Genetic Improvement of *Jatropha* for Biodiesel Production

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Abstract. *Jatropha curcas* L. is a perennial oilseed crop belonging to the Euphorbiaceae family, whose oil content in seeds varies from 33 to 38%, giving a yield potential of over 1200 kg of oil per hectare. However, it is a non-domesticated species and research is required for commercial exploration of this species for biodiesel production. The strategies of Embrapa's jatropha breeding program aim at developing cultivars with high yield and oil content, non-toxic (absence of phorbol esters), resistant to biotic and abiotic stresses and adapted to the main producing regions of Brazil. The program activities started with the enrichment and characterization of the germplasm bank, currently with over 200 accessions from different regions of Brazil. Depending on the specific objectives of the program, different selection and breeding methods are employed. In order to understand the genetic control of specific traits and to generate segregating populations, experimental designs such as diallel crosses, which allow the estimation of heterosis, general combining ability and specific combining ability among genotypes, have been adopted. In addition, molecular markers such as SSR and SNPs are being developed and may help in early selection for characters such as the absence of toxicity in the grains. The program also includes the study on genotype × environment interaction with the evaluation of the progenies/improved clones in different regions of Brazil, which is essential for recommending cultivars for specific or broad climatic conditions. In conclusion, considering that *J. curcas* is a perennial species and still not domesticated, approximately 5-7 years will be required to obtain improved cultivars and evidence-based information on crop production systems to support commercial cultivation.

Keywords: Breeding, crop improvement, *Jatropha curcas*.

Mejoramiento genético de *Jatropha* para producción de biodiesel

Resumen. *Jatropha curcas* L. es un cultivo oleaginoso perenne de la familia Euphorbiaceae, cuyo contenido de aceite en la semilla varía entre 33 y 38%, dando un rendimiento potencial de más de 1200 kg de aceite por hectárea. Sin embargo, es una especie no domesticada por lo que se necesita investigación para explorar comercialmente el uso de esta especie para la producción de biodiesel. Las estrategias de los programas de mejoramiento de jatropha de Embrapa apuntan a desarrollar cultivares con alto rendimiento y alto contenido de aceite, que no sean tóxicos (ausencia de ésteres de phorbol), con resistencia a estreses bióticos y abióticos y adaptados a las principales regiones productivas de Brasil. Las actividades del programa iniciaron con el enriquecimiento y caracterización del banco de germoplasma, que actualmente cuenta con más de 200 accesiones provenientes de diferentes regiones de Brasil. Dependiendo de los objetivos específicos del programa, se emplean diferentes métodos de selección y mejoramiento. Para entender el control genético de características específicas y generar poblaciones segregantes, se han adoptado diseños experimentales tales como cruces dialélicos, los cuales permiten la estimación de heterosis, la habilidad de combinación general y combinación específica entre genotipos. Adicionalmente, se están desarrollando marcadores moleculares tales como SSR y SNPs, ya que podrían ayudar en la selección temprana de características como la ausencia de toxicidad en la semilla. El programa también incluye el estudio de la interacción genotipo × ambiente con la evaluación de progenies/clones mejorados en diferentes regiones de Brasil, lo que es esencial para la recomendación de cultivares para condiciones específicas o amplias. En conclusión, considerando que *J. curcas* es una especie perenne y que aún no está domesticada, se requerirán aproximadamente 5-7 años para obtener cultivares mejorados e información sobre sus sistemas de cultivo basada en evidencia para apoyar su producción comercial.

Palabras clave: Fitomejoramiento, *Jatropha curcas*, mejoramiento de cultivo.

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Introduction

Jatropha curcas L., commonly known as Barbados Nut, Purging Nut or Physic Nut, is a perennial plant species, diploid (2n = 22 chromosomes), monoecious and belonging to the Euphorbiaceae family, the same as castor bean, cassava and rubber tree. Although the assumptions are that jatropha is not originated from Brazil, the plant has been growing spontaneously in various parts of the country since long time ago. In the past, jatropha oil was used for the production of homemade soap, and due to its feature of being odorless and burning with no smoke, it was also used for lighting lamps and in city public illumination. Historically, jatropha has also been planted to control erosion, in the restoration of degraded areas, containment of slopes and dunes, along canals, roads, railways and as a hedge in domestic fences or in rural property lines.

This oleaginous plant, of importance still not defined in the food chain, is considered as a potential source of feedstock for biofuel production in the National Program of Biodiesel Production and Use (Programa Nacional de Produção e Uso de Biodiesel - PNPB). The species has some desirable features making it interesting to the program, such as higher potential yield than traditional oilseeds, quality of oil for biodiesel production, perennial crop, an alternative for diversification and possibility of inclusion of family farming in the production chain. However, it is a non-domesticated plant species with yet unsolved scientific and technical challenges limiting its complete inclusion in the energy matrix of biofuel production.

Despite the lack of basic technical information, it is being disseminated and implemented in various regions of Brazil. Commercial plantations in Brazil are still in the early stages, with less than or equal to 3-year-old plantations. The perspective of high density plantations for a period longer than 3 years is still unknown. Besides the lack of conclusive assessments of its agricultural merit, the nonuniform production and unknown genetic nature of seeds and seedlings used in commercial plantations contribute to the current scenario where no guarantees can be provided on the potential and yield stability of the crop.

In modern agriculture, two main strategies are considered for increasing productivity per area: the use of genetically superior materials and improved cropping conditions. The isolation and quantification of the effect of each of these factors indicated an additive relationship between them, resulting from expression of superior genetic potential with improved environmental and planting conditions. Therefore, the improvement of economic, social and environmental viability of this crop depends on the selection and crossing of genetic materials to obtain genotypes with appropriate traits and adapted to different regions of the country.

Among the activities of characterization and selection of genotypes most suitable for cultivation, the breeding program led by the Brazilian Agricultural Research Corporation (Embrapa) aims to develop cultivars with high grain/oil yield, non-toxic, resistant to biotic and abiotic stresses and adapted to the main producing regions of Brazil. The program includes activities of enrichment and characterization of genetic resources, structuring breeding populations, selection of superior genotypes with high adaptability to different regions of the country and characterization with botanical and molecular descriptors for identification and cultivar registration.

The purpose of this review is to compile information on this oleaginous plant considering the botanical characteristics, latest research results and the challenges in developing materials and varieties most suitable for biodiesel production.

Botanical aspects

Jatropha is a fast-growing, deciduous bush, sometimes reaching over 8 m in height with stem diameter ranging from 20 to 30 cm. The stem is smooth, soft, gray-greenish in color, with weak xylem or wood and well developed pith. The phloem has long channels extended to the roots, in which the sap or latex circulates. The stem has a tendency to branch out from axillary buds since the base of the plant. Plants with a high number of branches are of interest. There is a wide variation in patterns of branching, from erect and unbranched to plants with many secondary branches (Saturnino *et al.* 2005).

The root system in jatropha has limited development, with short roots and few ramifications. In general, seedlings present five roots; four laterals and a central root that subsequently thickens forming the taproot, responsible for anchoring the plant and water uptake from subsoil layers (Heller 1996).

The leaves are alternate to sub-opposite, in spiral phyllotaxy, cordate at the base, with 3-5 acuminate lobes and long 10-15 cm petioles. In general, the young leaves are red-wine colored, turning bright green as they expand with whitish veins bulging in its abaxial surface (Saturnino *et al.* 2005). It is a deciduous species with leaf drop in dry and cold years (Figure 1A and 1B). At the beginning of the rainy and hot season, mobilizes reserves stored in the stem/root system and starts a vigorous vegetative growth.

The inflorescence is racemose with yellow-green flowers. The species is monoecious with male and female flowers on the same inflorescence (Figure 2A). Typically, the center of the inflorescence produces a female flower surrounded by a group of male flowers. In some cases, inflorescences with only male flowers may occur and hermaphrodite flowers are also observed (Saturnino *et al.* 2005). On average, the rate of occurrence is 1:20 female:male flowers and numerically 1-5 female flowers in a range of 25-100 male flowers may occur; however, this characteristic may vary according to the genetic material, season, region and crop management (Juhász *et al*. 2009). After the anthesis of the first flower, flowering occurs daily, lasting up to 11 days. The flower opens between 5:30 and 6:30 a.m. The stigmas become receptive after anthesis and remain so for about three days. The non-pollinated flowers fall after the fourth day, while pollinated flowers remain attached to the plant. The petals and sepals gradually increase in size to protect the fruit and persist until the fruit drops (Solomon and Ezradanam 2002; Saturnino *et al.* 2005).

The flowers of jatropha are self-compatible and fertilization of flowers may occur by geitogamy, xenogamy and self-fertilization (Solmonon and Ezradanam 2002; Juhász *et al*. 2009). Pollination is entomophilous involving several groups of insects including bees, flies, thrips, wasps and ants (Saturnino *et al*. 2005). Jatropha presents mixed reproduction system and the rate of autogamy and allogamy may vary depending on the region, presence of insect pollinators and cropping system. The knowledge of the crossing rate in different conditions is important to establish methods of breeding and varietal management.

A.

B.

Figure 1. Jatropha plants in the rainy season (A) and dry season (B).

The fruit is an ovoid capsule with 1.5 to 3.0 cm diameter (Figure 2B), trilocular, usually containing three seeds, one seed per locule. Fruit ripening is not uniform. Green fruits, yellow (ripened), brown and finally black fruits can be observed in the same bunch. Overall, 53-62% of fruit weight is represented by seeds and 38-47% by husk. The fruit weight varies from 1.5 to 3.0 g. The seeds are relatively large measuring between 1.5 to 2.0 cm long and 1.0 to 1.3 cm wide, with weight ranging from 0.5 to 0.8 g when dried. The endosperm, in the seed kernel, contains 33 to 38% oil, considering the total weight of the seed (Dias *et al*. 2007; Peixoto 1973).

A.

B.

Figure 2. (A) Inflorescence with male and female flowers. (B) Bunch with green fruits

Genetic resources

Origin and dispersal. The origin of jatropha is very controversial and to date there is still no scientific evidence to confirm the precise center of origin and/or the species diversity. Worldwide, jatropha can be found in higher proportions in intertropical areas (Peixoto 1973; Heller 1996), especially in countries from South America, Central America, Africa and Asia. Despite its presence in all these regions, the strongest evidence indicates that this species originated in tropical America (Jongschaap *et al.* 2007); more precisely Central America (Aponte 1978; Heller 1996). History reports that from the Caribbean, where the species was already being used by the Mayas, jatropha was probably dispersed by Portuguese ships. Around 1783, jatropha was introduced in the islands of the archipelago Cape Verde, subsequently reaching Africa and India, spreading later to the tropics (Heller 1996). The introduction of the species in the islands of Cape Verde was attributed to the interest of the Portuguese to utilize the so-called unfit lands of that archipelago, whose soils were of low fertility and could hardly be used for less rustic crops (Peixoto 1973).

In Brazil, the crop is scattered throughout the country, from coast to areas with more than 1000 m above sea level. It is found dispersed but not observed in large populations or as in natural vegetation. Its presence is usually related to human action. The plant can be found in backyards, gardens, vacant lots, as hedges and isolated in anthropic pastures, but not far from a residence. Since plants mainly occur isolated, most of the collected accession seeds originated from selfing. Even plants found in groups such as hedges, which were propagated by cross pollination, were usually closely related from families of siblings or half-siblings. Since its introduction, jatropha has been transported by man throughout sites and regions, via seed or cuttings, and plants from different regions have the same genetic origin (Laviola *et al.* 2011).

Germplasm bank. A germplasm collection is a recognized source of genetic variation useful in plant breeding, botanical research and conservation of plant diversity, especially useful for species in the process of domestication such as jatropha. According to Coelho and Barros (2005), the collection of accessions for germplasm banks must be conducted in accordance with a strategic execution plan, which must consider issues related to plant distribution, objectives and prospected sites. The collection period for most regions of Brazil vary from December to April, collecting ripen or dried fruits to remove the seeds or even cuttings, preferably from basal branches, which root more easily during the growth season. During the collection procedures, photographic record, georeferencing and description of aspects of the site and plant should be recorded in an index card to constitute the passport data.

In 2008, a germplasm bank with 200 accessions from various regions of Brazil was installed at Embrapa Agroenergy in partnership with Embrapa Cerrados. Such germplasm bank is currently in phase of enrichment and characterization.

Genetic diversity. In the addition of economic, social and environmental values to this oilseed crop, selection and crossing of superior genotypes is fundamental in the development of plants with characteristics for sustainable agricultural production. According to Clement (2001), the success in the domestication of new crops depends on pre-breeding activities that support suitable methods for screening and plant selection.

The knowledge of the ecology, reproductive system and dispersal patterns of this oilseed crop is relatively recent and incomplete. Recent studies on genetic diversity among accessions of jatropha using molecular markers showed low diversity, even among accessions from different continents. Basha *et al*. (2009) reported a low genetic diversity in accessions from China and India, and a higher diversity in accessions from Mexico; a region that has been considered as one of the centers of origin.

Genetic erosion, defined as loss of genetic variability within a species, resulting from the contribution of few individuals to subsequent generations, is a known effect and has been observed in other crops such as coffee (Lopez-Gartner *et al.* 2009). According to Achten *et al.* (2010), successive introductions of plants of the genus *Jatropha*, coupled with its simple vegetative propagation may have contributed to spread a few genotypes in the same region.

Molecular characterization of 192 accessions from different regions of Brazil with RAPD and SSR markers also showed a narrow genetic base. Rosado *et al.* (2010) found no pattern of dispersal of the accessions and accessions from different regions showed no diversity. Accessions from distant locations, such as from the States of Rio Grande do Sul and Pará, which are approximately 3,000 km apart, showed maximum similarity (100%), with high likelihood of being replicates in the germplasm bank. On the other hand, some accessions from nearby regions were grouped into genetically divergent groups. The clustering structure regardless of the geographic location does not suggest a dispersal pattern for this plant in the country. Specific areas with higher diversity which could be explored through new collections could not be observed in this study.

As a monoecious species with spatial separation of male and female flowers on the plant, the occurrence of moderate to high frequency of heterozygous loci resulting from cross-fertilization would be expected. However, the occurrence of high frequencies of microsatellite loci in homozygous genotypes indicate that the mating system of this plant should be better described (Rosado *et al*. 2010). Even in India, where jatropha cultivation is traditional, Sun *et al*. (2008) found homozygosity for 16 pairs of SSR loci in 58 accessions. As observed in other studies, the quantification of genetic diversity of accessions collected throughout the country indicate that, in Brazil, jatropha plants are breeding preferably by autogamy, which is facilitated by the occurrence of isolated plants (Rosado *et al*. 2010).

Few field works have quantified the variability among provenances and families of jatropha. However, in contrast to the results of analysis based on molecular markers which showed low genetic divergence among accessions, field evaluations indicated high genetic variation among accessions.

Heller (1996) observed significant differences in the evaluation of vegetative characteristics in genotypes from 13 provenances grown in Cape Verde and Senegal. The assessed genotype × environment interaction was significant only in Senegal. When comparing 6-24-month-old plants in India, Ginwal *et al.* (2004) observed significant variance among accessions, attributing more than 80% of the total phenotypic variance to the difference among plants.

Rao *et al.* (2008) observed a positive correlation between grain yield and plant height, number of branches and proportion of male and female flowers. The number of branches was the trait with the highest association with grain yield in accessions at 36 months from planting. Heller (1996) and Ginwal *et al.* (2005) observed the occurrence of high magnitude positive correlation between seed weight and oil content, and negative correlations between seed weight and fiber and ash content in seeds. Rao *et al.* (2008) also observed a significant correlation between seed weight and oil content, similar to results obtained by Rocha *et al*. (2008) which quantified higher variability of kernel weight, a major component of grain weight.

Plant breeding

Genetic parameters. Based on the available resources and the incipient degree of improvement, the characterization of divergent genetic materials with superior mean for production components for grain and oil is being conducted. Due to the concentration and complementarities of favorable alleles, the chances of selecting superior gene combinations in early generations increase. Subsequently, with the selection of elite genotypes, other traits of interest can be subsequently incorporated to improve the performance of genetic materials.

The complex inheritance pattern of production components in jatropha is due to segregation of many genes of little effect and highly influenced by environment. The mechanism of inheritance of these characteristics requires that an individual value must be assessed in comparison with the mean value of its progeny, hence estimating individual genetic values.

The understanding of the heritability of traits to be improved is an important predictor of earnings over the selection cycles and on decision-making about methods to be used to maximize the capitalization of existing genetic variance in the population. One of the most useful estimates for the development of new varieties is the narrow-sense heritability, which indicates the proportion of total variation that is transmitted to the next generation.

Ongoing research in the state of Rondônia, Brazil, has shown low heritability estimates for production in the first year after planting ($0.10 \leq h^2 \leq 0.20$), and average magnitude in the second year after planting ($0.22 \leq h^2 \leq 0.56$) (Rocha *et al*. 2010). Laviola *et al.* (2011), working on the characterization of a germplasm bank for 12 months found broad sense heritability of 27% for number of branches, 55% for plant height, 33% for stem diameter, 55% for juvenility and 51% for height of the first inflorescence. However, these heritabilities were estimated in plants at early developmental stage. According to Resende *et al*. (2001), in young perennial plants, it should be considered that the individual estimates of heritability might be inflated by the genotypes × years interaction.

Criteria for selection: ideotypes and yield components. The combined evaluation of the characteristics associated with oil yield allows the characterization of an ideal genotype, a plant model that presents a set of characteristics correlated with productivity. Therefore, the development of a commercial variety should consider: high yield, oil content, seed weight, no significant toxicity, synchronization of flowering, rusticity, drought tolerance and resistance to diseases and pests.

In general, low productivity coupled with uneven fruit ripening are among the main constraints to the viability. The initial expectation of production was six or more tons of grain per hectare; however, more accurate production estimates showing less than three tons per hectare were observed in different climate conditions in the country. The low productivity observed under water deficit environments, associated with high incidence of powdery mildew (*Oidium heveae*) and severe attack of leafhopper (*Empoasca* spp.) has limited the productivity in different regions. Sources of variability for resistance to powdery mildew, seed toxicity and production of hermaphrodite flowers were identified in accessions of the germplasm bank (Laviola *et al.* 2011). Plants with yield higher than 2.5 kg of seeds per tree were selected in different regions of Brazil (Drummond *et al.* 2009; Rocha *et al.* 2010).

The development of non-toxic or low toxicity commercial cultivars may contribute in adding value to the press cake from oil extraction, also reducing an environmental liability given by the current need of detoxification of this material. Furthermore, accessions with low concentrations of phorbol esters in the grains have shown a less vigorous growth that will probably be reflected in lower yield (Laviola *et al.* 2011). Currently, non-toxic genotypes are considered in crosses with productive genotypes in order to select plants and families with desirable traits.

Besides the manipulation of genetic variability among accessions, the identification of the most important vegetative characteristics for oil yield offers important information to the development of more appropriate criteria for selection. Spinelli *et al.* (2010) quantified by path analysis the direct and indirect effects of vegetative characteristics and feedstock quality on oil yield of jatropha. The characteristics of grain yield and canopy volume were the most important for the development of materials of high oil yield and the variability for other traits should be maintained in the population to obtain future gains.

Hybridization. The hybridization or artificial pollination in plants is one of the tools that allows the accumulation of desirable traits from different parents in a single individual, increasing the genetic variability for traits of interest to the breeding program. Experiments conducted by Juhász *et al.* (2009) to determine methods of hybridization demonstrated that all the fruits formed by natural or artificial pollination showed full development until maturity, and there was no abortion during fruit development. The artificial pollination is carried out in the early hours of the morning, placing the anthers previously collected from male flowers in contact with the stigma of emasculated female flowers in the inflorescence, which was subsequently protected with organza bag.

The strategy of the breeding program considers the use of genetic designs which is a crossing system planned to maximize the selection process by predetermined hybrid combinations and increase the efficiency in the use of additive variance. In addition, diallel crosses, which allow exploring combinations of groups of parents, the evaluation of full-sib, half siblings and unrelated families, are also planned. This methodology is recommended when using few parents, since the use of large numbers of parents (over 10) to form the base population makes the achievement of combinations impractical, hence hindering results.

Considering that the initial goal of the breeding program is to add vigor in more productive materials, diallel crosses are underway using accessions with higher grain yield, non-toxic, resistant to powdery mildew and small plant size.

Breeding methods. The domestication of new crops is a long and usually costly process, whose success depends on the knowledge of basic information on the species. The definition of breeding methods most suitable for jatropha depends, for example, on the knowledge of the reproductive system of this species. Dhillon *et al.* (2006) observed in plantations, in India, 89.7% rate of cross-pollination. Results of ongoing research, in Brazil, also showed a mixed reproduction system, predominantly allogamous.

When considering this oleaginous plant as a species with mixed reproduction system predominantly alogamous and tolerant to selfing, the manipulation of its variability can be achieved using the following breeding strategies:

1 - mass selection
2 - recurrent selection
3 - assisted selection
4 - exploring genotype × environment interaction.

Breeding methods aimed primarily to the gradual and continuous increase of the frequency of favorable alleles while maintaining genetic variability for constant gain. During the steps of the breeding program, genotypes should be selected with high mean value and large genetic variability to provide constant gains with selection over several generations. Elite materials from the selection process can be used directly in the release of commercial cultivars or in combination with other materials to obtain genotypes with superior performance or to introduce specific traits. Figure 3 shows the steps of the breeding program and schedule of activities to obtain cultivars.

Figure 3. Steps of the *Jatropha curcas* breeding program

Activity	Year 1		Year 2		Year 3		Year 4		Year 5		Year 6		Year 7	
	1º S	2º S	1º S	2º S	1º S	2º S	1º S	2º S	1º S	2º S	1º S	2º S	1º S	2º S
Collection	■													
Establishment of the germplasm bank		■												
Enrichment of the germplasm bank					■		■		■		■			
Agronomic characterization			■	■	■	■	■	■	■	■	■	■		
Genotypic characterization				■		■		■		■		■		
Crosses					■		■		■		■		■	
Mass/recurrent selection					■		■		■		■		■	
Evaluation of G × E interaction						■		■		■		■		■
Elite Cultivars / Clones														■

S: semester.

Conclusion

Jatropha is a promising oleaginous option as feedstock for biofuel production in different Brazilian regions. However, the crop is still not completely domesticated and research actions are needed for the development of cultivars and production systems.

In this review, we presented the strategies of the breeding program to develop cultivars with high grain/oil yield, non-toxic, resistant to biotic and abiotic stresses and adapted to the main producing regions of Brazil. The program includes enrichment activities and characterization of genetic resources, structuring breeding populations, selection of superior genotypes with high adaptability to different regions of the country and characterization of botanical and molecular descriptors to support the identification and registration of cultivars.

The development of production systems for different regions of Brazil is very important and complementary to the breeding program. Considering that phenotype is the result of genotype and environment, researches on planting systems, spacing, fertilization, integrated management of weeds, pests and diseases and other management practices are essential in order to allow improved materials to express and achieve their productive potential.

Since jatropha is a non-domesticated perennial species, several years are needed to obtain improved cultivars and evidence-based information on crop production systems in order to support a commercially competitive cultivation in different regions of Brazil.

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