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

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Accepted 1 April, 2011

*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.
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 Martínez, 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-Pamidimarri 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 Ceará, 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 Pinón 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 encountered limited morphological and chemical variability (Heller, 1991, 1996; Sukarín 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 (0.11 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 (Adewale and Adegbele; 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 germplasm 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 accessions, 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
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 (Korznik, 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
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 *Jatropha 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-Pamidiamarri 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 no close 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. multitida*, *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 non-toxic 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 Porteirinha. 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 subsequent 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.
the species is not a native, it exhibited a high genetic diversity; the methods used were sufficient to differentiate evaluation of “wild” accessions. They found that, although related to the other studies of Asian analysis (polymorphism information content, marker India with the long term goal of selecting appropriate widely collected germplasm of recommendations for more than one method on variations in Old World accessions. The same authors out groups) is not in agreement with the phenotypic dissimilarity with respect to other genotypes of different provenances, with wild accessions between 0.04 and 0.96 with RAPD and between 0.14 and 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 ongoing 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

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<th>Region</th>
<th>Genetic diversity (1-Jaccard's similarity index)</th>
<th>Type of marker used</th>
<th>Reference</th>
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Table 1. Comparison of genetic diversity in Jatropha curcas from several parts of the World studied with molecular markers.
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 $G_{st}$ 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 $F_{st}$ (an analog of $G_{st}$) 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 $F_{st}$ of 0.127 and the most of variation (87%) within groups. Xiang et al. (2007) reported a $G_{st}$ of 0.294, in populations of Yunnan, China. Another study of Chinese populations revealed a $G_{st}$ 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.

ACKNOWLEDGMENTS

To the Posgrado en Ciencias Biologicas of the Universidad Nacional Autonoma de Mexico, for the training given to the first author in his doctoral studies. To the Consejo Nacional de Ciencia y Tecnologia of Mexico (CONACyT), for the scholarship given to the first author. To the anonymous referees who provided valuable corrections to the manuscript and suggestions.

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