

## Biology and genetic improvement of *Jatropha curcas* L.: A review

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### ABSTRACT

Bio-diesel is a fast-developing alternative fuel in many developed and developing countries of the world. The bio-diesel production from vegetable oils during 2004–2005 was estimated 2.36 million tonnes globally. Of this, EU countries accounted for about 82% and USA about 6%. Global bio-diesel production is set to reach some 24 billion litres by 2017. Shortage of edible oil for human consumption in developing countries does not favour its use for bio-diesel production. Hence non-edible oil from crops like *Jatropha* (*Jatropha curcas*) and *Pongamia* (*Pongamia pinnata*) is favoured for bio-diesel production and the trend is expected to continue. Especially *J. curcas* has gained attention in tropical and sub-tropical countries and has spread beyond its centre of origin, because of its hardiness, easy propagation, drought endurance, high oil content, rapid growth, adaptation to wide agro-climatic conditions, and multiple uses of plant as a whole. The full potential of *J. curcas* has not been realized due to several technological and economic reasons. One of the major reasons is the lack of high yielding varieties with high oil content. In this review, we attempt to discuss the currently available information on *Jatropha* species identity, taxonomy and description, distribution and ecological requirements of the species, possibilities of exploitation of genetic potentiality, exploitation of existing diversity for yield and oil content by direct selection, hybridization and creation of diversity by mutation, and biotechnological interventions.

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## 1. Introduction

### 1.1. Global status of bio-diesel requirement

Biomass as a source of renewable energy is fundamental for development and sustenance of civilization. In view of growing interest for renewable energy sources, liquid bio-energy production from vegetable oils is one of the possible options to reduce greenhouse gas (GHG) emissions. Bio-diesel production from vegetable oils during 2004–2005 was estimated to be 2.36 million tonnes globally. Of this, EU countries (1.93 million tonnes) with expectation of 30% annual increase and the USA (0.14 million tonnes) together accounted for 88% and rest of the world (0.29 million tonnes) for the remaining 12% [1]. Global bio-diesel production is set to grow at slightly higher rate than bio-ethanol and will reach 24 billion litres by being the largest share by 2017 [2].

### 1.2. Bio-diesel crops

Bio-diesel is expanding very fast because of demand, necessary policy support and technological availability. India consumes approximately 40 million tonnes of diesel and ranked fifth in the world after the US, China, Russia and Japan in terms of fossil fuel consumption. Recently, Government of India launched “National Mission on Bio-diesel” with a view to find a cheap and renewable liquid fuel based on vegetable oils [3]. However, shortage of raw material to produce bio-diesel is a major constraint [4]. The total number of oil-bearing species range from 100 to 300, and of them 63 belonging to 30 plant families hold promise for bio-diesel production [5]. Many developed countries are using edible oil-seed crops such as soybean, rapeseed, groundnut, sunflower for production of bio-diesel. However, developing countries like India, having dearth of huge quantity of edible oil (6.31 million tonnes) for consumption, cannot afford to use edible oils for bio-diesel production and hence non-edible oil seeds such as *Pongamia* (*Pongamia pinnata*) and *Jatropha* (*Jatropha curcas*) are explored along with meeting additional criteria of greening the wastelands without compromising the food, fodder security and improve livelihoods [4,6]. Thus, *J. curcas* which meets the American and European standards, gained importance in tropical and sub-tropical countries [7].

### 1.3. *J. curcas* L. as a choice crop

*J. curcas* is native of tropical America, but is now found abundantly in many tropical and sub-tropical regions throughout Africa and Asia because of likely distribution by Portuguese ships via the Cape Verde islands and Guinea Bissau [8]. *J. curcas* has spread beyond its original distribution because of its hardiness, easy propagation, drought endurance, high oil content, low seed cost, short

gestation period, rapid growth, adoption to wide agro-climatic condition, bushy/shrubby nature and multiple uses of different plant parts [9–11]. Added to this, qualitative sustainability assessment, focusing on environmental impacts and strengthened by some socioeconomic issues, is quite favorable as long as *jatropha* is cultivated on wastelands/degraded lands [12]. In view of these advantages, many investors, policy makers and clean development mechanism (CDM) project developers are interested to tackle the twin challenges of energy supply and GHG emission reduction.

### 1.4. Reasons for review

For several reasons, both technical and economical, the full potential of *J. curcas* is far from being realized. Apart from agronomic, socioeconomic and institutional constraints, planned crop improvement programs are lacking globally. Earlier research programs involving large-scale plantations launched in Brazil, Nicaragua and India indicated that the crop productivity is far too low to be commercialized. In extreme cases, the plantations failed to produce fruits. There is limited information available on genetics and agronomy of *jatropha*. There is lack of bench mark descriptors and information on genetic variability, effects of environment and genotype  $\times$  environment ( $G \times E$ ) interaction [13]. Furthermore, there is a need for integration of the available scattered knowledge on, and experiences with crop performance of different *J. curcas* provenances in different environments and management interventions. Keeping this in view, an attempt is made to review available literature on genetic improvement of *J. curcas* from various journals, articles, reports and conference proceedings to cover wider range of information.

## 2. *Jatropha* naming and species

Current name is *J. curcas* Linnaeus (Euphorbiaceae). Linnaeus [14] was the first to name the physic nut *J. curcas* L. The genus name *Jatropha* derives from the Greek word *jatros* (doctor) and *trophe* (food), which implies its medicinal uses. According to Dehgan and Webster [15] and Schultze-Motel [16], synonyms of the physic nut are: *Curcas purgans* Medik.; *Ricinus americanus* Miller.; *Castiglionia lobata* Ruiz & Pavon.; *Jatropha edulis* Cerv.; *J. acerifolia* Salisb.; *Ricinus jarak* Thunb.; *Curcas adansoni* Endl.; *Curcas indica* A. Rich.; *Jatropha yucatanensis* Briq.; *Curcas curcas* (L.) Britton & Millsp. Table 1 presents vernacular names.

### 2.1. Taxonomy and description

#### 2.1.1. Taxonomic status

The genus *Jatropha* belongs to tribe Joannesieae of Crotonoideae in the Euphorbiaceae family, and contains approximately 175

**Table 1**  
Common/vernacular names *Jatropha curcas*.

Language/country	Name
English	purging nut
French	pourghère, pignon d'Inde
Dutch	purgeernoot
German	purgiernuß, Brechnuß
Portuguese	purgueira
Italian	fagiola d'India
Arab	dand barrî, habel meluk
Sanskrit	kanananaeranda, parvataranda
Hindi (India)	ratanjyot, bagbherenda, jangli arandi, safed arand, bagaranda
Nepal	kadam
Chinese	yu-lu-tzu
Thailand	sabudam
Philippines	túbang-bákod, Tuba-tuba
Indonesia	jarak budeg
Côte d'Ivoire	bagani
Togo	kpoti
Senegal	tabanani
Angola	mupuluka
Nigeria	butuje
Tanzania	makaen
Mexico	piñoncillo
Costa Rica	coquillo, template
Puerto Rico	tártago
Brazil	mundubi-assu
Peru	piñol
Guatemala	Pinón

Sources: [16,17].

known species. Dehgan and Webster [15] revised the subdivision made by Pax [18] and now distinguish two subgenera (*Curcas* and *Jatropha*) of the genus *Jatropha*, with 10 sections and 10 subsections to accommodate the Old and New World species. They postulated the physic nut (*J. curcas* L. [sect. *Curcas* (Adans.) Griseb., subg. *Curcas* (Adans.) Pax]) to be the most primitive form of the *Jatropha* genus. Species in other sections evolved from the physic nut or another ancestral form, with changes in growth habit and flower structures.

### 2.1.2. Botanical description of *J. curcas*

The physic nut, by definition, is a small perennial tree or large shrub, which can reach a height of three to 5 m, but can attain a height of 8 or 10 m under favorable conditions. The plant shows articulated growth [11] straight trunk, thick branchlets with a soft wood and a life expectancy of up to 50 years [19]. Flowering occurs during the wet season [20] often with two flowering peaks, i.e. during summer and autumn. In the permanently humid regions, flowering occurs throughout the year [8]. The inflorescence is axillary paniculate polychasial cymes formed terminally on branches and are complex, possessing main and co-florescences with paracladia. Flowers are unisexual, monoecious, greenish yellow colored in terminal long, peduncled paniculate cymes. Male flowers: calyx segments 5, nearly equal, elliptic or obviate; corolla is campanulate, lobes 5, connate, hairy inside, exceeding the calyx, each lobe bear inside a gland at the base, stamens 10 in two series, outer five filaments free, inner five filaments connate, anthers dithecous erect, opening by longitudinal slit. Female flowers: sepals up to 18 mm long, persistent; calyx as in male, corolla 4 scarcely exceeding the calyx lobes united, villous inside; ovary 3-locular, ellipsoid, 1.5–2 mm in diameter, style bifid, ovules solitary in each cell. The inflorescences form a bunch of green trilocular ellipsoidal fruits yielding approximately 10 or more ovoid fruits [21]. The exocarps remains fleshy until the seeds are mature. Wiehr [22] and Droit [23] described the microscopical anatomy of the seeds in detail, while Singh [24] described that of fruits.

## 2.2. Floral architecture and breeding system

### 2.2.1. Receptivity of male and female gametes

*J. curcas* is monoecious, and produces male and female flowers in the same inflorescence. Microsporogenesis and male gametogenesis of *J. curcas* was studied by Liu et al. [25]. Male flowers of *J. curcas* have ten stamens, each of which bear four microsporangia. The development of the anther wall is of the dicotyledonous type, and is composed of an epidermis, endothecium, middle layer(s) and glandular tapetum. Chang-wei et al. [26] studied the pollen viability, stigma receptivity and reproductive features and found that, the life span of the male flower is about two days. Its pollen viability is relatively high – 9 h after blooming and gets low 33 h later – and pollen hardly has any viability after 48 h. The life span of the female flower is about 5–12 days. The stigma receptivity is strong during 1st–4th day and begins to decline 5th day onwards and completely loses its receptivity on 9th day. There is no obvious secretion on the stigma. The higher proportion of green stigma indicates stronger receptivity. A few styles do not open after perianth's opening during rain and the stigma receptivity is quiet low. Normally, *J. curcas* shows protandry, while the female flowers open later, with 60% opening from the 3rd to the 5th day. There are still many unopen male flowers after all female flowers are opened. This provides time for receptive stigma to get pollens from male flowers, and enhance the opportunity of reproductive success. In a few racemes, and the female flowers opened firstly shows a tendency to promote xenogamy (cross-pollination) and minimize geitonogamy (self-pollination) [26]. Dhillon et al. [27] observed flowering at the terminal end of branches after rainy season in India. However, some plants flower even in spring season (March–April). The average male to female (M/F) flower ratio was 20:1, which changes drastically (108:1) with the fall in temperature. Usual peak period of flowering varies from 3 to 20 days. Prakash et al. [28] observed variation in the M/F ratio per inflorescence during the first year with average M/F ratio of 25:1, and it reduced to 13:1 in the second year indicating a positive trend towards productivity. Bhattacharya et al. [29] examined the forenoon pattern of anthesis with subsequent pollen release with each male flower producing  $1617 \pm 100$  pollen with Pollen:Ovule ratio as 539:1. The stigmas become receptive 2 h after anthesis, coinciding with nectar secretion and pollen presentation schedule. Each female flower ( $4.54 \pm 0.82 \mu\text{L}$ ) produces higher amount of nectar than male flower ( $1.92 \pm 0.44 \mu\text{L}$ ). Qing et al. [30] recorded 17 species of floral visitors among which 11 species were pollinators (*Apis dorsata*, *A. florea*, *A. mellifera*, *Eumenes conica*, *Vespa* sp.) with two floral visiting peaks at 10.00–12.00 and 16.00–17.00 h everyday. Among the different insect visitors *Apis* spp. were the most frequent. Sucrose level influence flower-visit duration, pollen removal and deposition on stigmas by honeybees [29]. Period of fruit development and maturity ranged from 55 to 61 days from date of first fruit initiation [27].

### 2.2.2. Nature of pollination

Breeding system plays a critical role in deciding the route of plant evolution and hence breeding [31]. There are three kinds of mechanisms in plant breeding systems; geitonogamy, xenogamy and apomixis [32]. In spite of the common existence of geitonogamy, the tendency to promote xenogamy is still universally significant way of evolution in angiosperms [33,34]. Plants have various adaptive ways to guarantee xenogamy, such as dioecism (separation between male and female sexual plants), dichogamy (timing separation of male and female sexual functions), opposite styles, styles of different lengths and self-incompatibility [35,36]. The results of breeding system indicated 32.9% fruit setting under selfing and 89.7% under natural pollination in *Jatropha*. The high fruit setting under open pollination revealed that the plant is capable of

producing fruits through selfing and cross-pollination. Such a breeding system represents facultative cross-pollination [27]. However the fruit sets of artificial self-pollination, artificial cross-pollination and natural cross-pollination were 87.93%, 86.66% and 76.42%, respectively, which indicated that *J. curcas* was self-compatible and tended to cross-pollinate [30]. The ability to self-pollinate through geitonogamy is considered to be adaptive for *J. curcas* for colonization [20]. Fifty percent of female flowers set fruit with 53% fecundity rate, 32% apomixis rate and 2:3 seed-ovule ratio [29]. Chang-wei et al. [37] and Abdelgadir et al. [38] suggest that fruit production can be boosted by manipulating biological processes of pollination and growth.

### 3. Genetic improvement

Very little is known about *Jatropha* genome. Chromosomes are of very small size (bivalent length 1–3.67  $\mu\text{m}$ ) with most species having  $2n = 22$  and base number of  $x = 11$  [39]. It is attractive candidate for genome sequencing with genome size (1C) to be 416 Mbp [40]. *J. curcas* is an introduced plant to many countries of Asia, Africa and Latin America and there have not been many systematic efforts for improvement of this crop. Improved varieties with desirable traits for specific growing conditions are not available, which makes growing *Jatropha* a risky business [13]. The objectives for genetic upgradation of the crop should aim at more number of female flowers or pistillate plants, high seed yield with high oil content, early maturity, resistance to pests and diseases, drought tolerance/resistance, reduced plant height and high natural ramification of branches. In addition to these targets, genetic improvement in general characteristics and methyl ester composition to make it more suitable for bio-diesel production as compared to *Pongamia pinnata* (Table 2) are also important. Correlation studies conducted by Mukta et al. [45] in *P. pinnata*

has indicated that, with increase in cetane number (ability of fuel to ignite), iodine number decreases, which means un-saturation decreases. Since iodine number is under strong genetic control (unpublished data), using genotype having low iodine number will prelude to efficient bio-diesel yielders with high cetane number. Sujatha [46] and Sujatha et al. [47] reported that genetic improvement and domestication of *J. curcas* should follow the same course as that of castor (*Ricinus communis* L.) belongs to the same family. Castor has been improved from a perennial wild to annual domesticate, having short internodes with varying flower sexuality ratios from completely pistillate to predominantly male types [48]. The success is due to the use of mutation techniques, selection of germplasm and identification of pistillate variants [49]. *J. curcas* can be improved through assessment of variation in wild source and selection of superior/elite genotypes and application of mutation, alien gene transfer through inter-specific hybridization and biotechnological interventions to bring the change in the desired traits. Enhancement of productivity can be achieved through development of pistillate plants and/or to identify divergent parents, which can later be exploited by heterosis. Development of pistillate plants through mutation and inter-specific hybridization techniques is time consuming. Meanwhile, physiological manipulation of sexuality, applying gametocides to enhance the M/F ratio and altering the expression levels of enzymes in the triacylglycerol biosynthetic (Kennedy) pathway [50] may also be considered to increase yield.

#### 3.1. Germplasm collection, characterization and availability

The key for success of any genetic improvement programme lies in the availability of genetic variability for desired traits [8]. Genetic resource through global exploration, introduction, characterization and evaluation will provide strong base for development of

**Table 2**  
Oil and methyl ester composition and general characteristics of *Jatropha curcas* and *Pongamia pinnata*.

	<i>Jatropha curcas</i>		<i>Pongamia pinnata</i>		EN 14214:2003
	Oil	Methyl esters	Oil	Methyl esters	
<i>Major fuel properties</i>					
Density ( $\text{g cm}^{-3}$ )	0.916	0.875	0.909	0.876	0.860–0.900
Flashpoint ( $^{\circ}\text{C}$ )	235	186	232	183	>120
Cetane number	46.3	57–62	54.0	55.0	>51
Carbon Residue (%)	0.38	0.18	1.51	–	<0.3
Sulfur content (%)	0–0.13	0.0036	0.007	–	<0.01
Calorific value ( $\text{MJ kg}^{-1}$ )	39.63	39.65	–	37.12	–
Acid Number ( $\text{mg KOH g}^{-1}$ )	3.71	0.27	5.91	0.42	<0.5
Iodine number ( $\text{mg iodine g}^{-1}$ )	101.7	95–106	89	91	<120
Saponification number ( $\text{mg g}^{-1}$ )	195	202.6	185	187	–
Free glycerol (%)	–	0.015–0.030	–	0.015	<0.02
Total glycerol (%)	–	0.088–0.100	–	0.797	<0.25
Viscosity at 40 $^{\circ}\text{C}$ ( $\text{mm}^2/\text{s}$ )	40.06	4.20	41.06	4.66	3.5–5.0
<i>General characteristics</i>					
Ecosystem	<i>Jatropha curcas</i> Arid to semi-arid, up to 500 m altitude		<i>Pongamia pinnata</i> Semi-arid to sub-humid, up to 1200 m altitude		
Rainfall	Low to medium up to 1200 mm		Medium to high up to 2500 mm		
Soil	Well drained soils		Tolerant to water logging, saline and alkaline soils		
Nitrogen fixation	Non fixer		$\text{N}_2$ – fixing		
Plant suitability	Wastelands, degraded lands, live fence for arable lands, green capping of bunds, shallow soils		Field boundary, river and stream banks stabilization, wastelands, tank foreshore		
Plant habit	Mostly bush, can be trained as small tree		Tree can be managed as bush by repeated pruning but will affect yield		
Leaves	Not palatable by livestock		Not palatable by livestock, used as green leaf mulch		
Gestation period	Short, 3rd year		Long, 7th year		
Yield	1.0 kg/plant		10–200 kg/tree		
Oil content	27–38% in seed		27–39% in kernel		
Protein	38%		30–40%		
Fire wood	Not useful		Good as firewood, high calorific value 4600 k cal/kg		

Sources: [19,41–44,58].

elite varieties by various improvement methods. Comprehensive work on collection, characterization and evaluation of germplasm for growth, morphology, seed characteristics and yield traits is still in its infancy. The fact that jatropha has adapted itself to a wide range of edaphic and ecological conditions suggest that there exists considerable amount of genetic variability to be exploited for potential realization [51]. Species and provenance trials contribute fundamental information for further breeding and genetic improvement [52]. Systematic provenance trials have not yet been carried out with the physiconut to the necessary extent, and material from the centre of origin has not been sufficiently screened. The genetic background of the physic nut grown in Africa and Asia is unclear. Certain provenances may differ relatively from others if cultivated at different sites, which is due to G × E interaction [53,54]. Priority should be given to assess intra- and inter-accessional variability in the available germplasm, selection of pure lines and then multiplication. Existence of natural hybrid complexes is reported in the genus *Jatropha*, such as, *J. curcas-canascentis* complex in Mexico [55], *J. integerrima-hastata* complex in Cuba and West Indian islands [18] and *J. curcas-gossypifolia* (*J. tanjorensis*) in India [56]. Hence, germplasm exhibiting gross morphological differences should be subjected for pollen studies and lines with pollen abnormality or poor seed set should be investigated in detail before drawing conclusions about the distinctness. Makkar et al. [57] reported large variations in contents of crude protein, crude fat, neutral detergent fiber and ash on 18 different provenances of jatropha from countries in West and East Africa, the Americas and Asia. Wani et al. [58] recorded variation in Indian accessions for oil content (27.8–38.4%) and 100 seed weight (44–77 g). Kaushik et al. [59] explored the variability in Haryana-India accessions to find wide variation in 100 seed weight (49–69 g) and oil content (28–39%). Similarly Rao et al. [51] found wide variation in 100 seed weight (57–79 g) and oil content (30–37%) for Andhra Pradesh, India, accessions.

### 3.2. Genetic studies of traits

Although selection based on seed characters would simplify the task, there still exist several limitations and doubts in these selected plants because of season and time bound expression of genes of interest. Knowledge of genetic variation in branching pattern, M/F flower ratio, pest resistance, drought resistance and yield attributes in wild jatropha germplasm can be of great potential in improvement programs. This is particularly important in selection of genotypes with more oil content and yield. Hence a large scale collection of germplasm from selected plus trees, their conservation and the evaluation program of various jatropha accessions is essential to understand patterns of variability. Estimation of correlation among characters, broad sense heritability and genetic advance will be useful to determine the extent to which improvement is possible through selection.

Kaushik et al. [59] recorded coefficients of variance between 24 Haryana provenances, India, which envisaged that environment has comparatively low influence on the seed traits and oil content. High broad sense heritability and high genetic advance for oil content in jatropha indicates potentiality of test material for further improvement through selection [51,59]. Positive significant association was observed between seed weight and oil content [59]. Rao et al. [51] evaluated genetic association and variability in seed and growth characters and found that M/F ratio had the highest positive direct relationship with seed yield (0.789), followed by number of branches (0.612) and number of days from fruiting to maturity (0.431) with high heritability for these traits. In another study using ten sources from central India, seed oil content was significantly correlated with seed weight (0.792), stem diameter (0.836) and total leaf area (0.883) [60]. Observation on influence

of regional climate [61] and altitudinal ranges [62] on seed oil content variation are recorded.

### 3.3. Phenotypic diversity studies

Analysis of phenotypic diversity in germplasm collections can facilitate reliable classification of accessions and its identification with future utility for specific breeding purposes. There are very few studies on phenotypic diversity involving a limited number of germplasm and their suggested use in hybridization. Sunil et al. [63] developed a methodology for identification of superior lines by assessing the phenotypic traits of plants recorded *in situ*. Further, they generated grid maps for the distribution pattern based on plant height, number of primary branches, collar length, number of fruits per cluster and oil content using Geographic Information System (GIS) to find the potential area for germplasm with high oil content based on rarefaction method of DIVA-GIS [64]. Heller [65] tested 13 provenances in multilocation field trials in 1987 and 1988 in two countries of the Sahel region: Senegal and Cape Verde. Significant differences in the vegetative development except leaf shape were detected among the various provenances at all locations. Kaushik et al. [59] studied divergence among 24 accessions using non-hierarchical Euclidian cluster analysis for seed traits in jatropha and suggested that the crossing between accessions of clusters IV and VI will result in wide spectrum of variability in subsequent generations. Gohil and Pandya [66] analysed diversity based on phenotypic traits of nine jatropha genotypes and suggested that for varietal improvement, hybridization among the genotypes of divergent clusters (clusters – III, IV and V) may be done in order to obtain better results in terms of variability and diversity. Rao et al. [51] observed four clusters with phylogeographic patterns of genetic diversity among 32 high yielding candidate plus trees of *J. curcas* for seed traits.

### 3.4. Selection and genetic improvement

The core activities of genetic improvement programs are selection and breeding. *J. curcas* is an often cross-pollinated crop and hence following genetic improvement methods can be applied to exploit genetic variation in jatropha: (i) mass selection; (ii) recurrent selection; (iii) mutation breeding; (iv) heterosis breeding and (v) inter-specific hybridization.

#### 3.4.1. Mass selection

Mass selection is the simplest, easiest and oldest method of selection where individual superior plants are selected based on their phenotypic performance and bulk seed is used to produce the next generation for genetic improvement (Fig. 1). To make genetic gains by this technique, there must be a positive offspring-parent regression which in turn depends greatly upon the magnitude of the environmental effects in the parental population. However, mass selection has a major drawback of lack of control of the pollen source, confusing effect of the environment and reduced population size leading to inbreeding depression. But these disadvantages can be taken care by de-tasselling/roughing of inferiors and crossing with of bulked pollens of superior plants. Mishra [67] devised a paired comparison method for selecting plus phenotypes of *J. curcas* trees with due importance to seed and oil yields. A global effort to evaluate the genetic variability in *J. curcas* was initiated by Montes et al. [68] using 225 accessions collected from 30 countries in Asia, Africa and Latin America and found low genetic variation in African and Indian accessions and high genetic variation in Guatemalan and other Latin American accessions. This is in support of earlier phenotypic and genotypic evaluation studied by Basha and Sujatha [69] on accessions of India. In India, *Jatropha* germplasm including a few wild species have been introduced

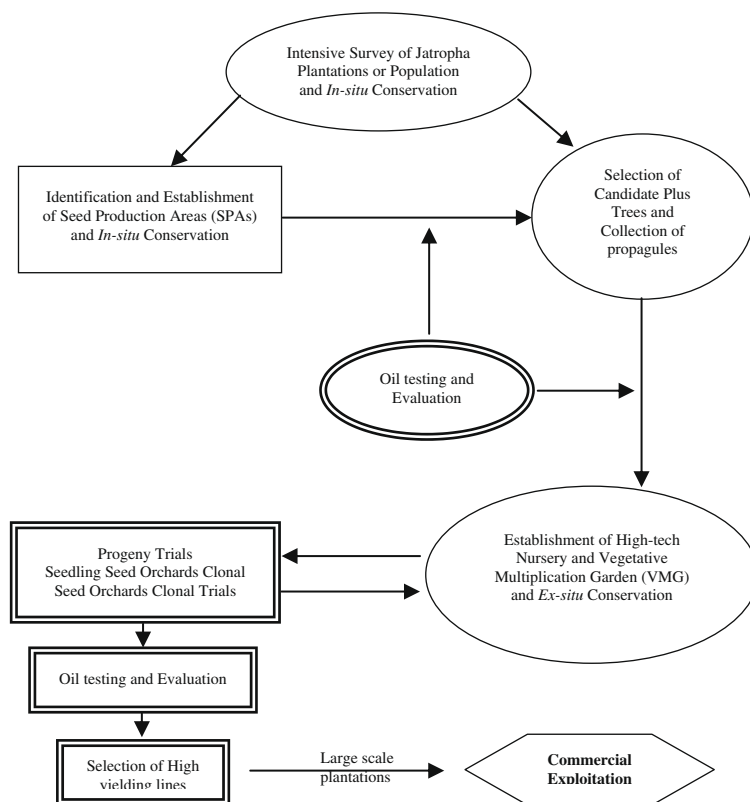


Fig. 1. Approach for genetic improvement of *Jatropha curcas* L.

from Brazil, Mali, Australia, Ghana, Nigeria, Mexico, Nicaragua and Nepal but these include only one or two accessions. Wild *J. curcas* germplasm is available in tropical America, Africa and South Asia [8,55]. This indicates the need for widening the genetic base of *J. curcas* germplasm as new source of genetic variation.

In India, the National Oilseeds and Vegetable Oils Development (NOVOD) Board (Ministry of Agriculture, Government of India) has identified 1855 candidate plus trees (CPTs) of jatropha and has over five thousand accessions collected with a network of 40 institutions. The oil content varies from 26.0% to 42.7% [70]. Department of Biotechnology (DBT), Government of India, launched micro-mission on production of quality planting material with 30–40% oil and 3–5 tonnes/ha seed yield, under this mission 1500 accessions have been collected [71]. Kaushik et al. [72] analysed one thousand samples of jatropha seeds representing twelve states of India for oil content and kernel seed coat ratio and reported that collection from Uttaranchal recorded highest percentage of high oil yielding plants (73%). Most of the *J. curcas* varieties spread all over the world viz. the variety – Cape Verde, variety from Nicaragua and a non-toxic variety from Mexico are developed from selections made in the natural populations [8,12]. The first variety, SDAUJ I (Chatrapati), has been identified for commercial cultivation in the semi-arid and arid regions of Gujarat and Rajasthan in India (<http://www.icar.org.in/pr/10052006.htm>). Indian Council of Agricultural Research (ICAR) launched network project in which, 28 seed sources were identified as the best among 496 seed sources [73].

#### 3.4.2. Recurrent selection

Recurrent selection schemes were devised in relation to heterosis breeding and may be useful in improving specific combining ability in jatropha by overcome all deficiencies of mass selection. The idea was to ensure the isolation of superior inbred from the

populations subjected to recurrent selection for their ultimate utilization in the production of hybrid and synthetic varieties. Theoretically, recurrent selection is a breeding procedure for increasing the frequency of desirable genes within a population while maintaining sufficient variability for continued selection. *Jatropha* being a cross-pollinated plant, 15 open-pollinated varieties have been developed using mass selection and recurrent selection methods in India and are under testing in the National Trials. After obtaining the required data on seed yield, oil content and oil quality, disease and insect pest resistance, the best performing genotypes will be released as new varieties of jatropha by adopting the standard procedure [70]. The importance of such considerations to tree breeding programs is obvious.

#### 3.4.3. Mutation breeding

Mutation is a sudden heritable change in a character as a result of change in gene or chromosome(s). Mutations occur in natural populations at low rate and are generally recessive and random. Mutation in tree crops is considered attractive because of lacunae in conventional breeding like time consuming, unpredictable results, long juvenile phase, high heterozygosity and fear of loss of the unique genotype. However, mutation breeding is not directed and long drawn but is one of the available options for genetic improvement of *J. curcas* with modest levels of variability. Mutation breeding studies in *J. curcas* carried out in Thailand using fast neutrons and isolated dwarf or early flowering mutants from the  $M_3$  generation, but the potential productivity of these variants under intensive cultivation conditions was not proved [74]. Dwimahyani and Ishak [75] used induced mutations in *J. curcas* for improvement of agronomic characters with irradiation dose of 10 Gy and identified mutant plants with early maturity, 100 seeds weight (30% over control) and better branch growth. In India, mutation breeding using chemical and physical mutagens has been

initiated to create genetic variation for various traits and developed mutants are being characterized using DNA markers [70]. Mutation studies undertaken at National Botanical Research Institute (NBRI), Lucknow, India has led to induction of cotyledonary variabilities in *J. curcas* [76]. The mutants themselves may not be suitable for direct release, but they do provide the necessary alleles for developing superior cultivars with desirable traits.

#### 3.4.4. Heterosis breeding

The exploitation of heterosis is a common objective in plant breeding [77]. Heterosis in tree growth is evident in many hybrids and perhaps best illustrated in studies of *Eucalyptus* and *Populus* (the genus of poplars and aspens). *J. curcas* as a facultative cross-pollinated crop shows heterosis, particularly when inbred lines are used as parent. Based on the earlier experience from other cross-fertilized crops, it appears that in *Jatropha* application of heterosis breeding could justify hybrid variety production. Seed and oil yield can be genetically enhanced through simple selection of superior germplasm and release as cultivars. In addition, hybrid cultivars could be bred to use the heterosis effect. Literature on *Jatropha* improvement through heterosis are scarce. Inter-specific hybrids utilizing *J. curcas* as the female parent and *J. integerrima* as the male parent indicated that the F<sub>1</sub> hybrids had a wide range of variation for vegetative, flowering and fruiting characters [78].

#### 3.4.5. Inter-specific hybridization

The early views that inter-specific hybridization would be a panacea for all tree improvement problems has largely been dispelled. Heterosis in hybrids, far from being a common phenomenon, is rare. Probably the more promising, and most useful contribution of inter-specific hybridization is in transferring desirable genes from one species to another, e.g. *Populus*, *Eucalyptus*. As any forest tree, *J. curcas* breeding is a time-consuming process due to perenniality. Since, most of the *Jatropha* species are insensitive to photoperiod and bear flowers continuously, selection and generation advancement can be accomplished without much time lapse. Other added advantage is the propensity of *J. curcas* towards inter-specific hybridization, supported by propagation both through seeds and vegetative propagule, which helps in rapid multiplication and acceleration of the breeding programs. Usually breeding objectives in *J. curcas* depend on solving specific problems of cultivation and intended application of economic output (seed and oil). Components that contribute to physic nut seed and oil yield per hectare are: more number of pistillate flowers per inflorescence and subsequent number of capsules per shrub, 1000-seed weight, oil content of seeds (%) and plants per hectare. As the maximum number of seeds per capsule is limited and the agronomic factor of planting density does not offer much flexibility for increasing yields, selection should focus on the other yield contributing components.

Dehgan and Webster [15] regarded *J. curcas* as the most primitive member of the genus because of its ability to interbreed with species of both the subgenera. Evolution of the genus *Jatropha* led to specialization of vegetative structures, culminating in a facultative annual growth habit in section *Jatropha* and in rhizomatous-sub-shrub habit in section *Mozzina*. Studies on phylogenetic significance of inter-specific hybridization in *Jatropha* by Dehgan [79] confining to identification of crossability barriers and morphological characterization revealed that all F<sub>1</sub> hybrids, except *J. curcas* × *J. multifida*, were more vigorous than the parental species. The species that could be crossed unilaterally with *J. curcas* as female parent include *J. macrorrhiza*, *J. capensis*, *J. cathartica*, *J. multifida*, *J. podagrica*, *J. cordata*, and *J. cinerea*. Inter-specific hybridization has immense scope for improving the genetic architecture and agronomic attributes of *J. curcas* [46]. Initial success in obtaining inter-specific F<sub>1</sub> and back cross (BC1) plants with low phorbol es-

ters and improved agronomic traits in *J. curcas* and further BC populations leading to varieties with targeted traits has been reported (<http://precedings.nature.com/documents/2782/version/1>). To increase genetic diversity and add new alleles, inter-specific cross-pollination was attempted between *J. curcas* and other *Jatropha* species by Parthiban et al. [80] to develop new hybrids with higher yield potential and resistance to diseases. Among all the crosses, the cross between *J. curcas* and *J. integerrima* produced successful hybrids with more seed set, while the other crosses failed to produce seeds due to existence of crossability barriers. In contrary Basha and Sujatha [81] produced artificial hybrids between *J. curcas* and all *Jatropha* species used in the study with the exception of *J. podagrica* without any crossability barriers. Evaluation of backcross inter-specific derivatives of cross involving *J. curcas* and *J. integerrima* indicate scope for prebreeding and genetic enhancement of *J. curcas* through inter-specific hybridization [80–83].

*J. curcas* has been considered to be less attacked by insect pests and diseases are merely based on solitary tree observation. The potential value of related species in genetic enhancement of resistance of *J. curcas* to various biotic stresses is of immense importance. Blue bugs and green stink bug will be sucking on fruits (*Scutellera nobilis*) and capsule-borer (*Pempelia morosalis*) will damage the fruits. However, incidence of pests (scutellarid bug, inflorescence and capsule-borer, blister miner (*Stomphastis thraustica*), semi-looper (*Achaea janata*), flower beetle (*Oxycetonia versicolor*), defoliator and pod borer and diseases (collar rot, black rot, leaf spots, root rot, damping off and Cassava mosaic virus) is frequently reported and particularly when the crop is raised as plantations. Screening of five *Jatropha* species against foliage feeders revealed resistance of *J. integerrima* in terms of maximum mortality, low larval weight gain with or without pupation [84]. Drought, soil salinity and water logging are major abiotic stresses in areas of *Jatropha* cultivation. Due to complex nature of these stresses and lack of appropriate screening technologies no systematic breeding program has undertaken. However, adaptability improvement of *J. curcas* to problematic sites can be achieved through grafting of *J. curcas* scions with *J. gossypifolia* stock since it thrives well on saline soils [46].

### 3.5. Breeding programs

#### 3.5.1. Global perspective

At global level, *J. curcas* evaluation, breeding and propagation programme is carried from 2006 to 2010 by Wageningen University and Research Centre, Plant Research International, the Netherlands ([www.jatropha.wur.nl](http://www.jatropha.wur.nl)). Efforts on screening of different genotypes and breeding has begun in order to produce high yielding plants at Lao Institute of Renewable Energy (LIRE) – Lao PDR (<http://www.lao-ire.org/j1.4-jatropha-breeding-experiments.html>). Surfactant and Bio-energy Resource Centre (SBRC) in Bogor has started *Jatropha* breeding activities by developing germplasm collection farm with about 200 accessions from various areas in Indonesia and overseas which will be used to engineer plants with preferred traits. Characterization of physical and morphological properties and genetic characterization for further variety engineering are in progress (<http://id.sbrc-ipb.com>). The Centro Hispaniola de Investigación en Bioenergías y Agricultura Sostenible (CHIBAS) – bi-national (Haiti and Dominican Republic) based in the Dominican Republic is improving the stocks of *Jatropha* by establishing an efficient *Jatropha* germplasm repository (live collection) and the development and release of germplasm (new improved varieties) adapted to the new needs for oil and bio-diesel production ([www.chibas-bio-energy.org/en-web-jatropha.pdf](http://www.chibas-bio-energy.org/en-web-jatropha.pdf)). The Agricultural Research Trust (ART), Zimbabwe has developed non-toxic varieties of *J. curcas*, which would make the seed cake following

oil extraction suitable as animal feed without its detoxification (<http://precedings.nature.com/documents/2658/version/1>).

### 3.5.2. Indian perspective

National Oilseeds and Vegetable Oils Development (NOVOD) Board (Ministry of Agriculture, Government of India) has over five thousand accessions collected with a network of 40 institutions and found 39 hybrid clones which showed wide variation in their morphological traits. Reproductive biological studies were undertaken in all hybrid clones. The preliminary results of this study showed that four species viz. *J. curcas*, *J. gossyfolia*, *J. multifida* and *J. podagrica* were found cross in-compatible to each other. Apomictic nature (fruit development without fertilization) of *J. podagrica* and *J. multifida* was detected by crossing five different species of *Jatropha* in all possible combinations and concluded not to use these two species as female parents, but their pollens from male flowers can be used for pollinating the other species. However, the crosses were successful when *J. multifida* (50% oil content) was used as male (♂) and *J. curcas* (23–38%) as female (♀) parent. The crossability barriers between these two species are weak and the cross combination aids in enhancement of oil content. A strong reciprocal compatibility was also found between *J. curcas* and *J. integerrima* and successful hybrid plants were developed [70].

### 3.6. Biotechnological intervention

The small genome size, chromosome number, ease of vegetative manipulation [85] and transformation [86] are favorable features for the use of biotechnological tools for *J. curcas* improvement. The potential benefit of biotechnological intervention in *Jatropha* improvement is possibility of gaining time in certain improvement process. Conventional propagation of *J. curcas* is beset with problems of poor seed viability, low germination, scanty and delayed rooting of seedlings and vegetative cuttings [8,87]. Plants propagated by cuttings show a lower longevity and possess a lower drought and disease resistance than those propagated by seeds [8]. Plants produced from cuttings do not produce true taproots (hence are less drought tolerant); rather, they produce pseudo-taproots that may penetrate only one-half to two-thirds the depth of the soil compared to taproots produced on seed grown plants [8]. Added to this, a large amount of quality planting material is required for future use. Considering its enormous potential, in depth studies need to be made for use of biotechnological tools in *Jatropha* genetic improvement.

#### 3.6.1. Micro-propagation application

The tissue culture protocols serve as a prelude for genetic improvement through biotechnological tools. Micro-propagation of *J. curcas* has been reported by various authors [85,88], using different tissues except nodal explants from the field grown plants. Regardless of the species and explant used, shoot regeneration occurred on medium supplemented with benzyladenine and indolebutyric acid but the concentration of growth regulators varied with the genotype and explant. Also in all of the cases the multiplication rate was low for application. These studies revealed that the scope for improvement of *J. curcas* through somatic hybridization [89] in inter-specific crosses limited by crossability barriers. Somatic embryogenesis not only helps to obtain a large number of plants year round, but also can act as a powerful tool for genetic improvement of any plant species because of its single cell origin [90].

#### 3.6.2. Molecular markers in genetic diversity analysis and selection

The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the significant achievements in the field of molecular genetics which accelerate breeding by establishment of molecular fingerprints

for distinct and most divergent accessions using diversity analysis [91]. DNA based markers such as Random Amplified Polymorphism DNA (RAPD), Enhanced Random Amplified Polymorphism DNA (ERAPD), Simple Sequence Repeat/Simple Sequence Repeat (Simple Sequence Repeat), Sequence Characterized Amplified Region (SCAR), Amplified Fragment Length Polymorphism (AFLP) and Inter-Simple Sequence Repeat (ISSR) can be used to assist breeding through Marker Assisted Selection (MAS) to select prospective varieties from seedling stage. Microsatellite markers are available for a few Euphorbiaceae members and are being developed for castor [92,93; <http://castorbean.tigr.org/>]. As the developmental costs for microsatellite markers are high, cross-taxa utility of molecular markers from hevea and cassava could be assessed as done in other plant species [94]. Majority of the studies are confined to characterization of accessions available in India (Table 3) except Basha and Sujatha [69] having one non-toxic accession from Mexico, Montes et al. [68] having accessions from 30 countries and Sun et al. [95] having accessions from south China. Regardless of the number of accessions used, the robustness of the primer and number of marker data points, all accessions from India clustered together. In general, diversity analysis with local germplasm revealed a narrow genetic base in India [69,97] and south China [95], indicating the need for widening the genetic base of *J. curcas* through introduction of accessions with broader geographical background and creation of variation through mutation and hybridization techniques. In contrary to the above studies, AFLP-based molecular characterization of *J. curcas* accessions from Andhra Pradesh were found diverse as these were scattered in different groups, showed occurrence of higher number of unique/rare fragments and had greater variation in percentage oil content [102]. However, careful understanding of the phylogeny and use of adequate number of molecular markers are essential prerequisites for drawing valid inferences about the genetic affinities [47]. However, Basha and Sujatha [81] characterized *Jatropha* species occurring in India using nuclear and organelle specific primers revealed high inter-specific genetic variation (98.5% polymorphism). Further characterization of both natural and artificially produced hybrids using chloroplast specific markers revealed maternal inheritance of the markers [81]. In support, genetic variation studies using RAPD, AFLP and combinatorial tubulin based polymorphism (cTBP) indicating higher possibilities of improving *J. curcas* by inter-specific breeding (<http://precedings.nature.com/documents/2782/version/1>). Hence, molecular diversity estimates combined with the datasets on other agronomic traits will be very useful for selecting the appropriate accessions.

**Table 3**  
Characterization of accessions using molecular markers.

Species/accessions	Primers	Number	References
142	AFLP	–	DBTIndia [96]
5	RAPD	18	GaneshRam et al. [97]
22	RAPD	7	Ranade et al. [98]
	DAMD	4	
13	RAPD	20	Gupta et al. [99]
	ISSR	14	
7	RAPD	52	Pamidiamarri et al. [100]
	AFLP	27	
20	RAPD	–	Reddy et al. [101]
	AFLP	–	
43	RAPD	400	Basha and Sujatha [69]
	ISSR	100	
225	AFLP	–	Montes et al. [68]
58	SSR	30	Sun et al. [95]
	AFLP	7	



### 3.6.3. Genetic engineering

Genetic engineering through transformation has been widely used to obtain transgenic plants through some stages in molecular and cellular biological techniques is another valuable method for the development of *J. curcas* variety. Recently a new full length cDNA of stearoyl-acyl carrier protein desaturase was obtained by RT-PCR and RACE techniques from developing seeds of *Jatropha* and the gene was functionally expressed in *Escherichia coli* [103]. It is an important enzyme for fatty acid biosynthesis in higher plants and also plays an important role in determining the ratio of saturated fatty acid to unsaturated fatty acids in plants [104]. For understanding the molecular mechanism of salt and drought tolerance, a new full length cDNA encoding aquaporin (JcPIP2) was isolated from seedling of *J. curcas*, the abundance of JcPIP2 was induced by heavy drought stress and it plays an important role in rapid growth of *Jatropha* under dry conditions [105]. Zhang et al. [106] reported a novel betaine aldehyde dehydrogenase gene (BADH) named *JcBD1* (cloned by RT-PCR and RACE techniques from *J. curcas*) is increasingly expressed in leaves undergoing environmental stress like drought (30% PEG), heat (50 °C) and salt (300 mM NaCl). The *JcBD1* protein was functionally expressed in *Escherichia coli* and conferred its resistance to abiotic stresses like salt. Li et al. [107] established highly efficient genetic transformation procedure for *J. curcas* for the first time via *Agrobacterium tumefaciens* infection of cotyledon disc explants. This helps in genetic modification and subsequent *in vitro* multiplication of cultivars for various uses. Further, the technology helps in better understanding and subsequent improvement of the oil biosynthesis pathway, which could have positive implications on reducing the world's dependence on fossil reserves.

### 3.6.4. *Jatropha* genome project

Synthetic Genomics Inc. (SGI) and Asiatic Centre for Genome Technology (ACGT) completed *Jatropha* genome project. The sequencing of the genome, using both traditional sanger sequencing and next generation sequencing, has revealed that the *Jatropha* genome is approximately 400 million base pairs in size, similar to the size of the rice genome. Annotation of genome to identify genes of interest helps in discovering genetic variation using marker-assisted breeding and provide information on factors controlling oil synthesis, maximizing yield, biotic and abiotic stress tolerance and low-curcun variants ([http://greenbio.checkbiotech.org/news/first\\_jatropha\\_genome\\_completed\\_synthetic\\_genomics\\_inc\\_and\\_asiatic\\_511\\_c\\_centre\\_genome\\_tech](http://greenbio.checkbiotech.org/news/first_jatropha_genome_completed_synthetic_genomics_inc_and_asiatic_511_c_centre_genome_tech)).

## 4. Future strategy for crop improvement

*J. curcas* is still in its infancy with respect to genetic improvement for increasing seed and oil yield per unit area. Genetic improvement activities so far involved only collection and selection in local germplasm and hence initiatives to evaluate global germplasm is necessary, to study pattern of diversity for various traits. Genetic improvement using conventional breeding approaches has to be initiated at more places and integrated with latest biotechnological techniques for reducing time and increasing efficiency of breeding. Potential of the new varieties developed has to be further tested for their performance, through multilocation trials. Development of techniques such as, somoclonal variants, mutations, doubled haploids, and gene transfer which support plant breeding activities should be emphasized. Similar to other crops, heritability of seed traits is the most common predictor of genetic gains for different breeding methods in *Jatropha*. Application of information from *Jatropha* genome project can be used in marker-assisted breeding to accelerate and complement conventional breeding. Genetic transformation and gene transfer

is particularly important for traits for which variability is unavailable in the cultivated species.

## 5. Conclusions

*J. curcas* has spread beyond its original distribution because of its hardiness, easy propagation, drought endurance, high oil content, low seed cost, short gestation period, rapid growth, adoption to wide agro-climatic condition, bushy/shrubby nature and multiple uses of different plant parts. Although *Jatropha* is well known for having wide adaptability and plethora of uses, the full potential of *J. curcas* is far from being realized because of several reasons. Improved varieties with desirable traits for specific growing conditions are not available, which makes growing *Jatropha* a risky business. Apart from agronomic, socioeconomic and institutional constraints, planned crop improvement programs are lacking globally. Hence, *J. curcas* can be improved through assessment of variation in wild sources and selection of superior/elite genotypes and application of mutation, alien gene transfer through inter-specific hybridization and biotechnological interventions to bring the change in the desired traits. Enhancement of productivity can be achieved through development of pistillate plants and exploiting by heterosis.

## References

- [1] Parikh J. Growing our own oils. *Biofuels India* 2005;3(3):7.
- [2] OECD-FAO. Agricultural outlook 2008–2017. 2008; <[www.oecd.org/publishing/corrigenda](http://www.oecd.org/publishing/corrigenda)>.
- [3] Shukla SK. Experiences of Chattisgarh biofuel development authority. *Biofuels India* 2005;3(4):12–3.
- [4] Wani SP, Sreedevi TK, Reddy BVS. *Biofuels: status, issues and approaches for harnessing the potential*. Hyderabad, India; 2006. <[www.indianjournals.com/ijor.aspx?target=ijor:in&volume=3and4&issue=4and1&article=004](http://www.indianjournals.com/ijor.aspx?target=ijor:in&volume=3and4&issue=4and1&article=004)>.
- [5] Hegde DM. 2003. Tree oilseeds for effective utilization of wastelands. In: Compendium of lecture notes of winter school on wasteland development in Rainfed areas, Central Research Institute for Dryland Agriculture, September 1–30, Hyderabad, India; 2003. p. 111–9.
- [6] Reddy BVS, Ramesh S, Ashok kumar A, Wani SP, Ortiz R, Ceballos H, et al. Bio-fuel crops research for energy security and rural development in developing countries. *Bioenergy Res* 2008;1:248–58.
- [7] Tiwari AK, Kumar A, Raheman H. Biodiesel production from *Jatropha (Jatropha curcas)* with high free fatty acids: an optimized process. *Biomass Bioenergy* 2007;31:569–75.
- [8] Heller J. *Physic nut Jatropha curc* as L. Promoting the conservation and use of underutilized and neglected crops. Institute of Plant Genetic and Crop Plant Research, Gatersleben/International Plant Genetic Resource Institute, Rome, Italy 1996. <<http://www.ipgri.cgiar.org/Publications/pdf/161.pdf>>.
- [9] Jones N, Miller JH. *Jatropha curcas*: a multipurpose species for problematic sites. ASTAG Technical paper – Land resources, vol. 1. Washington (DC, USA) World Bank; 1992. p. 1–12.
- [10] Francis G, Edinger R, Becker K. A concept for simultaneous wasteland reclamation, fuel production, and socioeconomic development in degraded areas in India: need, potential and perspectives of *Jatropha* plantations. *Nat Resour Forum* 2005;29:12–24.
- [11] Kumar A, Sharma S. An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): a review. *Ind Crops Prod* 2008;28(1):1–10.
- [12] Achten WMJ, Mathijs E, Verchot L, Singh VP, Aerts R, Muys B. *Jatropha* biodiesel fueling sustainability? *Biofuels Bioprod Biorefin* 2007;1:283–91.
- [13] Jongschaap REE, Corré WJ, Bindraban PS, Brandenburg WA. Claims and facts on *Jatropha curcas* L. Wageningen, The Netherlands: Plant Research International. 2007. <[http://www.fact-fuels.org/media\\_en/Claims\\_and\\_Facts\\_on\\_Jatropha\\_WUR?session=isgsklbn58j7grfst888n5r7](http://www.fact-fuels.org/media_en/Claims_and_Facts_on_Jatropha_WUR?session=isgsklbn58j7grfst888n5r7)>.
- [14] Linnaeus C. *Species plantarum*. In: *Jatropha*. Impensis Stockholm: Laurentii Salvii; 1753. p. 1006–7.
- [15] Dehgan B, Webster GL. Morphology and infrageneric relationships of the genus *Jatropha* (Euphorbiaceae). University of California Publications in Botany; 1979.
- [16] Schultze-Motel J. *Rudolf Mansfelds Verzeichnis landwirtschaftlicher and gärtnerischer Kulturpflanzen (ohne Zierpflanzen)*. Berlin: Akademie-Verlag; 1986.
- [17] Münch, E. *Die Purgiernuß (Jatropha curcas L.) – Botanik, Ökologie, Anbau*. Diploma Thesis. University Hohenheim, Stuttgart; 1986.
- [18] Pax F. *Euphorbiaceae–Jatrophaeae*. In: Engler A, editor. *Das Pflanzenreich IV*, vol. 147(42). Leipzig: Verlag von Wilhelm Engelmann; 1910.
- [19] Achten WMJ, Verchot L, Franken YJ, Mathijs E, Singh VP, Aerts R, et al. *Jatropha* bio-diesel production and use. *Biomass Bioenergy* 2008;32(12):1063–84.

- [20] Raju AJS, Ezradanam V. Pollination ecology and fruiting behaviour in a monoecious species, *Jatropha curcas* L. (Euphorbiaceae). *Curr Sci* 2002;83:1395–8.
- [21] Tewari DN. *Jatropha* and biodiesel. 1st ed. New Delhi: Ocean books Ltd.; 2007.
- [22] Wiehr E. Beiträge zur Kenntnis der Anatomie der wichtigsten Euphorbiaceensamen unter besonderer Berücksichtigung ihrer Erkennungsmerkmale in Futtermitteln. Ph.D. Dissertation, Mathematical-Natural Scientific Faculty, University Hamburg; 1930.
- [23] Droit S. Recherches sur la graine et l'huile de Purgère ou Pignon d'Inde (*Jatropha curcas* L.). Dissertation, Université de Paris, Faculté de Pharmacie, Paris; 1932.
- [24] Singh RP. Structure and development of seeds in Euphorbiaceae, *Jatropha* species. *Beitr Biol Pflanz* 1970;47:79–90.
- [25] Liu HF, Kirchoff BK, Wu GJ, Liao JP. Microsporogenesis and male gametogenesis in *Jatropha curcas* L. (Euphorbiaceae). *J Torrey Bot Soc* 2007;134(3):335–43.
- [26] Chang-wei L, Kun L, You C, Yong-yu S, Wen-yun Y. Pollen viability, stigma receptivity and reproductive features of *J. curcas* L. (Euphorbiaceae). *J Northwest Plant* 2007;27(10):1994–2001.
- [27] Dhillon RS, Hooda MS, Handa AK, Ahlawat KS, Kumar Y, Subhash, et al. Clonal propagation and reproductive biology in *Jatropha curcas* L. *Indian J Agroforest* 2006;8(2):18–27.
- [28] Prakash AR, Patolia JS, Chikara J, Boricha G.N. Floral biology and flowering behaviour of *Jatropha curcas*. In: Expert seminar on *Jatropha curcas* L. agronomy and genetics, March 26–28. Wageningen, The Netherlands: Published by FACT Foundation; 2007.
- [29] Bhattacharya A, Datta K, Datta SK. Floral biology, floral resource constraints and pollination limitation in *Jatropha curcas* L. *Pak J Biological Sci* 2005;8(3):456–60.
- [30] Qing Y, Ping PD, Biao DZ, Liang WZ, Xiang SQ. Study on pollination biology of *Jatropha curcas* (Euphorbiaceae). *J South China Agric Univ* 2007;28(3):62–6.
- [31] Grant V. *Plant speciation*. 2nd ed. New York: Columbia University Press; 1981.
- [32] Les DH. Breeding systems, population structure and evolution in hydrophilous angiosperms. *Ann Mo Bot Gard* 1988;75:819–35.
- [33] Stebbins GL. Adaptive radiation in angiosperms, pollination mechanisms. *Ann Rev Ecol Syst* 1970;1:307–26.
- [34] Faegri K, Pijl VDL. *The principles of pollination ecology*. 3rd ed. Oxford: Pergamon Press; 1979.
- [35] Wyatt R. Pollinator plant interactions and the evolution of breeding systems. In: Real L, editor. *Pollination biology*. Florida: Academic Press; 1983. p. 51–95.
- [36] Fang YM. *Plant reproductive ecology*. Jinan: Shandong University Press; 1996.
- [37] Chang-wei L, Kun L, You C, Yong-yu S. Floral display and breeding system of *Jatropha curcas* L. *Forum Stud China* 2007;9(2):114–9.
- [38] Abdelgadira HA, Johnson SD, Stadena JV. Approaches to improve seed production of *Jatropha curcas* L. *South Afr J Botany* 2008. doi:10.1016/j.sajb.2008.01.023.
- [39] Soontornchainaksaeng P, Jenjittikul T. Karyology of *Jatropha* (Euphorbiaceae) in Thailand. *Thai For Bull* 2003;31:105–12.
- [40] Carvalho CR, Clarindo WR, Praça MM, Aratijo FS, Carels N. Genome size, base composition and karyotype of *Jatropha curcas* L., an important biofuel plant. *Plant Sci* 2008;174:613–7.
- [41] Singh RK, Kumar AK, Sethi S. Preparation of karanja oil methyl ester; 2006. <http://dspace.nitrkl.ac.in/dspace/bitstream/2080/303/1/ow-rksingh-1.pdf>.
- [42] Gubitz GM, Mittelbach M, Trabi M. Exploitation of the tropical oil seed plant *Jatropha curcas* L. *Bioresour Technol* 1999;67:73–82.
- [43] Francis G, Edinger R, Becker K. A concept for simultaneous wasteland reclamation, fuel production, and socio-economic development in degraded areas in India: need, potential and perspectives of *Jatropha* plantations. *Nat Resour Forum* 2005;29:12–24.
- [44] Kalbande SR, Vikhe SD. *Jatropha* and Karanj bio-fuel: an alternate fuel for diesel engine. *ARPN J Eng Appl Sci* 2008;3(1):7–13.
- [45] Mukta N, Murthy IYLN, Sripal P. Variability assessment in *Pongamia pinnata* (L.) Pierre germplasm for biodiesel traits. *Industrial Crops Prod* 2008. doi:10.1016/j.indcrop.2008.10.002.
- [46] Sujatha M. Genetic improvement of *Jatropha curcas* L. possibilities and prospects. *Indian J Agroforest* 2006;8(2):58–65.
- [47] Sujatha M, Reddy TP, Mahasi MJ. Role of biotechnological interventions in the improvement of castor (*Ricinus communis* L.) and *Jatropha curcas* L. *Biotechnol Adv* 2008;26:424–35.
- [48] Singh D. Castor *Ricinus communis* (Euphorbiaceae). In: Simmonds NW, editor. *Evolution of crop plants*. London: Longman; 1976. p. 84–6.
- [49] Hegde DM, Sujatha M, Singh NB. Castor in India. Hyderabad, India: DOR; 2003.
- [50] King AJ, He W, Cuevas JA, Freudenberger M, Ramiamanana D, Graham IA. Potential of *Jatropha curcas* as a source of renewable oil and animal feed. *J Exp Botany* 2009. doi:10.1093/jxb/erp025.
- [51] Rao GR, Korwar GR, Shanker AK, Ramakrishna YS. Genetic associations, variability and diversity in seed characters, growth, reproductive phenology and yield in *Jatropha curcas* (L.) accessions. *Trees* 2008. doi:10.1007/s00468-008-0229-4.
- [52] Burley J, Wood PJ. A manual on species and provenance research with particular reference to the Tropics. *Tropical Forestry Papers* No. 10. Department of Forestry, Commonwealth Forestry Institute, University of Oxford; 1976.
- [53] Namkoong G, Kang HC, Brouard JS. *Tree breeding: principles and strategies*. Monographs on theoretical and applied genetics, vol. 11. New York: Springer Verlag; 1988.
- [54] Zobel BJ, Wyk GV, Stahl P. *Growing exotic forests*. New York: John Wiley & Sons; 1988.
- [55] Dehgan B, Webster GL. Three new species of *Jatropha* (Euphorbiaceae) from Western Mexico. *Madrono* 1978;25:30–9.
- [56] Prabhakaran AJ, Sujatha M. *Jatropha tanjorensis* Ellis and Saroja, a natural interspecific hybrid occurring in Tamil Nadu, India. *Genet Resour Crop Evol* 1999;46:213–8.
- [57] Makkar HPS, Becker K, Sporer F, Wink M. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. *J Agri Food Chem* 1997;45:3152–7.
- [58] Wani SP, Osman M, D'silva E, Sreedevi TK. Improved livelihoods and environmental protection through biodiesel plantations in Asia. *Asian Biotechnol Develop Rev* 2006;8(2):11–29.
- [59] Kaushik N, Kumar K, Kumar S, Kaushik N, Roy S. Genetic variability and divergence studies in seed traits and oil content of *Jatropha* (*J. curcas* L.) accessions. *Biomass Bioenergy* 2007;31:497–502.
- [60] Ginwal HS, Rawat PS, Srivastava RL. Seed source variation in growth performance and oil yield of *Jatropha curcas* Linn. in Central India. *Silvae Genet* 2004;53:186–92.
- [61] Kun L, Wen-yun Y, Li L, Chun-hua Z, Yong-zhong C, Yong-yu S. Distribution and development strategy for *J. curcas* L. in Yunnan Province, Southwest China. *For Stud China* 2007;9(2):120–6.
- [62] Pant KS, Khosla V, Kumar D, Gairola S. Seed oil content variation in *Jatropha curcas* Linn. in different altitudinal ranges and site conditions in H.P. India. *Lyonia* 2006;11(2):31–4.
- [63] Sunil N, Varaprasad KS, Sivaraj N, Kumar TS, Abraham B, Prasad RBN. Assessing *Jatropha curcas* L. germplasm in-situ – a case study. *Biomass Bioenergy* 2008;32:198–202.
- [64] Sunil N, Sivaraj N, Anitha K, Abraham B, Kumar V, Sudhir E, et al. Analysis of diversity and distribution of *Jatropha curcas* L. germplasm using Geographic Information System (DIVA-GIS). *Genet Resour Crop Evol* 2008. doi:10.1007/s10722-008-9350-x.
- [65] Heller J. Untersuchungen über genotypische Eigenschaften und Vermehrungs- und Anbauverfahren bei der Purgiernuß (*Jatropha curcas* L.) [Studies on genotypic characteristics and propagation and cultivation methods for physic nuts (*Jatropha curcas* L.)]. Dr. Kovac, Hamburg; 1992.
- [66] Gohil RH, Pandya JB. Genetic diversity assessment in physic nut (*Jatropha curcas* L.). *Int J Plant Production* 2008;2(4):321–6.
- [67] Mishra DK. Selection of candidate plus phenotypes of *Jatropha curcas* L. using method of paired comparisons. *Biomass Bioenergy* 2008. doi:10.1016/j.biombio.2008.08.004.
- [68] Montes LR, Azurdia C, Jongschaap REE, Van Loo EN, Barillas E, Visser R and Mejia L. Global evaluation of genetic variability of *Jatropha curcas*; 2008. <http://www.pri.wur.nl/NR/rdonlyres/90AF26A1-47D5-4F2F-9E96-D413C2933685/70112/PosterMontesHR.pdf>.
- [69] Basha SD, Sujatha M. Inter and intra-population variability of *Jatropha curcas* (L.) characterized by RAPD and ISSR markers and development of population-specific SCAR markers. *Euphytica* 2007;156:375–86.
- [70] Punia MS. Current status of research and development on *Jatropha* (*Jatropha curcas*) for sustainable biofuel production in India. In: USDA Global Conference on Agricultural Biofuels: Research and Economics, 20–22 August, Minneapolis, Minnesota; 2007.
- [71] Swarup R. Quality planting material and seed standards in *Jatropha*. In: Singh B, Swaminathan R, Ponraj V, editors. *Proceedings of the biodiesel conference toward energy independence—focus on Jatropha*, June 9–10, Rashtrapati Bhawan, Hyderabad, India; 2006. p. 129–35.
- [72] Kaushik N, Roy S, Biswas GC. Screening of Indian germplasm of *Jatropha curcas* for selection of high oil yielding plants. *Indian J Agroforest* 2006;8(2):54–7.
- [73] Paramathma M, Hegde DM, Parthiban KT, Mukta N, Guptha VK, Abraham Y, et al. Scope of tree borne oilseeds as source of vegetable oil and biofuels. In: Singh H, Reddy BN, Murthy IYLN, editors. *Souvenir of the national symposium on vegetable oils scenario: approaches to meet the growing demands*, January 29–31. Hyderabad, India: Directorate of Oilseeds Research; 2009. p. 47–9.
- [74] Sakaguchi S, Somabhi M. Exploitation of Promising Crops of Northeast Thailand Siriphan Offset. Thailand: Khon Kaen; 1987.
- [75] Dwimahyani I, Ishak. Induced mutation on *Jatropha* (*Jatropha curcas* L.) for improvement of agronomic characters variability; 2004. <http://www.digilib.batan.go.id/atom-indonesia/fulltex/v30-n2-7-2004/Ita-Dwimahyani-Ishak.pdf>.
- [76] Pandey RK, Datta SK. Gamma ray induced cotyledonary variabilities in *Jatropha curcas* L. *J Nucl Agric Biol* 1995;24:62–6.
- [77] Mayo O. *The theory of plant breeding*. 2nd ed. Oxford: Oxford University Press; 1987. p. 334.
- [78] Paramathma M, Reeja S, Parthiban KT, Malarvizhi D. Development of inter-specific hybrids in *Jatropha*. In: Singh B, Swaminathan R, Ponraj V, editors. *Proceedings of the biodiesel conference toward energy independence—focus on Jatropha*, June 9–10, Rashtrapati Bhawan, Hyderabad, India; 2006. p. 136–42.
- [79] Dehgan B. Phylogenetic significance of interspecific hybridization in *Jatropha* (Euphorbiaceae). *Syst Bot* 1984;9(4):467–78.

- [80] Parthiban KT, Kumar RS, Thiagarajan P, Subbulakshmi V, Vennila S, Rao MG. Hybrid progenies in jatropha – a new development. *Curr Sci* 2009;96(6):815–23.
- [81] Basha SD, Sujatha M. Genetic analysis of *Jatropha* species and interspecific hybrids of *Jatropha curcas* using nuclear and organelle specific markers. *Euphytica* 2009;168:197–214.
- [82] Rupert EA, Dehgan B, Webster GL. Experimental studies of relationships in the genus *Jatropha*. *L. J. curcas* x *J. integerrima*. *Bull Torrey Bot Club* 1970;97:321–5.
- [83] Sujatha M, Prabakaran AJ. New ornamental jatropha through interspecific hybridization. *Genet Resour Crop Evol* 2003;50:75–82.
- [84] Lakshminarayana M, Sujatha M. Screening of *Jatropha* species against major defoliators of castor. *J Oilseeds Res* 2001;18:228–30.
- [85] Wei Q, Lu WD, Loao Y, Pan SL, Xu Y, Tang L, et al. Plant regeneration from epicotyl explants of *Jatropha curcas*. *J Plant Physiol Mol Biol* 2004;30:475–8.
- [86] Li MR, Li HQ, Wu GJ. Study on factors influencing agrobacterium-mediated transformation of *Jatropha curcas*. *Fen Zi Xi Bao ShengWuXue Bao* 2006;39:83–9.
- [87] Openshaw K. A review of *J. curcas*: an oil plant unfulfilled promise. *Biomass Bioenergy* 2000;19:1–15.
- [88] Sujatha M, Mukta N. Morphogenesis and plant regeneration from tissue cultures of *J. curcas* L. *Plant Cell Tiss Organ Cult* 1996;44:135–41.
- [89] Jha TB, Mukherjee P, Datta MM. Somatic embryogenesis in *Jatropha curcas* Linn an important biofuel plant. *Plant Biotechnol Rep* 2007;1:135–40.
- [90] Bhansali R. Somatic embryogenesis and regeneration of plantlets in pomegranate. *Ann Bot* 1990;66:249–54.
- [91] Caetano-Anolles G, Gresshoff PM. DNA markers protocols applications and overviews. New York: Wiley-Liss; 1997.
- [92] Okogbenin E, Marin J, Fregene M. An SSR-based molecular genetic map of cassava. *Euphytica* 2006;147:433–40.
- [93] Lespinasse D, Rodier-Goud M, Grivet L, Leconte A, Legnate H, Seguin M. A saturated genetic linkage map of rubber tree (*Hevea* spp.) based on RFLP, AFLP, microsatellite and isozyme markers. *Theor Appl Genet* 2000;100:127–38.
- [94] Rossetto M. Sourcing of SSR markers from related plant species. In: Henry RJ, editor. *Plant genotyping; The DNA fingerprinting of plants*. UK: CAB International; 2001. p. 211–24.
- [95] Sun Qj-Bao, Li Lin-Feng, Li Yong, Wu Guo-Jiang, Ge Xue-Jun. SSR and AFLP markers reveal low genetic diversity in the biofuel plant *Jatropha curcas* in China. *Crop Sci* 2008;48:1865–71.
- [96] DBTIndia; 2007. <[http://dbtindia.nic.in/Energy\\_bioscience/Presentations/TS\\_4\\_Dr%20Nutan%20Kaushik\\_TERI\\_N%20Delhi.pdf](http://dbtindia.nic.in/Energy_bioscience/Presentations/TS_4_Dr%20Nutan%20Kaushik_TERI_N%20Delhi.pdf)>.
- [97] Ganesh Ram S, Parthiban KT, Kumar RS, Thiruvengadam V, Paramathma M. Genetic diversity among *Jatropha* species as revealed by RAPD markers. *Genet Resour Crop Evol* 2008. doi:10.1007/s10722-007-9285-7.
- [98] Ranade SA, Srivastava AP, Rana TS, Srivastava J, Tuli R. Easy assessment of diversity in *Jatropha curcas* L. plants using two single-primer amplification reaction (SPAR) methods. *Biomass Bioenergy* 2008. doi:10.1016/j.biombioe.2007.11.006.
- [99] Gupta S, Srivastava M, Mishra GP, Naik PK, Chauhan RS, Tiwari SK, et al. Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different *Jatropha curcas* genotypes. *African J Biotechnol* 2008;7(23):4230–43.
- [100] Pamidiamarri DVNS, Pandya N, Reddy MP, Radhakrishnan T. Comparative study of interspecific genetic divergence and phylogenetic analysis of genus *Jatropha* by RAPD and AFLP: genetic divergence and phylogenetic analysis of genus *Jatropha*. *Mol Biol Rep* 2008. doi:10.1007/s11033-008-9261-0.
- [101] Reddy MP, Chikara J, Patolia JS, Ghosh A. Genetic improvement of *Jatropha curcas* adaptability and oil yield. In: Expert seminar on *Jatropha curcas* L. agronomy and genetics, 26–28 March. Wageningen, The Netherlands, Published by FACT Foundation; 2007.
- [102] Leela T, Wani SP, Kannan S, Naresh B, Hoisington DA, Devi P, et al. AFLP-based molecular characterization of an elite germplasm collection of *Jatropha curcas* L. a biofuel plant. *Plant Sci* 2009. doi:10.1016/j.plantsci.2009.01.006.
- [103] Tong L, Peng SM, Deng WY, Ma DW, Xu Y, Xiao M, et al. Characterization of a new stearoyl-acyl carrier protein desaturase gene from *Jatropha curcas*. *Biotechnol Lett* 2006;28:657–62.
- [104] Lindqvist Y, Huang W, Schneider G, Shanklin J. Crystal structure of delta 9 stearoyl-acyl carrier protein desaturase from castor seed and its relationship to di-iron proteins. *EMBO J* 1996;15:4081–92.
- [105] Ying Z, Yunxiao W, Luding J, Ying X, Yingchun W, Daihua L, et al. Aquaporin JcPIP2 is involved in drought responses in *Jatropha curcas*. *Acta Biochim Biophys Sin* 2007;39:787–94.
- [106] Zhang F, Niu B, Wang Y, Chen F, Wang S, Xu Y, et al. A novel betaine aldehyde dehydrogenase gene from *Jatropha curcas*, encoding an enzyme implicated in adaption to environmental stress. *Plant Sci* 2008;174:510–8.
- [107] Li M, Li H, Jiang H, Pan X, Wu G. Establishment of an *Agrobacterium*-mediated cotyledon disc transformation method for *J. curcas*. *Plant Cell Tiss Organ Cult* 2008. doi:10.1007/s11240-007-9320-6.