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VARIACIÓN GENÉTICA EN POBLACIONES DOMESTICADAS DE *JATROPHA CURCAS* L. DE CHIAPAS, SUR DE MÉXICO

T E S I S

QUE PARA OBTENER EL GRADO ACADÉMICO DE

DOCTOR EN CIENCIAS

P R E S E N T A

ISIDRO OVANDO MEDINA

TUTOR PRINCIPAL DE TESIS: DR. MIGUEL SALVADOR FIGUEROA
COTUTOR DE TESIS: DR. FRANCISCO JAVIER ESPINOSA GARCÍA

COMITÉ TUTOR: DR. JUAN SERVANDO NÚÑEZ FARFÁN

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Dr. Isidro Ávila Martínez
Director General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 1º de agosto de 2011, se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del alumno **OVANDO MEDINA ISIDRO** con número de cuenta **508014598** con la tesis titulada: "**Variación genética en poblaciones domesticadas de** *Jatropha curcas* **L. de Chiapas, Sur de México**", realizada bajo la dirección del **DR. MIGUEL SALVADOR FIGUEROA**:

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Coordinadora del Programa

c.c.p. Expediente del (la) interesado (a).

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Resumen

Jatropha curcas L. es una planta de la familia Euphorbiaceae cuya importancia económica actual radica en que el aceite de sus semillas es de alta calidad para la fabricación de biodiesel. Derivado de esto, se está convirtiendo en un cultivo extensivo del que existen ya varios millones de hectáreas sembradas en Asia, África y América Latina, lo que implica retos tecnológicos porque no es una planta totalmente domesticada, se carece de variedades seleccionadas y de procesos agronómicos. Se conoce poco sobre su biología y ecología, así como sobre la diversidad genética y el origen geográfico de sus poblaciones. Estudios realizados con germoplasma asiático, africano y sudamericano han encontrado baja diversidad genética, pero no existen estudios sobre diversidad fenotípica y genotípica en poblaciones de Mesoamérica, de donde probablemente es nativa.

Considerando que en el Estado de Chiapas existe la mayor extensión sembrada con *J. curcas* en México, en la presente investigación se caracterizaron poblaciones de distintas regiones del estado y de otros sitios del sur de México. La investigación inició con un análisis sobre la sustentabilidad de la producción de biodiesel de *J. curcas*, así como sobre su centro de origen y diversidad. Asimismo, se hizo una revisión de la información disponible sobre variación genética de la especie para identificar oportunidades de investigación. Con la finalidad de caracterizar la diversidad de las poblaciones, se utilizaron como marcadores caracteres con valor adaptativo directo (ácidos grasos de la semilla), así como marcadores moleculares neutros AFLP (polimorfismo en la longitud de fragmentos amplificados de ADN, por sus siglas en inglés).

Los estudios químicos mostraron que el contenido de aceite de la semilla en 135 accesiones de 38 sitios varió entre 8.02% y 54.28%, siendo los ácidos grasos mayoritarios el ácido oleico (18:1) y linoleico (18:2); la proporción de ácidos grasos insaturados varió entre 74.5% y 83.7%. Un estudio con plantas clonadas crecidas en jardín común mostró que tanto el contenido de aceite como los ácidos grasos son altamente heredables (h^2_{bs} 70.3% y $h^2_{bs}\bar{x}$ 88.1%, respectivamente), por lo que son útiles como marcadores químicos para estimar la diversidad genética de la especie. Un análisis de componentes principales mostró que los ácidos grasos que contribuyen más a la varianza son: esteárico, oleico, linoleico, metilpalmítico,

gadoleico y ricinoleico. Las poblaciones fueron clasificadas en diez grupos, mostrando elevada variación genética en poblaciones de esta especie en Mesoamérica. Un análisis discriminante separó las poblaciones de acuerdo a su origen geográfico, lo cual se verificó con una prueba de Mantel. Usando el algoritmo de Monmonier se identificaron dos barreras genéticas entre las poblaciones. Los resultados se discuten a la luz del significado evolutivo de la composición de ácidos grasos de la semilla de esta especie tropical.

Los estudios de genética molecular encontraron 152 marcadores útiles con polimorfismo global de 81% y heterocigocidad de 0.192. La población más diversa fue la denominada "Fronteriza" por ser colindante con Guatemala, ubicada en la depresión central de Chiapas (*He*: 0.245, *I*: 0.378). Un análisis de conglomerados reveló el más alto coeficiente de disimilitud basado en accesiones reportado hasta ahora, lo que evidencia la elevada diversidad del germoplasma de Chiapas. Para analizar la estructura genética de las poblaciones se realizó un análisis de varianza molecular (AMOVA), encontrando que la mayor parte de la variación estuvo dentro de poblaciones (87.8%), seguida por la variación entre poblaciones (7.9%). El valor de Φ_{ST} fue de 0.121 indicando diferenciación moderada entre poblaciones, aunque un AMOVA espacial (SAMOVA) detectó mayor estructuración (Φ_{ST} = 0.176). Para entender la estructura fina de poblaciones, se realizó un análisis de datos con estadística Bayesiana. El número de poblaciones genéticas (K) fue de 5, con ancestría mezclada en la mayoría de los individuos, excepto en la población "Soconusco" (también colindante con Guatemala, pero en la costa de Chiapas), donde hubo solo una pequeña fracción de los alelos observados en otras poblaciones. En contraste, el SAMOVA agrupó a las poblaciones en cuatro unidades. Para corroborar los hallazgos, se buscaron posibles barreras genéticas, determinando que la principal barrera separa a Fronteriza del resto de poblaciones. Los resultados se discuten con base en la posible ancestría de las poblaciones.

Finalmente, se integraron los resultados en una discusión general sobre los marcadores fenotípicos y neutros utilizados, destacando la relevancia de los hallazgos en los ámbitos científico y productivo.

Abstract

Nowdays, the importance of the Euphorbiaceous plant *Jatropha curcas L.* lies in its high-quality seed oil, ideal for the manufacture of biodiesel fuel. As a result, an extensive cultivation has already reached several million hectares in Asia, Africa and Latin America, and implies certain challenges since it is not a fully domesticated plant, and there are not agronomic process and selected varieties for its cultivation. In addition, little is known about its biology, ecology, genetic diversity and geographic origin of its populations. Despite the growing body of knowledge on *J. curcas*, there are no studies on phenotypic and genotypic diversity in populations of Mesoamerica, from where it is probably native.

Whereas the greatest extension of *J. curcas* sown in Mexico is located in the state of Chiapas, this investigation describes the characteristics of the populations in different regions of this state and other sites in southern Mexico.

The present dissertation starts with a reflection on the sustainability of biodiesel production from *J. curcas;* then the basic aspects of the species, such as its center of origin and/or diversity, were discussed. The state-of-the-art of the genetic variation research of the plant was reviewed in order to identify research opportunities. To broadly understand the diversity of populations, we used two types of markers: direct adaptive value characteristics, such as fatty acids in the seed, and neutral molecular markers such as amplified fragment length polymorphisms (AFLP).

The results of the chemical study showed that the contents of seed oil in 135 accessions from 38 sites ranged from 8.02% to 54.28%, with the most abundant fatty acids being oleic acid (18:1) and linoleic (18: 2); the proportion of unsaturated fatty acids ranged from 74.5% to 83.7%. A study with cloned plants grown in a common garden experiment showed that both the oil content as well as the proportion of fatty acids were highly inheritable, making them useful as chemical markers to estimate genetic diversity. An analysis of principal components showed that the compounds contributing most to the variance were stearic, oleic, linoleic, methylpalmitic, gadoleic and ricinoleic acids. The Mesoamerican populations surveyed were classified into ten groups, all of them showing a high genetic variation. A discriminant analysis separated populations according to their

geographical origin, which was verified with a Mantel test. Using the Monmonier algorithm, two genetic barriers were identified between the populations. The results are discussed according to the putative evolutionary significance of the seeds fatty acid composition for this tropical species.

In the molecular study, 152 useful markers were found with a global polymorphism of 81% and a heterozygosity of 0.192. The most diverse population was that called "Fronteriza", adjacent to Guatemala, located in the central depression of Chiapas (*He*: 0.245, I: 0.378). A conglomerate analysis revealed the highest coefficient of dissimilarity based on accessions reported so far, which demonstrates the high diversity of Chiapas germplasm. In order to detect a genetic structure, an analysis of molecular variance (AMOVA) was performed, finding that most of the variation was located within populations (87.8%), followed by variation between populations (7.9%). The Φ_{ST} value was 0.121 indicating a moderate differentiation between populations, although a spatial AMOVA (SAMOVA) detected a stronger structure (Φ_{ST} = 0.176). In order to survey the fine structure of populations, a Bayesian analysis was additionally performed. The number of genetic populations (K) was 5, with mixed ancestry in most individuals, except in the Soconusco population (also adjacent to Guatemala, but on the coast of Chiapas), where there was only a small fraction of alleles shared with other populations. In contrast, the SAMOVA grouped the populations into four units. To corroborate these findings, a search was conducted for possible genetic breaks, and determined that the principal barrier is that separating "Fronteriza" from the other populations. The results are discussed with reference to the possible ancestry of the populations.

The results were finally integrated into a general discussion on the phenotypic and neutral markers used, highlighting the relevance of the findings in the scientific and productive realms.

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INTRODUCCIÓN

Jatropha curcas L. es una planta de la familia Euphorbiaceae conocida en la región mesoamericana como "piñón". Sus semillas son tóxicas para los mamíferos y tienen diveros usos tradicionales; entre ellos su uso medicinal, el cual podría tener una antigüedad de dos milenios en el sur de México (Leonti *et al*., 2003). Aunque su principal utilidad es como cerco vivo de las fincas agrícolas, esta especie ha ganado importancia económica en la última década debido a la extracción de aceite de sus semillas para la fabricación de biodiesel (Gubitz *et al*., 1999; Openshaw, 2000; Pramanik, 2003; Fairless, 2007; Escobar *et al*., 2008). Diversos autores han destacado las características ventajosas que *J. curcas* tiene sobre otras oleaginosas, particularmente su rápido crecimiento y desarrollo (Sujatha *et al*., 2005), su adaptación a suelos con fertilidad marginal (Jones y Miller, 1992; Heller, 1996; Henning, 1997; Carels, 2009; Behera *et al*., 2010) y su tolerancia al estrés hídrico, lo que le permite crecer en sitios semi-áridos (Henning, 1997; Zhang et al., 2008; Abou-Kheira y Atta, 2009; Maes *et al*., 2009). Además, tiene capacidad para controlar la erosión del suelo (Reubens *et al*., 2011) y potencial para la fitorremediación (Mangkoedihardio *et al*., 2008; Kumar *et al*., 2008).

J. curcas se está convirtiendo rápidamente en un cultivo extensivo (Achten *et al*., 2007; Fairless, 2007), y se proyecta que para el año 2015 habrán sembradas alrededor de 15 millones de hectáreas en el mundo, principalmente en el sur de Asia y en América Latina (Renner y Zelt, 2008). Lo anterior implica grandes retos tecnológicos, sobre todo si se considera que no es una planta totalmente domesticada, que carece de proceso agronómico, que no tiene material genético seleccionado, que no hay conocimiento preciso sobre su potencial productivo ni de su fenología, y se desconoce su número de variedades.

Paralelo al interés de aprovechamiento económico de la planta como cultivo, está el interés científico sobre la biología y ecología de la especie, la diversidad genética y el origen geográfico de las poblaciones actualmente distribuidas en muchos lugares tropicales de América, África y el sur de Asia. Aunque no hay consenso acerca del origen de esta especie, investigadores sugieren que la región mesoamericana (México y Centroamérica) es el centro de origen (Heller, 1996; Openshaw, 2000; Ranade *et al*., 2008), mientras que otros postulan a Sudamérica (Martin y Mayeux, 1984; Basha y Sujatha, 2009; Carels, 2009). De lo que no hay duda es que Mesoamérica es el centro de diversificación del género *Jatropha* (Dehgan y Webster, 1979; Martínez *et al*., 2002) y que el germoplasma de esta región ha sido la fuente para el establecimiento de plantaciones en África y Asia (Heller, 1996).

El estudio de la variación genética en poblaciones junto con el análisis de la variación fenotípica son dos prioridades de investigación en *J. curcas*, ya que en conjunto contribuirían a la identificación de loci asociados a características cuantitativas de interés productivo. Aunque existe un creciente cuerpo de conocimiento relativo a la diversidad de *J. curcas*, se detectaron dos carencias importantes de información en la literatura revisada: a) la mayoría de los reportes de diversidad se basan en un enfoque de accesiones o individuos (Basha y Sujatha, 2007; Gupta *et al*., 2008; Ranade *et al*., 2008; Sun *et al*., 2008; Abdulla *et al*., 2009; Basha *et al*., 2009; Kumar *et al*., 2009; Subramanyam *et al*., 2009; Tatikonda *et al*., 2009; Ikbal *et al*., 2010; Pamidiamarri *et al*., 2010; Shen *et al*., 2010; Umamaheswari *et al*., 2010; Wen *et al*., 2010), faltando estudios con enfoque de poblaciones, y b) se han estudiado principalmente accesiones asiáticas, aunque en menor grado también sudamericanas (Hartmann-Neto *et al*., 2006; Oliveira et al., 2006; Rosado *et al*., 2010) y africanas (Basha *et al*., 2009; Ambrosi *et al*., 2010; Ricci *et al*., 2011), por lo que hacen falta estudios del germoplasma mesoamericano. Por otra parte, poca atención se ha puesto al estudio de la composición de ácidos grasos de las semillas como marcadores químicos para estimar la diversidad genética. Estudios con este tipo de caracteres permitirían contar con información básica a la vez que se pueden utilizar para la selección de genotipos para la siembra.

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Los estudios realizados con germoplasma colectado en el Viejo Mundo y en Sudamérica han encontrado baja diversidad y una base genética reducida (Sun *et al*., 2008; Popluechai *et al*., 2009; Rosado *et al*., 2010; Ricci *et al*., 2011). El entendimiento de la diversidad genética y de la estructura de las poblaciones en el posible centro de origen y/o diversidad, Mesoamérica, permitirá identificar material genético útil en el futuro mejoramiento de la especie. Por ejemplo, posibilitaría el diseño de cruzas entre plantas de grupos genéticamente distanciados.

Considerando que en el Estado de Chiapas existe la mayor extensión sembrada de cercos vivos tradicionales de *J. curcas* en México, la presente investigación se centró en el estudio de la diversidad en poblaciones de dicho Estado.

Un análisis conceptual y proposicional (Ford, 2000) ayudó a identificar los axiomas y a proponer los postulados de la presente investigación. Los axiomas son proposiciones que se asumen verdaderas y que son la base de un estudio. No obstante, se redactan de manera tal que puedan ser retados o respaldados por la investigación que se lleva a cabo. El reto a un axioma no es directo ni completo, sino a través de sus postulados correspondientes y a través de más de una investigación (Ford, 2000).

El trabajo se fundamentó en tres axiomas propuestos a partir de la literatura revisada: 1) *Jatropha curcas* L. es nativa de Mesoamérica; 2) *J. curcas* es una planta en proceso de domesticación; 3) En la región mesoamericana, *J. curcas* es propagada principalmente por la vía clonal.

El primer axioma se basó en la revisión de investigaciones sobre sistemática y fitogeografía del genéro *Jatropha* (Dehgan y Webster, 1979; Dhegan, 1984; Dhegan y Schutzman, 1994) y en general de la familia Euphorbiaceae (Wurdack *et al*., 2005; Martínez-Gordillo *et al*., 2002; Steinmann, 2002). En esas investigaciones se ha postulado que *J. curcas* es la especie más primitiva o plesiomórfica del género *Jatropha*, idea que se ha robustecido con investigaciones recientes basadas en datos moleculares (Sujatha *et al*., 2005; Basha y Sujatha,

2007; Ganesh-Ram *et al*., 2008); asimismo, se encontró que el género está dividido en dos subgéneros (*Curcas* y *Jatropha*) y diez secciones (Dehgan y Webster, 1979; Dhegan, 1984). Una hipótesis filogenética bastante robusta es que las especies del subgénero *Curcas*, y específicamente las de la sección Curcas, a la cual pertenece *J. curcas*, se originó primero que el subgénero *Jatropha* (Dhegan y Schutzman, 1994). En dicha reconstrucción filogenética el clado más plesiomórfico estuvo formado por especies de la sección Curcas: *J. curcas*, *J. andrieuxii*, *J. bartlettii*, *J. hintoni*, *J. mcvaughii*, *J. pseudocurcas* y *J. yucatanensis*, las mayoría de las cuales son endémicas de México y todas son endémicas de Mesoamérica (dos especies de la sección Curcas son de África: *J. afrocurcas* y *J. macrophylla*, y una de la India: *J. villosa*; Heller, 1996). El axioma 1 de la presente investigación descansa en el hecho que Mesoamérica alberga a la mayoría de los parientes silvestres de *J. curcas* y que es el centro de diversificación del género *Jatropha* (cerca de 100 de 175 especies son nativas de dicha región, además tan solo en México existen entre 35 y 39 especies estrictamente endémicas; Martínez-Gordillo *et al*., 2002; Steinmann, 2002).

Respecto del segundo axioma se puede argumentar que, a diferencia de otras especies de *Jatropha*, el "piñón" no forma poblaciones, en ecosistemas naturales. Esta planta se cultiva, tanto en América como en el Viejo Mundo, como cerco vivo, y, ocasionalmente, como planta de traspatio (Anzueto y De MacVean, 2000; Openshaw, 2000; Jongschaap *et al*., 2007; Toral *et al*., 2008). En algunos casos se han encontrado plantas en distintos tipos de vegetación natural, pero los autores que los reportan mencionan que probablemente se trata de plantas derivadas de semilla que han "escapado" de sistemas de cultivo (Heller, 1996). En los recorridos de campo en la costa del Pacífico Sur de México que el autor de esta tesis ha hecho no se han encontrado poblaciones en condiciones silvestres. La situación descrita podría ser un indicio de domesticación de esta planta, ya que, en general, las especies vegetales domesticadas no son exitosas reproductivamente sin manejo antrópico (Elias y McKey, 2000). Otro síntoma de domesticación, es la existencia en México de genotipos cultivados o fomentados no tóxicos, con ausencia total o con niveles mínimos de ésteres de forbol y otras moléculas tóxicas (Martínez-Herrera, 2007; Martínez-Herrera *et al*., 2010;

Parawira, 2010), existen genotipos no tóxicos en varios estados de México, aunque la mayor parte del germoplasma es tóxico (Ovando *et al*., 2009). Sin embargo, la planta no muestra el llamado "síndrome de domesticación", que incluye modificaciones morfológicas y fisiológicas (Poncet *et al*., 1998; Pujol *et al*., 2005; McKey *et al*., 2010). Esto último probablemente se debe a que el uso tradicional histórico de *J. curcas* ha sido como cerco vivo y más limitadamente como medicinal y alimento, en varios estados de México se consumen semillas tostadas de las variedades no tóxicas, incluso en el norte de Veracruz forma parte de la gastronomía tradicional local (Makkar *et al*. 1998; Martínez-Herrera *et al*., 2010). Por su uso como cerco vivo es reproducida vegetativamente, por lo que es probable que tenga habilidades para reproducirse por organogénesis superiores a las de sus relativos silvestres, aunque no se encontraron investigaciones sobre este tema. La comparación fitoquímica entre *J. curcas* y otras especies relacionadas silvestres también podría arrojar luz sobre el grado de domesticación, como se ha demostrado en otros taxa (Lindig-Cisneros *et al*., 2003). Debido a lo anteriormente expuesto y puesto que no existen "variedades" mejoradas genéticamente por cualquier método, varios autores consideran que esta especie está en proceso de domesticación (Achten *et al*., 2010; Divakara et al., 2010).

Con relación al tercer axioma, las observaciones del autor en el campo en el sur de México y en Centroamérica señalan que los agricultores de esta región sólo propagan a la planta de manera vegetativa. En el mismo sentido, Granados-Galván (2008) menciona que aunque las semillas germinan fácil y rápidamente, los cercos vivos de *J. curcas*, los cuales pueden implicar cientos de kilómetros de siembras lineales en Chiapas, se propagan exclusivamente por medios clonales. Recientemente se han iniciado viveros en Chiapas, otros Estados de México y en Guatemala para propagar "piñón" mediante semillas, pero estas plantas están siendo sembradas en plantaciones de monocultivo fácilmente identificables. En Centroamérica los cercos vivos de "piñón" son establecidos a partir de estacas enraizadas (Budowski, 1987).

Las hipótesis del proyecto fueron redactadas en forma de declaraciones a ser investigadas, como se enlista a continuación:

1) Existe variación genética en poblaciones de *J. curcas* de Chiapas detectable mediante métodos basados en el ADN de la planta.

2) Existen diferencias en la composición química de la semilla dentro y entre poblaciones de *J. curcas* de Chiapas.

3) En general, la diversidad genética en poblaciones de *J. curcas* de la región mesoamericana es más elevada que la del germoplasma del Viejo Mundo.

Cada una de las hipótesis se redactó considerando los axiomas, los cuales representan hechos que potencialmente pueden favorecer la diversificación infraespecífica en *J. curcas* de Chiapas, pero que también pueden restringirla.

El hecho que las poblaciones de Chiapas se encuentren en el centro de diversidad del género *Jatropha* representa la posibilidad de encontrar mayor diversidad genética que la de poblaciones de otras regiones del mundo donde *J. curcas* es una especie exótica. Los centros de diversidad son sitios donde la evolución ha tenido más tiempo para dirigir a las especies, mediante cambios genéticos, a adaptarse a una variedad de ambientes (Carels, 2009). Un centro de diversidad contiene un gran número de razas o variedades de una especie cultivada (CONABIO, 2006).

Por otra parte, el hecho que *J. curcas* sea una especie en proceso de domesticación puede implicar menor diversidad genética si los agricultores han realizado una selección intensiva (Doebley *et al*., 2006). Por el contrario, la simpatría de *J. curcas* con sus relativos silvestres en su centro de diversificación puede ser una situación favorable para el intercambio genético entre individuos y conducir a la producción de nuevos genotipos sobre los cuales puede operar la selección natural y antrópica (Jarvis y Hodgkin, 1999).

Finalmente, la propagación clonal de *J. curcas* en la región mesoamericana podría suponer una restricción de la diversidad genética. La ausencia de recombinación

sexual bajo clonalidad exclusiva conduce a la pérdida de diversidad (Obeso, 2002). Debe considerarse que todos los procesos de domesticación son graduales y mixtos, es decir, la reproducción sexual y clonal de las plantas se presenta al mismo tiempo en las primeras etapas de la domesticación de plantas que finalmente se propagan clonalmente, lo que introduce cierto grado de variación en las poblaciones (McKey *et al*., 2010); además, también se puede producir variación a través de la fijación de mutaciones somáticas o por medio de variación epigenética heredable (Gerrish y Lenski, 1998).

El objetivo de la presente investigación fue analizar, bajo el enfoque de la genética de poblaciones, la variación infraespecífica de *J. curcas* de distintas regiones del Estado de Chiapas y, con fines de comparación, de otros sitios del sur de México. La finalidad fue generar conocimiento para dar respuesta a la pregunta global siguiente: ¿Cuánta variación genética, caracterizada mediante caracteres neutrales y adaptativos, existe en poblaciones del centro de diversidad de *J. curcas*, considerando que es una especie en proceso de domesticación y propagada clonalmente?

La investigación dio inicio con una reflexión acerca de la sustentabilidad de la producción de biodiesel de *J. curcas*, como se presenta en el Capítulo 2 de esta tesis.

A continuación se hizo una discusión acerca de aspectos básicos de la especie, como su centro de origen y/o diversidad. Asimismo, se hizo una revisión del estado del arte del estudio de la variación genética de la planta para identificar oportunidades de investigación. Esto se describe con amplitud en el Capítulo 3.

Con la finalidad de conocer de manera amplia la diversidad de las poblaciones, se utilizaron dos tipos de marcadores: caracteres con valor adaptativo directo, como son los ácidos grasos de la semilla, y marcadores moleculares neutros como los polimorfismos en la longitud de fragmentos de DNA amplificados (AFLP).

En el Capítulo 4 se presenta el estudio químico, el cual analizó poblaciones de *J. curcas* de Chiapas, así como de las regiones costeras de Oaxaca, Guerrero y Michoacán. Se estudiaron las plantas tanto *in situ* como sus clones cultivados en jardín común.

En el Capítulo 5 se expone el estudio molecular basado en los AFLP, el cual incluyó solo poblaciones de Chiapas.

Finalmente, se integran los resultados en una discusión general sobre los marcadores fenotípicos y neutros utilizados, destacando la relevancia de los hallazgos en los ámbitos científico y productivo.

2

IMPORTANCIA DE *JATROPHA CURCAS* EN LA PRODUCCIÓN SUSTENTABLE DE BIODIESEL

El presente capítulo de la tesis analiza la importancia global actual que tiene *Jatropha curcas* (el "piñón") para la producción de biodiesel. Se arguye que el origen fotosintético del biodiesel es suficiente para justificar su investigación como combustible renovable, no sólo como simple desarrollo tecnológico, sino situándolo como un elemento a tener en cuenta en la llamada crisis energética global, teniendo en cuenta la multitud de factores que afectan la sustentabilidad de su uso. En ese sentido, se plantea que el súbito interés mundial por la producción de biodiesel con base en *J. curcas*, puede conducir a prácticas no sustentables. Por lo mismo, se propone dar atención a cuatro aspectos mínimos por su importancia en la sustentabilidad de la cadena productiva cultivo de piñón/consumo de biodiesel: a) el área destinada al cultivo, b) el uso de insumos en el cultivo, c) los procesos de conversión del aceite a biodiesel, y d) el destino final del biocombustible.

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Article

Does Biodiesel from *Jatropha Curcas* Represent a Sustainable Alternative Energy Source?

Isidro Ovando-Medina [1,4,*], **Francisco Espinosa-García** [2], **Juan Núñez-Farfán** [3] and **Miguel Salvador-Figueroa** [1]

[1] Centro de Biociencias, Universidad Autónoma de Chiapas, Carretera a Puerto Madero Km 2.0, Tapachula, 30700, Chiapas, Mexico

[2] Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, Morelia, Michoacán, Mexico; E-Mail: espinosa@oikos.unam.mx

[3] Instituto de Ecología, Universidad Nacional Autónoma de México, Ciudad Universitaria, México, D.F., Mexico; E-Mail: farfan@servidor.unam.mx

[4] Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, México, D.F., Mexico; E-Mail: msalvad@hotmail.com

* Author to whom correspondence should be addressed; E-Mail: ibtmedina@gmail.com; Tel.: +52-962-6427972; Fax: +52-962-6427972.

Abstract: Various government agencies around the world have proposed vegetable oils and their conversion to biodiesel as a renewable alternative to fossil fuels. Due to its adaptability to marginal soils and environments, the cultivation of *Jatropha curcas* is frequently mentioned as the best option for producing biodiesel. In the present work the current situation of proven and potential reserves of fossil fuel, and the production and consumption model for the same are analyzed, in order to later review the sustainability of the production process which begins with the cultivation of *J. curcas*, and culminates with the consumption of biodiesel. A review of the following topics is proposed in order to improve the sustainability of the process: areas destined for cultivation, use of external (chemical) inputs in cultivation, processes for converting the vegetable oil to biodiesel, and, above all, the location for ultimate consumption of the biofuel.

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1. Introduction

The consumption of fossil fuels, above all petroleum and mineral coal, has been the foundation of the economic growth and the betterment of general levels of well-being for the so-called leading economies throughout the last century. At the same time, in the peripheral economies, like those of Latin America, a phenomenon has been presented of production of material primarily for exportation and to a lesser extent for internal transformation and consumption. There are two essential concerns which put at risk the viability of the model of growth based on export and/or excessive consumption of energy, both related with the lack of sustainability in said model: the apparent decreasing availability of fossil fuels and the notable increase in greenhouse gasses in the atmosphere caused most probably by human activity.

With regard to the proven and potential reserves of petroleum in the world, undoubtedly there exists a decline in the former since the decade of 1970–1980 [1-3], although apparently this is not significant [4,5]. During 2008 the international prices of petroleum rose to historically high levels, a situation utilized as an argument for the near-term emptying of the fuel reserves and for planning for the so-called energy transition, which includes, among other topics, the investigation and use of alternative sources of energy. Nevertheless, at the beginning of 2009, said prices fell substantially, which indicates that the price does not depend exclusively on the availability of petroleum, but also on multiple financial and geopolitical interests [5]. Over the potential reserves (possible and probable), the exploration in various parts of the world [5,6] and the application of new technologies [7-9] shows that it will be possible to extract petroleum at competitive costs for at least all of the 21st century.

On the other hand, the significant emission of carbon dioxide (and other greenhouse gasses) into the atmosphere by the combustion of petroleum in various human activities and its repercussion on global climate is a fact which only in the last two decades has been taken into account [10], but which recently has become a preoccupation even for petroleum exporting countries [5]. Proposals exist to reduce the emissions of CO_2, which include the design and use of more efficient machines and processes. Without considering if the foregoing may be achieved, the environmental economy postulates that technological solutions do not exist *ad infinitum* for the problems of the environment, and the second law of thermodynamics teaches that no completely efficient machines can exist.

The two discussed preoccupations have driven the investigation of alternative sources of energy, of which there exists a large list [11], although, since the substitutes most akin to petroleum are sought after, the options most prominent in the last decade are the liquid biofuels, specifically ethanol [12] and biodiesel [13-15]; these also have the advantage of being of photosynthetic origin (simple or complex sugars in the case of ethanol and greases or oils in the case of biodiesel) and, also, renewable, as part of the carbon cycle.

This present essay is focused on the biodiesel product from the oil of the tropical plant *Jatropha curcas* L. (Euphorbiaceae). There are a lot of oleaginous plants with potential for producing biodiesel, but *J. curcas* is frequently mentioned as the best option. Among several advantageous characteristics of this species is important to underline its adaptability to marginal soils and environments [13].

2. Regarding the Sustainability of Biodiesel

From the above, one concludes that governments and energy companies are not formulating profound changes in the model of consumption of petroleum fuels, but only technological solutions for (a) extending as much as possible the extraction of petroleum at non-prohibitive costs, (b) minimizing the emission of CO_2, and (c) substituting for petroleum with liquid biofuels.

Furthermore, biodiesel cannot replace petroleum, in the first place because it cannot be produced, without causing environmental damages greater than those for which it intends to give a solution [16], on a scale similar to that of petroleum, in accordance with the present and projected demand; in the second place because from biodiesel is not possible to extract the multitude of by-products which are produced by the petrochemical industry.

One fact which exemplifies the contradiction between a preoccupation with CO_2 emissions into the atmosphere and the plan to continue the model of economic development is that the leading economies, principally the United States, postulate that biofuels, in particular biodiesel, will permit a reduction in imports of petroleum [17], while the peripheral economies, especially those located in tropical zones, plan for the production of biodiesel for export [18] to countries like the United States and China, which are the largest emitters of CO_2 into the atmosphere. Another pattern in growth is the re-exportation of biodiesel [19], the vegetable oil is exported to one country, converted into biodiesel, and then re-exported to another country.

In any case, the photosynthetic origin of biodiesel is sufficient to justify its investigation as a renewable fuel, not only as a simple technological development, but also situating it as an element to play its part in the so-called global ecological crisis (*sensu* Iranzo [20]), taking into account the multitude of factors which affect the sustainability of its use. In this sense, Ovando *et al.* [21], put forward that the rising world interest in biodiesel, specifically that produced from "piñón" (*J. curcas*), can lead to non-sustainable practices. For this reason, they propose giving attention to at least four aspects for their importance in sustainability of the production process of "piñón"-consumption of biodiesel: (a) the area dedicated for cultivation, (b) the use of inputs in the cultivation, (c) the processes for converting vegetable oil to biodiesel, and d) the final destination of the biofuel.

Achten *et al.* [22] performed a qualitative evaluation of the future sustainability of cultivating *J. curcas*, focussing on the environmental impacts and socioeconomic aspects; they determined that the cultivation is sustainable when practiced in marginal or degraded lands, but not when fertile areas are dedicated which could serve to cultivate foodstuffs or other crops with greater profitability. One logical conclusion from the work of Achten *et al.* is that if areas of forest are clear-cut to convert them into "piñón" cultivation, the sustainability of the process is nonexistent. Nevertheless, it is possible to use areas considered as agriculturally high quality, if only one takes advantage of the periphery of the principal crops. It has been calculated, for example, that in the Mexican state of Chiapas, the cultivation of "piñón" could occupy a maximum of 230,000 hectares on the perimeters of the cultivated areas or used for cattle-grazing [21]. It was mentioned before that biodiesel cannot substitute for petroleum, not even for diesel, at the actual and projected levels of consumption; for example, the National Mission for Biodiesel in India has proposed the planting, by the year 2020, of an area of ten million hectares with *J. curcas* to produce 7.5 million tons of biodiesel each year; this volume represents only 20% of the demand for diesel in that country [3]. To substitute biodiesel for

the actual demand for diesel in Mexico (17.4 million metric tons [23]) would require close to 23 million hectares planted with *J. curcas*, which is equivalent to more than 90% of the cultivable ground surface. In this country there is a recent legislation that promotes the investigation and use of biofuels [24]; however, the corresponding bylaw has not yet been published.

To minimize the use of fossil energy and improve the energy balance it is required that the cultivation not include the use of chemical fertilizers, since these represent an elevated level of energy consumption. The data shows that 45% of commercial energy used in global agricultural production is due to the consumption of chemical fertilizers [25]; for that reason, implementing a bioenergy cultivation consuming a huge quantity of energy is, at the least, contradictory. For example, according to the same author, ammonia, the principal source of nitrogenous fertilizers, is produced from natural gas, and the petrochemical industry, which synthesizes it, consumes 1.2% of fossil fuels extracted on the global level.

The post-harvest processes for the majority of agroindustrial products imply a high consumption of energy derived from the use of indispensable machines; doubtless, in this case one should pay special attention to the energy efficiency of the machines for separating the husk of the seed, the extraction of the oil and the conversion to biodiesel. In this way, the husk can be used for fuel to heating the cauldrons if one opts for extraction of oil by extrusion by heat, or for heating the transesterification reactors for conversion to biodiesel. Regarding this last, it is greatly relevant to mention that although the reaction of transesterification has a high yield level (80%, that is to say that 800 mL of biodiesel are obtained from every liter of vegetable oil), the use of methanol reduces the energy gain. Methanol, which industrially is obtained from the distillation of petroleum, requires a proportion of 200 mL for each liter of processed oil. Among the alternatives one encounters the use of ethanol, which derives from the fermentation of sugar, although it has the inconvenience that on production it is dissolved in water with a distillation yielding a maximum of 96% alcohol to 4% water. The conversion of vegetable oil to biodiesel is favored by the absence of water, or by minimal quantities thereof. The production of completely anhydrous ethanol raises both costs and energy consumption.

Other topic requiring attention is the content of toxic substances of the *J. curcas* seed. Several toxic molecules have been reported in the seed [26-28], but the protein curcin and the phorbol esters are the most hazardous for human and animal health. After the oil extraction, the seed cake still contains those substances, representing a potential risk for the *J. curcas* biodiesel workers [29]. The potential of phorbol esters as carcinogens is known [30]. Although non-toxic genotypes of "piñón" exist in several parts of Mexico or improved non-toxic varieties could be obtained (by conventional breeding or by transgenic methods), toxic genotypes are being used for establishing extensive plantations around the world. However, a dilemma exists: if non-toxic genotypes are used, problems with pests could be a limitation, as the plant-herbivores interaction would be substantially modified. Alternatives to use the press cake are the physical or enzymatic detoxification for using as fodder, and the composting for using in the same plantation.

3. Conclusions

The aspects mentioned only refer to challenges to the sustainability of production, which can be overcome by means of technological development, while the true challenge is a reflection over the

viability of the model of exporting peripheral economies *vs.* leading economies in the consumption of fuel: namely, that given that the transport of biodiesel by whatever means entails the consumption of energy, then in order to maximize the energy efficiency of the process, the production of biodiesel derived from "piñón" should have a focus on local use in the rural environment, or in urban areas close to the sites for cultivation and industrialization of *J. curcas*, more than any export focus. The energy balance for a biofuel is a comparison of the energy contained in the fuel with the energy required to produce, process, and distribute it.

It does not mean that the proposal tends toward the so-called "Arcadian ecology" (*sensu* Iranzo [20]), but this could provide rural communities with energy to stimulate processes of improvement which at present they do not possess, whether due to lack of services or energy products or due to the high costs of these; for example, in some cases it would improve the preservation of foodstuffs at lower temperatures. Since transporting biofuels across large distances is not a sustainable result, the production for export, and later re-export, should be limited.

Our criticism to current approaches for the production and consumption of *J. curcas* biodiesel is not intended to disqualify the efforts of many people, but to contribute to a more sustainable productive chain. Of course, as Khan and Islam [31] explain, sustainability is not achieving only by minimizing risks and remediating problems engendered by the introduction of a given process or technology, but visualizing future potential problems, that is, having the time as main variable.

Acknowledgements

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ESTADO DEL ARTE DE LA INVESTIGACIÓN EN VARIACIÓN GENÉTICA DE *JATROPHA CURCAS*

En este capítulo de la tesis se sintetizan los avances de la investigación de la diversidad genética de *J. curcas*, discutiendo previamente el origen geográfico de la especie. A partir de esto se identificaron prioridades de investigación, que incluyeron la colecta y caracterización de germoplasma alrededor del mundo. Estas comprenden la más probable región de origen, el estudio de la variación genética usando marcadores morfológicos, químicos y moleculares, el uso de información del genoma de otras Euphorbiaceae, y el mejoramiento genético del cultivo para incrementar la producitividad de aceite.

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Review Paper

State of the art of genetic diversity research in *Jatropha curcas*

I. Ovando-Medina[1,4]*, F. J. Espinosa-García[2], J. S. Núñez-Farfán[3] and M. Salvador-Figueroa[1]

[1]Centro de Biociencias, Universidad Autónoma de Chiapas, Carretera a Puerto Madero Km 2.0, Tapachula, 30700, Chiapas, Mexico.
[2]Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, Morelia, Michoacán, Mexico.
[3]Instituto de Ecología, Universidad Nacional Autónoma de México, Ciudad Universitaria, Mexico, D. F.
[4]Posgrado en Ciencias Biológicas. Universidad Nacional Autónoma de México.

Jatropha curcas (Euphorbiaceae) is a mesoamerican plant. However, till date, it is extensively grown in tropical and subtropical regions of the world. The seeds have high oil content, which can be transformed into biodiesel. In the present review, the geographical origin of *J. curcas* is discussed, then, advances of research in the genetic diversity are summarized and contrasted. Proposed future research in this species include: (a) the collection and characterization of germplasm around the world, including the center of origin, (b) the study of the genetic variation within the context of population genetics, using morphological, chemical and molecular markers, (c) the use of genome information from other Euphorbiaceae, and subsequently (d) crop breeding to increase oil productivity.

Key words: *Jatropha*, Mexico, molecular markers, population genetics.

INTRODUCTION

Jatropha curcas L., also known in Southern Mexico and Guatemala as "Piñón" (pronounced Pinyon), is a euphorbiaceous plant with many uses across the world and a great potential in several fields. This plant is probably native to Mesoamerica; however, it currently exists as a crop in both the Old and the New World, with excellent adaptation to both tropical and subtropical conditions. It is a multi-use species, for example, different parts of the plant are used for medicine or for pharmacological studies (Marroquin et al., 1997; Panigrahi et al., 1984), as live fences (Anzueto and De MacVean, 2000) and, principally, to extract oil from its seeds to produce fatty acid methyl esters or biodiesel (Gubitz et al., 1999; Fairless, 2007; Martin and Mayeux, 1985; Openshaw, 2000; Pramanik, 2003; Takeda, 1982).

J. curcas has several advantages over other oleaginous species, because it is fast growing (Sujatha et al., 2005), easily adapts to marginal lands (Jones and Miller, 1992), tolerates drought and, therefore, can be grown in semi-arid areas (Henning, 1997). Its oil is inedible and toxic to human beings and animals (Joubert et al., 1984) and hence the rational production of biodiesel from *J. curcas* would not compete with human food security (Ovando-Medina et al., 2009a).

During the last three decades, many reports have mentioned the potential of this plant for diesel fuel production and studies have been carried out in different parts of the world to establish new plantations. However, scientific research has been fragmented and only recently have institutional programs been set up. Although more than 300 articles, either or not peer reviewed, concerning *J. curcas* exist in journals and internet, most of them are concentrate on the socio-economic aspect of *J. curcas* as a crop and on the energy-source potential of the plant. There are excellent reviews on these topics (Divakara et al., 2010; Gubitz et al., 1999; Heller, 1996; Openshaw, 2000; Parawira, 2010), however, the genetic aspects of the plant are barely mentioned.

This review highlights the advances in the genetic research of *J. curcas* and identifies priorities for research

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*Corresponding author. E-mail: isidro.ovando@unach.mx. Tel: +52+962 6427972.

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in the immediate future.

THE DEBATE ON THE ORIGIN OF *J. CURCAS*

Euphorbiaceae is a pantropical family with three subfamilies: Acalyphoideae, Crotonoideae and Euphorbioideae (Webster, 1994; Wurdack et al., 2005). The genus *Jatropha*, with 66 representatives in the Old World, is pantropical too. The center of diversity of the genus *Jatropha* is the Mesoamerican region (Mexico and Central America), which is illustrated by the fact that more than 100 out of 175 species of *Jatropha* are native to that region (Dehgan and Webster, 1979). In addition, in Mexico there are 41 native species, of which 31 are strictly endemic (Jiménez and Martinez, 1994). Several species of *Jatropha* are native to South America.

It is possible that *J. curcas* originates in Mesoamerica and many authors coincide with this idea (Abdulla et al., 2009; Ambrosi et al., 2010; Basha and Sujatha, 2007; Basha et al., 2009; Dehgan and Webster, 1979; Ginwal et al., 2005; Heller, 1996; Kumar et al., 2009; Lin et al., 2010; Openshaw, 2000; Parawira, 2010; Saikia et al., 2009; Sudheer-Pamidiamarri et al., 2009; Tatikonda et al., 2009; Umamaheswari et al., 2010; Zubieta et al., 2009). Nowadays this species is distributed throughout the tropical world as a result of European colonialism, the plant was introduced to Caribbean islands, Africa and Southeast Asia where it is grown as a hedge plant. Nevertheless, there are disagreements and some authors considering Brazil as the origin of *J. curcas*, as suggested by Arruda et al. (2004), Bomfim-Gois et al. (2006) and Oliveira et al. (2006). Melo et al. (2006) recommend the extensive culture of *J. curcas* in that South American country for the reason that "it is a species native of Brazil" and Martin and Mayeux (1984) mention the State of Ceara, in Brazil, as the centre of origin of the plant. In the same way, Basha et al., (2009), Sudheer-Pamidimarri et al. (2009b) and Sudheer et al. (2010b) mention that this plant is a native of South America. Basha and Sujatha (2007), in the introduction of their paper, declare that *J. curcas* is a native of Mexico and the Central American region, but in their final comments they said that, up till date, the "true" centre of origin of *J. curcas* has not been established. Other authors prefer a more conservative point of view stating that the origin of the plant is "tropical America" (Ambrosi et al. 2010; Divakara et al., 2010; Ganesh-Ram et al., 2008; Ranade et al., 2008).

In 1979, Dehgan and Webster suggested that *J. curcas* was the most primitive form within the *Jatropha* genus, because it posses morphological characters shared by both subgenera, *Curcas* and *Jatropha*, including palmately lobed leaves, arborescent habit, presence of a co-florescence and occasional hermaphroditic flowers; supported by comparative microscopic examination of several anatomical and morphological features (Dehgan and Craig, 1978; Dehgan, 1980, 1982) and interspecific hybridizations demonstrating the ability of the species to interbreed as maternal parent with species of the two subgenera (Dehgan, 1984; Sujatha and Prabakaran, 2003). It is reasonable, therefore, to deduce that, if the Mesoamerican region is the centre of diversity of the genus and if *J. curcas* is the most plesiomorphic species in the *Jatropha* genealogical tree, then it must be a Mesoamerican originative species.

However, a center of diversity not necessarily is a center of origin. An additional complication in determining the center of origin of *J. curcas* is its presence, "in the wild", in South America. Further, the existence of African species of *Jatropha*, represents an opportunity to explain the diversification of the genus by vicariance (Dehgan and Webster, 1979). Dehgan and Schutzman (1994) analyzed the evolution of 32 morphological characters in 77 New World *Jatropha* species. Their results revealed a morphological continuum from South to North, with Southern species possessing the most primitive characteristics (arborescent habit, presence of a co-florescence, monoecy, diploidy, ten uniseriate and connate stamens, three locules and three style branches). Although they did not include geographic data, they postulated that the current distribution of *Jatropha* is as a result of the separation of the ancient continent of Gondwana (*ca.* 100 millions of years ago –m. y. a.) and the subsequent spreading of *Jatropha* in Africa and America. In summary, another possibility is that the center of origin of *J. curcas* was South America, from where it spread to Mesoamerica (after the closure of the Isthmus of Panama, *ca.* 3 m. y. a.), a site with optimal conditions for its diversification.

A more accurate conclusion may only be drawn from a complete revision of the Old and New World *Jatropha* species using both morphological and molecular characters, and conducting phylogeographic research on *J. curcas* in the American continent.

MORPHOLOGICAL AND CHEMICAL DIVERSITY IN THE SPECIES

Due to the interest of scholars and governments in the use of *J. curcas* as a crop for biodiesel production, many programs aimed at the collection and selection of elite genotypes have been undertaken (Openshaw, 2000; Ovando-Medina et al., 2009b; Sujatha et al., 2005), and an understanding of the degree of genetic variation in native populations of *J. curcas* is critical for the success of such programs.

There are a few recognized varieties of *J. curcas* in the world and their differentiation is based upon the size or the content of toxic molecules (phorbol esters and curcin) within the seed. However this classification has an element of arbitrariness. For example, three varieties are frequently mentioned by researchers: the Cape Verde variety that has spread all over the world, the Nicaraguan variety with few but larger fruits and a non-toxic Mexican variety that only has traces of phorbol esters in the fruit

(Heller, 1996; Henning, 1997; Sujatha et al., 2005). Recently, some commercial varieties have been released. These include SDAUJ1, from an Indian program of selection of germplasm (Basha and Sujatha, 2007) and JMAX, derived of Guatemalan germplasm (www.sgbiofuels.com). Comparative agronomic studies of such varieties have not been reported. In addition, there is limited information with regard to the number of introductions and the genetic diversity of *J. curcas* populations grown in different parts of the tropics.

In various studies, some potentially important variations in Piñón trees have been detected, however, the initial variations in fruit and seed yield of the candidate trees were found to be insignificant when the plants were grown on a common site, indicating low genetic variability. Sakaguchi and Somabhi (1987) found no intra-specific morphological variations between forty *J. curcas* clonal lines from different locations in Thailand. Other records of systematic provenance trials have en-countered limited morphological and chemical variability (Heller, 1991, 1996; Sukarin et al., 1987). Conversely, Kaushik et al. (2007) reported the variability in seed traits and oil content of 24 accessions of *J. curcas* collected from different zones of India. There were significant differences ($P<0.05$) in seed size, 100 seed weight and oil content between accessions. However, the coefficient of variation was higher for phenotype than genotype, indicating a predominant role of the environment. Similar results were found by Sunil et al. (2008) and Mishra (2009), who selected promising accessions of *J. curcas* from India, correlating morphological characteristics (plant height, collar height and thickness, number of primary branches, petiole length, number of fruits per cluster, pedicel length and seed yield) with the oil content of the seed. Gohil and Pandya (2008, 2009) studied fourteen characters in Indian accessions finding moderate genetic diversity and none of the morphological variables had heritability of over 75%. In another study of Indian accessions, Saikia et al. (2009) compared 34 sources, finding moderate variation in plant height, stem girth, branches per plant and seed weight.

In general, it appears that the environment has a predominant role in the morphological variation among provenances, which could be interpreted as a narrow genetic base of *J. curcas*, at least in the Old World germplasm. Contrary to this idea, Ginwal et al. (2005), studying plants from Central India, observed that characteristics of seed morphology, germination and seedling growth were highly variable and significant among sources, and were under strong genetic control (broad sense heritability values over 75%).

Studies concerning chemical variation have been focused on the seed oil content, with minimal attention to other molecules potentially useful as markers for the estimation of genetic diversity. Makkar et al. (1998) compared the content of toxic compounds of four types of *J. curcas*, which originated from Nicaragua, Cape Verde, Nigeria and Mexico. The concentrations of phorbol esters in the kernels of Cape Verde, Nicaragua and Nigeria types were 2.70, 2.17 and 2.30 mg/g, respectively, whereas kernels of non-toxic Mexican types had a very low concentration (O.11 mg/g); but the variation among sites was not evaluated.

Ferrao and Ferrao (1984) found variations in Asian clones ranging from 23 to 43%, based on the complete seed. Heller (1996) reported that the crude fat content of seeds from ten different origins ranged from 28.4 to 42.3% (Mean = 35.6%). Ovando-Medina et al. (2009b) investigated variations in seed oil content between populations of *J. curcas* from the coastal zone of the Mexican State of Chiapas. Results showed that seed oil content varied from 12.09 to 44.28%, apparently related to the aridity of the sites, with the higher contents corresponding to zones with lower rainfall. Ginwal et al. (2004) reported similar associations between oil content and rainfall. The variation in oil content can be generated by genetic and environmental factors, including rainfall and soil fertility (Escobar et al., 2008; Mishra, 2009), however, several authors have reported high heritability values for this characteristic, 99% (Kaushik et al., 2007), 89.7% (Gohil and Pandya, 2009), >75% (Ginwal et al., 2004) and 70.3% (Ovando-Medina et al., In Press).

There are reports on the composition of *J. curcas* oil (fatty acids, sterols and other molecules), but researchers have concentrated on the potential of the oil as food/feed or as biocide (Adebowale and Adedire, 2006; Martínez-Herrera et al., 2006). Limited attention has been paid to the chemical diversity as indicator of genetic variation. An exception is the work of Wang et al. (2008), who compared the oil content and fatty acid composition in samples of *J. curcas* collected from three regions of China and one from India. They found 12 fatty acids and reported differences among accessions, concluding that attention should be given to these chemical markers in the introduction of germoplasm to that country and in the breeding of *J. curcas*.

There are two main explanations for the genetic diversity (estimated with morphological and chemical characters) found in Old World *Jatropha* accesions, the first, as Ginwal et al. (2005) and Saikia et al. (2009) suggested, is related to the fact that this species grows over a wide range of climatic conditions and populations must have experienced marked differences in selective pressure in their natural habitat. The problem with this postulate is that *Jatropha* populations are relatively new in Asia and Africa. The second explanation is that the variation was introduced with the seeds from tropical America, centuries ago.

RECENT ADVANCES IN MOLECULAR STUDIES OF *J. CURCAS*

Conventional methods have shown that morphological
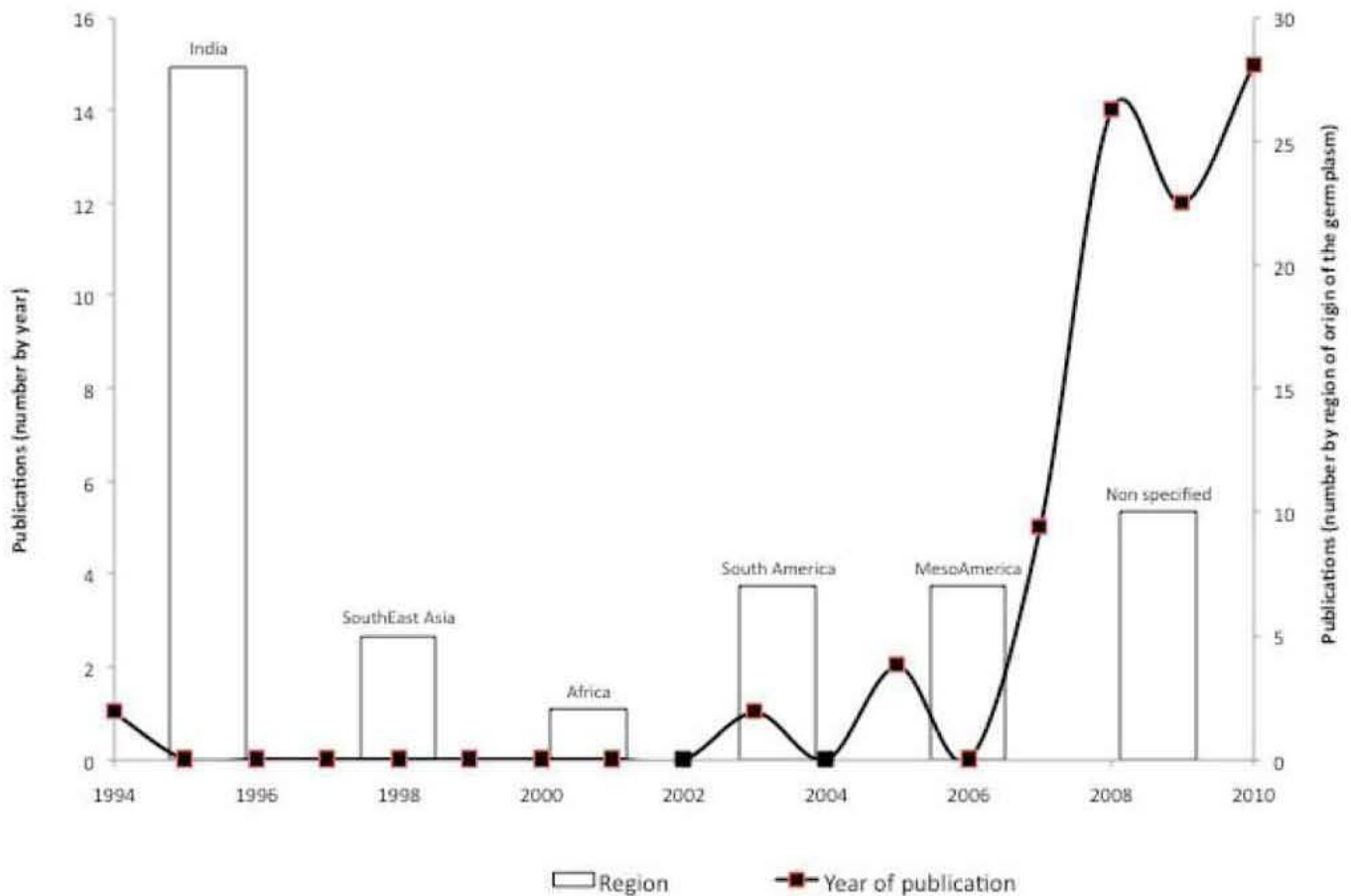
Figure 1. Evolution of the investigations on *Jatropha curcas* genetic diversity, published in indexed journals the last fiftheen years, by region of origin of the germplasm analyzed.

characteristics are useful to establish phylogenetic relationships at the genus level, but are insufficient to define genetic diversity and relationships among accessions of *J. curcas*, due to the strong influence of the environment on traits like seed weight, seed protein and oil content (Heller, 1991, 1996; Sakaguchi and Somabhi, 1987; Sukarin et al., 1987). It is, therefore, clear that evaluation of genetic variation is more feasible using neutral molecular markers (Basha and Sujatha, 2007). Conventionally, the identification of markers linked to useful traits has been based on complete linkage maps and hybridization experiments. However, alternative methods, such as the construction of partial maps and combination of pedigree and marker information, are also useful in identifying marker/trait associations (Korzun, 2003).

Molecular markers have been employed for determining genetic diversity in species of family Euphorbiaceae, especially in *Hevea brasiliensis* (Willd. ex A. Juss.) Müll.Arg. (Lakawipat et al., 2003) and *Manihot esculenta* Cranz (Asante and Offei, 2003).

The number of *J. curcas* molecular marker studies has increased remarkably in the last ten years (Figure 1).

however, there are limitations, for example, there are only a few reports about the use of AFLPs to analyze genetic variations in populations of *J. curcas*.

Isoenzymes

Among the variety of molecular marker systems, isoenzymes are a good option for rapid evaluation of plant materials because the analysis is simple, fast and cheaper than DNA-based methods. Unfortunately, besides Sathaiah and Reddy (1985), who used isoenzymes to determine phylogeny of *Jatropha* and *Ricinus*, only one report mentioned the use of isozymes in *Jatropha*. Bomfim-Gois et al. (2006) compared the isoenzymes of 15 accessions of *J. curcas* from four States of Brazil. Peroxidase, esterase and glutamate oxaloacetate transaminase expressions were used to estimate genetic similarities between genotypes. They observed differences in electrophoretic profiles of accessions for the different enzymatic systems but they did not find extensive divergence, except in the genotype named JCUFLA001, which presented only 55% of
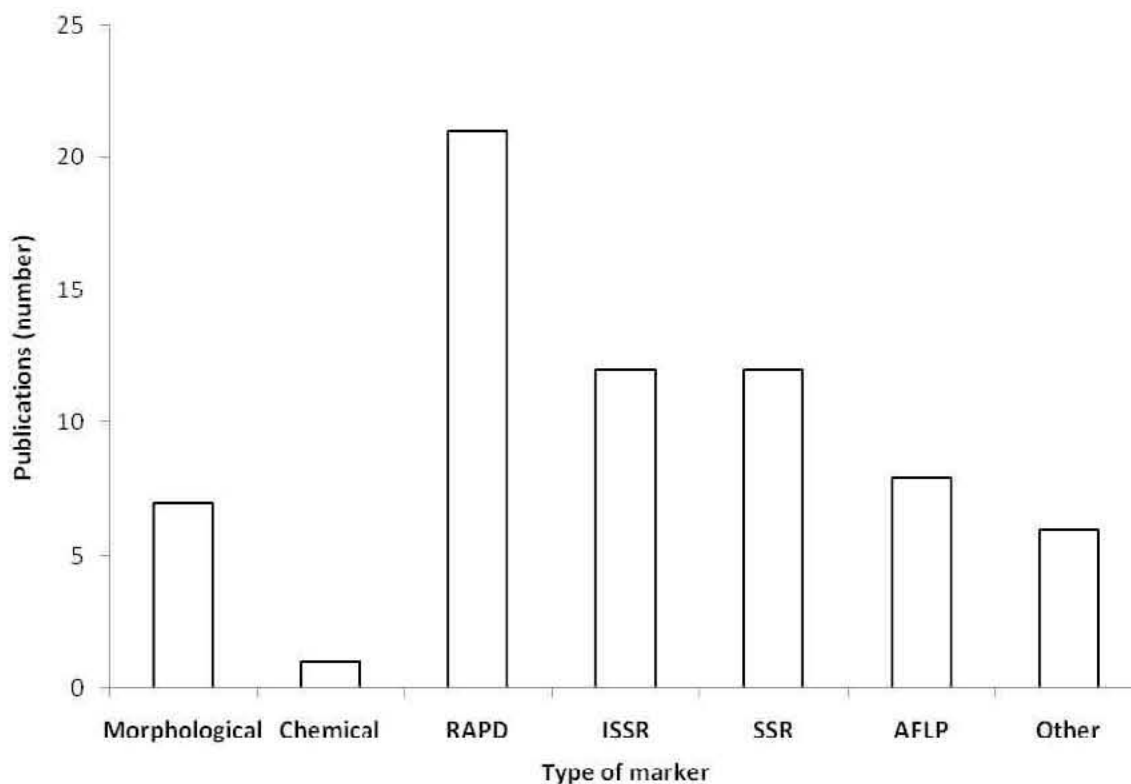
Figure 2. Frequency of use of markers to study genetic diversity in *Jatropha curcas* extracted from a survey in public scientific databases.

similarity with the rest of the biological materials tested.

DNA-based markers

A number of DNA markers are being used to study *Jatropha curcas* germplasm around the world. However, the method of fingerprinting most frequently used is the random amplified polymorphic DNA, RAPD (Figure 2). An exhaustive revision of studies on molecular markers yielded two types: (a) those exploring the usefulness of markers to elucidate phylogenetic relations in the genus *Jatropha*, and (b) those studying the variability in accessions of *J. curcas* from different origins, with the long term goal of marker-assisted germplasm improvement. There are also a few reports with a population genetics approach.

Studies of the first type have shown that *J. curcas* can hybridize with other species of *Jatropha*, for example Sujatha and Prabakaran (2003) used RAPD markers to confirm hybridity between *J. curcas* and *Jatroph integerrima*. By using the primers OPA-04 and OPA-08, they identified five fragments specific to *J. curcas*.

Sudheer-Pamidiamarri et al. (2009), using RAPD and AFLP markers, indicated the relatedness between *J. curcas* and *J. integerrima*. The two markers showed comparable results in elucidating that *J. curcas* is closely related to *J. integerrima*, which could be the possible reason of their intercrossing. In another study, Sudheer-Pamidimarri et al. (2009) focused on the understanding of phylogenetic relationships between seven species of *Jatropha*, sequencing a nuclear ribosomal DNA ITS (nrDNA ITS). Their results indicated close relationship between *J. curcas* and *J. integerrima*. Sudheer et al. (2010a) reported a phenogram of several *Jatropha* species using SSR markers, showing a grouping more congruent with results of RAPD and AFLP than with nrDNA ITS.

On the contrary, other studies showed noclose relationship between the two species. The genetic relationships of eight species of *Jatropha* were assessed by Ganesh-Ram et al. (2008) using RAPD markers. They selected the primers OPA-04, OPF-11 and OPD-14 (from Operon Technologies, USA) for the final screening due to their high polymorphism detection. The main phenogram revealed three clusters: five *Jatropha curcas* accessions were separated from the rest of *Jatropha*; a second cluster was formed by *Jatropha ramanadensis*, *Jatropha gossypifolia*, *Jatropha podagrica*, *Jatropha tanjorensis*, *Jatropha villosa* and *J. integerrima*, the last cluster consisted only of *Jatropha glandulifera*. On average, *J. integerrima* had a genetic distance of 0.525 with respect to *J. curcas*, indicating a lack of close relatedness between them. Infrageneric relations within *Jatropha*

were explored by Senthil-Kumar et al. (2009), who compared ISSR markers of nine species. A cluster analysis separated three distinct clusters: The first one comprising all accessions of *J. curcas*, the second cluster including *J. tanjorensis*, *J. gossypifolia*, *J. podagrica* and *J. maheshwarii*, and the last cluster containing *J. villosa*, *J. multifida*, *J. integerrima* and *J. glandulifera*.

Unfortunately, these researchers did not include outgroups (wild relatives) in the studies, and used phenetic instead of parsimonious (phylogenetic) methods. Nevertheless, these results support the view of Dehgan and Webster (1979) in their classical article on the taxonomy of genus *Jatropha*, in which they concluded that *J. curcas* is the most plesiomorphic species in the genus. Ganesh-Ram et al. (2008) concluded that the molecular distinctness of *J. curcas* accessions as revealed by the formation of a distinct cluster also supports the view that *J. curcas* is the most primitive form of *Jatropha*. There is a generalized view that *J. curcas* is the maternal parent of the natural hybrid *J. tanjorensis*, however, Basha and Sujatha (2009) demonstrated, using consensus chloroplast microsatellite primers that *J. gossypifolia* is the maternal parent.

Studies of the second type include that of Sujatha et al. (2005), who used RAPD analysis to determine the similarity index between toxic Indian accessions and nontoxic Mexican genotypes. Of the 120 primers tested, amplification was observed with 95 primers. The number of bands per primer varied between one and 13; the maximum polymorphism was generated with primers from OPJ and OPM series while no polymorphism was detected with primers from OPL series. The similarity index between the two genotypes based on 435 bands scored was 96.3%. The polymorphism generated with these primers served as reference fingerprints for distinguishing the non-toxic variety from the toxic Indian cultivars.

Inter Simple Sequences Repeats or ISSRs are other marker tool with a great potential to analyze genetic diversity in *J. curcas* but, similar to SSRs, a previous step is needed to select primers for the blank sequences. Hartmann-Neto et al. (2006) selected ISSR primers to evaluate their potential as markers in accessions of *J. curcas* from the Meio-Norte region in Brazil. They found five primers (UBC 816, UBC 821, UBC 822, UBC 830 and UBC 880) that resulted in acceptable levels of polymorphism and robustness of bands. Genotypes used to test the primers were collected from Teresina-Pi and Nova Porteirihna. Palmieri and Maia (2006) used bioinformatic tools to identify microsatellite markers for *J. curcas* and *Ricinus communis* from genomic sequences of *R. communis* available from public databases.

RAPDs and ISSRs are perhaps the most developed molecular markers used for *J. curcas*, to explore genetic diversity in accessions of germplasm banks. Oliveira et al. (2006) investigated the genetic similarities between 24 accessions of *J. curcas* from different origins of Brazil,

using the RAPD technique with 14 10-bp random primers. The amplification yielded 36 polymorphic fragments, with maximum similarity between genotypes of 83% and the highest divergence of 90%, indicating the existence of high genetic diversity. Nevertheless, most studies of the genetic variation of *J. curcas* have found only modest levels of diversity (Sujatha et al., 2005) even with Brazilian provenances (Bomfim-Gois et al., 2006), for that reason it is necessary to explore a broader range of biological material and to use highly repeatable methods. The main constraint of the RAPDs is its low reproducibility (Korzun, 2003).

Basha and Sujatha (2007) evaluated the genetic diversity of *J. curcas* germplasm from India and a non-toxic genotype from Mexico using RAPD and ISSR techniques. With the aim to describe the genetic structure of *J. curcas* germplasm in India, 43 accessions from different locations were analyzed. That study was one of the most complete attempts at assessing the genetic diversity in *J. curcas* using molecular markers, and the development of SCAR markers to distinguish Indian accessions from the Mexican genotype. The study identified polymorphic RAPD markers that distinguished between these two geographically isolated genotypes but the polymorphism detected with 400 RAPD and 100 ISSR primers was low (42.0 and 33.5%, respectively), indicating a narrow genetic base of the studied accessions. Furthermore, the intra-population variation as determined by RAPD primers was 36.0%, similar to the genetic variation detected between populations. SCAR markers developed included a fragment of 543 bp (GenBank EF012272), which is specific to Indian accessions, and a fragment of 1096 bp named ISPJ2 (GenBank EF012273), present exclusively in the Mexican genotype.

Sudheer-Pamidimarri et al. (2009b) evaluated the efficacy of RAPD, AFLP and microsatellites in the detection of polymorphism in *J. curcas* from India, with the subsecuent objective of developing a methodology for marker assisted selection. They found genetic similarity indices of 0.92 and 0.90 with RAPD and AFLP, respectively, between Indian accessions and a non toxic variety from Mexico. Seven out of 12 microsatellite markers resulted polymorphic. In general, they detected low genetic variation in Indian germplasm of *J. curcas*.

Accessions from China have a narrow genetic base too, the main explanation resides in the fact that the species could have been introduced to Africa and Asia in reduced amounts as vegetative propagules. Sun et al. (2008) studied the genetic relationships of 58 *J. curcas* accessions located in the South China Botanical Garden; they used microsatellites and AFLP. Only one out of 17 microsatellite markers was polymorphic with two alleles, and from 70 generated AFLP fragments only 14% were polymorphic. In another study of Chinese germplasm, the polymorphism was 27% and the Jaccard's similarity coefficients ranged between 0.866 and 0.977 (that is,

Table 1. Comparison of genetic diversity in *Jatropha curcas* from several parts of the World studied with molecular markers.

Region	Genetic diversity (1-Jaccard's similarity index)			Type of marker used	Reference
	Minimum	Maximum	Mean		
India	0.05	0.51	0.28	RAPD-ISSR	Basha and Sujatha (2007)
India	-	-	0.12	AFLP	Pamidimarri et al. (2010a)
India	0.03	0.57	0.30	AFLP	Tatikonda et al. (2009)
India	0.16	0.37	0.27	ISSR	Umamaheswari et al. (2010a)
India	0.04	0.37	0.20	RAPD	Kumar et al. (2009)
India	0.08	0.46	0.27	RAPD	Ikbal et al. (2010)
India	0.11	0.59	0.35	ISSR-RAPD	Gupta et al. (2008)
India	0.00	0.19	0.09	RAPD	Abdulla et al. (2009)
India	0.00	0.80	0.40	RAPD	Subramanyam et al. (2009)
China	0.03	0.14	0.085	AFLP	Shen et al. (2010b)
China	0.02	0.15	0.085	ISSR	Cai et al. (2010)
Brazil	0.17	0.67	0.42	RAPD	Oliveira et al. (2006)
India, Nigeria, Thailand	0.07	0.27	0.17	RAPD	Popluechai et al. (2009)
Mexico, Asia, Africa	0.08	0.52	0.30	RAPD-ISSR	Basha et al. (2009)
Mexico, SouthAmerica, Asia, Africa	0.01	0.23	0.12	SSR	Ambrosi et al. (2010)
Indonesia, China, Grenada, South America	0.08	0.45	0.27	EST-SSR	Wen et al. (2010)
China, Indonesia	0.06	0.21	0.135	SRAP	Shen et al. (2010a)
China, Indonesia, Thailand	0.05	0.49	0.27	ISSR	Duan and Guo (2010)

genetic diversities as low as 0.03 to 0.14), using AFLP markers (Shen et al., 2010a).

To the contrary, two investigations report a high genetic variation in *J. curcas* from India. Ranade et al. (2008), evaluated the genetic diversity of accessions of *J. curcas* from four regions of India using RAPD's and directed amplification of minisatellites. An important difference in relation to the other studies of Asian *J. curcas* is the evaluation of "wild" accessions. They found that, although the species is not a native, it exhibited a high genetic diversity; the methods used were sufficient to differentiate genotypes of different provenances, with wild accessions from the North East region being clearly dissimilar to the rest. However, it should be noted that even in the probable centre of origin there are no wild populations of *J. curcas* and therefore "wild" should be clearly defined. Deghan, cited by Heller (1996) mentioned the existence of plants from seeds "escaped" to natural environments from live fences or other domesticated forms of culture. However, the extremely high variation (similarities between 0.04 and 0.96 with RAPD and between 0.14 and 1.00 with minisatellites; three accessions were more dissimilar with respect to other *J. curcas* than one of the out groups) is not in agreement with the phenotypic variations in Old World accessions. The same authors recommend the application of more than one method on widely collected germplasm of *J. curcas*.

Tatikonda et al. (2009) used AFLP's to assess the diversity in a collection of *J. curcas* from six states of India with the long term goal of selecting appropriate "elite" accessions for plant improvement through conventional and molecular breeding approaches. The study was chiefly centered in the robustness of the method of analysis (polymorphism information content, marker index and resolving power) and secondarily in the genetic variation of the collection. Results showed that 680 out of 770 fragments generated were polymorphic, revealing high diversity among genotypes from the State of Andra Pradesh. Similarity coefficients ranged from 0.43 to 0.97, which could denote a broad genetic base of *J. curcas* in India. These results imply that Asian accessions of *J. curcas* are almost as diverse as their Guatemalan counterparts. On another hand, they tried to correlate genetic data with phenotypic characteristics as oil content and seed weight, but no significant trends were observed.

A research group of Plant Research International (Wageningen University) started a program of global evaluation of the diversity of *J. curcas*, including accessions of Guatemala. In preliminary results, they found low genetic diversity in African and Asian genotypes in contrast to the high variation in Guatemalan materials, detected by AFLP (Montes-Osorio et al., 2008, Van Loo et al., 2008). Studies to correlate genetic markers with oil content and quality are on going with promising results.

In Table 1, we compare the genetic diversity found in the molecular studies reporting Jaccard's index. There is a lack of agreement between the results of diversity analysis obtained for a common region, which appears to depend on the type of marker used; for example, in India mean diversity can be as low as 0.09 (Abdulla et al., 2009) or as high as 0.40 (Subramanyam et al., 2009), both analyzed with RAPD. AFLP analysis appears to detect moderate levels of diversity (Pamidimarri et al., 2010a; Shen et al., 2010b; Tatikonda et al., 2009), whereas SSRs are the most stringent markers for the detection of variability (Ambrosi et al., 2010; Basha et al., 2009; Sudheer et al., 2010b; Wen et al., 2010; Zubieta et

al., 2009).

In general, studies have shown a low genetic variation in *J. curcas* from different provenances using both morphological and molecular methods, which is not common in an allogamous species like *J. curcas*. Possible explanations for this phenomenon could be the fact that most of studies were done with materials from India, Africa and South America while germplasm from the most probable centre of origin remains little explored. Plants from the Mesoamerican region have been used mainly for comparative purposes. According to Basha and Sujatha (2007), low variability of samples from India could be due to the few introductions of "Piñón" that have spread across the country; this could be true also for other Old World countries where *J. curcas* was introduced centuries ago. Another possible explanation is that people from rural areas propagate the plant mainly through vegetative propagation.

Population genetics studies are important because they determine not only the degree of diversity but also how that variation is distributed (among regions, among populations or within populations). The great majority of investigations are focused on the diversity among individuals (that is, accessions) and a few are centered on populations. For example, Wen et al. (2010) obtained a genetic diversity index of 0.557 in average, studying populations of Indonesia, China and South America, which represent a broad genetic stock. However, the total gene diversity was higher than the gene diversity within groups, which means that most of diversity is not within populations. A value of *Gst* of 0.186 indicated a significant differentiation between geographical regions and a gene flow index of 2.18 indicated that an elevated flow of genes (pollen or individuals) could have occurred in the past. Ambrosi et al. (2010) reported values of *Fst* (an analog of *Gst*) of 0.200 reflecting large genetic differentiation among the geographic groups (populations) studied (America, Asia and Africa). Nevertheless, when Bayesian methods to study the structure of populations were applied, a high genetic homogeneity was observed within each population. Furthermore, Cai et al. (2010) found moderate differentiation between Chinese populations, with an *Fst* of 0.127 and the most of variation (87%) within groups. Xiang et al. (2007) reported a *Gst* of 0.294, in populations of Yunnan, China. Another study of Chinese populations revealed a *Gst* of 0.539 (He et al., 2007). Values of differentiation among populations greater than 0.25 can be considered very high while values higher than 0.5 should be taken with caution, since it means that populations are isolated between them.

FUTURE RESEARCH SUGGESTIONS

In conclusion, based on this review of current knowledge, further or continued research is needed in the following areas:

Germplasm banks

The majority of programs that promote "Piñón" as a crop have several common objectives: selection of candidate plus phenotypes, establishment of seed production areas, evaluation, establishment of state-of-the-art nurseries and progeny trials of high yielding plants. To achieve such objectives, the establishment of germplasm banks is an essential first step. Presently, germplasm collections of *J. curcas* contain a reduced number of accessions and represent only a fraction of the potential of India, Africa and South America. There are no collections covering the foremost part of the germplasm from the center of origin of the species. An exhaustive collection in Southern Mexico and Central America is needed along with a systematic monitoring of domesticated populations of *J. curcas* across the tropics. Not only traditional germplasm banks, where the entire plant or seeds are conserved *ex situ*, but also micropropagated plantlets stocks and DNA banks are needed. These two last suggestions will facilitate the international interchange of materials.

Population genetics studies

Although the study of accessions is valuable for the selection of elite individuals, is required to analyze the genetic variation with a focus on populations using morphological, chemical and molecular markers.

Morphological markers

The genus *Jatropha* is morphologically diverse (Dehgan, 1982), but *J. curcas* apparently is not. However, the leaf architecture, number and arrangement of primary veins and anatomy of the petiole and the fine structure of the flower have not been studied in accessions. Those characters could exhibit sufficient diversity to the intra-specific level. It is important to mention that the morphology, in contrast with the neutral molecular markers, has an adaptive value; for that reason, the study of genetic diversity needs a combination of markers.

Chemical markers

Several studies have been carried out on the phytochemistry of *J. curcas* (Van Den Berg et al., 1995; Makkar et al., 1998) but have not focused on the degree of variation among different provenances of *J. curcas*. This kind of study could provide useful information, in particular the level and composition of fatty acids of the

seed oil.

Molecular markers

As mentioned in this review, molecular markers are powerful tools to characterize *J. curcas* accessions and increased effort is required to achieve comparable results of genetic variation between regions. AFLPs, for their high reproducibility, moderate cost and for yielding the highest number of polymorphic loci, and SSRs, because of their being co-dominant and moderate in cost, are the techniques recommended for *J. curcas*. An agreement between *J. curcas* researchers from different parts of the world would be highly desirable in order to use some common basic techniques in the analysis of diversity in the species and in the type of plant materials to be studied; we emphatically recommend the use of vegetative parts instead of seeds, because the plant is allogamous and the origin of the paternal parent is unknown. Financing of those projects could come, in part, from international agencies and from local governments.

Genomics and bioinformatics

Even taking into account the relatively small genome of *J. curcas* ($C = 0.416 \times 10^9$ bp; Carvalho et al., 2008) it would be difficult from the economic point of view to develop a project to sequence the genome of *J. curcas*. Few groups are working in this area, for example Lin et al. (2003) reported the cloning and expression of the gene of curcin. Until now, only the chloroplast genome sequencing has been reported (Asif et al., 2010). A private enterprise has announced the completion of the sequence of *J. curcas* genome (www.sgbiofuels.com), but no information exists to date in public databases. It is possible that genome sequencing will be started for other economically important Euphorbiaceae. Valuable information will be obtained to understand many processes in *J. curcas* using bioinformatics tools.

Breeding

No reports are available so far on breeding *J. curcas* for productivity. There is an immediate need to systematize research for widening the genetic base of *J. curcas* through selection of superior genetic stocks, mutagenesis, transgenesis and inter-specific hybridization.

CONCLUSION

Programs launched in several tropical countries for introduction of *J. curcas* for oil production, have had limited success due to poor seed and oil yields. Breeding

for productivity has been restricted by the lack of genetic information; for that reason it is necessary to evaluate the genetic diversity in domesticated populations from the Mesoamerican region and from others parts of the so called "*J. curcas* belt". The understanding of the genetic structure of populations of *J. curcas* will allow us to have genetic material available for future improvement of this important biofuel species.

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VARIACIÓN GENÉTICA EN *JATROPHA CURCAS* DE MÉXICO ESTIMADA CON ÁCIDOS GRASOS DE LA SEMILLA

En este capítulo de la tesis se presenta el estudio de la diversidad química de ácidos grasos de la semilla de *J. curcas* del sur de México. La mayoría de las muestras provinieron del Estado de Chiapas debido a que en este estado existe la más vasta extensión de cultivo tradicional de "piñón" en México. Además de abordar aspectos básicos como la utilidad de los ácidos grasos como marcadores químicos de diversidad genética, el grado de variación, las relaciones entre poblaciones, y las implicaciones adaptativas de la composición de ácidos grasos, se encontró información que podría ser de utilidad en el aprovechamiento económico de la planta. Esta incluye la identificación de genotipos con cantidad y calidad de aceite incrementados.

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Genetic Variation in Mexican *Jatropha curcas* L. Estimated with Seed Oil Fatty Acids

I. Ovando-Medina[1, 4*] , FJ. Espinosa-García[2] , J. Núñez-Farfán[3] and M. Salvador-Figueroa[4]

[1] *Posgrado en Ciencias Biológicas. Universidad Nacional Autónoma de México (Ciudad Universitaria, Distrito Federal, MEXICO)*
[2] *Centro de Investigación en Ecosistemas. Universidad Nacional Autónoma de México (Morelia, Michoacán, MEXICO)*
[3] *Instituto de Ecología. Universidad Nacional Autónoma de México (Ciudad Universitaria, Distrito Federal, MEXICO)*
[4] *Centro de Biociencias. Universidad Autónoma de Chiapas (Tapachula, Chiapas, MEXICO)*

Abstract: The genetic diversity of Mesoamerican populations of the biofuel plant *Jatropha curcas*, using the fatty acids of the seeds as chemical markers was studied. The oil content of the whole seed in 135 accessions from 38 sites varied between 8.020% and 54.28%. The prevalent fatty acids were oleic acid (18:1) and linoleic acid (18:2), and the proportion of unsaturated fatty acids varied between 74.5% and 83.7%. A study with cloned plants grown in common garden showed that both the content of oil as well as the proportion of fatty acids are highly inheritable, therefore these chemical markers are valid for estimating the genetic diversity of the species. An analysis of principal components showed that the fatty acids that contribute more to the variance are stearic, oleic, linoleic, methylpalmitic, gadoleic and ricinoleic. The populations were classified in ten groups when the data were analyzed for fatty acids by analysis of clusters, showing the elevated genetic variation in natural populations of this native species of Mesoamerica. A discriminant analysis separated the populations in accordance with their geographic origin, which was verified with a Mantel test. Using the Monmonier's algorithm two genetic barriers between the populations were identified. The results are discussed in light of their microevolutionary significance.

Key words: seed oil, *Jatropha*, Mesoamerica, Mexico, genetic diversity

1 INTRODUCTION

Jatropha curcas L. is perhaps currently the plant of greatest importance for the extraction of oil for the fabrication of biodiesel. Various authors have mentioned the advantageous characteristics of this plant from the family Euphorbiaceae, in comparison with other oleaginous plants, highlighting particularly its adaptation to marginal environments[1-4].

Although there is no consensus about the origin of this species, many researchers point to the Mesoamerican region (Mexico and Central America) as its center of origin[5-7], while others mention South America[8, 9]; but there is no doubt that Mesoamerica is the center of diversification of the genus *Jatropha*[10] and that this region held the source material for establishment of present populations in Africa and Asia. Moreover, only in Mexico do domesticated genotypes exist of low or null toxicity[11, 12].

In the last decade, the number of studies and publications on *J. curcas* have grown geometrically, driven by the growing interest of governmental agencies in many parts of the world, but above all in Southeast Asia, in the cultivation of the plant. Notoriously, the majority of these reports used Asian accessions to study the agronomic performance, the genetic variation through molecular markers, and of course, the content and composition of the seed oil[13-18]. To a lesser extent, accessions from South America[19, 20] and from Africa[21] have been studied. When samples from the Mesoamerican region have been used, it has been only for comparison. On the other hand, little attention has been paid to the composition of fatty acids of the seeds as chemical markers for estimating the genetic diversity of this plant; instead, the studies have been focused on determining the proportion of saturated or unsaturated fatty acids due to their importance in the production of biodiesel, as foodstuff and for usage in industrial processes[22-25], with the exception of the work of Wang *et al.*[26], who studied some accessions of *J. curcas* introduced in China.

In the present study the content of oil in the seeds was investigated along with its composition of fatty acids in Mexican populations of *J. curcas*, with the purpose of estimating the level of chemical variation and relating this with its evolutionary meaning.

2 EXPERIMENTAL PROCEDURES

2.1 Sample collection

Plants of *J. curcas* were studied in the Mesoamerican region, covering a total of 38 sites which were a *priori* grouped into 6 populations from areas in the south and southeast of Mexico, and in Guatemala (Table 1, Fig. 4). Each population was represented by at least five trees in the stage of seed production (rainy season, August-September of 2008), from which were collected close to 100 g of seed from each plant. Cuttings of 80 cm in length were also collected, which were rooted in a greenhouse and taken in as 135 accessions of the Bank of *Jatropha* in the Center for Biosciences at the Autonomous University of Chiapas (Tapachula, Chiapas, Mexico).

2.2 Extraction of oil and preparation of FAMEs

The seeds from each site were dried at room temperature for at least 30 days, until they possessed less than 2% moisture. The whole seeds (including seed coat, endosperm and embryo) were crushed in a mortar washed with hexane, homogenized, and sampled in triplicate 10 g of the resulting paste for determining the content of oil. The method utilized was Soxhlet 920.39 of the Association of Official Analytical Chemists[27]. To prepare Fatty Acid Methyl Esters (FAMEs), the method of Ichihara *et al.*[28] was followed -with modifications. In a screw-top test tube previously washed with hexane and dried for 2 h at 300℃, 2 mL of n-hexane were introduced and 40 mg of crude oil were added. The sealed test tubes were brought to 40℃ in a bath of water for 1 min and 200 μL of a solution of methanolic NaOH 2.0 M were added. These were agitated in vortex for 2 min, returned to heat for 1 min and centrifuged at 5000 rpm for 10 minutes. The upper hexane phase was separated containing the prepared FAMEs.

2.3 Determination of the composition of fatty acids by gas chromatography

The fatty acids were analyzed by gas chromatography/mass spectrometry (Chromatograph Agilent 6890), injecting 0.5 ÕL of the FAMEs from each accession into a column of intermediate polarity (Equity-1, Supelco 28046-U, 30 m × 250 μm × 0.30 μm), with the following program of temperatures: 100℃, 25℃ min^{-1} to 200℃, 2.5℃ min^{-1} to 230℃ per one minute, 10℃ min^{-1} to 250℃). The chromatograph was operated using helium as carrier gas (7.48 psi). The injector was programmed in mode Split 60:1 to a temperature of 280℃. A selective mass detector was used (Agilent 5973 Network) operated at a voltage of ionization of 70 eV, with a temperature interface of 280℃, Scan mode and mass rank of 50 to 550 m/z. The compounds from each one of the samples were identified by comparing their mass spectra with those of Library of the National Institute of Standards and Technology (NIST98). The concentration of each compound was obtained comparing its abundance with that of the internal standard, methyl ester of nonadecanoic acid, and considering the total concentration of the fatty acids as 100%. The relative proportions for each compound were reported in percentage.

2.4 Study in common garden with cloned plants

To study if the composition and proportion of fatty acids in the oil of *J. curcas* of Mesoamerica are fixed characters, 15 accessions clonally reproduced and situated in the Bank of *Jatropha* were evaluated under similar conditions (cultivated during a year in rain-fed andosol approximately 2500 mm raifall in the 2008-2009 cycle-, average annual temperature of 30℃, without agronomic management). The plants initiated their flowering in May 2009 and the production of seeds in July, so that 100 g of seeds were collected from three clones per accession during August-September of 2009. The preparation of samples, extraction of oil, obtaining of FAMEs and chromatographic analysis were made under the conditions described above.

2.5 Statistical analysis

The data for oil content were processed through an analysis of variance. The proportion of saturated/unsaturated fatty acids was compared between sites, and an exploration was made for possible Pearson correlations (α 0.05) between the altitude and latitude of the collection sites and the content and composition of the oils. For this, the software XLStat© 2010.3.03 (AddinSoft, USA) was utilized.

With the data derived from plants cloned and grown in common garden the broad sense heritability was studied for the fatty acids and the oil content, utilizing the online software PBStat© 1.02[29]. With the data of proportions for fatty acids in seeds collected from the sites, analyses of principal components, discriminant and clusters (utilizing Euclidean distances and grouping by average linkage) were made. These analyses were done with XLStat© 2010.3.03.

Isolation by distance was tested through the correlation of matrices of Euclidean and geographic distances using the Mantel test with 10000 permutations. The geographic distances were obtained by means of latitude/longitude of the sites, using GenAlEx 6.0[30]. To test the existence of possible genetic barriers between populations the software Barrier 2.2[31] was utilized, correlating a Fisher distance matrix, as generated in a discriminant analysis, with a matrix of average decimal geographic distances of each population.

3 RESULTS

3.1 Content and composition of the seed oil

The quantity of oil in the seed of Mesoamerican *J. curcas* by individual varied between 8.02 and 54.28%, while the variation per site was between 12.09 and 44.27% (Ta-

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Table 1 Location data, oil content seed and proportion of unsaturated fatty acids in accessions of *Jatropha curcas* L. from 38 sites in the Mesoamerican region.

Population	Site	Number of accessions	Latitude / Longitude	Altitude (masl)	Seed Oil Content (%)	Unsaturated Fatty Acids (%)
Coast of Chiapas	ACA	5	15.1040/-92.3511	51.0	37.22	78.96
Coast of Chiapas	CAC	2	14.5901/-92.5941	472.0	25.13	82.29
Coast of Chiapas	HUX	5	15.0402/-92.2922	23.0	37.11	74.52
Coast of Chiapas	MAP	5	15.2540/-92.5384	34.8	39.88	79.62
Coast of Chiapas	PC	5	14.4371/-92.2578	5.4	33.59	81.05
Coast of Chiapas	SCH	5	14.3957/-92.1032	24.3	34.65	78.31
Coast of Chiapas	TAP	5	14.5453/-92.1925	74.4	32.23	78.91
Coast of Chiapas	TON	1	16.0343/-93.5078	12.0	39.29	83.42
Coast of Chiapas	ARR	5	16.1141/-93.6359	53.0	40.25	82.50
Coast of Chiapas	PIJ	5	15.5552/-92.5956	79.0	33.80	79.99
Center of Chiapas	BERR	4	16.4760/-93.1625	895.8	35.76	82.85
Center of Chiapas	CIN	4	16.4113/-93.4265	574.8	17.51	80.44
Center of Chiapas	JIQ	4	16.4004/-93.3922	533.3	40.69	79.69
Center of Chiapas	OCZ	3	16.4623/-93.2372	803.3	19.99	82.10
Center of Chiapas	TYL	1	16.2269/-93.4918	689.0	14.18	77.84
Center of Chiapas	TUX	1	16.4233/-93.0670	894.0	12.20	78.72
Center of Chiapas	CCR	4	16.0664/-92.4104	537.5	12.09	83.02
Center of Chiapas	PUJ	3	16.1569/-92.1747	511.0	37.71	78.22
Center of Chiapas	RV	3	16.0993/-93.0453	560.7	43.12	81.02
Center of Chiapas	VCR	1	16.1831/-93.0454	830.0	18.90	77.69
Center of Chiapas	VCO	1	16.1169/-93.1563	579.0	26.21	77.72
Center of Chiapas	VIF	1	16.1482/-93.1578	564.0	31.16	78.43
Center of Chiapas	VLR	1	16.1924/-92.2057	1304.0	21.14	83.78
Center of Chiapas	CHIC	5	15.4465/-92.1672	587.8	14.52	83.44
Center of Chiapas	CDCU	5	15.4050/-92.0012	700.8	26.76	82.71
Center of Chiapas	COM	5	15.3918/-92.0801	677.4	27.12	83.20
Center of Chiapas	LACO	2	15.5029/-91.5422	635.0	44.27	79.25
Center of Chiapas	LAGOS	5	15.5034/-91.5437	631.0	30.04	80.26
Center of Chiapas	RIZ	3	15.5794/-92.2884	561.0	27.90	81.77
Center of Chiapas	IXT	4	16.4722/-92.5463	813.0	32.17	77.31
Guatemala	GUA	5	14.3627/-90.2908	1245.6	31.88	80.97
Oaxaca	ECO	4	15.4339/-96.3386	61.8	28.41	80.44
Oaxaca	ZIM	1	15.5056/-96.0015	66.0	22.39	79.55
Oaxaca	PA	5	16.1125/-97.4476	37.8	32.00	80.71
Oaxaca	PIN	3	16.1931/-97.5355	263.7	25.24	81.96
Guerrero	COP	4	16.3747/-99.0074	8.5	24.77	79.46
Guerrero	ACAP	5	16.4996/-99.4688	19.2	24.73	80.49
Michoacán	APA	5	19.0424/-102.2210	312.4	29.33	77.97

ble 1), which was significant (F = 8.205, $P <$ F 0.0001). It was found that the oil content is negatively correlated with the altitude at which the plants were growing (Fig. 1a). There was no significant correlation with the latitudes of collection sites.

Eleven transesterified fatty acids were found: Decanoic Acid (Capric Acid 10:0), Tetradecanoic Acid (Myristic Acid 14:0), Hexadecanoic acid (Palmitic Acid 16:0), 9-Hexadecenoic Acid (Palmitoleic Acid 16:1), 14-Methyl Hexadecanoic Acid (Methyl Palmitic Acid 17:0), Octadecanoic Acid

(Stearic Acid 18:0), 9-Octadecenoic Acid (Oleic Acid 18:1), 7-Hydroxy,9-Octadecenoic Acid (Ricinoleic Acid 18:1), 9,12-Octadecadienoic Acid, (Linoleic acid 18:2), Eicosanoic Acid (Arachidic Acid 20:0), Eicosenoic Acid (Gadoleic Acid 20:1). In addition, several accessions showed 3-Octyl,Oxirane-Octanoic Acid (16:0) and / or Decanedioic Acid (11:0, Sebacic Acid, Dicarboxylic), which could be intermediary products of synthesis or degradation of other fatty acids.

The most abundant fatty acids were oleic (average 38.7%), linoleic (average 40.7%), palmitic (average 12.8%) and stearic (6.1% in average). The proportion of unsaturated fatty acids varied between 59.14 and 86.76%, considering the individuals, while the variation per site was between 74.5 and 83.8%, which was significant (F = 1.633, P = 0.029; Table 1). The proportion of unsaturated fatty acids is weakly but positively correlated with the altitude (Fig. 1b). There was no significant correlation with the latitude of the sites. The oil content and the unsaturated fatty acid proportion are negatively correlated (Fig. 1c).

3.2 Most informative and heritability of characters

The principal components analysis (PCA) showed that the variables stearic, oleic and linoleic acids contributed mainly to the first principal component; consequently, those variables can be considered the most informative of the variation detected (Table 2). The study with cloned plants showed that almost all fatty acids had very high broad sense heritabilities (around 90%), except for the case of palmitoleic acid, of which it was not possible to estimate the heritability, since statistically significant differences between genotypes were not detected; whereas the
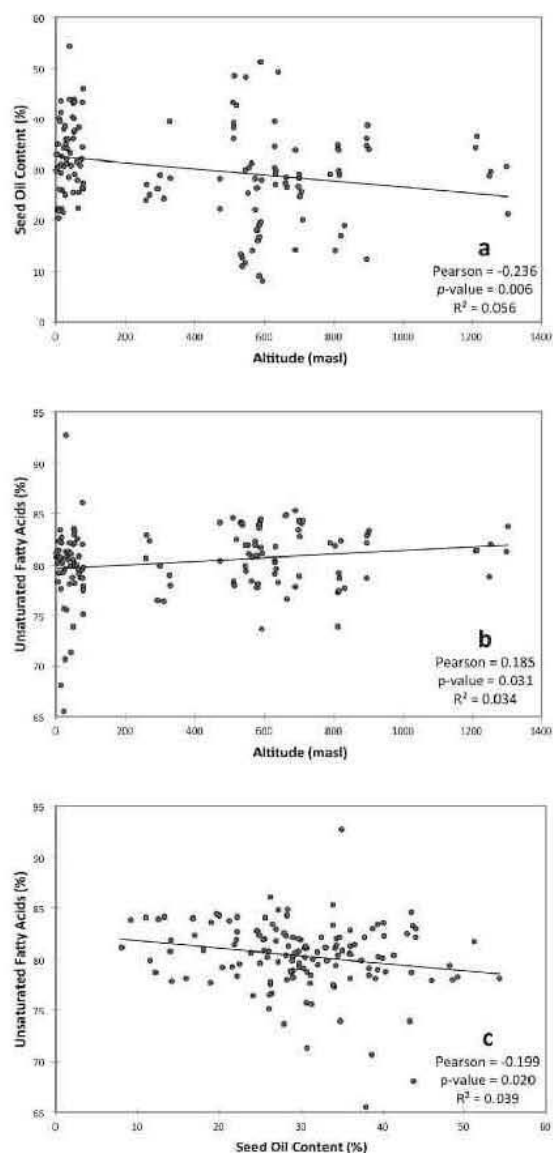
Fig. 1 Significant correlations between parameters of the seed oil of Mesoamerican *Jatropha curcas*; a) Seed oil content vs. altitude, b) Unsaturated fatty acids vs altitude, c) Unsaturated fatty acid vs seed oil content.

Table 2 Contributions of the variables (%) to the first two principal components (F1 and F2).

Variable	F1	F2
Myristic Acid (14:0)	0.033	0.463
Palmitic Acid (16:0)	6.767	10.802
Palmitoleic Acid (16:1)	0.504	17.875
Methyl-Palmitic Acid (17:0)	1.288	17.468
Stearic Acid (18:0)	18.666	0.493
Oleic Acid (18:1)	24.353	6.621
Linoleic Acid (18:2)	31.988	3.672
Arachidic Acid (20:0)	4.827	0.452
Gadoleic Acid (20:1)	2.690	23.049
Capric Acid (10:0)	0.874	0.595
Ricinoleic Acid (18:1)	1.777	18.332
Seed Oil Content (%)	6.234	0.176

Bold values correspond, for each variable, to the factor for which the squared cosine is the largest.

Table 3 Estimation of the broad sense heritability of the characters studied.

Variable	V_G	V_P	h^2_{bs} (%)
MYRISTIC	0.0056	0.0063	89.23
PALMITIC	29.8427	30.4945	97.86
PALMITOLEIC	0.0000	0.5984	0.00
MPALMITIC	0.0028	0.0031	92.08
STEARIC	9.7004	10.1895	95.20
OLEIC	24.0714	25.2182	95.45
LINOLEIC	36.0180	37.2547	96.68
ARACHIDIC	0.0509	0.0515	98.82
GADOLEIC	0.0032	0.0037	86.48
RICINOLEIC	0.0089	0.0215	41.35
OILCONTENTS	17.9423	25.5240	70.30

V_G: Genotypic variation; VP: Phenotypic variation;
h^2_{bs}: broad sense heritability, in percentage.

oil content had heritability of 70% (Table 3).

3.3 Description of the variation

Having confirmed the high heritability of fatty acids of the seed, we described the genetic variation using these characters as chemical markers. The analysis of ascending hierarchical classification grouped the 135 accessions into 10 groups, as shown in the dendrogram of **Fig. 2**, where the dotted line indicates the limit for the formation of groups. In general, it was observed that accessions are grouped according to their geographical origin, but some that belong to a same town or locality are in a different group.

It is noteworthy that the groups I, II and III with one, one and four accessions of the Coast of Chiapas, respectively, are clearly separated from the other accessions, including those of the rest of the mentioned population, i.e. they were outliers (**Fig. 2**). Groups IV to VIII contained accessions from the Center of Chiapas and populations of Guatemala, Oaxaca, Guerrero and 3 accessions of Michoacán, along with some accessions from the Coast of Chiapas. Groups IX and X contained almost exclusively accessions from the Coast of Chiapas, except ZIM1 (Oaxaca) and APA2 and APA5 (Michoacán).

The genetic variation encountered can be considered high given that the line forming groups stood at a Euclidean distance of 7.5, while the maximum distance between groups was 33. In addition, the variation within classes accounted for only 10.7%, while the variation among classes was 89.3%.

Discriminant analysis, it showed that the first two factors explain 89% of the variation and separated the accessions into three major groups in the bidimensional geometric space: the accessions of Michoacán, the Coast of Chiapas

and Center of Chiapas; accessions of the other populations were mixed with those of the last two populations (**Fig. 3a**). To confirm the trends in spatial location, the centroids were plotted for each population (**Fig. 3b**), confirming that the population of Michoacán is isolated and that those of Chiapas are clearly far apart. The centroids of the populations of Guatemala and Guerrero overlap the Center of Chiapas. The Oaxacan population is related to that of Guerrero.

3.4 Isolation of populations

To evaluate the apparent ranking of accessions according to their origin, a Mantel test of association was performed, with significant results ((r(AB) = −0.082); P < 0.0001)), which indicates that there was a correlation between genetic distance (Euclidean) and geographic distance in kilometers. Isolation was also shown by the existence of genetic barriers detected with Barrier vers. 2.2. The major barrier (a) separates the population of Central Chiapas of the rest of the populations, and the second barrier (b) isolates the population of Michoacán (**Fig. 4**).

4 DISCUSSION

The storage of oils in seeds is a generalized feature in higher plants, which serve as a source of energy for the embryo during the heterotrophic stage[32]. This stage is crucial in the success or failure of the embryo to germinate, emerge and establish itself as a new plant[33], and therefore, the content of the endosperm, at least in part, determines reproductive success or "fitness" of the plant. As a result, the total content and composition of seed oil should be

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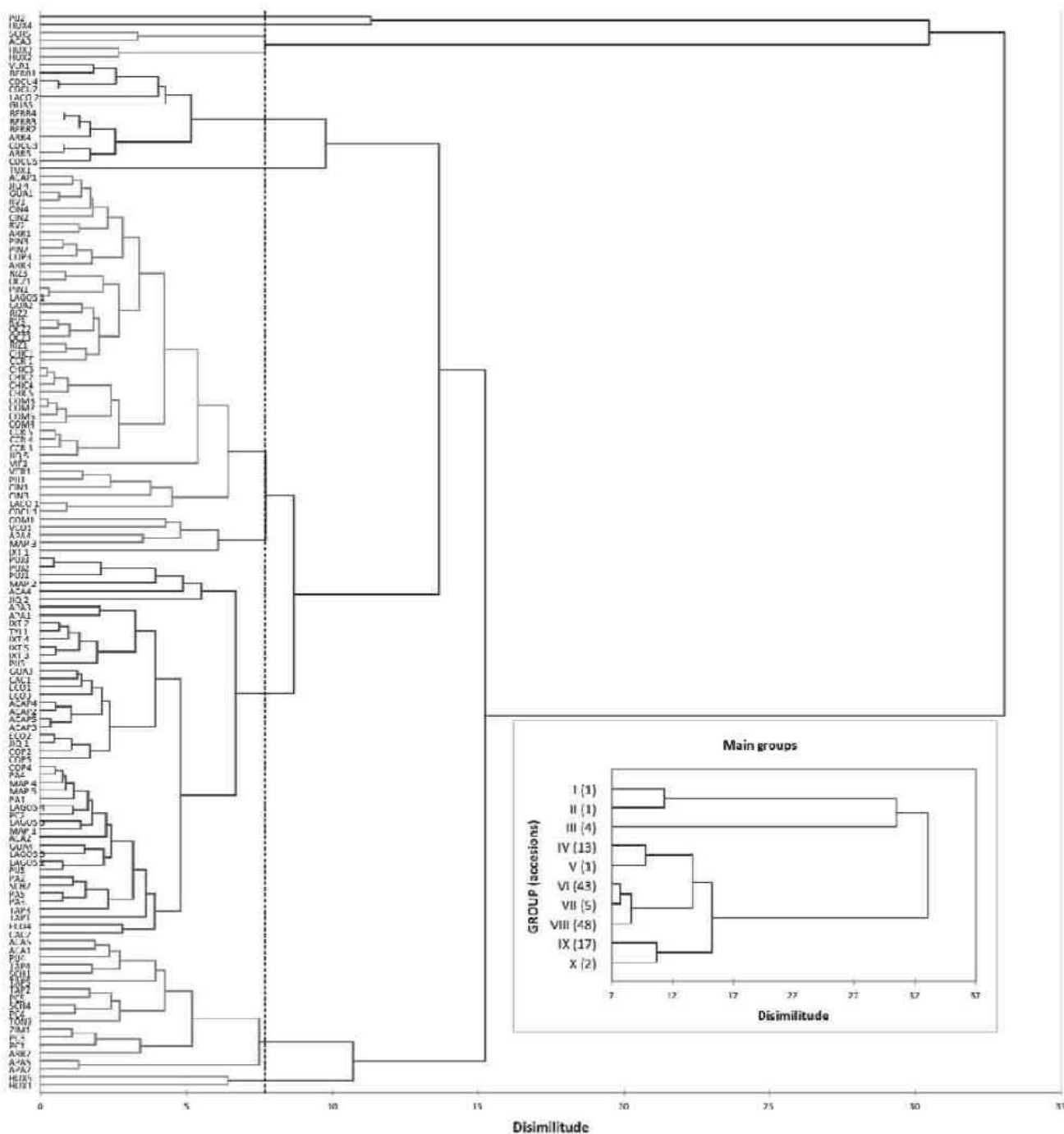
Fig. 2 Dendrogram of Euclidean distances for 135 accesiones de *Jatropha curcas* L. in the Mesoamerican region based on the proportion of 11 fatty acids in the seed. The box shows the ten groups, which were formed, and the number of accessions in each of them.

considered characters subject to natural selection. The present study, in a microevolutionary and not phylogenetic context, showed that the variation in oil content and fatty acid composition of the seed in populations of *J. curcas* in Mesoamerica is very high (Table 1) and that the chemical

markers used are highly heritable (Table 3).

4.1 Pattern of total oil content

The quantity of oil determined by sites (between 12 and 44% of the mass of the whole seed) is consistent with pre-

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Fig. 4 Map showing the sites of collection of *Jatropha curcas* evaluated in their composition of oil from Southern Mexico and from Guatemala, and the two main genetic barriers (lines "a" and "b" in yellow) found by the algorithm of Monmonier (Barrier vers. 2.2), as based on Fisher distances of fatty acid composition of the seed. Map kindly prepared by Francisco Javier Pérez Racancoj.
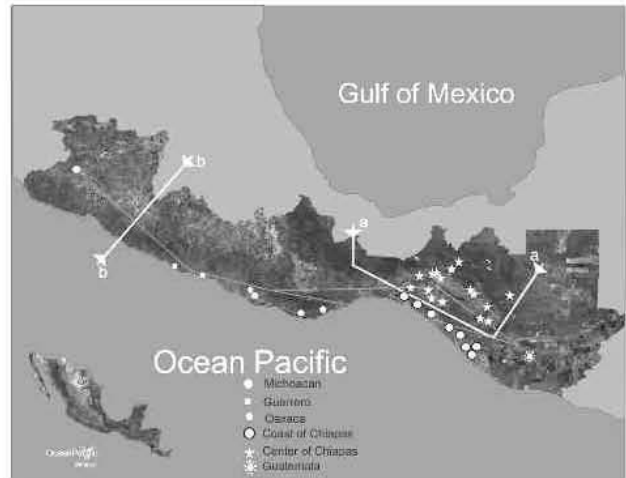
Fig. 3 Discriminant analysis of 135 accessions of *Jatropha curcas* L. from six Mesoamerican populations, based on the proportion of fatty acids in the seed; a) distribution in the bidimensional geometric space based in factors 1 and 2, b) distribution based on the centroids.

vious reports for this species, either cultivated in Asia or Africa[5, 34] or collected in the Mesoamerican region[11, 12]. Similarly, the fact that there is a pattern of negative correlation with altitude confirm the findings of Pant *et al.*[35] who determined the variation in yield and oil content of Indian accessions of *J. curcas* in two soil conditions and three altitudinal ranges, finding that there is a higher content of oil when the plant is found in non-arable soils and low elevation.

It has been documented that there is considerable variation in oil content of this species that can be generated by genetic and environmental factors, including rainfall and soil fertility[36, 37]. Heller[5] reported that variability in this characteristic might be influenced by the origin of the accessions studied and genotype-environment interactions. Contrary to this, Kaushik *et al.*[38] reported that the oil content of twenty-four accessions of Indian *J. curcas* had a broad sense heritability of 99%, implying a limited influence of the environment. However, they also reported a variation of only 10% among accessions and very low coefficients of phenotypic and genotypic variation, which con-

firms the narrow genetic basis of *J. curcas* in Asia. In the same way, Gohil and Pandya[39] reported 89.7% of broad sense heritability for this character.

Unlike the explanation of that phenomenon for Asian accessions, where *J. curcas* was introduced a few centuries ago and where the variation might be due to strong environmental effects by having a narrow genetic base[40], in Mesoamerican populations the reason could be the selection of genotypes adapted to adverse conditions (higher temperature) with increased production of energy substances for the embryo during germination. This increases seedling vigor and thus, the fitness of the species.

It is noteworthy that the oil content is a characteristic that had a high heritability (70%), while it had a high coefficient of genotypic variation and 32% of variation among accessions (**Tables 1** and **3**), which shows that populations in Mesoamerica, where most likely *J. curcas* is a native of, are more variable than in other parts of the world.

Although there have been determinations of the oil content in higher plants for a long time, perhaps the first study to look for relationships between ecological parameters and accumulation of oil was that of Levin[41], who studied the relationship between oil content and the habit and habitat of over a thousand species of angiosperms, finding that the oil content, evolutionarily, has increased with the development of woody stems and shade tolerance, and there is no relation to the latitudinal origin. In the case of the tropical semi-domesticated plant *J. curcas*, in this study there was no significant correlation with latitude of

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collection sites. In a thorough review, no reports were found which examine this relationship, possibly conforming to that postulated by Levin. The high oil content is also consistent with the hypothesis of Levin because *J. curcas* can be considered a woody tree shrub. To test the hypothesis of variation according to the habits of growth, it would be of great scientific interest to know the correlation between the accumulation of seed oil and the degree of stem lignification of the 175 species of genus *Jatropha*.

4.2 Pattern in the fatty acid composition

Except for ricinoleic acid, which is characteristic of *Ricinus communis* L., all other fatty acids have been reported for *J. curcas*; although this fatty acid has been reported recently in the species *J. gossypifolia* and other Euphorbiaceae[42]. Either way, it was only found in seven of the 135 accessions studied and its heritability was low (Table 3); however, the PCA revealed that it is a relatively informative variable by having the highest percentage of variability in the second principal component (Table 2).

The fatty acid profile is consistent with that reported in other studies, which report the same four main fatty acids[5, 11, 12, 43, 44]. Behenic (22:0) and linolenic (18:3) fatty acids, which have been reported for *J. curcas*[45] were not found in the evaluated accessions. The proportion of unsaturated fatty acids is an important variable in the biodiesel manufacturing, since quality standards indicate it must be minimized[46]. Interestingly, this study found that oil content and the proportion of unsaturated fatty acids are negatively correlated, which, if confirmed in a larger study of genetic association with outstanding genotypes, will permit genetic improvement for increased oil yield while decreasing unsaturated fatty acids.

To date great progress has been made in molecular understanding of the origin of the fatty acids in seeds and their diversification mechanisms[47, 48]. However, it is surprising how little attention has been given to the selection factors that drive the evolution of the fatty acid composition of seeds. In this regard, Linder[49] suggested that the temperature of germination is an important selective agent that causes the seed oils of plants native to high latitudes (or altitudes) to have a higher proportion of unsaturated fatty acids that plants in lower latitudes/altitudes. The explanation is that in cooler environments (high latitudes and altitudes) the catabolism of unsaturated fatty acids is more feasible compared to saturated, thus the seeds with more unsaturated fatty acids germinate and grow faster at lower temperatures, increasing their fitness, even at the expense of having less total energy available. On the other hand, in warm environments, such as the tropical, seeds with more saturated fatty acids are selectively favored because they have more energy and they do not need to germinate quickly, since the conditions in the tropics are more or less stable throughout the year.

However, although the proportion of unsaturated fatty acids was positively correlated with the altitude of the sites, the fatty acid composition of *J. curcas* seems to be in disagreement with the hypothesis described, because, being a tropical species, selection has favored a greater proportion of unsaturated fatty acids.

The same author mentions that the lack of association between germination temperature and the proportion of unsaturated fatty acids in one species could be due to the lack of genetic variation in oil composition, which, as described in this paper, is not the case of *J. curcas*.

Based on the foregoing, it is hypothesized that, in the case of *J. curcas*, soil moisture has exerted selection pressure to select a higher proportion of unsaturated fatty acids. It is well documented that this species is tolerant to drought, although adapting to different environments, from humid to semi-arid; however, the plant is susceptible to flooding, for example, according to Dehgan & Schutzman[50], *J. curcas* is found in South America in seasonally dry tropical areas, but is completely absent in the always wet Amazon region. In the Mesoamerican tropical region, the onset of flowering and seed production coincides with the onset of the rainy season, so seeds, which do not exhibit dormancy, should germinate quickly, usually within five days (for which they require higher proportion of unsaturated fatty acids) and establish themselves as seedlings before soil moisture levels increase to waterlogging.

4.3 Patterns of diversity of Mexican *J. curcas*

The analysis of clusters showed that genetic diversity of Mesoamerican *J. curcas* is high, as shown by the 7.5 units of Euclidean distance that marked the line of formation of the 10 clusters (Fig. 2); unfortunately, there are no reports of similar works with which to compare the variation using fatty acids as markers. Except for the work of Wang *et al.*[26], who compared the oil content and fatty acid composition in samples of *J. curcas* collected from three regions of China and India, finding 12 fatty acids and reporting differences between accessions. They concluded that attention should be given to these chemical markers to introduce new Chinese germplasm and for the genetic improvement of the plant.

Discriminant analysis separated the populations more or less according to their geographical origin (Fig. 3); this was checked with the Mantel test, which indicated a correlation between chemical and geographical distance. In contrast to these results, Kaushik *et al.*[38] evaluated 24 accessions of *J. curcas* from India to establish the genetic variability, where the pattern of clustering in the Euclidean cluster analysis revealed that geographic diversity does not necessarily represent the genetic diversity.

In our study two genetic barriers could be identified (Fig. 4) that separated populations of the Center of Chiapas and Michoacán. Possible explanations are related to the exis-

tence of the mountain chain "Sierra Madre del Sur" which functions as a geographic barrier; the population of Center of Chiapas is the only one that is to the North of this mountain chain. Other possible reason is the geographical remoteness of the population in Michoacán from the rest of the populations. Studies with molecular markers based on DNA may provide information about the rate of gene flow between populations and confirm or reject the existence of the genetic barriers mentioned in Mesoamerican *J. curcas*.

5 CONCLUSION

The results of the work showed that the variation in the oil content and eleven fatty acid was very high with heritabilities of about 90%, which demonstrate that they are useful markers in estimating the genetic diversity of *J. curcas*. The populations of *J. curcas* from the Mesoamerican region represent valuable genetic resources for the future establishment of extensive plantations of this oil producing plant.

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DIVERSIDAD GENÉTICA EN POBLACIONES DE *JATROPHA CURCAS* DE CHIAPAS, MÉXICO, CARACTERIZADA CON AFLPs

En este capítulo de la tesis se describe el estudio de la diversidad genética en poblaciones domesticadas de *J. curcas* de Chiapas, mediante marcadores polimórficos en la longitud de fragmentos amplificados (AFLP), un método de muestreo del ADN genómico. Aunque existe una literatura creciente acerca de la diversidad genética de esta especie, el aquí presentado es el primer estudio con un enfoque poblacional que se realiza dentro de la más probable región de origen del "piñón" (Mesoamérica). Después de listar los descriptores de diversidad genética de cinco poblaciones de *J. curcas* en Chiapas, se realizó un análisis de conglomerados que reveló el más alto coeficiente de disimilitud entre accesiones reportado hasta ahora (Jaccard = 0.893). Tanto un análisis de varianza molecular, como una variante que usa datos espaciales de dicho análisis, detectaron moderada estructura genética de las poblaciones, con la mayor variación ubicada dentro de las poblaciones (87.8%). Un análisis bayesiano mostró que la mayoría de los individuos tiene ancestría mezclada, excepto los de la población "Soconusco". Se detectaron dos barreras genéticas; la más importante de ellas separa a la población "Fronteriza" del resto de poblaciones, indicando un posible papel de la Sierra Madre de Chiapas como barrera geográfica. La elevada variación genética encontrada sugiere que la región mesoamericana podría ser un centro de diversidad de *J. curcas*.

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Article

Genetic Diversity in *Jatropha curcas* Populations in the State of Chiapas, Mexico

I. Ovando-Medina[1,2]*, A. Sánchez-Gutiérrez[1], M. L. Adriano-Anaya[1], F. J. Espinosa-García[3], J. Núñez-Farfán[4] and M. Salvador-Figueroa[1]

[1] Centro de Biociencias, Universidad Autónoma de Chiapas/Carretera a Puerto Madero Km 2.0, Tapachula, 30700, Chiapas, Mexico; E-Mails: isidro.ovando@unach.mx (I.O.); saga_9387@hotmail.com (A.S.); maria.adriano@unach.mx (L.A.); msalvad@hotmail.com (M.S.)

[2] Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México/Av. Ciudad Universitaria 3000, C.P. 04360, Coyoacán, Distrito Federal, Mexico; E-Mails: isidro.ovando@unach.mx (I.O.)

[3] Centro de Investigación en Ecosistemas, Universidad Nacional Autónoma de México/Morelia, Michoacán, Mexico; E-Mails: espinosa@oikos.unam.mx (F.E.)

[4] Instituto de Ecología, Universidad Nacional Autónoma de México. Ciudad Universitaria, Mexico, D.F.; E-Mails: farfan@unam.mx (J.N.)

* Author to whom correspondence should be addressed; E-Mail: isidro.ovando@unach.mx; Tel.: +52-962-642-7972; Fax: +52-962-642-7972.

Abstract: *Jatropha curcas* L. has become an important source to produce oil for biodiesel fuel. Most genetic studies of this plant have been conducted with Asian and African accessions, where low diversity was encountered. There are no studies of this kind focusing in the postulated region of origin. Therefore, five populations of *J. curcas* were studied in the state of Chiapas, Mexico, using amplified fragment length polymorphism (AFLP) markers. One hundred and fifty-two useful markers were obtained: overall polymorphism = 81.18% and overall Nei's genetic diversity (*He*) = 0.192. The most diverse population was

Border [*He*: 0.245, Shanon's information index (I): 0.378]. A cluster analysis revealed the highest dissimilarity coefficient (0.893) among accessions reported until now. An analysis of molecular variance (AMOVA) revealed that the greatest variation is within populations (87.8%), followed by the variation among populations (7.88%). The *PhiST* value (0.121) indicated moderate differentiation between populations. However, a spatial AMOVA (SAMOVA) detected a stronger genetic structure of populations, with a *PhiST* value of 0.176. To understand the fine structure of populations, an analysis of data with Bayesian statistics was conducted with software Structure©. The number of genetic populations (K) was 5, with mixed ancestry in most individuals (genetic migrants), except in the Soconusco, where there was a tiny fraction of fragments from other populations. In contrast, SAMOVA grouped populations in four units. To corroborate the above findings, we searched for possible genetic barriers, determining as the main barrier that separating the Border from the rest of the populations. The results are discussed based on the possible ancestry of populations.

Keywords: AFLP, biodiesel, populations, genetic structure, differentiation.

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1. Introduction

Jatropha curcas L. is a shrubby plant, which has seeds with a high content of oil capable of being transformed into biodiesel. This plant grows well on marginal soils and is drought resistant [1,2]. Moreover, it has been shown that this species has the capacity to control soil erosion [3, 4] and potential for phytoremediation [5,6]. It is expected that in 2015 about 13 million hectares will have been planted with *J. curcas* in tropical regions of Asia, Africa and America [7]. It is widely distributed throughout the tropics, in Central and South America, Asia and Africa. Nevertheless, many studies mention that its center of origin is Mesoamerica [8-10]. For a discussion about the debate of the center of origin, see our review article [11]. In Mexico, *J. curcas* is known as Piñón and can be found as living fences in various states, both on the Pacific slope, as well as along the Gulf of Mexico [12]. Seeds of this plant are toxic due to their high content of phorbol esters, but in Mexico there are a few non-toxic varieties used as food in some rural areas [7,13,14].

Researchers are studying many aspects of the biology of this species across the world, including its genetic diversity. Recently, the nuclear [15] and chloroplast [16] genomes of this species were sequenced, thus facilitating the identification of genes of productive

interest, such as those related to the synthesis and accumulation of oil. Complementary to this, the study of genetic variation in populations with analysis of the phenotypic variation will contribute to the identification of loci associated with quantitative traits of agronomic interest. The understanding of the genetic diversity and structure of populations of *J. curcas* in their postulated center of origin will permit to identify genetic material useful for future improvement of the species. For example, it will be possible to design crosses between plants from groups genetically distanced.

Most genetic studies of this plant have been conducted with Asian and African accessions, with low diversity found therein. Furthermore, most Mexican germplasm studied for these purposes has been limited to varieties with low or no toxicity at all [10,17-22]. The results of these studies, although based on individual accessions, not populations, suggest that the center of diversity is in Mesoamerica. However, there are no molecular studies focusing on this region.

The state of Chiapas, southern Mexico, has a huge population of *J. curcas* distributed over most of its territory. Previous studies have shown that populations of *J. curcas* from Chiapas have a high variability in oil content and fatty acid composition [23], in toxic compounds of the seed [7] and in floral characteristics (data collected by the authors, Unpublished). Genetic relationships of these populations are still unknown. It should be noted that, while *J. curcas* is a semi-domesticated species (there are not "true" wild populations and is cultivated as fence, although, apparently, it does not exhibit the domestication syndrome), interchange of germplasm only exists at the local level and not between regions of the state [24].

Genetic diversity of this species is influenced by some aspects of its biology (*J. curcas* is a diploid, $n = 11$, with a genome relatively small, 416 Mb [25]; it is monoecious producing male and female flowers in the same inflorescence [26]; a small proportion of its reproduction is via apomixis [26,27]). Ecological aspects are also important, for example, its main mating system is by xenogamy or cross-pollination [27], being pollinated by insects [28]). Anthropic management of populations can affect diversity since *J. curcas* is a plant in process of domestication and it is propagated mainly clonally. Based in the antecedents, we propose the follow postulate: the genetic diversity in *J. curcas* populations in the Mesoamerican region is more elevated than those reported of Old World germplasm. Since there are no studies of these plant populations in Mesoamerica, the present work aims to study the genetic diversity of populations of *J. curcas* of the state of Chiapas, Mexico, using AFLP molecular markers.

2. Experimental Section

2.1 Plant material.

One hundred and thirty-four individuals of *J. curcas* were collected from living fences in five populations of Chiapas, Mexico: Soconusco, Isthmus, Center, Frailesca and Border. Individuals were entered as accessions to the Germplasm Bank, in the Center for Biosciences at the Autonomous University of Chiapas (CenBio-UNACH, abbreviation in Spanish).

The criteria to group plants from different sites in determinate populations were climate (annual mean precipitation and temperature) and soil residual humidity (Table 1). Those sites are clearly differentiated in the mentioned criteria [29-33].

2.2 Isolation of total DNA.

Total DNA extraction was performed by the method described by Doyle and Doyle [34] and modified in the Research Laboratory CenBio-UNACH; young leaves were collected, transported on ice to the laboratory, were washed with sterile water and ethyl alcohol 70%, and kept at -30 °C until processing. An amount of 0.2 g of leaves was ground with liquid nitrogen with 60 µg of polyvinyl pyrrolidone and 1 mL of buffer CTAB (hexadecyltrimethylammonium bromide 0.1% w/v, 5mm EDTA, 1.5M NaCl, 50 mM Trizma Base pH adjusted to 8 with HCl and 2 - mercaptoethanol 0.1% v / v). Extractions were made with chloroform-isoamyl alcohol and precipitation with isopropanol. The extracted DNA was purified with a mixture of phenol: chloroform: isoamyl alcohol (25:24:1). The integrity of the DNA, dissolved in 60 µL milli-Q water, was verified by gel electrophoresis on 1% agarose and quantified spectrophotometrically at 260 nm (GBC Cintra 10e™ spectrophotometer, Australia).

2.3 AFLP Analysis.

AFLP was performed with the procedure proposed by Invitrogen™ (AFLP™ Core Reagent Kit, USA) using 500 ng of total DNA restricted with enzymes *EcoRI* and *MseI* and changing the time of ligation of adapters to 4 hours. In the pre-amplification primers used were: 5'-GACTGCGTACCAATTC + C-3', complementary to the *EcoRI* adapter, and 5'-GATGAGTCCTGAGTAA + C-3' to the adapter *MseI* in a reaction mix containing: 5 µL of the restricted and ligated products, 2 µL MgCl$_2$ 25 mM, 0.5 µL dNTP mix 10 mM, 5 U of Taq DNA polymerase, 4 µL 10 X buffer and 10 pmol of each primer, adjusted to 25 µL with milli-Q water. The pre-selective amplification was conducted at an initial temperature of 92 °C for 2 minutes, 40 cycles at 92 °C for 2 minutes, 1 minute at 38 °C for alignment and 2 min at 72 °C extension, 1 cycle of final

extension at 72 °C for 10 minutes and finally at 4 °C indefinitely. The selective amplification was performed using primers 5'GATGAGTCCTGAGTAA+CAT3' for the adapter *MseI* 5'GACTGCGTACCAATTC+CAT3' for *EcoRI* adapter, which was labeled with the fluorogenic D2WellRed™ (Genoma Lab™, USA), under conditions previously described.

Table 1. *Jatropha curcas* L. accessions collected in the state of Chiapas located in the Germplasm Bank of the Center for Biosciences-University of Chiapas, Mexico.

Locality	Number of Accessions	Latitude (North)	Longitude (West)	Population	Environmental Data
Arriaga	16	16°11.231'	93°54.816'	Isthmus	Coast of Chiapas. Climate Aw0 (w), warm sub-humid with summer rains; mean annual precipitation of 1500 mm; mean annual temperature: 29 °C; five months with soil humidity; 50 m a.s.l.
Pijijiapan	6	15°55.561'	92°59.842'	Isthmus	
Tonalá	6	16°03.430'	93°50.782'	Isthmus	
Acapetahua	5	15°10.300'	92°35.100'	Soconusco	Coast of Chiapas. Climate Aw2 (w) Ig, most humid of the warm sub-humid tropical; mean annual precipitation of 2500 mm; mean annual temperature: 28 °C; eight months with soil humidity; 150 m a.s.l.
Cacahoatán	8	14°59.022'	92°19.433'	Soconusco	
Huixtla	8	15°05.115'	92°29.146'	Soconusco	
Mapastepec	10	15°25.505'	92°53.854'	Soconusco	
Puerto Chiapas	13	14°43.742'	92°25.935'	Soconusco	
Suchiate	9	14°40.057'	92°10.071'	Soconusco	
Berriozabal	3	16°47.562'	93°16.191'	Center	Central Valleys of Chiapas. Climate Aw0(w)(i)g, semi-warm sub-humid with summer rains; mean annual precipitation of 900 mm; mean annual temperature: 22 °C; five months with soil humidity; 800 m a.s.l.
Jiquipilas	7	16°40.012'	93°39.242'	Center	
Ocozocuatla	3	16°46.243'	93°23.641'	Center	
La Concordia	5	16°06.663'	92°41.035'	Frailesca	Central Valleys of Chiapas. Climate Aw1(w)(i)g, warm sub-humid with summer rains; mean annual precipitation of 1200 mm; mean annual temperature: 25 °C; five months with soil humidity; 600 m a.s.l.
Villa Corzo	3	16°10,171'	93°16,059'	Frailesca	
Villa Flores	3	16°19,475'	93°20,976'	Frailesca	
Revolución Mexicana	3	16°09.675'	93°04.742'	Frailesca	
Pujiltic	8	16°16.430'	92°17,850'	Frailesca	
Ciudad Cuauhtémoc	7	15°40.473'	92°00.129'	Border	Central Valleys of Chiapas. Climate Aw1(w)(i)g, warm sub-humid with summer rains; mean annual precipitation of 1400 mm; six months with soil humidity; mean annual temperature: 28 °C; 700 m a.s.l.
Comalapa	7	15°39.030'	92°08.170'	Border	
Rizo de Oro	4	15°57.981'	92°28.824'	Border	

Source of environmental data: [29-33].

The amplified products were resolved by capillary electrophoresis in a CEQ8000™ (Beckman Coulter™, USA) sequencing equipment, for which a 2 µl sample and 0.125 µl standard 400 bp -labeled with the fluorogenic D1WellRed™ (Genoma Lab™)- were mixed, adjusting to 25 µl with sample loading solution (SLS). The electrophoretic conditions were: capillary temperature 50 °C, denaturation temperature 90 °C, an injection voltage of 2.0 kV and separation voltage of 5.0 kV, for one hour. To determine the size of the fragments, the electropherograms were calibrated with molecular weight marker (calibration curve fitted with cubic model, with correction to the mobility of marker AE.Ver2; confidence level >99%). The electropherograms obtained were taken into account only when the peaks obtained good resolution and if the correlation coefficient of the marker was at least 0.99, with cubic correction. Moreover, to accept the minor peaks, the intensity of its signal had to have at least 2% of the intensity of the second highest peak in the electropherogram in question. The findings were made in duplicate and were accepted only when the repetitions have the same result.

2.4 Statistical analysis.

Data from the electropherograms were transformed into a matrix: presence (1), absence (0) of the fragments, using the CEQ Genetic Analysis System© software version 9.0. We performed a cluster analysis of the populations, built with the coefficient of Nei's genetic identity.

Genetic diversity within each population was measured by calculating the percentage of polymorphism (% P), effective number of alleles (Ne), Shannon index of information (I) and expected heterozygosity or Nei's genetic diversity (*He*), using the program GenAlEx© version 6.3 [35]. The format of data selected was Binary (Diploid). Results were compared with those yielded by the program AFLP-SURV 1.0© [36], using the approach of Lynch and Milligan [37] and the Bayesian method with non-uniform prior distribution to compute allelic frequencies [38].

To determine the degree of differentiation within and between populations and between regions, an analysis of molecular variance (AMOVA) was performed, with 100000 permutations using the program Arlequin© version 3.11 [39]. Results of AMOVA were compared with those obtained with the software SAMOVA 1.0© [40]. SAMOVA runs were made for 2 to 10 groups to find the number of homogeneous populations, using the *PhiCT* value as indicator.

Genetic structure of the populations was searched using the software Structure© version 2.3.2 [41]. The program was run with 30,000 iterations, 50,000 iterations after burn-in and 10 repetitions of each number of genetic populations (K1-K9). It was assumed, as prior information for the populations, a migration rate of 0.001, so that the

option was selected USEPOPINFO with the model of ancestry Admixture Model (GENSBACK = 3, MIGPRIOR = 0.001) proposed by Falush *et al.* [42]. The value of K was estimated following the procedure described by Evanno *et al.* [43].

In order to find possible isolation by distance, a test of the Mantel correlation between Nei's genetic distance and geographical distance was performed with 10,000 permutations using the program GenAlEx© version 6.3.

Finally, possible genetic barriers were searched for using the program Barrier© version 2.2 [44], found with the Monmonier algorithm based on *Fst* genetic distances generated in AFLP-SURV 1.0, using the Bayesian method with non-uniform prior distribution to compute allelic frequencies [36,38]. To estimate the robustness of barriers, the analysis was performed using one hundred bootstrapped distance matrices. Considering the potential genetic discontinuities according to the differences among regions, five barriers were initially searched for, but only the two more robust (more than 50% of bootstrap support) were plotted.

3. Results and Discussion

3.1. Genetic Diversity

Two hundred and nine AFLP markers were obtained from electropherograms. Figure 1 shows a typical electropherogram of an accession of *J. curcas*. After elimination of six monomorphic fragments and a process of pruning of data (to avoid fragment size homoplasy), by the methods proposed by Lynch and Milligan [37] and Vekemans *et al.* [45], remained 152 useful markers for diversity analysis.

Polymorphism rates were found ranging between 71.7% and 92.1%, while the average polymorphism was 81.1%; the effective number of alleles (Ne) was between 1.181 and 1.398 with an average of 1.303; the Shannon diversity index (I) ranged from 0.202 to 0.378, the average was 0.306; the genetic diversity of Nei (*He*) ranged from 0.121 to 0.245 with an average of 0.192. These results reveal high genetic diversity in the populations studied. The population with greatest genetic diversity was Border and the least diverse was Soconusco (Table 2).

Parameters of genetic diversity obtained with AFLP-SURV 1.0 were slightly different, but with the same tendency. For example, the most diverse population, Border, had a value of *He* of 0.271, while the least diverse, Soconusco, had a *He* value of 0.123. Global gene diversity within populations was 0.207.

Figure 1. Electropherogram of a typical sample of *J. curcas* from Chiapas obtained by capillary electrophoresis. The marker of molecular size, 400 bp labeled with the fluorogenic D1WellRed®, is showed in red. PCR products were resolved in a CEQ8000® (Beckman Coulter®) sequencer.
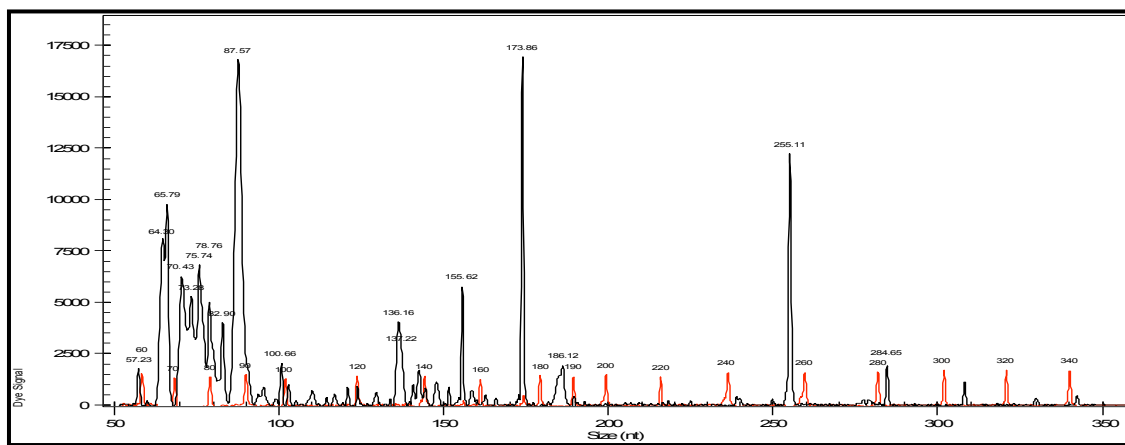
Table 2. Genetic diversity parameters of 134 accessions of *J. curcas* of Chiapas, Mexico, grouped in five populations.

Population	N	Na	Ne	I	*He*	%P
Soconusco	53	1.434	1.181	0.202	0.121	71.71%
SE		0.073	0.021	0.018	0.012	
Isthmus	28	1.842	1.328	0.335	0.208	92.11%
SE		0.044	0.026	0.018	0.013	
Center	13	1.480	1.347	0.331	0.213	73.68%
SE		0.071	0.027	0.021	0.015	
Frailesca	22	1.684	1.263	0.285	0.173	84.21%
SE		0.059	0.024	0.018	0.013	
Border	18	1.697	1.398	0.378	0.245	84.21%
SE		0.058	0.027	0.019	0.014	
Mean	26.8	1.628	1.303	0.306	0.192	81.18%
SE	0.508	0.028	0.012	0.009	0.006	3.77%

N: number of individuals; Na: number of different alleles; Ne: effective number of alleles; I: Shannon Information Index; *He*: Expected heterozygosity; % P: percentage of polymorphism; SE: standard error.

Very little research on genetic diversity of *J. curcas* has had a focus on populations [22,46,47]. Ambrosi *et al.* [22] analyzed plants from different geographical regions, nine accessions of Jalisco, Mexico, and 17 commercial varieties from South America, Asia and Africa, using SSR markers. They found higher diversity values than those obtained in this investigation (Ne average of 1.843, an average of I of 0.661 and *He* average of

0.345), being the Mexican population the most diverse. Wen *et al.* [46] used EST-SSR markers to study populations from Indonesia, South America, Grenada and China, reporting average Ne values of 1.686, an average of I of 0.557 and *He* average of 0.381. Another study, with ISSR markers, analyzed a total of 219 accessions from China and five from Myanmar, divided into seven populations and obtained Ne average of 1.317, an average of I of 0.292 and *He* average of 0.190 [47]. In a study with ISSR markers with 158 individuals from 8 semi-wild populations of Yunnan, China, were found polymorphism of 55.04%, Ne average of 1.382, *He* average of 0.217 and a mean of I of 0.317 [48]. Another study in China (nine populations) reported *He* average of 0.235 and a mean of I of 0.376 [49].

Table 3. Comparison of genetic variation in *Jatropha curcas* collected in different parts of the world.

Collection site	Maximum dissimilarity between accessions	Mean dissimilarity between accessions	Molecular marker used	Reference
India	0.51	0.28	RAPD-ISSR	Basha y Sujatha [17]
India	N.R.	0.12	AFLP	Pamidiamarri *et al.* [18]
India	0.57	0.30	AFLP	Tatikonda *et al.* [53]
India	0.37	0.27	ISSR	Umamaheswari *et al.* [54]
India	0.37	0.2	RAPD	Kumar *et al.* [55]
India	0.46	0.27	RAPD	Ikbal *et al.* [56]
India	0.59	0.35	ISSR-RAPD	Gupta *et al.* [57]
India	0.19	0.09	RAPD	Abdulla *et al.* [58]
India	0.8	0.40	RAPD	Subramanyam *et al.* [59]
China	0.14	0.08	AFLP	Shen *et al.* [20]
China	0.15	0.08	ISSR	Cai *et al.* [47]
Brazil	0.67	0.42	RAPD	Oliveira *et al.* [60]
Brazil	0.86	0.11	RAPD SSR	Rosado *et al.* [61]
India, Nigeria, Thailand	0.27	0.17	RAPD	Popluechai *et al.* [21]
Asia, Africa, Mexico	0.52	0.30	RAPD-ISSR	Basha *et al.* [10]
Asia, Africa, South America, Mexico	0.23	0.12	SSR	Ambrosi *et al.* [22]
Africa, Asia, Mesoamerica	0.55	0.30	SSR	Sato *et al.* [15]
China, Indonesia, Suriname, Tanzania, India	0.00	0.00	AFLP, RAPD, DAMD	Yi *et al.* [62]
Indonesia, China, Granada, South America	0.45	0.27	EST-SSR	Wen *et al.* [46]
China, Indonesia	0.21	0.13	SRAP	Shen *et al.* [63]
China, Indonesia, Thailand	0.49	0.27	ISSR	Duan y Guo [64]
Chiapas (Mexico)	0.89	0.72	AFLP	This study

N.R.: Not reported.

Although individual-based trees are not useful to infer population structure or other population attributes [50,51], we compared the Jaccard's dissimilarity index of our accessions with those of other studies (Table 3). According to Bonin *et al.* [52], coefficients of similarity are accepted band-based metrics of diversity for dominant data. It is important to note that the type of marker used can bias values of diversity index. For example, the use of SSR markers has the advantage of obtaining observed heterozygosity values, but entails the risk of overestimating the diversity indexes, especially when using a low number of markers due to the high allelic variability in the sequences of SSR [48].

Cluster analysis among the 134 accessions showed a Jaccard's dissimilarity coefficient of 0.893, indicating high genetic diversity. That value is over the 0.360 reported by Basha and Sujata [17], which analyzed 42 accessions of different geographical locations of India and a non-toxic variety of Veracruz, Mexico, using RAPD- ISSR markers. However, Tatikonda *et al.* [53] found a maximum dissimilarity coefficient of 0.570, which indicated relatively high percentage of diversity, in studying 48 accessions from India with AFLP markers.

3.2 Differentiation of populations.

The populations were grouped into two regions: 1) the coast of Chiapas (Soconusco and Isthmus), and 2) the central part of the state (Center, Frailesca and Border). AMOVA detected that the highest proportion of variation was found within populations (87.8% of the total molecular variation, Table 4).

Phi statistics, which are equivalent to *F* statistics [65,66], indicated the presence of structuring and possibly genetic barriers. *PhiST* differentiation index (analogous to *Fst* and *Gst* indexes) had a value of 0.121, which was significant, indicating moderate differentiation (12.1% of total genetic variation is due to differentiation among populations). The significant value of *PhiCT* showed that 4.3% of global genetic variation is due to differentiation among regions.

Table 4. Analysis of molecular variance of *Jatropha curcas* populations collected in Chiapas, Mexico.

Source of Variation	Degrees of Freedom	Sum of Squares	Estimated Variance	Variation (%)	Differentiation Indexes	Significance
Among Regions	1	119.09	0.868	4.29	*PhiCT* = 0. 0429	*p < 0.000*
Among Populations	3	166.69	1.593	7.88	*PhiSC* = 0. 0823	*p < 0.000*
Within Populations	129	2290.81	17.75	87.83	*PhiST* = 0. 1217	*p < 0.000*
Total	133	2576.59	20.21	100%		

PhiCT: Measure of genetic differentiation among regions for the total populations; *PhiSC*: Measure of genetic differentiation among populations within a region; *PhiST*: Measure of genetic differentiation among populations.

Wen *et al*. [46] obtained *Gst* of 0.18 indicating a significant differentiation between geographical regions studied in Indonesia, China and South America. Ambrosi *et al*. [22] reported values of *Fst* of 0.20 reflecting large genetic differentiation among the geographic groups studied (America, Asia and Africa). Furthermore, Cai *et al*. [47] found moderate differentiation between populations with a *Fst* of 0.12. Xiang *et al*. [48] reported a *Gst* = 0.2944, in populations of Yunnan, China. Another study among populations of China, revealed *Gst* = 0.539 [49]. Index values of differentiation among populations greater than 0.25 are very high and higher than 0.5 should be taken with caution, since it means that populations are so different that they could even are in the process of speciation, which is not likely to be happening with *J. curcas*. Contrary to the values of *He*, the *Fst* values calculated with different types of molecular markers are frequently proportional [67]. Compared to these investigations, the degree of differentiation found in Chiapas populations is lower, which could indicate that previous to anthropic management the species had a high gene flow through pollen and seed dispersal [68].

The spatial analysis of molecular variance showed that the maximum partitioning of the genetic diversity is obtained when sites are arranged in four groups (*PhiCT* = 0.15021, *p* < 0.000): 1) Villa Flores, 2) Ciudad Cuauhtémoc, Comalapa, Jiquipilas, Ocozocuautla and Rizo de Oro, 3) Arriaga, Tonalá, Acapetahua, Cacahoatán, Huixtla, Mapastepec, Puerto Chiapas, Suchiate, Berriozabal, Pujiltic, La Concordia, Villa Corzo and Revolución Mexicana, 4) Pijijiapan. By this arrangement, the program SAMOVA 1.0 detected a stronger genetic structure of populations, with a *PhiST* value of 0.176 (Table 5). According to the International Plant Genetic Resources International [69], *PhiST* values below 0.05 indicate small genetic differentiation, between 0.05 and 0.15 indicate moderate differentiation, between 0.15 and 0.25 the differentiation is high and over 0.25 is very high.

**Table 5.** Spatial analysis of molecular variance of *Jatropha curcas* collected in Chiapas, Mexico.

Source of Variation	Degrees of Freedom	Sum of Squares	Estimated Variance	Variation (%)	Differentiation Indexes	Significance
Among Groups	3	245.10	3.188	15.02	$PhiCT = 0.150$	$p < 0.000$
Among Populations	16	340.97	0.560	2.64	$PhiSC = 0.031$	$p < 0.000$
Within Populations	114	1992.39	17.47	82.34	$PhiST = 0.176$	$p < 0.000$
Total	133	2578.47	21.22	100%		

The program SAMOVA 1.0 defines groups of populations that are geographically homogeneous and maximally differentiated from each other [40]. SAMOVA maximizes the proportion of total genetic variance among groups (*PhiCT*) and minimizes the variance among populations within groups (*PhiSC*) to obtain the most probable grouping. A constraint of SAMOVA, as its authors recognize, is that the algorithm assign populations to groups based in the adjacency taking into account linear geographic distances, without consider ecological factors. For example, grouped populations of the coast of Chiapas with those of Frailesca, which are geographically adjacent, but there is a mountain chain between them. A method considering "real" geographic distances (resistance distances) is needed. A resistance distance or circuit take into account all possible pathways connecting populations pairs [70].

The study of the genetic relationships among the populations showed that populations Frailesca and Isthmus are the closest, and that despite the geographic proximity between the Border and Frailesca populations, the Border was the most distant of all (Figure 2). In this case, the similarity coefficient was 0.96; this result shows that most genetic variation is within populations rather than between populations, as shown by the above results of AMOVA and SAMOVA.

Since the populations showed moderate differentiation among them, possible isolation by distance was looked for, and the outcome of the Mantel test of correlation between the Nei genetic distance and geographic distance proved to be not significant with a value of $r^2 = 0.00146$ and $p = 0.056$, but it would be interesting to make this test with resistance distances, rather than linear distances. Therefore, the existence of genetic barriers, and not the isolation by distance, may be the reason for the differentiation found in the populations of *J. curcas* in Chiapas.

Figure 2. Dendrogram constructed with the coefficient of genetic identity of Nei from AFLP data of five populations of *Jatropha curcas* of Chiapas, Mexico.
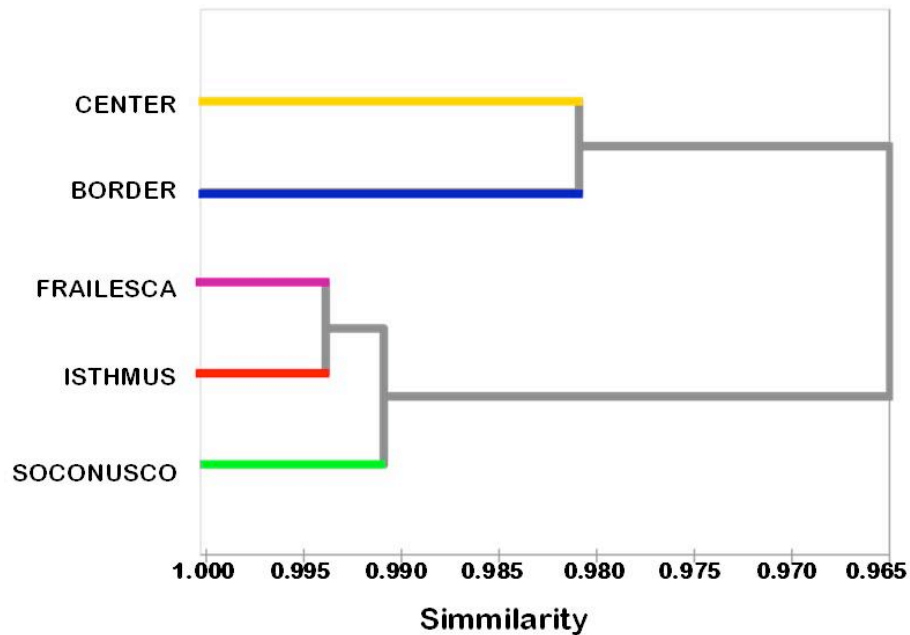

3.3 Structure of populations.

The study of genetic structure revealed five genetic groups (K = 5) using the package Structure© with a migration rate of 0.001 and a model of ancestry admixed. The five groups identified using Structure© are consistent with the declared geographical populations, as can be concluded from Figure 3. The five colors used represent the five groups identified by Structure©. Each individual is represented by a vertical bar. The proportion of a color in the bar indicates the proportion of alleles coming from one of the five groups identified by Structure© in that individual genotype. Results should be interpreted with caution because although Structure© it is a valuable tool to study individuals whose population of origin is unknown, the program is not designed to describe relationships between populations [71].

Mixed ancestry was found in most individuals (probably genetic migrants), except in Soconusco, where there was a small fraction of alleles from other populations. This clearly shows that Soconusco is source of migrant bands for populations of both Coast of Chiapas and Central Valleys of Chiapas, with the exception of Border where most migrants belong to Center and Frailesca. To explain the origin and spreading of *J. curcas* individuals in Chiapas, we must take into account the study of the natural distribution in time and space, which is based on the biogeography and also considers the processes that led to such a distribution [72].

Figure 3. Genetic structure of five populations of *J. curcas* of Chiapas, Mexico. Obtained with the software Structure© 2.3.2. with 30000 burn-in, 50000 replications after burn-in and 10 runs for each K (K 1-9). Ancestry model: GENSBACK = 3, MIGPRIOR = 0.001, Admixture Model. Map kindly prepared by F. Pérez-Racancoj (University of Chiapas).
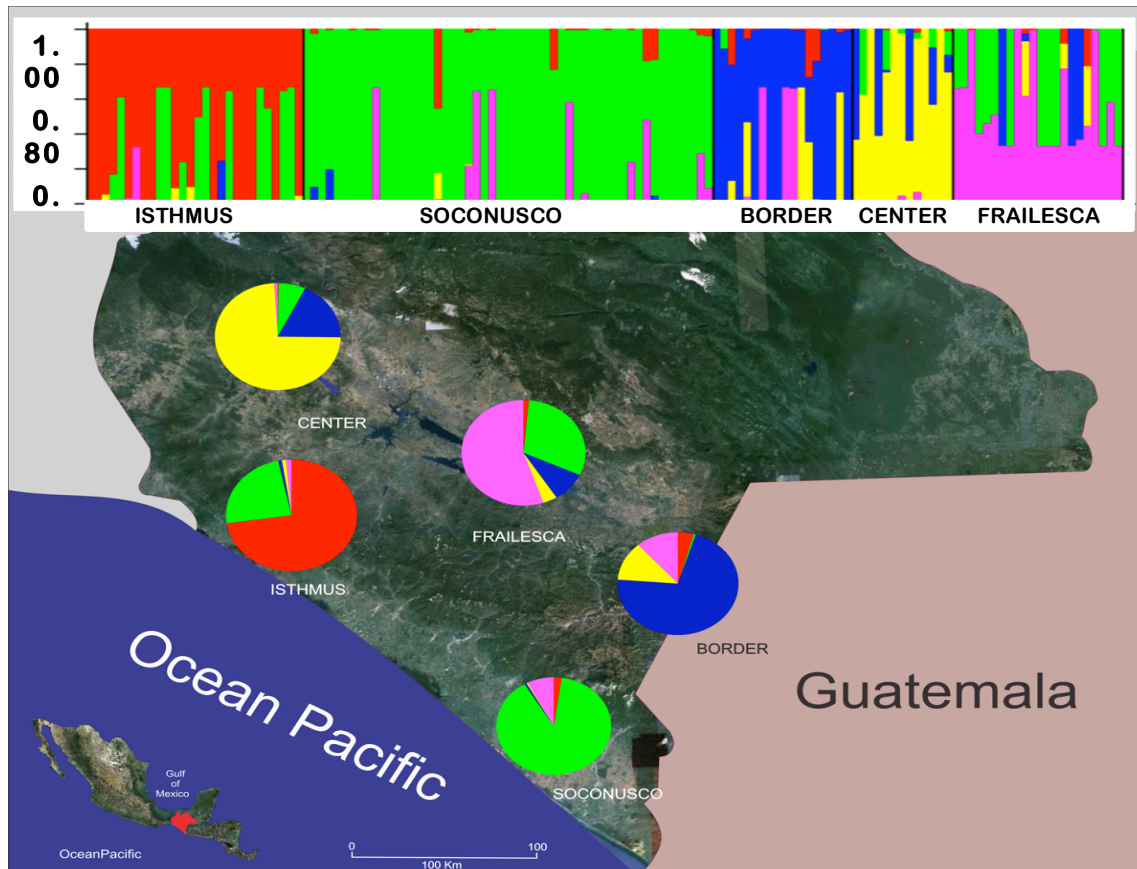
Within the biogeography there is a school of thought, which raises centers of origin of species (sympatric speciation), from which they are scattered at random, crossing preexisting barriers and colonizing new areas, this is called dispersalism. On the contrary, vicariance theory assumes that populations are separated or fragmented by the formation of geographical barriers leading to allopatric speciation [73]. On an infraspecific level, it is likely that individuals of the Soconusco population, with patterns of differentiated bands, have been dispersed in the past into other populations, prior to anthropic management and the emergence of significant barriers. The other possibility is that populations of *J. curcas* in Chiapas had patterns of similar fragments, which then differed after the rise of the Sierra Madre, and those genes that best adapted to areas on each side of the barrier persisted. To determine with more certainty the origin of

populations, phylogeography studies are needed for this Mesoamerican plant, using conserved markers such as mitochondrial or chloroplast.

The loci of Border (in blue) and those of Center (in yellow) were found in very low proportions in populations of the Coast of Chiapas, and despite the proximity, migrant alleles from Border and Center were found in low proportions in Frailesca. Despite the closeness between Soconusco and Border, there is scarce exchange of alleles between these populations, suggesting the existence of a genetic barrier. These two facts suggest that the alleles of Border could have come from Guatemala and then dispersed into Chiapas via two likely pathways, Soconusco and Border. It is possible that in Border, individuals with alleles in blue found appropriate conditions for their reproductive success. However, it is necessary to perform population genetic studies in Guatemala. The high diversity in Chiapas is remarkable, contrary to the homogeneity results found in the populations studied by Ambrosi *et al.* [22].

Figure 4. Map of five populations of Chiapas showing the two main genetic barriers (*a* and *b* yellow lines) found by the Monmonier algorithm (Barrier © vers. 2.2), based on *Fst* distances. Thickness and the number on the side of the barriers indicate the percentage of bootstrap support. Map kindly prepared by F. Pérez-Racancoj (University of Chiapas).
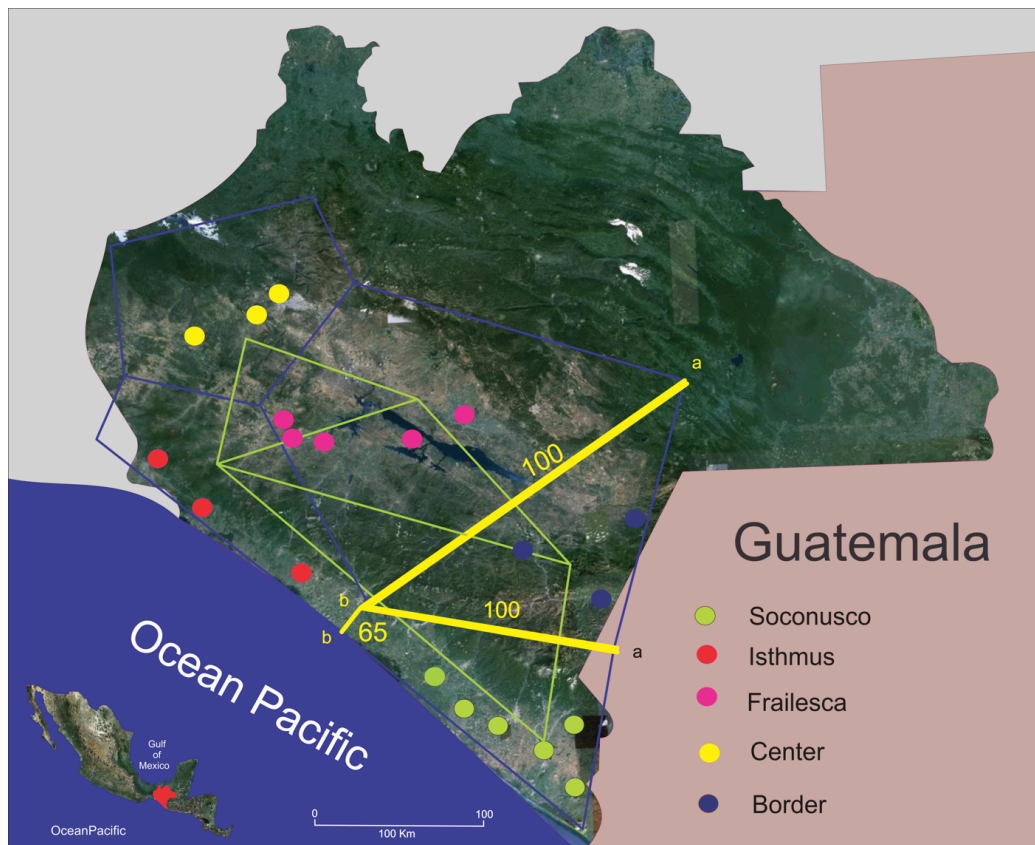
To corroborate the above findings, we searched for possible genetic barriers and found that both Border and Soconusco are isolated from the rest. The main barrier isolates the Border (yellow line "a" in Figure 4). It is clear that the Sierra Madre is a strong physical barrier between the populations of Soconusco and the Border, and is possibly the main cause of differentiation found among populations, keeping them separate right from the emergence of this mountain chain. The Sierra Madre mountain chain arose probably from the medium Miocene to the early Pliocene (between 13 and 4.5 million years ago –m.y.a.-) [73,74], while *J. curcas* probably exists from more than 70 m.y.a. [75].

The second most important barrier (65% of bootstrap support) separates Soconusco from the Isthmus, although apparently on the coast of Chiapas there are no major geographical barriers separating these two populations, so it may be the climate, which could be taking this role. Soconusco has an average annual precipitation of 2500 mm, relative humidity of 79.4% and average annual temperature of 27 °C [32]. Its climate is Aw2 (w) Ig [33], which corresponds to the most humid of the warm sub-humid tropical climates. For its part, the Isthmus has a climate type Aw0 (w), which corresponds to a warm sub-humid with summer rains climate. In this region is recorded an average annual precipitation of 1500 mm, with less than one hundred days of rain per year [33].

4. Conclusions

High genetic diversity was found within and among populations of *J. curcas* in Chiapas, the highest being found among individuals. The population with greatest diversity was Border and the least diverse was Soconusco. Depending of the method of analysis, from moderate to high differentiation was detected among populations, which is attributed to the existence of genetic barriers between populations. If the dissimilarity among accessions is considered, in Chiapas this species has greater genetic diversity than in other parts of the world. Results showed that the Mesoamerican region could be a center of diversity of this plant. It is possible that previous to anthropic handling of *J. curcas* its genetic base was sufficiently broad to avoid the erosion of the diversity by the process of domestication and by the clonal propagation.

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6

DISCUSIÓN

La investigación científica parte de la observación del contexto para identificar problemas teóricos a estudiar y resolver, convirtiendo incluso los problemas prácticos en objetos de estudio teóricos. Por esa razón esta investigación inició con un ensayo acerca de los problemas de sustentabilidad de la producción de biodiesel a partir de *J. curcas*.

Se identificó que existen dos preocupaciones esenciales que ponen en riesgo la viabilidad del modelo de crecimiento basado en la exportación/consumo excesivo de energía, ambas relacionadas con la carencia de sustentabilidad de dicho modelo: el aparentemente mermado *stock* disponible de combustibles fósiles y el incremento notable de gases de efecto invernadero en la atmósfera, muy probablemente ocasionado por las actividades humanas (Karl y Trenberth, 2003). Esto ha impulsado la investigación de fuentes alternativas de energía, de las cuales existe una lista grande (Douglass, 2005), sin embargo, debido a que se han buscado sustitutos parecidos al petróleo, las opciones que más han destacado en la última década son los biocarburantes líquidos, específicamente el etanol (Pimentel y Patzek, 2005) y el biodiesel (Gubitz *et al*., 1999; Openshaw, 2000; Pramanik, 2003).

El origen fotosintético del biodiesel es suficiente para justificar su investigación como combustible renovable, no sólo como simple desarrollo tecnológico, sino situándolo como un elemento a tener en cuenta en la llamada crisis ecológica global (*sensu* Iranzo, 1993). Se identificó que, en el caso de *J. curcas*, se debe dar especial atención al área destinada al cultivo, al manejo de las plantaciones, incluyendo el germoplasma usado y los insumos externos, a los procesos de conversión del aceite a biodiesel, y, especialmente, al destino de consumo final del biocombustible.

A partir de este contexto, la investigación se centró en el germoplasma de *J. curcas* y se identificó que existen al menos tres enfoques sobre el manejo del material de siembra para las futuras plantaciones de "piñón". Un primer punto de vista es que deberían conocerse y caracterizarse a varios niveles, incluyendo el molecular, los genotipos locales y aprovechar la variación natural para establecer plantaciones con varios genotipos que presenten características deseables, independientemente de su toxicidad. Estos podrían ser propagados por varias vías, incluyendo la micropropagación. Otra vía es la selección de unos cuantos genotipos élite (Sunil *et al*., 2008; Mishra, 2009) para su clonación (Sujatha *et al*., 2005; Datta *et al*., 2007) y siembra extensiva, promoviendo la siembra de súper variedades únicas, preferentemente las no tóxicas o de baja toxicidad. Los interesados en este enfoque consideran que los recursos genéticos son la materia prima para el mejoramiento por hibridación convencional y asistido por marcadores, inclusive interespecífico, es decir, entre *J. curcas* y otras especies de *Jatropha* (Popluechai *et al*., 2009). El enfoque final considera imperativa la producción de plantas transgénicas élite (Gressel, 2008; Sujatha *et al*., 2008) que expresen proteínas que les confieran ventajas o para bloquear la síntesis de los ésteres de forbol, los cuales confieren la toxicidad a las semillas. Recientemente se ha reportado la transformación genética exitosa de *J. curcas* (Li *et al*., 2008; Trivedi *et al*., 2009).

Independientemente del enfoque que se elija (o una combinación de ellos), es de primordial importancia conocer la diversidad de las poblaciones de *J. curcas* en la región mesoamericana, sea o no el centro de origen. Respecto a este último aspecto, lo descrito en la Introducción de esta tesis y lo revisado en el Capítulo 3 muestra que aún no pueden hacerse conclusiones sólidas sobre el centro de origen. Lo anterior, a pesar de que autores como Carels (2009) aportan argumentos valiosos que postulan a la región tropical de Sudamérica como el centro de origen.

Al tratarse *J. curcas* de una planta en proceso de domesticación [para revisiones al respecto ver a Carels (2009) y Achten *et al*. (2010)], los estudios de diversidad se han realizado con germoplasma cultivado como cercos vivos o como plantas de

traspatio. Las plantas domesticadas presentan lo que se conoce como síndrome de domesticación, que incluye pérdida de latencia en las semillas, germinación epigea favorecida, pérdida de capacidad de dispersión, gigantismo en la parte u órgano de interés y reducción o desaparición de metabolitos tóxicos (Poncet *et al*., 1998; Pujol *et al*., 2005). Aunque *J. curcas* ha sido cultivada como cerco vivo o como planta medicinal durante siglos por los agricultores mesoamericanos (Leonti, 2003; Granados-Galván, 2008), no muestra con claridad este síndrome, excepto por la existencia de genotipos no tóxicos en varios sitios de Mesoamérica (Martínez-Herrera *et al*., 2007, 2010).

En general, los estudios de diversidad infraespecífica utilizando germoplasma de Asia (particularmente de India) y África (como se documenta en el Capítulo 3) han encontrado baja variación genética usando marcadores moleculares, lo cual no es común en una especie alógama (Bhattacharya *et al*., 2005; Rosado *et al*., 2010). No obstante, se ha detectado moderada variación usando caracteres fenotípicos (Kaushik *et al*., 2007; Saikia *et al*., 2009; Yi *et al*., 2010). La falta de variación en el germoplasa del Viejo Mundo puede explicarse por posibles introducciones limitadas de *J. curcas* provenientes de Mesoamérica, y a partir de las cuales fue propagada clonalmente y luego distribuida. Incluso, un trabajo reciente (Yi *et al*., 2010) reportó variación fenotípica, pero ausencia total de variación molecular en poblaciones de *J. curcas* de África, Asia y Sudamérica, aún cuando se usaron varios marcadores moleculares, dominantes y codominantes. Una posible explicación a la presencia de variación fenotípica es la existencia de fenómenos epigenéticos. Llama la atención que también el germoplasma de Sudamérica tenga baja diversidad molecular. En ese sentido, Rosado *et al*. (2010) encontraron limitada diversidad en *J. curcas* de Brasil, usando microsatélites. Esos datos indican que Sudamérica no es un centro de diversidad actual de *J. curcas* y que existe la posibilidad de que en dicha región la selección artificial de los materiales actualmente cultivados haya sido intensiva.

Un punto importante es que la mayoría de los estudios de diversidad se han realizado con individuos y no con un enfoque de poblaciones. Por esa razón se

estudió la diversidad química y molecular de poblaciones de *J. curcas* de Chiapas, como se describió en los Capítulos 4 y 5 de esta tesis.

Variación en el contenido y composición del aceite de la semilla

El almacenamiento de aceites en las semillas es una característica generalizada en plantas superiores, ya que son fuente de energía para el crecimiento del embrión, antes de que este fotosintetice (Pujar *et al.*, 2006). Esta etapa es crucial en el éxito o fracaso del embrión para germinar, emerger y establecerse por sí mismo (Bewley y Black, 1994). Por lo tanto, el contenido del endospermo determina, al menos en parte, el éxito reproductivo de las plantas y este debería estar sujeto a la acción de la selección natural.

Como se describe con detalle en el Capítulo 4, se encontró que la variación en el contenido de aceite y la composición de ácidos grasos de la semilla en poblaciones de *J. curcas* en Mesoamérica es muy alta y que dichos marcadores químicos son altamente heredables.

La cantidad de aceite determinada varió entre 12% y 44%, lo cual es consistente con reportes previos para esta especie, ya sea cultivada en Asia o África (Heller, 1996; Pant *et al*., 2006), o colectada en la región mesoamericana (Makkar *et al*. 1998; Martínez-Herrera *et al*., 2010). Se ha documentado que esta variación es generada tanto por factores genéticos como ambientales, como la precipitación y la fertilidad del suelo (Heller, 1996; Escobar *et al*., 2008; Mishra *et al*., 2007). Sin embargo, otros estudios mostraron limitada influencia del ambiente y alta heredabilidad del contenido de aceite (Kaushik *et al.*, 2007; Gohil y Pandya, 2008). En nuestro estudio fue puesta a prueba la hipótesis de que el ambiente influye en el contenido de aceite en *J. curcas* de Mesoamérica, encontrando que este carácter tiene alta heredabilidad (70%), alto coeficiente de variación genotípica y 32% de variación entre accesiones. Es decir, aunque este carácter sea fijo tiene una alta variabilidad entre poblaciones. La razón de lo anterior podría ser la selección de genotipos adaptados a condiciones variables.

Es conocido que existen relaciones entre el contenido de aceite y el hábito y hábitat de las angiospermas (Levin, 1974). Evolutivamente, la cantidad de aceite de la semilla se incrementó con el desarrollo de tallos leñosos y con la tolerancia a la sombra, sin haber relación con el origen latitudinal (Levin, 1974). En nuestra investigación se tuvieron poblaciones ubicadas en un rango latitudinal reducido, lo que puede ser la causa de que no se encontrara correlación entre la cantidad de aceite y la latitud de los sitios de colecta.

La temperatura de germinación es un importante agente selectivo que causa que los aceites de la semilla de plantas nativas de altas latitudes o altitudes tengan mayor proporción de ácidos grados insaturados (Linder, 2000). La explicación es que en ambientes fríos el catabolismo de ácidos grasos insaturados en más factible que el de los saturados, así las semillas germinan y crecen más rápido a menor temperatura, incrementando su adecuación, aún a costa de tener menor energía total disponible para el embrión. Por el contrario, en ambientes calurosos, como los tropicales, las semillas con más ácidos grasos saturados son favorecidas poque tienen más energía disponible y no necesitan germinar rápidamente, ya que las condiciones en los trópicos son más o menos estables a lo largo del año. *J. curcas* es una especie tropical para la que no aplica la hipótesis de Linder, ya que, si bien la proporción de ácidos grasos insaturados estuvo positivamente correlacionada con la altitud de los sitios de colecta, en general la selección ha favorecido un mayor proporción de ácidos grasos insaturados (oleico y linoleico). Entonces se podría postular que, en el caso de *J. curcas*, la humedad del suelo ha ejercido presión de selección para una mayor proporción de ácidos grasos insaturados. Está bien documentado que la especie es tolerante a la sequía, aunque también se adapta a ambientes que pueden ir de húmedos a semi-áridos; sin embargo, es susceptible al exceso de agua (Dehgan y Schutzman, 1994). En la región tropical mesoamericana el inicio de la floración y producción de semillas coincide con el inicio de la temporada de lluvias; las semillas germinan y se establecen rápidamente (para lo cual requieren una alta proporción de ácidos grasos insaturados) antes de que la humedad se incremente hasta la inundación. Para probar la hipótesis de que la humedad del suelo es un agente selectivo de la proporción de ácidos grasos, se requieren estudios de germinación de semillas de

genotipos con distinta composición de aceite (obtenidas por autopolinización para evitar variación genética no controlada) en medios con un gradiente de actividad de agua. Los genotipos con mayor proporción de ácidos grasos insaturados germinarían con mayor velocidad y en más cantidad en los medios con elevada actividad de agua que los genotipos con más ácidos grasos saturados.

Variación genética detectada mediante AFLP

Pocas investigaciones sobre diversidad genética de *J. curcas* han tenido un enfoque de poblaciones (Ambrosi *et al*., 2010; Wen *et al*., 2010; Cai *et al*., 2010), las cuales han encontrado baja a moderada diversidad. En el presente estudio se encontró alta variación y moderada estructuración (ver Capítulo 5 para detalles). Aunque los métodos de agrupación basados en individuos no son útiles para inferir estructura u otros atributos poblacionales, se comparó el índice de disimilitud de Jaccard de las accesiones de Chiapas con lo reportado en otros estudios, encontrando el valor más alto (0.893) reportado hasta ahora.

Puesto que las poblaciones mostraron moderada diferenciación entre ellas, se exploró la existencia de aislamiento por distancia, aunque la prueba de Mantel resultó no significativa al correlacionar las distancias genética y geográfica. Este resultado contrastó con el estudio de ácidos grasos, ya que un análisis discriminante encontró que las poblaciones de *J. curcas* del sur de México están separadas acorde a su origen geográfico, lo cual se verificó con una prueba de Mantel, encontrando correlación entre la distancia química y geográfica. La falta de consenso entre dichos resultados puede tener dos posibles explicaciones, una biológica y otra técnica. La primera es que los marcadores moleculares AFLP al ser neutrales pueden no estar reflejando la diferenciación real entre poblaciones; mientras que los caracteres químicos, como ya se argumentó, son adaptativos y tienen mayor poder de discriminación de poblaciones. La segunda explicación tiene que ver con el número de poblaciones estudiadas en cada caso, ya que el estudio molecular se enfocó en las poblaciones de Chiapas, mientras que en el estudio de ácidos grasos se incluyeron, además, poblaciones de otros sitios de

Mesoamérica (Guatemala, Oaxaca, Guerrero y Michoacán). Considerando lo anterior, se compararon los resultados de ambos estudios, pero solo para poblaciones de Chiapas. El objetivo fue conocer el grado de consenso entre la ordenación producida por la matriz de datos de AFLPs (marcadores neutrales binarios) y la obtenida a partir de datos de proporción de ácidos grasos (marcadores adaptativos contínuos). Para esto se hizo un Análisis de Procrustes Generalizado, utilizando el software InfoStat 2008 (Di Rienzo *et al*., 2008). Debido a que los datos de AFLPs fueron numerosos (154 variables o bandas) se hizo una reducción previa de variables por análisis de coordenadas principales. Se encontró que hubo 68.9% de consenso entre la ordenación con datos genéticos y fenotípicos, lo cual puede considerarse bastante alto.

El estudio de estructura genética reveló cinco grupos genéticos, mediante el software Structure, los cuales son consistentes con las poblaciones geográficas declaradas *a priori*. Se encontró ancestría mezclada en la mayoría de los individuos, excepto en la población Soconusco, donde solo hubo una pequeña fracción de alelos de otras poblaciones, aunque parece ser fuente de alelos a las demás poblaciones, excepto Fronteriza. Asimismo, cuando se buscaron posibles barreras genéticas se encontró que la principal de ellas aisla a la población Fronteriza, mientras que la segunda más importante separa a las poblaciones Soconusco e Istmo.

Para explicar la falta de intercambio de alelos entre Fronteriza y Soconusco, las cuales son colindantes con Guatemala y cercanas geográficamente en línea recta, se propone que la Sierra Madre ha funcionado como una fuerte barrera física entre ellas desde su emergencia. La Sierra Madre de Chiapas surgió probablemente desde el Mioceno medio hasta el Plioceno temprano, hace entre 13 y 4.5 millones de años (Aguayo y Trápaga, 1996; Burkart, 1978). Por otra parte, *J. curcas* puede tener una antigüedad aproximada de 70 millones de años (Dehgan y Schutzman, 1994; Carels, 2009), considerando que es la especie más plesiomórfica de su género y que *Jatropha* existe de manera natural tanto en el Viejo como en el Nuevo Mundo, por lo que debió existir antes de la separación de África y Sudamérica (hace aproximadamente 65 millones de años; Carels, 2009). Esto

último hace pensar en que el posible centro de origen de *J. curcas* sea el antiguo continente de Gondwana, pero persiste la duda debido a que el clado más primitivo del género *Jatropha* está formado por especies endémicas de Mesoamérica (ver la Introducción de esta tesis).

Dos explicaciones de la diferenciación entre Soconusco y Fronteriza son posibles, dependiendo del centro de origen de la planta. Por un lado, si *J.curcas* se originó en Mesoamérica, las poblaciones existentes en el Este de Chiapas fueron separadas por el surgimiento de la Sierra Madre y han evolucionado diferenciadamente. Por otro lado, si la especie es nativa de Sudamérica solo pudo arribar a Chiapas después del cierre del Istmo de Panamá (hace aproximadamente 3 millones de años), por lo que las poblaciones colonizaron Chiapas por al menos dos vías de entrada desde Guatemala: por ambos lados de la Sierra Madre. Para probar esas dos hipótesis se requieren estudios adicionales que incluyan muestras de Centro y Sudamérica, usando marcadores conservados o de lenta evolución como el ADN mitocondrial o de cloroplasto, al mismo tiempo que se analicen marcadores hipervariables o de rápida evolución como los microsatélites. El ADN conservado puede ayudar a discernir las relaciones filogeográficas de *J. curcas* de las Américas y a identificar posibles patrones de distribución de linajes. Los microsatélites revelarían dónde las poblaciones tendrían un origen reciente, ya que donde no haya alta variación alélica probablemente las secuencias hipervariables no han tenido tiempo suficiente para evolucionar a nuevos alelos.

El resultado anterior coincide con el del estudio químico en la identificación de discontinuidades o barreras genéticas. La principal de ellas separa las poblaciones del centro de Chiapas del resto de poblaciones, mientras que la segunda aisla a la población de Michoacán. Las posibles explicaciones se relacionan con la existencia de la Sierra Madre del Sur, que funciona como barrera geográfica; la población del centro de Chiapas es la única que está al Norte de dicha cadena montañosa. Otra posible razón es la lejanía geográfica de la población de Michoacán del resto de poblaciones.

La segunda barrera encontrada con datos AFLP separa la población Soconusco de Istmo. Aunque aparentemente no hay barreras geográficas que las dividan. El clima podría estar funcionando como barrera, ya que el clima de Soconusco es mucho más húmedo que el de Istmo (García, 1973; CNA, 1998). No obstante, esta discontinuidad no fue validada por los datos de ácidos grasos, por lo que habría que colectar datos de otro tipo de caracteres adaptativos (morfología y fisiología floral, por ejemplo) para verificar o desechar la hipótesis del clima de la costa de Chiapas como barrera ambiental. Un experimento de interacción genotipo-ambiente, cultivando plantas de Soconusco en Istmo y viceversa, ayudaría a identificar si la selección natural o antrópica ha sido lo suficientemente intensa como para influir en el desempeño de las plantas.

Consideraciones finales

La investigación tuvo dos aportaciones, por un lado se encontró que *J. curcas* colectada en la región mesoamericana tiene alto nivel de variabilidad química y molecular, comparada con el germoplasma del Viejo Mundo y de Sudamérica. Esto apoyaría la hipótesis de que Mesoamérica podría ser el centro de diversidad de esta especie. Asimismo, se encontró estructuración genética de las poblaciones aunque la variación está ubicada mayoritariamente dentro de las mismas.

Por otra parte, se encontró información que puede servir en el aprovechamiento económico de la planta como cultivo. Por ejemplo, se identificaron grupos de individuos con cantidad y calidad de aceite incrementados (cap. 4, p. 43-44); se halló que el contenido de aceite y la proporción de ácidos grasos insaturados están correlacionados negativamente, situación que facilita el mejoramiento genético para disminuir los ácidos grasos saturados sin disminuir la cantidad de aceite; se encontró que existe una débil pero significativa correlación negativa entre el contenido de aceite y la altitud del sitio de colecta, lo cual coincide con lo reportado por Pant *et al*. (2006) y, que de confirmarse con un estudio de

asociación con genotipos sobresalientes cultivados en un gradiente altitudinal, podría ayudar a diseñar planes de siembra de *J. curcas*.

Los resultados del estudio molecular permitirán diseñar planes de mejoramiento genético utilizando el germoplasma de Chiapas. Se pueden realizar cruzamientos usando como parentales individuos de poblaciones divergentes para tener máxima variabilidad en la descendencia, por ejemplo entre Soconusco y Fronteriza. Al realizar cruzas entre genotipos divergentes se puede poner a prueba la hipótesis de obtención de heterosis o vigor híbrido en la descendencia (Mayo, 1987). Además, al generarse poblaciones segregantes para caracteres de interés productivo (cantidad de frutos, semillas y/o aceite, calidad de aceite, tolerancia a diferentes fuentes de estrés, etc.), se podría dar seguimiento con métodos moleculares a los parentales y a la descendencia buscando marcadores asociados y estudiando su herencia. El objetivo a largo plazo sería realizar mejoramiento genético y/o selección asistidos por marcadores moleculares.

En la presente investigación se aportan datos que contribuyen a identificar los marcadores mencionados. Por ejemplo, se identificaron bandas AFLP privadas o específicas para algunas poblaciones (Cuadro 1).

Cuadro 1. Patrón de bandas AFLP encontradas en cinco poblaciones de *Jatropha curcas* L. de Chiapas, México.

Tipo de banda	Población				
	Istmo	Soconusco	Fronteriza	Centro	Frailesca
Bandas diferentes (No.)	140	109	130	113	128
Bandas frecuentes (No.)[1]	118	76	130	113	99
Bandas privadas (No.)[2]	6	2	1	0	2
Bandas comunes localmente (No.)[3]	16	8	16	8	13

[1]Bandas con frecuencia >= 5%
[2]Bandas únicas de una población
[3]Bandas encontradas en 50% o menos poblaciones

Los individuos de la población Istmo denominados ARR3 y TON7 tienen el fragmento de 189 pb, el individuo PIJ1 tiene de manera exclusiva las bandas de 282 pb, 342 pb y 344 pb, y el individuo ARR6 tiene las de 320 pb y 369 pb. En la población Fronteriza solo el individuo COM13 tuvo una banda privada (181 pb). En la población Frailesca la planta PUJ7 tuvo el fragmento de 204 pb y la CCR3 el de

309 pb. Finalmente dos individuos de la población Soconusco tuvieron bandas privadas: PC8 (198 pb) y SCH7 (351 pb). Dichas bandas, aunque no son útiles en el estudio de relaciones genéticas entre poblaciones, podrían ser candidatas para monitorearse en la descendencia de cruzas con el fin de conocer si se heredan junto con algún carácter de interés.

Relacionado con lo anterior están los fragmentos AFLP que en esta investigación se encontraron asociados, débil pero significativamente, a la cantidad de aceite de la semilla. Para esto se hizo un análisis de componentes principales conjuntando los datos moleculares con los fenotípicos, siguiendo lo propuesto por Van-Loo *et al.* (2008), y se identificaron las bandas que presentaron correlación estadísticamente significativa con las variables Contenido de Aceite de la Semilla y Porcentaje de Ácidos Grasos Saturados. Posteriormente se hicieron análisis de regresión múltiple teniendo como variable dependiente a las características fenotípicas y como variables explicativas a las bandas mencionadas (Cuadro 2).

Cuadro 2. Efectos de bandas AFLP en el contenido de aceite de la semilla de *Jatropha curcas* L. de Chiapas, México, obtenidos por análisis de regresión múltiple.

Parámetro	Valor	Desviación típica	*t* de Student	*p*
Intersección	32.665	2.512	13.001	< 0.0001
Banda 120 pb	5.619	3.804	1.477	0.146
Banda 121 pb	0.239	2.620	0.091	0.928
Banda 129 pb	5.214	2.265	2.303	0.025
Banda 131 pb	-6.114	2.395	-2.553	0.014
Banda 163 pb	0.843	3.055	0.276	0.784
Banda 172 pb	5.579	4.368	1.277	0.207
Banda 174 pb	10.754	10.032	1.072	0.289
Banda 175 pb	-9.731	10.833	-0.898	0.373
Banda 179 pb	-7.562	5.097	-1.484	0.144
Banda 180 pb	-3.248	2.395	-1.356	0.181
Banda 292 pb	-2.452	4.553	-0.538	0.593
Banda 349 pb	-21.115	8.022	-2.632	0.011
Banda 375 pb	-7.360	8.403	-0.876	0.385

Los resultados mostraron que no hubo correlación estadísticamente significativa para la variable ácidos grasos saturados (lo cual es un indicador de calidad de

aceite para la fabricación de biodiesel), pero sí la hubo para el contenido de aceite (Cuadro 2).

El modelo obtenido con la regresión permitió obtener valores predichos de contenido de aceite para los individuos estudiados, los cuales se compararon mediante regresión lineal simple con los datos observados en el estudio químico (Figura 1). Se encontró una asociación débil (coeficiente de determinación r^2 de 0.462), sin embargo, es una muestra de que podrían resultar de utilidad estudios de selección genómica que utilicen marcadores moleculares densos (de miles de loci), como los polimorfismos de un solo nucleótido obtenidos mediante secuenciación masiva (Goddard, 2009; Crossa *et al*., 2010; Jannink *et al*., 2010).
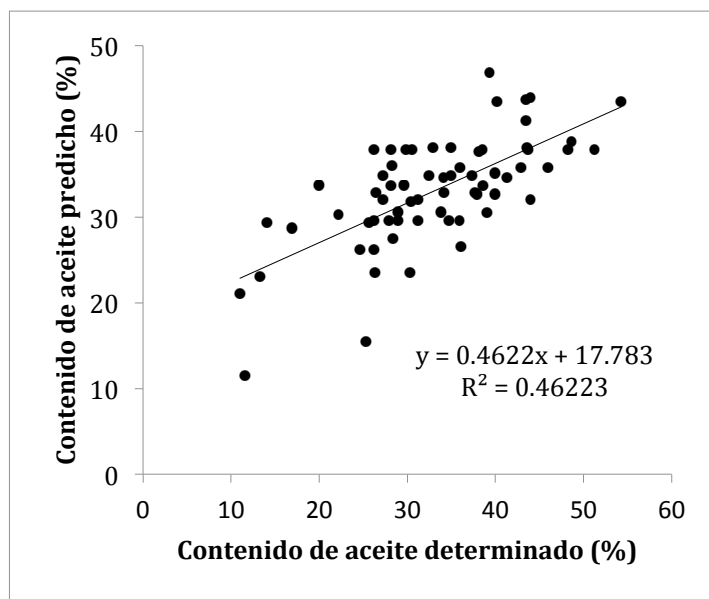

Figura 1. Correlación entre el contenido de aceite determinado en semillas de *Jatropha curcas* L. colectada en Chiapas, México y el contenido de aceite predicho con un modelo de regresión múltiple basado en 13 marcadores AFLP.

Las bandas privadas o específicas de poblaciones, así como aquellas asociadas al contenido de aceite deberían ser aisladas, secuenciadas y convertidas a marcadores robustos de tipo regiones amplificadas de secuencia caracterizada (SCAR, por sus siglas en inglés). La disponibilidad de marcadores SCAR facilitaría la identificación de genotipos eventualmente mejorados, ya que funcionarían como etiquetas específicas.

Los resultados de la presente investigación muestran que es posible que la base genética de *J. curcas* de la región mesoamericana, específicamente de Chiapas, haya sido lo suficientemente amplia previo a su manejo antrópico como para que la propagación asexual y el proceso de domesticación no hayan erosionado la diversidad intraespecífica.

7

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