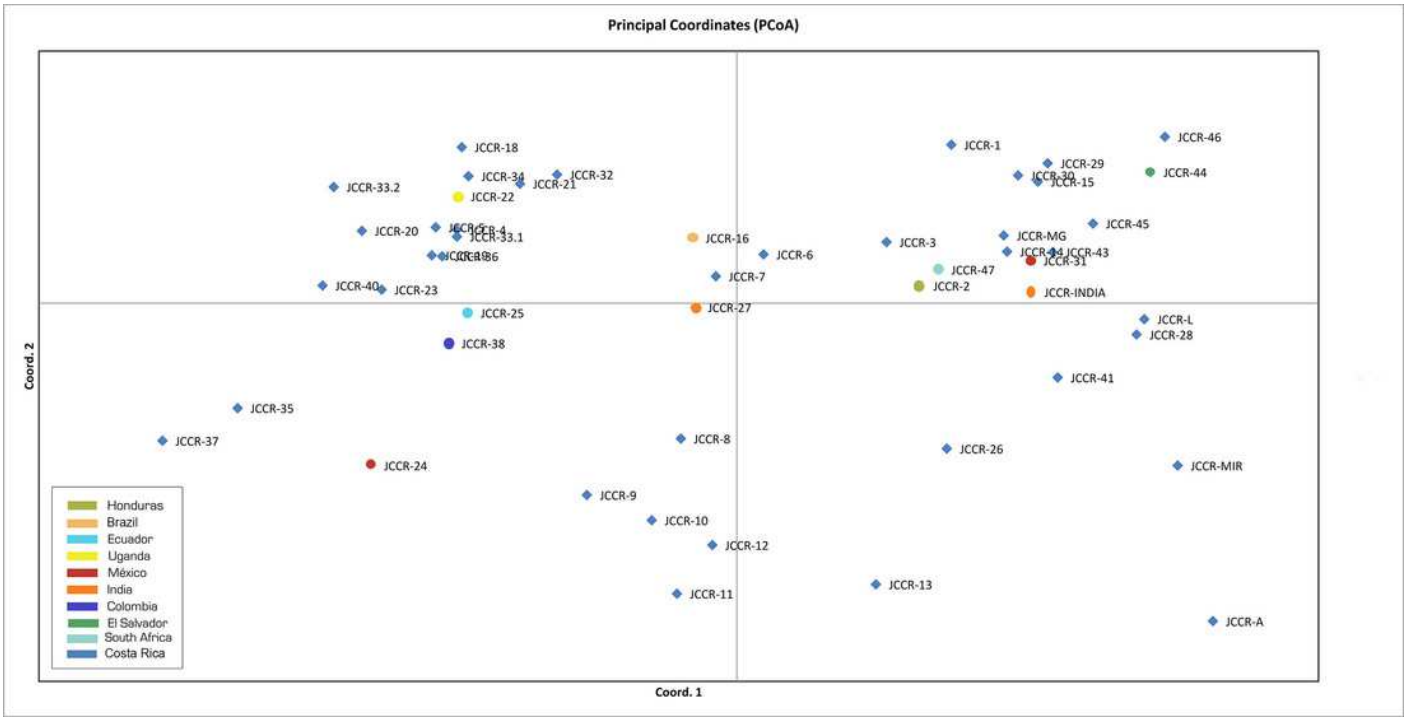

Figure 4

Maximum likelihood phylogenetic tree

Maximum likelihood phylogenetic tree generated with 60 *Jatropha curcas* samples. Analyzed sequences represent the nrDNA-ITS region. The tree was constructed with the GTR model using a jModelTest analysis with 3000 bootstraps and uniform substitution rates in the MEGA 6.0 software. Only bootstrap values higher than 50 % are shown. The bar indicates the substitutions per site. Blue boxes show the sequences obtained from the current work in Costa Rica. Other accessions were obtained from Genbank for comparison.

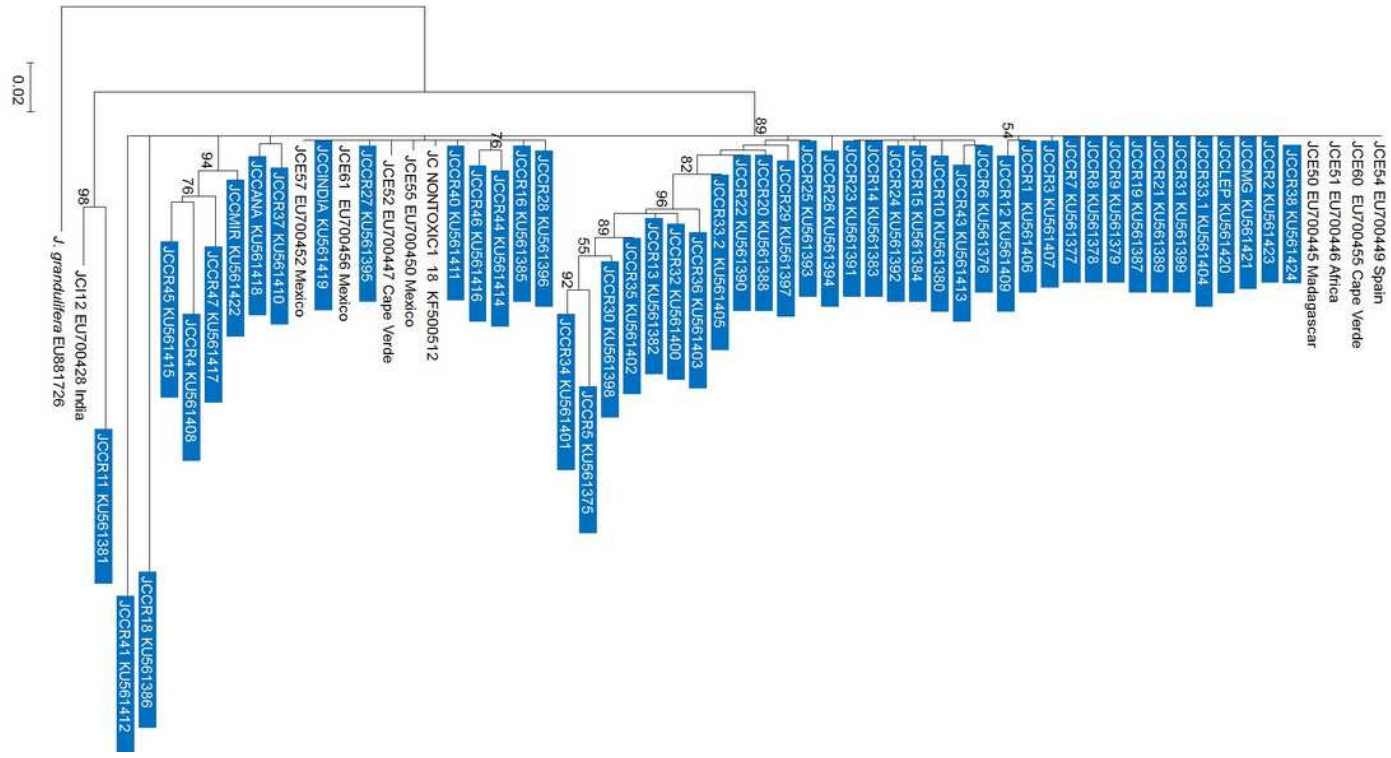

Table 1(on next page)

Germplasm bank information

Germplasm bank identification number, geographical collection sites and Genbank Accession number of nrDNA-ITS region for each of the *J. curcas* accessions in the Fabio Baudrit Experimental Station used in the study.

1

Germplasm code	Country	Location	Genbank accesion number
JCCR-1	Costa Rica	Orotina	KU561406
JCCR-3	Costa Rica	Orotina	KU561407
JCCR-4	Costa Rica	San Mateo	KU561408
JCCR-5	Costa Rica	Cañas	KU561375
JCCR-6	Costa Rica	San Antonio	KU561376
JCCR-7	Costa Rica	Cañas	KU561377
JCCR-8	Costa Rica	Abangares	KU561378
JCCR-9	Costa Rica	Guapinol	KU561379
JCCR-10	Costa Rica	Cañas	KU561380
JCCR-11	Costa Rica	San Antonio	KU561381
JCCR-12	Costa Rica	San Mateo	KU561409
JCCR-13	Costa Rica	Orotina	KU561382
JCCR-14	Costa Rica	Turrubares	KU561383
JCCR-15	Costa Rica	Abangares	KU561384
JCCR-18	Costa Rica	Bagaces	KU561386
JCCR-19	Costa Rica	Cañas	KU561387
JCCR-20	Costa Rica	San Vito	KU561388
JCCR-21	Costa Rica	Capulin	KU561389

JCCR-23	Costa Rica	San Antonio	KU561391
JCCR-26	Costa Rica	Capulin	KU561394
JCCR-28	Costa Rica	Upala	KU561396
JCCR-29	Costa Rica	Bagaces	KU561397
JCCR-30	Costa Rica	Coto 54	KU561398
JCCR-32	Costa Rica	Bagaces	KU561400
JCCR-33.1	Costa Rica	Los Santos	KU561404
JCCR-33.2	Costa Rica	Los Santos	KU561405
JCCR-34	Costa Rica	FabioBaudrit	KU561401
JCCR-35	Costa Rica	Abangares	KU561402
JCCR-36	Costa Rica	Turrubares	KU561403
JCCR-37	Costa Rica	Unknown	KU561410
JCCR-40	Costa Rica	Lagunilla	KU561411
JCCR-41	Costa Rica	CATIE	KU561412
JCCR-43	Costa Rica	Turrubares	KU561413
JCCR-45	Costa Rica	Unknown	KU561415
JCCR-46	Costa Rica	Diquis	KU561416
JCCR-MIR	Costa Rica	Miramar	KU561422
JCCR-ANA	Costa Rica	Anabel	KU561418
JCCR-MG	Costa Rica	Montaña Grande	KU561421
JCCR-LEP	Costa Rica	Lepanto	KU561420

JCCR-2	Honduras	Unknown	KU561423
JCCR-16	Brazil	Unknown	KU561385
JCCR-22	Uganda	Unknown	KU561390
JCCR-24	Mexico	Unknown	KU561392
JCCR-31	Mexico	Unknown	KU561399
JCCR-25	Ecuador	Manabí	KU561393
JCCR-27	India	Unknown	KU561395
JCCR-INDIA	India	Unknown	KU561419
JCCR-38	Colombia	Unknown	KU561424
JCCR-44	El Salvador	Unknown	KU561414
JCCR-47	South Africa	Unknown	KU561417

2

Table 2(on next page)

Primers used for evaluation of J. curcas

EST-SSR and G-SSR primers used for evaluation of *Jatropha curcas* germplasm.

1

ID	Forward Primer	Reverse Primer	T_A (°C)*	Expected size (bp)
JESR-001	AACCACAGGAGTTGGTAATG	GAAAGAAGCAACAGAAATGG	50	307
JESR-028	ACTTCCTTCAGATCATGCAC	CTGGGTAATCTTGTTCCAAA	52	292
JESR-047	GTTGATACTGGAAGTGAGCC	TGTGTTCAAAGGTGATGAGA	52	398
JESR-086	TCCCTCTCCTTCAGATTAAA	ATGATAGCCAAACAGCAACT	54	333
JESR-092	CTCTGAGAATTGAACCATCC	GGGAACAAAGAAATTACTGG	54	378
JESR-093	CACCTCCCATTAGGGTTT	CTAATCGACGCTGATAATCC	54	239
JESR-095	AATGAGTCTGACAATCAGGG	GCATGCTCTGTTCTGCTT	54	336
JESR-096	ACACAAACACAATCAACAGC	CGCGACTCACTTTGTATGTA	54	244
JESR-098	AGATCACAAGGATCACAAGG	GCAGTTGTCAAACACTAGCA	54	290
JESR-099	ATAATGGCAAACAAGTGGTC	TGGTAGTGTTGTTCTTGCAG	54	305
JESR-101	ATCCTAACACAGTTGCCATC	AAACTCAACCAAACCACAAC	54	230
JESR-102	ATCCTTCTGCAGTAGCCATA	TTATATGCTACACATCAACCTG	54	278
JESR-103	CAAGTTCGAGGAGTACAAGG	TGTTACAACGAGATGAGTGC	54	292
JESR-104	CCACAGTTCATCCTCAATTT	GATATTCACTCTGGAACCCA	54	308
JESR-118	CTAAAGGCTGTGAAGAAGGA	TCCGAGCCAATTTCTTATTA	54	276
JESR-161	AAGAAGTGTATGGGTTGCAC	TACGATACCTAGGGCTACGA	56	323
JESR-162	ACTGATGGGTATGTGAGAGG	TTCTTCATCATGGCTACCTT	56	220
JESR-163	CAGAAACGGAGAGGTCTG	AGATTGGAAGAGGAGAGGAG	56	144
JESR-164	AGCCCAGTCTCGCGGAAG	CAGTTCCCTTCAGAAGCTC	56	231
JESR-178	CTTTAGTCCACCTCAAGTGC	TGCAGCAATCAACTCTACTG	56	375
JSSR-203	ATCCTTGCCTGACATTTTGC	TTCGCAGAGTCCAATTGTTG	55	210

2 * T_A = annealing temperature

3

4

5

Table 3(on next page)

Primers used to evaluate toxicity of accessions

Primers used to evaluate toxicity of accessions in the germplasm bank.

Primer ID	Primer sequences	T_m (°C)	Expected size non-toxic (bp)	Expected size toxic (bp)
JCT27	F: 5′-CATTAGAATGGACGGCTA-3′ R: 5′-GCGTGAAGCTTTGATTTGA-3′	60	259	253
JcSSR-26	F: 5′-CATACAAAGCCTTGTCC-3′ R: 5′-AACAGCATAATACGACTC-3′	55	210	230
JCT31	F: 5′-TGGAAAACGAATGAGGCTCT-3′ R: 5′-GGACACTCTGGAAAGGAACG-3′	59	214	208
ISPJ1	F: 5′-GAGAGAGAGAGAGAGGTG-3′ R-5′-GAGAGAGAGAGAGAAAACAAT-3′	54	NA*	543
ISPJ2	F- 5′GAGAGAGAGAGTTGGGTG-3′ R-5′AGAGAGAGAGAGCTAGAGAG-3′	54	1096	NA

*NA: No amplification expected

16

17

18

Table 4(on next page)

Parameters of genetic diversity information obtained

1

SSR name	He*	PIC*	f*
JEST-01	0.375	0.551	0.411
JEST-28	0	0.043	0.001
JEST-47	0.050	0.048	-0.0129
JEST-86	0	0.336	0.001
JEST-92	0.814	0.370	-0.654
JEST-93	0.327	0.250	-0.160
JEST-95	0.217	0.175	-0.111
JEST-96	0.380	0.260	-0.225
JEST-98	0.043	0.114	0.650
JEST-99	0.280	0.236	-0.110
JEST-101	0.500	0.357	-0.062
JEST-102	0.325	0.235	-0.182
JEST-118	0.313	0.228	-0.175

JEST-161	0.043	0.042	-0.011
JEST-162	0.500	0.677	0.300
JEST-163	0.245	0.192	-0.129
JEST-164	0.721	0.355	-0.555
JEST-178	0.512	0.364	-0.058
JSSR-203	0.933	0.375	-0.862
Mean	0.346	0.274	-0.102
SD	0.062	0.165	0.346

* He: Heterozygosity, PIC: Polymorphism information content, f: inbreeding coefficient