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REVIEW

Molecular approaches to improvement of *Jatropha curcas* Linn. as a sustainable energy crop

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Abstract With the increase in crude oil prices, climate change concerns and limited reserves of fossil fuel, attention has been diverted to alternate renewable energy sources such as biofuel and biomass. Among the potential biofuel crops, *Jatropha curcas* L, a non-domesticated shrub, has been gaining importance as the most promising oilseed, as it does not compete with the edible oil supplies. Economic relevance of *J. curcas* for biodiesel production has promoted world-wide prospecting of its germplasm for crop improvement and breeding. However, lack of adequate genetic variation and non-availability of improved varieties limited its prospects of being a successful energy crop. In this review, we present the progress made in molecular breeding approaches with particular reference to tissue culture and genetic transformation, genetic diversity assessment using molecular markers, large-scale transcriptome and proteome studies, identification of candidate genes for trait improvement, whole genome sequencing and the current interest by various public and private sector companies in commercial-scale cultivation, which highlights the revival of *Jatropha* as a sustainable energy crop. The information generated from molecular markers, transcriptome profiling and whole genome sequencing could accelerate the genetic upgradation of *J. curcas* through molecular breeding.

Keywords Abiotic stress tolerance · Energy crop · Fatty acid biosynthesis · Genetic transformation · Genome · Molecular markers · Transcriptome

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Introduction

Global warming, spurt in population growth, increase in demand of transport fuels in developing economies coupled with limited reserves of fossil fuels underline the importance of interest in renewable and alternate sources of energy such as biofuels. Currently, most biofuels are obtained from either carbohydrate-based feedstock (sugar, starch, cellulose) or oil-based feedstock. Oil-based feedstock or biodiesel can be produced from vegetable oils of agricultural plants such as rapeseed, sunflower, soybean, oil palm and groundnut. The European Union is currently the global leader in biodiesel production and use, with Germany and France accounting for 88% of the world production, followed by the USA, which produces 8% of global production (Hazell and Pachauri 2006; Nass et al. 2007). As a feedstock, about 84% of the world's biodiesel production is derived from rapeseed oil. However, the use of edible oils in developing countries for biofuel purpose will lead to shortage of food and escalation of food prices; it would encourage the use of agricultural lands, which are valuable for biodiversity, for cultivation of biofuel crops creating severe competition with food crops.

For sustainable biodiesel production, plant species capable of growing in marginal lands with minimum agricultural inputs would be the choice. Driven by such a situation, focus is shifting from edible to non-edible oil crops. There are

many non-edible oleaginous plants such as neem (*Azadirachta indica* A.), karanja (*Millettia pinnata* L.), mahua (*Madhuca* sp), castor bean (*Ricinus communis* L.), simarouba (*Simarouba glauca* DC.), wild apricot (*Prunus armeniaca*), jojoba (*Simmondsia chinensis*), kokum (*Garcinia indica*), mahua (*Madhuca indica*), *Calophyllum ionophyllum*, physic nut (*Jatropha curcas* L.), etc. (Carels 2009; http://www.novodboard.com/Jatropha-english.pdf). Among these crops, *J. curcas* (Fig. 1) has gained importance primarily because it is a non-food plant, does not compete with food supply and can be cultivated in wastelands (Kumar et al. 2011). According to Carels (2009), the commercial yield in *J. curcas* plantations is 2,000–4,000 kg seeds/ha/year. However, the yields may vary with the type of planting model. The plant is adapted to grow in arid and semi-arid regions and shows resistance to drought. The environmental advantages for *Jatropha*-based biofuels are great when the plant is cultivated on degraded and marginal soils. Under these conditions, *J. curcas* is capable of completion of life cycle without commercially acceptable yields (250–600 g/plant/year). Moreover, there are risks to the sustainability of *Jatropha* cultivation in terms of economic viability (Achten et al. 2010). There are also risks to the environment and to society. The level of economic returns anticipated by the investor may not be attainable on degraded lands without the best agricultural practices. Economic returns to labor for *Jatropha* depends much on the levels of yields. The negative impact on biodiversity is to be anticipated where *Jatropha* cultivation replaces the natural ecosystem (Abhilash et al. 2010). This may be mitigated to some extent by intercropping with other biofuel, food and fodder crops (Fig. 1). Thus, if *J. curcas* is planted on degraded land, the risk to biodiversity is likely to be minimum.

Therefore, establishment of good agricultural practices, using improved cultivars with intercropping and plantation on degraded lands can help optimize input use and offer higher productivity and returns with minimal environmental risks. Thus, there is an immediate need for systematic breeding approaches to develop varieties suitable for different agro-climatic zones and stress conditions. Because of several industrial and medicinal uses, initial investments toward commercial-scale plantations of this plant are in progress.

In the past few years, a wealth of information has been published on biotechnological approaches for improvement of the crop with particular reference to molecular markers, genetic transformation, genomics, transcriptomics, etc. Several laboratories all over the world are working in parallel on various aspects to contribute to better understanding of the crop. On the contrary, there is also a lot of criticism that the crop has not lived up to expectations. Many of the investments on developing *Jatropha* as an oil crop are made without the backing of sufficient science-based knowledge. This review is an attempt to present an up-to-date status of the research on molecular breeding approaches toward improvement of *J. curcas* highlighting the advances and limitations of the existing studies and future perspectives thereon for making this crop with unexploited potential economically sustainable.

Fig. 1 *J. curcas* with maize plantation as intercrop. *J. curcas* can be intercropped with other food and fodder crops. Intercropping with maize as shown above at one of the large-scale plantations can help optimize input and offer higher economic returns with minimal environmental risks

Challenges for commercial-level cultivation

Large-scale cultivation of *J. curcas* remains the single most important issue that will ultimately decide the success of the crop. Low and unpredictable yields are reported from established plantations. The challenges of developing viable market for *Jatropha* oil are considerable, but by no means insurmountable. According to an analysis by Weyerhaeuser et al. (2007), the challenges are:

- Ensuring sufficient quantity and quality of available, non-agricultural and non-forest land to meet a reasonable scale of feedstock demand.
- Building institutions that facilitate between smallholder farmers upstream and the oil and biodiesel processing industries downstream.
- Determining minimum efficient scales for *Jatropha* growing and processing.
- Coordinating the timing of government investments in *Jatropha* research with the speed of the *Jatropha* biodiesel industry's development, and ensuring that the scale of implementation matches the appropriate scale suggested by research results.

Consequences of introducing non-domesticated *J. curcas* in large-scale culture

Large-scale monoculture plantations of non-domesticated *J. curcas* have their own consequences. The economies of scale favored by biofuels encourage acquisitions of large chunks of fertile land meant for food crops. This risk can be reduced by using land unsuitable for food crops. Simultaneously, large-scale farming is expected to contribute to on-farm and off-farm employment generation. The more specific problem in monocultures is management of insect pests and diseases that prompts uncertainties in yields. Achten et al. (2010) suggested that such problems could be overcome by initiating community-based small-scale plantations in multiple zones. This proposal can form a robust and sustainable base for *J. curcas* domestication. Yet another alternative is the practice of intercropping with seasonal and annual crops. The key requirement for large-scale plantations is the availability of high-quality, uniform planting material, which is still a major bottleneck in *J. curcas* cultivation. Since *J. curcas* is a non-domesticated crop, there is a risk of the crop becoming a weed; however, this still needs to be scientifically ascertained. Further, occurrence of vivipary was reported in *J. curcas* plantations in tropical, humid conditions (Deore and Johnson 2008a). If harvesting is delayed by few days, most of the seeds germinate under humid conditions. This reduces the seed yield per unit area with a threat to sustainable farming in large-scale plantations. Most tropical tree species have not been domesticated except for few species such as mango, tea, coffee and eucalyptus. Experiences with previous tree domestication efforts are useful to increase the efficiency of sustainable *J. curcas* plantation. The authors have listed the important targets for successful large-scale plantations (Fig. 2).

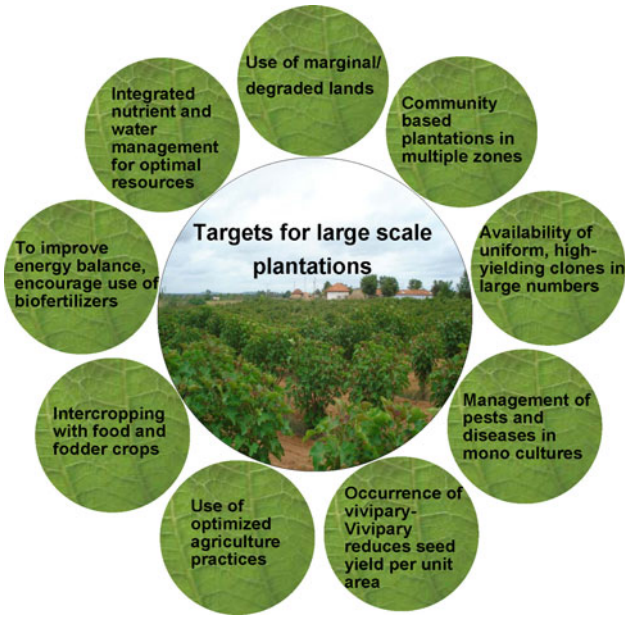

Fig. 2 Targets for large-scale *J. curcas* plantations. Sustainability of cultivation of *J. curcas* in large scale can be achieved by minimizing risks to environment and society. Genotypes with consistent yields, better agricultural practices and availability of uniform clones in large numbers might ensure success of large-scale plantation

Breeding targets for *J. curcas* crop improvement

A number of traits such as seed yield, oil content, seed toxicity, female to male flower ratio, increased branching, early flowering, synchronous maturity and adaptation to biotic and abiotic stresses are considered relevant for commercial exploitation of the crop (Fig. 3). Improvement in seed yield could be achieved by increasing the ratio of female flowers, number of branches, number of days from fruiting to maturity, stem diameter, total leaf area and modifying plant architecture (Abdelgadir et al. 2009). Increasing the oil content can be achieved by altering the expression levels of genes involved in fatty acid and triacylglycerol synthesis. Further, developing non-toxic varieties by blocking the expression of curcin and phorbol esters is important to make the crop environment friendly. Curcin and other toxic compounds are known to be toxic to both human handlers and environment in the long term. Increased seed yield was achieved conventionally by increasing the ratio of lateral branches (Abdelgadir et al. 2009) and by application of paclobutrazol, a growth regulator (Ghosh et al. 2010). Knowledge of pest resistance and drought resistance in wild *Jatropha* germplasm can be of great potential in improvement programs (Divakara et al. 2010; Shanker and Dhyani 2006). Further, FAO in its recently released review (Brittaine and Lutaladio 2010) highlighted several pro-poor breeding objectives, which will benefit small *Jatropha* growers, especially the small and marginal farmers. Improving drought resistance and productivity under abiotic stress conditions, enhanced resistance to pests and diseases, altered plant architecture to allow intercropping and mechanization, enabling easier shelling and seed crushing, increased adaptability and productivity under low-to-medium soil nutrient conditions, and plants with inedible leaves to ensure utility as a livestock hedge need immediate attention.

Breeding strategies

In vitro regeneration and genetic transformation

J. curcas has been extensively studied to develop an optimized regeneration protocol from different explants. Direct regeneration from leaf (Sujatha and Mukta 1996; Sujatha

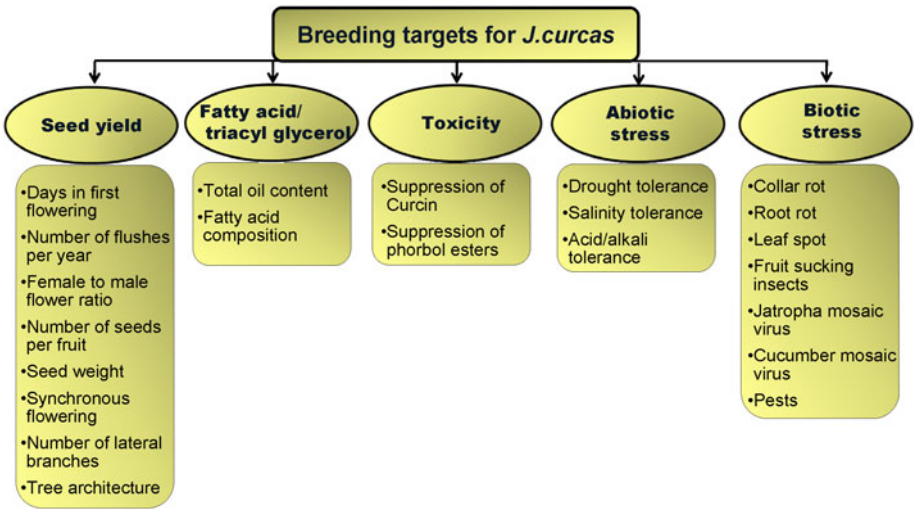

Fig. 3 Breeding goals for *J. curcas*. For maximizing yield, priority breeding objectives such as seed yield, oil content and quality, addressing biotic, abiotic stress tolerance and toxicity need to be considered

et al. 2005; Deore and Johnson 2008b; Khurana-Kaul et al. 2010), petiole (Sujatha and Mukta 1996; Kumar and Reddy 2010; Kumar et al. 2011), nodal segments (Datta et al. 2007), axillary nodes (Sujatha et al. 2005), shoot tips (Rajore and Batra 2005; Purkayastha et al. 2010), hypocotyls (Sujatha and Mukta 1996; Kaewpoo and Te-chato 2010), epicotyl (Qin et al. 2004; Kaewpoo and Te-chato 2010) and cotyledons (Kumar et al. 2010; Varshney and Johnson 2010; Khemkladngoen et al. 2011) have been reported. There are very few reports on regeneration of *J. curcas* through the intermediary callus phase. The explants tested for morphogenic studies were immature cotyledons and embryos (Varshney and Johnson 2010) and mature cotyledonary leaf (Sujatha and Mukta 1996; Jha et al. 2007; Khurana-Kaul et al. 2010). However, in vitro regeneration methods are beset with problems such as low root induction, high microbial contamination and high cost of production.

Genetic transformation is a pre-requisite for plant improvement through genetic engineering techniques. Li et al. (2008a) and Pan et al. (2010) established an efficient *Agrobacterium*-mediated gene transfer protocol in cotyledon explants of *J. curcas*. Purkayastha et al. (2010) and Joshi et al. (2011) used in vitro regenerated shoot apices for gene transfer through particle bombardment. Subsequently, in a concerted effort to develop an efficient protocol, Mazumdar et al. (2010) and Kumar et al. (2010) independently reported *Agrobacterium*-mediated gene transfer using leaf explants. These protocols have potential to facilitate genetic modification and subsequently to generate improved varieties of *J. curcas*. A more detailed review has been published by Mukherjee et al. (2011).

Molecular markers and genetic diversity analysis

The key for success of any genetic improvement program lies in the availability of adequate genetic variability for desired traits (Heller 1996). Genetic resources through global exploration, introduction, characterization and evaluation will provide a strong base for the development of elite varieties by various improvement methods. The fact that *J. curcas* has adapted itself to a wide range of edaphic and ecological conditions indicates the existence of considerable amount of genetic variability, which could be exploited for potential realization (Rao et al. 2008). Comprehensive work on collection, characterization and evaluation of germplasm for growth, morphology, seed characteristics and yield traits has been initiated by several independent groups in India, China, Brazil and Mexico with locally available germplasm and at Plant Research International (PRI), Netherlands on the global germplasm.

Genetic diversity based on morphological traits such as oil content, 100-seed weight, seed yield per plant, etc. has been studied. The studies indicated the existence of considerable genetic divergence among the accessions. The accessions having intermediate inter-cluster distance could be used in hybrid development for achieving maximum heterosis (Rao et al. 2009). Ginwal et al. (2004) and Kaushik et al. (2007) analyzed the genetic variability associated with growth performance, oil yield and seed traits of *J. curcas* accessions collected from the Indian states of Madhya Pradesh and Haryana. A study of biochemical traits for assessment of genetic relationships between 72 *J. curcas* accessions from 13 different countries (India, Cape Verde, Mexico, Madagascar, El Salvador, Uganda, Africa, Egypt, Vietnam, China, Malaysia, Philippines and Thailand) was carried out by Basha et al. (2009). Seed kernel protein, oil content, ash content and phorbol esters revealed variation between accessions from Mexico containing low phorbol esters. Based on morphological variations, several accessions were assembled *ex situ*, but evaluation of plants on a common site failed to show the initial variation, and differences in qualitative and

quantitative traits remained insignificant (http://www.novodboard.com/Jatropha-english.pdf). Diversity studies based on morphological characters are not very reliable as *J. curcas* exhibits enormous phenotypic plasticity and is strongly influenced by environment.

Molecular markers have been considered to have great potential for plant breeding in enhancing the efficiency of selection of desirable traits via marker-assisted breeding and understanding the genetic relationships, evolutionary trends and fingerprinting of varieties. Among the various molecular markers employed to study diversity, PCR-based markers such as RAPD (random amplified polymorphic DNA), ISSR (inter simple sequence repeats), SSR (simple sequence repeats), EST-SSRs and AFLP (amplified fragment length polymorphism) were the most preferred and have been used to study the extent of genetic diversity and phylogeny in *J. curcas* (Table 1). The studies on genetic diversity and phylogeny were carried out on *J. curcas* in many growing countries worldwide, while those with *Jatropha* species were confined to species native to India.

The earliest report of using RAPD markers to study the similarity between toxic Indian variety and non-toxic Mexican variety was by Sujatha et al. (2005), and the similarity index between these two accessions was 96.3%. The polymorphism generated by these primers served as unique fingerprints for identifying the India toxic variety and the non-toxic variety. Following this, Basha and Sujatha (2007) evaluated the genetic diversity among the *J. curcas* germplasm collected from various geographic locations in India along with a non-toxic genotype from Mexico using RAPD and ISSR markers, and the study indicated a narrow genetic base in the local germplasm. Studies carried out in different laboratories (Table 1) using different marker systems substantiated the fact that the local germplasm in India lacks adequate variability for exploitation in the breeding programs (Gupta et al. 2008; Umamaheswari et al. 2010).

Genetic variation reported in the molecular studies was mainly due to inclusion of wild species (Ganesh Ram et al. 2008; Ranade et al. 2008; Senthil Kumar et al. 2009) or geographically isolated germplasm (Sujatha et al. 2005; Basha and Sujatha 2007; Pamidimarri et al. 2009a). Some of the studies were confined to few accessions (<10) and limited number (<10) of primers. It is important to have a cautious approach while carrying out the genotyping assays, and there is need to consider the minimum population size, the number of data points and the polymorphism information content of the markers being employed, besides understanding the population structure in terms of its geographic isolation and mode of reproduction to draw meaningful conclusions. Regardless of the number of accessions used, the robustness of the primer and number of marker data points, all accessions from India clustered together confirming the existence of low genetic variation in the *J. curcas* ecotypes being genotyped. Similar results were obtained with germplasm from China and Brazil (Sun et al. 2008; Grativol et al. 2010; Rosado et al. 2010; Shen et al. 2010). Diversity analysis with local germplasm, thus, indicate the need for widening the genetic base of *J. curcas* through introduction of accessions with broader geographical background (Basha and Sujatha 2007; Ranade et al. 2008; Tatikonda et al. 2009; Sun et al. 2008; Shen et al. 2010). Cross-pollinating species have a significantly higher genetic diversity compared to self-pollinating species. Although a predominantly outbreeding species, *J. curcas* exhibited lower genetic variation in local populations, which could probably be due to its propagation through vegetative cuttings and or apomixis.

Based on the observations of existence of low genetic variation in local populations in different countries indicating a common ancestry, the need for assessment of genetic diversity in global germplasm has been realized. The studies of Sujatha et al. (2005), Basha et al. (2009), and Pamidimarri et al. (2009b) unequivocally established the fact that the non-toxic Mexican accessions (with low/nil phorbol esters) are genetically different from the toxic accessions. Ambrosi et al. (2010) analyzed 27 accessions of *J. curcas* from different geographic locations in the world using flow cytometric seed screening (FCSS) and ten RAPD primer markers to study the genetic diversity and reproductive strategy. The study reiterated the fact that the germplasm from different geographical regions had limited genetic variation with the exception of accessions from Mexico. Under the GJEP program at PRI, Netherlands, 60 accessions were studied for genetic variation using AFLP and NBS (motif binding) markers (Montes Osorio et al. 2008; Van Loo et al. 2008). High diversity was detected in accessions from the Central American region and a low diversity in accessions from Africa and India. Further, there is rich allelic diversity in germplasm from the Central American region (Basha et al. 2009; Popluechai et al. 2009), which could be utilized in the genetic improvement programs.

Genetic diversity present in *Jatropha* species was assessed using RAPD and ISSR markers to a great extent, but the studies were mostly limited to *Jatropha* species found in India (Table 1). The studies showed that the molecular diversity corroborates with the extensive morphological diversity available in the genus indicating higher possibilities of improving *J. curcas* by interspecific breeding (Pamidimarri et al. 2009a; Popluechai et al. 2009; Basha and Sujatha 2009; Senthil Kumar et al. 2009; Vijayanand et al. 2009; Tanya et al. 2010). Molecular markers such as RAPD were also used to confirm the hybridity in interspecific crosses (Dhillon et al. 2009). Recently, a new marker technique based on intron length

Table 1 A consolidated list of intraspecies and interspecies genetic diversity studies conducted in *J. curcas*

No.	Reference	Marker type used for analysis	Location of collection	Number of accessions/ species
1.	Sujatha et al. (2005)	RAPD	Andhra Pradesh	02
2.	Basha and Sujatha (2007)	RAPD, ISSR, SCAR	Tamil Nadu, Rajasthan, Kerala, Andhra Pradesh, Haryana, Madhya Pradesh,	42
3.	Ranade et al. (2008)	RAPD, DAMD	Uttar Pradesh, Uttaranchal, Sikkim, Arunachal Pradesh, Meghalaya, Orissa	12
4.	Gupta et al. (2008)	ISSR, RAPD	Uttaranchal, Rajasthan, Orissa, Uttar Pradesh	13
5.	Subramanyam et al. (2009)	RAPD	Andhra Pradesh, Orissa, Tamil Nadu, Karnataka, Jarkhand, Bhihar, Kerala, Madhya Pradesh, Uttaranchal, Uttar Pradesh, Haryana, Punjab, Assam, West Bengal, New Delhi, Gujarat, Meghalaya, Tripura, Chatthisgarh, Maharashtra	40
6.	Pamidimarri et al. (2009b)	RAPD, AFLP, SSR	Gujarat	07
7.	Tatikonda et al. (2009)	AFLP	Uttar Pradesh, Gujarat, Rajasthan, Andhra Pradesh, Chhattisgarh, Madhya Pradesh	48
8.	Jubera et al. (2009)	RAPD	Tamil Nadu, Madhya Pradesh, Maharashtra, Karnataka	07
9.	Umamaheshwari et al. (2010)	ISSR	Tamil Nadu	17
10.	Ikbal et al. (2010)	RAPD	Rajasthan, Punjab, Haryana, Madhya Pradesh, Gujarat	40
11.	Rao et al. (2009)	Morphological traits	Karnataka	–
12.	Pamidimarri et al. (2009d)	nrDNA ITS sequence	Gujarat	07
13.	Pamidimarri et al. (2009a)	RAPD, AFLP	Gujarat	07
14.	Ganesh Ram et al. (2008)	RAPD	Tamil Nadu, Andhra Pradesh	12
15.	Dhillon et al. (2009)	RAPD	Haryana	23 hybrids from cross of two species
16.	Kumar et al. (2009)	ISSR	Tamil Nadu	09
17.	Vijayanand et al. (2009)	ISSR, Morphological traits	Tamil Nadu	09
18.	Basha and Sujatha (2009)	RAPD, ISSR, SSR	Andhra Pradesh, Tamil Nadu	08
19.	Sujatha and Prabakaran (2003)	RAPD	Andhra Pradesh	Hybrids of two species
20.	Prabakaran and Sujatha (1999)	Isozyme	Tamil Nadu	03
21.	Basha et al. (2009)	RAPD, ISSR, SSR	India, Cape Verde, Mexico, Madagascar, Uganda, Africa, El Salvador, Egypt, Vietnam, China, Malaysia, Phillippines, Thailand	72
22.	Sun et al. (2008)	SSR, AFLP	South China Botanical Garden	58
23.	Wen et al. (2010)	EST-SSR, genomic SSR	Indonesia, Grenada, South America, Yunnan China, Hainan China	45
24.	Ambrosi et al. (2010)	RAPD, ISSR	Mexico, Brazil, South America, Africa, Western Africa, Sri Lanka, Jordan, South-east Asia, India	27
25.	Shen et al. (2010)	AFLP	Hainan China	38
26.	Xiang et al. (2007)	ISSR	Yunnan China	158
27.	Pamidimarri et al. (2009c)	SSR	Different parts of the globe	6 species of *Jatropha*
28.	Cai et al. (2010)	ISSR	China, Myanmar	224
29.	Tanya et al. (2010)	ISSR	Mexico, China, Vietnam, Thailand	39 accessions and 4 species
30.	Rosado et al. (2010)	RAPD, SSR	Brazil	192 accessions
31.	Subramanyam et al. (2010)	RAPD	India	10 accessions

The table depicts different marker types used for the studies (RAPD, SSR, AFLP, ISSR SCAR and ITS, apart from morphological traits), the location of collection and the sample size

polymorphism, termed as combinatorial tubulin-based polymorphism, producing codominant bands, was used to reliably detect the interspecific hybrids (Popluechai et al. 2009). In the genus *Jatropha*, existence of natural hybrids is reported and organelle-specific markers in combination with nuclear markers can be used to unravel the putative progenitors and also the direction of the cross (Basha and Sujatha 2009).

Initial studies on molecular markers for estimation of the extent of genetic diversity in *J. curcas* were carried out with markers such as, RAPD, ISSR and AFLP, which do not require prior sequence of the genome. With the advent of genomics and vast genomic databases for *J. curcas* and other economically important members of Euphorbiaceae, focus is now on sequence-tagged sites because these markers are highly polymorphic, codominant and reliable. Accordingly, SCAR markers have been developed for distinguishing the toxic and non-toxic accessions (Basha and Sujatha 2007; Basha et al. 2009). Among the different markers, microsatellite markers or simple sequence repeat markers are the choice due to their abundance, co-dominance, multi-allelic nature, easy transferability and amenability for multiplexing. Pamidimarri et al. (2009b) and Basha et al. (2009) identified SSR markers specific to the toxic and non-toxic varieties. However, the allelic diversity in local populations as disclosed by SSR markers remained low (Sun et al. 2008; Rosado et al. 2010; Ambrosi et al. 2010).

Owing to the high cost involved in the development of genomic SSR markers, the EST databases have been mined to select SSR-rich EST sequences. Also, the EST-SSRs from other Euphorbiaceae members were checked for their cross-taxa transferability to *J. curcas.* A total of 187 EST-SSR and 54 genomic SSR markers from *Manihot esculenta* (cassava) were found to be polymorphic in *J. curcas* (Wen et al. 2010). Out of these, 36 EST-SSRs and 20 genomic SSRs were used to study genetic diversity among 45 *J. curcas* accessions collected from different countries, such as Indonesia, South America, Yunnan and Hainan provinces in China and Grenada. The results suggested that genetic diversity between groups is greater than within groups and the cross-taxa transferability of ESTs from *M. esculenta* to *J. curcas* was demonstrated. Yadav et al. (2010) obtained 702 sequences containing 786 SSRs EST-derived SSR markers (from NCBI database) from *J. curcas* to study the transferability of these markers to other species and genera. Out of these, 92.4% were simple repeats and the remaining 7.6% were compound repeats. Twenty-one SSR markers were polymorphic and they were transferable across *Jatropha* species and also across the genera to *Ricinus communis*. In spite of extensive diversity, *J. curcas* is said to have a narrow genetic base, suggesting the need for further research on widening genetic base through mutations and intra/inter specific hybridization programs.

Analysis of whole genome and chloroplast genome

Since *J. curcas* has received tremendous attention as an oil source for biodiesel, transcriptome analysis has also gained importance worldwide to understand the functions of various genes involved in fatty acid biosynthesis and abiotic stress tolerance. Chromosomes of *J. curcas* are of very small size (bivalent length 1–3.67 μm) with most species having $2n = 22$ and base number of $x = 11$. It is an attractive candidate for genome sequencing with genome size (1C) estimated to be 416 Mbp (Carvalho et al. 2008). Recently, the Synthetic Genomics Inc. (SGI) and Asiatic Centre for Genome Technology (ACGT) have announced completion of the first draft of *Jatropha* genome project (http://www.acgt.asia/press/pdf/20May2009.pdf. Accessed date 15 Feb 2011). The sequencing of the genome, using both traditional Sanger sequencing and next-generation sequencing, has revealed that the *Jatropha* genome is approximately 400 Mbp in size, similar to the size of the rice genome. Further, Sato's group (Sato et al. 2011) recently announced whole genome sequencing using a combination of the Sanger method and new-generation multiple sequencing methods. The strategy is superior in that shortcomings of the respective methods are compensated for by each other. Moreover, it enables acquisition of sequences of higher quality at a lower cost within a short period of time. This is a breakthrough report and, for the first time, the whole genome sequence was made publicly available. The total length of non-redundant sequences was reported to be 285,858,490 bp constituting 120,586 contigs and 29,831 singlets, which is 70 and 75% of the whole genome of 410^5 and 318 Mb, respectively, estimated by flow cytometry. The coverage of gene space has been estimated to be 95%. From the sequencing, a total of di-, tri- and tetra-nucleotide SSRs were identified. The frequency of occurrence was one in every 7.0 Kb. The protein encoding genes were found to be 9,870. The basic structure of protein encoding genes in *J. curcas* are similar to those of *A. thaliana*. The authors declared set of genes that are involved in synthesis of triacylglycerols, phorbol ester biosynthesis, curcin, disease resistance; flowering related genes and several MADS-box genes were also present. The information generated from whole genome sequencing is expected to fill the current knowledge gaps and might accelerate the process of molecular breeding in *J. curcas.*

Simultaneously, the chloroplast genome of *J. curcas* has also been successfully sequenced (Asif et al. 2010). The complete nucleotide sequence of *J. curcas* chloroplast genome (cpDNA) was determined by pyrosequencing and gaps filled by Sanger sequencing. The cpDNA is a circular molecule of 163,856 bp in length and codes for 110 distinct genes (78 protein coding, 4 rRNA and 28 distinct tRNA).

The availability of the *Jatropha* chloroplast genome will be useful for designing vectors for efficient transformation to obtain better improved varieties (Asif et al. 2010).

Proteomics

Proteomic analysis in *J. curcas* is mainly confined to characterization of oil bodies and understanding oil biogenesis. Yang et al. (2009) studied oil mobilization during seed germination and post-germination development through proteomic analysis of endosperm in germinating seeds. Results showed that initiation of oil mobilization occurs during germination and subsequently the oil gets consumed during early seedling development. Several pathways such as β-oxidation, glyoxylate cycle, glycolysis, TCA cycle, gluconeogenesis and pentose phosphate pathways were found to be involved in oil mobilization. Proteomic analysis of the soluble proteins derived from embryo and endosperm of mature seeds of *J. curcas* was compared (Liu et al. 2009). The results indicated that both the tissues include proteins related to stress and signal transduction. The proteins in endosperm were predominantly the catabolism-related enzymes and reserves that provided nutrition for the growing embryo while the embryo-specific proteins were related to anabolism and utilized the nutrition from the endosperm for further growth. Popluechai et al. (2011) studied the proteomic composition of the oil bodies of *J. curcas* and related *Jatropha* species. The oil bodies revealed oleosins as the major components and three oleosins (*JcOle1, JcOle2, JcOle3*) were isolated and characterized at the gene, transcript and protein level. The transcript level of *JcOle3* was about fivefold higher as compared to the other two oleosins. Interestingly, this oleosin (*JcOle3*) showed allelic variation and single nucleotide polymorphism in its intron region, which could serve as marker in phylogenetic and molecular breeding studies.

Transcriptome analysis by expressed sequence tags (EST)

ESTs are short subsequences derived from randomly isolated cDNAs. EST databases provide comparative data for analyses of organisms that lack comparable genomic resources. EST data are also useful to identify differentially expressed genes from different parts of the plants. With respect to the vast amount of sequence data that is being generated, EST databases seem to be a practical alternative to whole genome sequencing, which is very expensive and not feasible for many organisms with large genomes.

A total of 42,747 ESTs and 14,007 partial and full length genes are available till date for *J. curcas* in the public domain (http://ncbi.nlm.nih.gov.in). This includes the most recent study by Costa et al. (2010) where they have generated 13,249 ESTs (dbEST accession numbers GT: 969394–982642) from two cDNA libraries constructed using developing and germinating *J. curcas* seeds. This EST library was constructed to detect the genes related to lipid synthesis and their degradation and also to identify ESTs coding for proteins involved in toxicity of *J. curcas* seeds. The study showed a large number of transposable element-related sequences in the developing seed library (800) as compared to germinating seed library (80).

Zhitao et al. (2007) had also constructed and analyzed ESTs from endosperm cDNA library of *J. curcas* to identify genes related to fatty acid synthesis and signal transduction. The remaining *Jatropha* ESTs submitted in NCBI were obtained from seed kernel and leaf cDNA libraries (http://ncbi.nlm.nih.gov.in). The sequence databases will be useful in understanding the oil biosynthesis pathway and obtaining improved varieties of *J. curcas* having higher oil yield and improved oil quality.

Our group at the Reliance Life Sciences is one of the few earliest groups that has deposited large-scale ESTs from *J. curcas* (http://www.ncbi.nlm.nih.gov/nucest), from which we obtained 1240 ESTs (dbEST accession numbers GO: 246457–247696) (http://jatrophagenomics.rellife.com/Est/ViewEST.aspx. Accessed date 15 Feb 2011) from salt-stressed roots of *J. curcas* (manuscript communicated). Our aim was to understand the differential expression of genes involved in various abiotic stress tolerance mechanisms. The gene expression analysis of few genes reported to be involved in abiotic stresses was studied. Validation of abiotic stress-responsive genes identified in the study could provide an ideal platform for improving *J. curcas* and other plant species to stress tolerance. The list of EST libraries currently available in NCBI database is presented in Table 2 .

There are various advantages and applications of EST libraries of which deriving microsatellite markers from ESTs is extensively exploited. Yadav et al. (2010) obtained EST-derived SSR markers (from NCBI database) in *J. curcas* to study the transferability to other species and genera. A total of 12,080 sequences, including 5,851 transcriptome contigs developed at NBRI and 5,002 singlets and 1,227 contigs assembled from 13,201 expressed sequence tags (ESTs) of *J. curcas,* downloaded from NCBI database were used to search for simple sequence repeats (SSRs). A total of 702 sequences containing 786 SSRs were obtained, of which 92.4% were simple repeats and the remaining 7.6% were compound repeats. Fifty randomly selected EST-SSR markers were tested on 25 accessions of *J. curcas* collected from different parts of India and on *Ricinus communis*. Twenty-one SSR markers were polymorphic and also showed transferability to five species of *Jatropha* and across the genera to *R. communis*. These EST-derived SSR markers will be useful in future studies

Table 2 List of EST libraries in *J. curcas* submitted to NCBI dbEST database

No.	Library	Tissue source	Number of ESTs	Main author	Year
1.	cDNA from phenotypic mutant	Leaves	09	Milella L.	2008
2.	cDNA from phenotypic wild type	Leaves	04	Milella L.	2008
3.	Root cDNA library	Root	1,240	T. Sudhakar Johnson	2009
4.	Total leaf cDNA library	Leaf from healthy plant	999	Rekha Singh	2008
5.	Salinity-stressed root cDNA	Root	64	T. Sudhakar Johnson	2009
6.	Seed cDNA library	Seed kernel	102	Rajani S. Nadgauda	2009
7.	cDNA of seeds from fruits of three stages of maturation	Seed	~10,000	Carels N.	2009
8.	Germinating and developing seed cDNA library	Seed	13,249	Marcio J. Da Silva	2010
9.	Seed-specific full-length normalized library	Seed	~7,000	Parani M	2010
10.	Flower and seed mixed library	Flower and seed	9,289	Ning Li	2010

Source: NCBI database, accessed on 13th Feb 2011

EST libraries were constructed from leaves, root, flower and seeds. Seed EST libraries account for maximum transcripts

in *Jatropha* such as genetic mapping, quantitative trait loci (QTL) analysis, marker-assisted molecular breeding, phylogenetic analysis, etc.

Identification of fatty acid biosynthesis genes

Although *J. curcas* is known for its wide adaptability and multiple uses, the full potential is far from being realized, the reason being the non-availability of improved varieties for specific growing conditions. *Jatropha* seed oil is similar to fossil diesel and has higher C18 fatty acid content (oleic, linoleic and stearic acid contents are 34.3–45.8%, 29–44.2% and 3.7–9.8%, respectively) and a lower C16 fatty acid content (14.1–19.5%). As part of the *J. curcas* improvement program, we initiated in vitro propagation (Deore and Johnson 2008b; Varshney and Johnson 2010; Varshney et al. 2011) and large-scale gene expression studies to understand the mechanisms of tolerance to biotic and abiotic stresses besides fatty acid biosynthesis (Eswaran et al. 2010).

Several reports are now available on isolation and characterization of novel genes, which are involved in the fatty acid biosynthesis pathway as a prelude to produce transgenic *Jatropha* plants with increased oil content (Fu et al. 2010; Gomes et al. 2010; Natarajan et al. 2010; Costa et al. 2010; Sato et al. 2011; Xu et al. 2011). Increasing the seed oil content can be achieved by altering the expression levels of enzymes in the triacylglycerol (TAG) biosynthetic (Kennedy) pathway. Overexpression of diacylglycerol acyltransferases has been shown to increase oil content in *Arabidopsis* (Jako et al. 2001) and soybean (Lardizabal et al. 2008). Genetic control of seed development and maturation and TAG biosynthesis in seeds has also been studied (Santos-Mendoza et al. 2008). Recently, a new full-length cDNA of stearoyl-acyl carrier protein desaturase was obtained by RT-PCR and RACE techniques from developing *Jatropha* seeds and the gene was functionally expressed in *E. coli* (Tong et al. 2006). This is an important enzyme involved in fatty acid biosynthesis in higher plants, and it also plays a key role in determining the ratio of saturated fatty acids to unsaturated fatty acids. Besides this, the full-length gene encoding a putative β-ketoacyl-acyl carrier protein (ACP) synthase III (*JcKAS III*) was cloned and sequenced from seeds of *J. curcas* (Li et al. 2008b). This gene was found to be expressed in all tissues with the highest expression in roots, which increased during seed development and is believed to be involved in carbon chain elongation. This study helps to provide an insight into the regulation of fatty acid biosynthesis and carbon chain elongation, which may ultimately help to improve oil production of *J. curcas* by producing transgenic plants through deployment of this gene (Li et al. 2008b). A number of studies have indicated that it is possible to increase the oil content of seeds via manipulation of the expression levels of key regulators of seed oil accumulation. For example, disruption of the homeobox gene *GLABRA2* led to increased oil content in *Arabidopsis* (Shen et al. 2006), and overexpression of soybean transcription factor *GmDOF4* and *GmDOF11* in *Arabidopsis* has also been shown to result in increased oil content (Wang et al. 2007).

For further understanding the fatty acid biosynthesis, a gene from *J. curcas* designated *JcFATB1,* which encodes a putative acyl-ACP TE (likely involved in the termination of carbon chain elongation), was cloned and sequenced (Wu et al. 2009). Seed-specific overexpression of this gene was studied in *Arabidopsis*. To elucidate the substrate specificity of *JcFATB1 in planta*, the gene was expressed in *Arabidopsis* under the control of a seed promoter. It was observed that the transcript levels of *JcFATB1* in the

transgenic *Arabidopsis* plants were higher as compared to controls. The saturated fatty acid content of C16:0, C18:0 and C20:0 was also found to be increased while the unsaturated fatty acid content was decreased.

Seed development in *J. curcas* has always been an important field of study. Annarao et al. (2008) have carried out the lipid profiling of developing *J. curcas* seeds using ^{1}H NMR spectroscopy. They studied the variations in free fatty acid (FFA), triglycerol esters (TAG), polyunsaturated fatty acids (PUFA) and MUFA apart from methyl esters of fatty acids (FAME) and sterols at various stages in development of the seeds. These findings will be helpful in understanding oil biosynthesis and are useful in the efforts to improve biosynthesis of TAG and reduce the FFA content.

A full-length cDNA of the carboxyltransferase (*accA*) gene of acetyl-coenzyme A (acetyl-CoA) carboxylase from *J. curcas* was cloned and sequenced (Xie et al. 2010). Fluorogenic real-time polymerase chain reaction (RT-PCR) studies revealed increased expression levels of the *accA* gene in leaves at early, middle and late stages under pH 8.0 stress compared to pH 7.0. Similarly, the expression levels in fruits showed a significant increase under dark conditions compared to the control. Under light stress, the expression levels of the *accA* gene in the fruits at early, middle and late stages showed the largest fluctuations compared to those of the control. The findings suggested that the expression levels of the *accA* gene are closely related to the growth conditions and developmental stages in the leaves and fruits of *J. curcas*. Similarly, Xu et al. (2011) investigated the temporal expression of 21 lipid genes involved in fatty acid and triacylglycerol synthesis in developing seeds of *J. curcas* by quantitative real-time PCR. This study provided a glimpse of the global pattern of gene expression and regulation that are critical to understand the molecular basis of lipid synthesis. The list of genes involved in fatty acid biosynthesis being cloned from *J. curcas* is presented in Table 3.

Ye et al. (2009) used virus induced gene silencing (VIGS) to investigate possible functions of 13 *J. curcas* genes of several functional categories, including fatty acid biosynthesis, developmental regulation and toxin biosynthesis. It was found that *KASII* and *FATB* genes played key role in regulating fatty acid chain length and the saturated/unsaturated ratio. It was also found that *SAD1* (stearoyl-ACP desaturase-1) was highly expressed in seeds during the triglycerides accumulation stage. In parallel, oil mobilization in germinating seeds was studied by ultrastructural observation and proteomic analysis of endosperm in germinating seeds of *J. curcas* (Yang et al. 2009). Results showed that oil mobilization was initiated during germination, and the oil was consumed for early seedling development. The authors related several pathways including β-oxidation, glyoxylate cycle, glycolysis, citric acid cycle, gluconeogenesis and pentose phosphate pathway in oil mobilization.

Molecular approaches to biotic and abiotic stress tolerance

Understanding plant responses to abiotic stress is of particular interest to develop improved varieties that can tolerate harsh environmental conditions such as salinity, drought and oxidative damage (Yoshida 2002). Although known to thrive under adverse climatic conditions, the productivity of *J. curcas* on marginal and sub-marginal lands is not impressive. Therefore, efforts were made by several researchers to understand the mechanism of abiotic stress tolerance as a prelude to develop stress-tolerant crops. Eswaran et al. (2010) developed a fast approach to screen a number of genes for abiotic stress tolerance using *shs* (salt hypersensitive) mutants of yeast. Initially, three *shs* mutants were generated by random mutagenesis that exhibited growth retardation beyond 500 mM NaCl, which were used to screen the cDNA library generated from salt-stressed roots of *J. curcas*. From 20,000 yeast transformants with *J. curcas* cDNA libraries, 31 full-length genes were obtained that can confer salt tolerance (Table 4). The authors demonstrated that the method of yeast functional screening is rapid and serves a universal assay system for large-scale screening of genes involved in abiotic stress tolerance.

During abiotic stress, plants undergo a series of physiological changes to cope up with external stress. Protective mechanism against oxidative damage during drought and heat stress was studied by Silva et al. (2010a). It was observed that the leaf CO_2 assimilation rate, stomatal conductance and instantaneous carboxylation efficiency were significantly decreased under stress conditions. The oxidative enzymes, CAT and superoxide dismutase were stimulated only under heat conditions, whereas APX activity increased in drought and heat conditions. The authors noted that at high temperature, *J. curcas* plants do not have efficient mechanism for protection against drought-induced oxidative stress. The same group studied the effects of salinity and water stress on photosynthesis and water relations (Silva et al. 2010b). It was found that PEG-stressed plants suffered higher restrictions in leaf growth compared to salt-stressed plants. Furthermore, only PEG treatment caused pronounced effect on leaf membrane integrity. The authors concluded that the physiological alterations could represent adaptive mechanisms employed by *J. curcas* to cope with stress.

Accumulation of organic and inorganic solutes have a major role in plant adaptation to stress via adjusting osmoticum. In *J. curcas* plants subjected to water deficiency, K^+ ions followed by Na^+ and Cl^- ions participated in osmotic adjustments in both leaves and roots (Silva et al. 2010c). Of the organic solutes studied, soluble sugars showed highest participation in osmotic adjustment of the

Table 3 Genes cloned and characterized in *J. curcas*

No.	Name of gene	Length (bp)	References
1.	Aquaporin	843	Zhang et al. (2007)
2.	3-Hydroxy-3-methylglutaryl coenzyme A	1,950	Lin et al. (2009)
3.	Geranylgeranyl diphosphate synthase	1,110	Lin et al. (2010)
4.	Curcin 2	927	Qin et al. (2005)
5.	JcERF	774	Tang et al. (2007)
6.	Betaine aldehyde dehydrogenase	1,509	Zhang et al. (2008)
7.	Curcin	882	Lin et al. (2003)
8.	B-ketoacyl-acyl carrier protein (ACP) synthase III	1,203	Li et al. (2008b)
9.	JcFATB1	1,257	Wu et al. (2009)
10.	Stearoyl-acyl carrier protein desaturase	1,191	Tong et al. (2006)
11.	Plastidial ω3 fatty acid desaturase	1,368	Guo et al. (2008)
12.	Superoxide dismutase (SOD)		Gao et al. (2008a), Yan et al. (2008), Luo et al. (2010)
13.	Peroxidase (POD)		Gao et al. (2008a), Yan et al. (2008), Luo et al. (2010)
14.	Catalase (CAT)		Gao et al. (2008a), Yan et al. (2008), Luo et al. (2010)
15.	Phenylalaline ammonia lyase (PAL)		Gao et al. (2008b), Yan et al. (2008), Luo et al. (2010)
16.	Carboxyltransferase of ACCase	1,149	Xie et al. (2010)
17.	Allene oxide cyclase	924	Liu et al. (2010a)
18.	Phospholipase D	2,427	Liu et al. (2010b)
19.	AcylCoA: Diacylglycerol acyltransferase-1 (DGAT1)		Xu et al. (2011)
20.	Micosomal 3 fatty acid desaturase		Xu et al. (2011)
21.	Glycerol-3-phosphate dehydrogenase (GPD)		Xu et al. (2011)
22.	Oleosin 1		Xu et al. (2011)
23.	Oleosin 2		Xu et al. (2011)
24.	Homomeric acetyl-CoA carboxylase		Sato et al. (2011)
25.	Acyl-CoA synthetase		Sato et al. (2011)
26.	Malonyl-CoA: ACP acyltrasferase		Sato et al. (2011)
27.	Acyl-ACP Thioesterase A		Sato et al. (2011)

A select list of genes cloned with an aim to understand abiotic stress tolerance mechanism and fatty acid biosynthesis. Majority of the genes listed here have role in fatty acid biosynthesis

cell. In addition, free amino acids and glycine betaine also effectively contributed to the reduction in osmotic potential. These results suggest that *J. curcas* young plants adjust their cellular osmoticum by participation of inorganic and organic solutes and stomatal closure during stress conditions. Likewise, Wang et al. (2011) studied the expression of myo-inositol during abiotic stress. They have cloned and characterized *J. curcas D*-myo-inositol-3-phosphate synthase (*JcMIPS*) invloved in myo-inositol biosynthesis by mRNA differential display technology (DDRT) and RACE. It was found that the transcripts of *JcMIPS* were high in seed and leaves and low in stem and flowers. The transcripts of *JcMIPS* were up-regulated when exposed to abscisic acid (100 μM), drought (30% PEG-6000), NaCl (200 mM) and low-temperature (4°C) treatments indicating their role in abiotic stress responses.

To understand and correlate the gene expression pattern in stress determination, the expression analysis of five selected genes, *viz.,* late embryogenesis abundant protein-5 (*LEA-5*), cytosolic ascorbate peroxidase (*Apx-1*), metallothionein, profilin and annexin, in leaf and root tissues of *J. curcas* was performed (Eswaran et al. 2010). Gene expression data indirectly suggest varied modes of gene regulation between the root and leaf tissues in *J. curcas* and indicate a complex framework for gene regulation during adaptation to salt stress in different tissues (Eswaran et al. 2010).

Aquaporins are membrane proteins or major intrinsic proteins (MIPs) that form water channels or pores in biological cell membranes, controlling transmembrane water movement in plants and are thought to be involved in plant adaptation to drought stress. These channels are widely distributed in all kingdoms of life including bacteria, plants and mammals (Amiry-Moghaddam et al. 2005). In plants, aquaporins are present in multiple isoforms. The sequence relationship between all plant MIPs like cDNAs indicates that the encoded proteins fall into four sequence subclasses: (1) tonoplast intrinsic proteins (TIP) localized to the

Table 4 Cloning of full-length genes derived from salt-stressed root cDNA library of *J. curcas*[*]

Sr. no.	GenBank accession no.	Function of the gene	Length of gene (bp)
1.	FJ: 489601	Allene oxide cyclase	777
2.	FJ: 489602	Thioredoxin H-type (*TRX-h*)	357
3.	FJ: 489603	Metallothionein	234
4.	FJ: 489604	Heterotrophic ferredoxin	492
5.	FJ: 489605	Defensin	234
6.	FJ: 489606	Calmodulin-7 (*CAM-7*)	810
7.	FJ: 489607	Major allergen Pru ar1-like protein	495
8.	FJ: 489608	S18.A ribosomal protein	495
9.	FJ: 489609	60S ribosomal protein L18a	537
10.	FJ: 489610	Protease inhibitor/seed storage/lipid transfer protein family	348
11.	FJ: 619041	Membrane protein-2	189
12.	FJ: 619042	Late embryogenesis abundant protein 5 (*LEA-5*)	267
13.	FJ: 619043	Cold-induced plasma membrane protein	174
14.	FJ: 619044	Cytosolic ascorbate peroxidase-1 (*Apx-1*)	753
15.	FJ: 619045	Profilin-like protein	384
16.	FJ: 619046	Caffeoyl-CoA-O-methyltransferase (*CCoAOMT*)	741
17.	FJ: 619047	Eukaryotic translation initiation factor SUI1	381
18.	FJ: 619048	Copper chaperone	282
19.	FJ: 619049	Ubiquitin conjugating enzyme 2 (*JcE2*)	447
20.	FJ: 619050	Mitochondrial ATP synthase 6 KD subunit (*JcMtATP6*)	171
21.	FJ: 619051	Ferritin-2, chloroplast precursor	771
22.	FJ: 619052	Annexin-like protein	945
23.	FJ: 619053	Al-induced protein	711
24.	FJ: 619054	Avr9/cf-9 rapidly elicited (*JcACRE*) gene	231
25.	FJ: 619055	60S ribosomal protein L39	156
26.	FJ: 619056	Ribosomal protein L37	291
27.	FJ: 619057	Ribosomal protein L15	729
28.	FJ: 623457	40S ribosomal protein S15	456
29.	FJ: 623458	40S ribosomal S18	459
30.	FJ: 623459	Plant lipid transfer/seed storage/trypsin-alpha amylase inhibitor	306
31.	FJ: 623460	Low-molecular weight cysteine rich 69	234

The genes with role in abiotic stress tolerance have been obtained from yeast functional genetic screen using *shs* (salt hypersensitive strains)

[*] All the genes have role in abiotic stress tolerance (Eswaran et al. 2010)

tonoplast; (2) plasma membrane intrinsic proteins (PIP) that are localized to the vacuolar/plasma membrane; (3) nodulin-26, which is expressed in the peribacteroid membrane of root symbiotic nodules; and (4) small basic intrinsic proteins (SIP). PIPs are further classified on the basis of their amino acid sequence into PIP1 and PIP2 subgroups. As compared to PIP1, PIP2 proteins possess a shorter N-terminal extension and a longer C-terminal end containing a putative phosphorylation site (Fetter et al. 2004; Johanson and Gustavsson 2002). Recently, a new full-length cDNA encoding aquaporin (*JcPIP2*) was isolated from seedlings of *J. curcas* and its role in drought responses was studied. Severe drought conditions were imposed in *J. curcas* through addition of PEG6000 in different concentrations of 10, 20, 30 and 40%. It was observed that with increasing levels of drought stress, the level of *JcPIP2* increased indicating its role in drought resistance (Zhang et al. 2007). The presence of aquaporins is considered to play an important role in the rapid growth of *J. curcas* in dry weather conditions.

A novel betaine aldehyde dehydrogenase gene (*BADH*) called *JcBD1* has been isolated from *J. curcas.* It was observed that *JcBD1* gene transcript levels were 79% higher in case of plants exposed to drought (30% PEG), salt (300 mM NaCl) and heat (50°C) stress as compared to control plants, indicating its role in conferring tolerance to all the major abiotic stresses. The gene was also expressed in *E. coli* and was shown to confer resistance to salt stress (Zhang et al. 2008). A ribosome inactivating protein (*Curcin 2*) gene was isolated from cDNA of *J. curcas*

leaves under drought stress, temperature stress and biotic stress. Biotic stress was mitigated by injecting pathogens for fungal infections of *Pestalotia funereal*, *Curvularia lunata* (Walk) Boed and *Gibberelle zeae* (Schw.) Petch. It is known that curcin gene cannot be expressed in leaves whether under stress or non-stress conditions. But it was found that *Curcin 2* gene expresses in leaves of seedlings under biotic and abiotic stresses (Qin et al. 2005).

The role of antioxidant enzymes, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in NaCl-mediated stress was recently conducted on seedlings of *J. curcas* (Gao et al. 2008a). SOD is one of the several important antioxidant enzymes with the ability to repair oxidative damage caused by reactive oxygen species (ROS), while POD is involved in various processes including lignification, auxin metabolism, salt tolerance and heavy metal stress. CAT is considered to be the most effective antioxidant enzyme against oxidative damage, which is involved in the degradation of hydrogen peroxide into water and oxygen. Increased activity of POD, SOD and CAT in cotyledon, hypocotyls and radicles of *J. curcas* under salt stress indicated ROS-mediated oxidative tolerance during salt stress. Another enzyme phenylalanine ammonia lyase (PAL), a marker in environmental stress, also showed increased activity in cotyledon, hypocotyls and radicles during NaCl-mediated stress (Gao et al. 2008a).

Similarly, a study was conducted to assess the effect of high concentrations of nickel (100, 200, 400 and 800 μmol) (heavy metal stress) on *J. curcas* seedlings and the correlations with the activities of SOD, POD, CAT and PAL enzymes were observed (Yan et al. 2008). Results showed a negative correlation of the activity of SOD, POD and CAT with nickel concentrations, with the activity highest at 400, 200 and 200 μmol, respectively, of nickel. However, PAL had a positive correlation with the highest activity at 400 μmol of nickel. Hence, lower nickel concentrations and higher SOD, POD, CAT and PAL activities suggest the tolerance capacity to protect the plant from oxidative damage due to heavy metal stress (Yan et al. 2008). The effect of different zinc concentrations (0, 0.25, 0.5, 1, 2 and 3 mM) on *Jatropha* seedlings and its influence on antioxidant enzymes such as SOD, POD, CAT and PAL have also been reported (Luo et al. 2010). In this study, the biomass of cotyledons, hypocotyls and radicals was increased with increasing concentrations of zinc. The activities of SOD and POD increased gradually with an increase in the zinc concentration. CAT activity in cotyledons, hypocotyls and radicles reached the largest increments at concentrations of 0.5, 2 and 0.5 mM, respectively, and a similar trend was observed in case of PAL activity. The study indicated an important role of SOD, POD, CAT and PAL enzymes in the defense mechanisms of *J. curcas* exposed to excess metal. Another study on similar lines reported that increased SOD, POD and PAL activities are involved in the defense mechanism of *J. curcas* radicles against lead toxicity (Gao et al. 2009). Changes in protein content, SOD, CAT, POD and PAL activities in *J. curcas* seedlings were positively correlated with copper concentrations (Gao et al. 2008b). The results confirm that the ability of *J. curcas* to sustain metal stress depends on oxidative stress defense mechanisms.

The role of transcription factors and regulatory elements in the manipulation of the expression of key genes during abiotic stress tolerance has also been studied. Tang et al. (2007) isolated and functionally characterized *JcERF* gene, a putative *AP2/EREBP* domain containing transcription factor from *J. curcas*. The *AP2/EREBP* proteins in plants are the largest transcription factors family having a role in plant development and responses to ethylene, disease and other biotic and abiotic stresses. In Jatropha, this gene was isolated from seedlings under high salt stress. It was seen that expression of the *JcERF* gene was upregulated upon imposing stress to salinity, drought, ethylene and mechanical wounding proving that this gene has a role in tolerance to abiotic stresses in *J. curcas* as well. Photosynthetic characteristics and chlorophyll fluorescence parameters have been studied in *J. curcas* under drought stress (Dou et al. 2008) and cold stress (Liang et al. 2007). Under drought stress with lower concentrations (≤15%) of PEG, the photosynthetic rate (P_n), stomatal conductance (G_s) and intercellular carbon dioxide concentration (C_i) of the seedlings decreased with increasing PEG concentration, while the chlorophyll fluorescence parameters did not change. Interestingly, there was a drop in PSII activity under drought stress, which recovered immediately when drought was relieved indicating that *J. curcas* has strong tolerance to drought stress (Dou et al. 2008). Similarly under cold stress (4°C), eight photosynthetic-related proteins significantly changed (Liang et al. 2007). The chlorophyll fluorescence parameters were also sensitive to cold stress. There was correlation between photosynthetic-related proteins and chlorophyll fluorescence parameters indicating that the early stage (0–12 h) acclimation of PS II and the late stage (after 24 h) H_2O_2 scavenging might be involved in cold response mechanisms in *J. curcas*.

Guo et al. (2008) identified and cloned a plastidal ω3 fatty acid desaturase gene from leaves of *J. curcas,* which is involved in the synthesis of trienoic fatty acids. It is known that high ω3 fatty acid content in leaves can increase the plant's tolerance to cold stress. Hence, by identifying this gene in *Jatropha*, it will be useful to understand the molecular mechanism of cold tolerance in *J. curcas*. It is known that soil alkalinity can also be a stress to plants. When the effect of alkalinity (Na_2CO_3–0.1, 0.2, 0.3, 0.4 and 0.5%) was studied on *J. curcas* seedlings, it

was observed that with an increase in alkalinity there was reduction in growth. But when some beneficial microbes (*Azotobacter microfoss* and arbuscular mycorrhizal fungi, AMF) were added in combination, it was observed that different combinations of these microbes with 0.4% sodium carbonate increased the survival percentage over control plants. The combination of AM fungi and *Azotobacter* increased plant height, shoot diameter, shoot dry weight, leaf relative water content and soluble sugar content and decreased the level of soluble protein at 0.4% of Na_2CO_3 over other treatments (Kumar et al. 2009), indicating the beneficial effects of AMF during plant's exposure to stress conditions.

Allene oxide cyclase (AOC) is a key enzyme in the jasmonate biosynthetic pathway. A cDNA encoding *AOC*, named *JcAOC*, has been cloned from *J. curcas* (Liu et al. 2010a). Phylogenetic analysis indicated that *JcAOC* belonged to the *AOC* superfamily. Semi-quantitative RT-PCR analysis revealed that *JcAOC* mRNA was expressed in roots, stems, leaves, young seeds, endosperms and flowers, but that the expression level was highest in leaves and lowest in seeds, and mRNA expression of *JcAOC* could be induced by salt stress (300 mM NaCl) and low temperature (4°C). Furthermore, the full-length coding region of *JcAOC* excluding signal peptide sequence was inserted into pET-30a and was successfully expressed in *E. coli*. Overexpression of *JcAOC* in *E. coli* conferred its resistance to salt stress and low temperature (Liu et al. 2010a).

Phospholipase D *(PLD)* is a key enzyme in plants involved in phospholipid catabolism, initiating a lipolytic cascade in membrane deterioration during senescence and stress. Liu et al. (2010b) by semi-quantitative RT-PCR analysis revealed its abundance in root, stem, leaf, endosperm and flower, and its weak expression in seed. Further, the *JcPLDα* was increasedly expressed in leaf undergoing environmental stress such as salt (300 mM NaCl), drought (30% PEG), cold (4°C) and heat (50°C). The *JcPLDα* protein was successfully expressed in *E. coli* and showed high enzymatic activities**.**

Expression of LEA genes, major intrinsic proteins, ROS-mediated oxidative tolerance, transcription factors and regulatory elements during abiotic stress response in *J. curcas* can pave the way to understanding the stress responses and can be a target for plant improvement under abiotic stress conditions.

Biotic stresses

Significant yield losses due to insect pests (Fig. 4), fungi and viruses have been reported in plantations under humid conditions. Reported diseases are 'collar rot' (*Macrophomina phaseolina* or *Rhizoctonia bataticola*) at juvenile stages or waterlogging at adult stage, leaf spots (*Cercospora jatrophae-curcas*, *Helminthosporium tetramera),* root rot (*Fusarium moniliforme*) and damping off (*Phytophthora* spp.), etc. Fruit-sucking damage caused by insects such as *Calidea* and *Nezara viridula* was also reported. Narayana et al. (2006) reported the occurrence of Jatropha mosaic virus and cucumber mosaic virus disease in a number of plantations in India. In Nicaragua, *Heteroptera* spp. have been reported to affect the *J. curcas* nut. Damage by bugs such as *Scutellera nobilis* and *Pempelia morosalis* have also been observed in some *Jatropha* monocultures. Pest and pathogen-resistant varieties can considerably increase yields. While most success with transgenics for insect resistance is through deployment of crystal toxin genes from the bacterium, *Bacillus thuringiensis* (ISAAA 2010 http://www.isaaa.org/resources/publications/pocketk/6/default.asp), there is an immediate need to identify suitable candidate genes for the major biotic agents. The transcription factor, *AP2/EREBP*, is known to have a role in the plant's response to biotic and abiotic stress and has been identified by Tang et al. (2007). In addition, several antioxidant enzymes such as CAT and PAL are also known to be expressed during biotic stress.

Molecular biological approaches toward regulation of secondary plant metabolites and phorbol esters

Apart from the seed oil, *J. curcas* is a source of many phytochemicals of which some of them are found to be toxic. The plant contains alkaloids, lignans, essential amino acids, cyclic peptides and terpenes. The toxicity is due to the presence of curcin, a ribosome inactivating protein (RIP), phorbol esters, saponins and trypsin inhibitors in the seeds. Chemical detoxification procedures are available for reducing/eliminating most of these compounds with the exception of phorbol esters. The full-length curcin has been isolated, cloned and sequenced (Lin et al. 2003; Qin et al. 2009). The phorbol esters, which are tetracyclic diterpenoids, are known for their tumor-promoting and cytotoxic activities (Devappa et al. 2010). The toxicity of phorbol esters limits the use of by-products to be used as animal and poultry feed. As diterpenes, phorbol esters are formed from the isoprenoid pathway. The 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) probably acts as one of the key regulatory enzymes for phorbol ester biosynthesis in the mevalonic acid pathway (MVA). Lin et al. (2009) cloned and characterized the HMGR gene from *J. curcas*. The functional expression was carried out in *E. coli* where the authors have shown accumulation of *β*-carotene, a product of MVA pathway. Further, the same group studied the functional analysis of another gene encoding geranylgeranyl diphosphate synthase (GGPPS) from *J. curcas* (Lin et al. 2010). GGPPS is involved in the biosynthesis of

Fig. 4 Infestation of red hairy caterpillar in *J. curcas* plantations. (*inset*), caterpillar feeding on *J. curcas* leaf. Several red hairy caterpillars feed in clusters on leaves which affect productivity in large-scale monocultures of *J. curcas*

diterpenes. The study by Lin's group involving genes of the MVA pathway might pave the way to map and regulate phorbol ester biosynthesis in *J. curcas*. Further characterizing the genes will be useful in understanding the regulation of phorbol ester biosynthesis and to provide insights into decreasing the toxin content using biotechnology (Lin et al. 2010).

Conclusion and future perspectives

As the scale of *J. curcas* cultivation increases, it is imperative to develop improved varieties that could be commercially exploited. Major research efforts are focused on the development of crop management practices, cultivation, genetic improvement, canopy management and mutant development. To fulfill the requirements of a successful energy crop, concerted efforts should be on producing *J. curcas* feedstock with better water-use efficiency, greater net energy gain and low recalcitrance. There has been considerable progress in identifying key genes involved in fatty acid synthesis. The information obtained can be successfully utilized to develop varieties with improved oil content.

Abiotic stress factors such as sub-optimal water levels limit optimal production. Therefore, stress tolerance traits are important to enable production of *J. curcas* on marginal or sub-marginal lands that are generally not favorable for food crops. Stresses due to drought, metal, salt, cold and heat induce similar physiological and transcriptional responses in plants, despite the induction of different set of genes in response to various stimuli. Understanding the upstream pathways and expression profiles in combination with measurement of physiological parameters will lead to success in translation research. Likewise, insect pests and diseases have become a major limitation under monoculture. Suitable candidate genes conferring resistance to the major biotic stresses need to be deployed through biotechnological tools.

The extensive genetic diversity studies carried out on different accessions from several geographical locations indicate that *J. curcas* has narrow genetic base. There is a need for widening the genetic base through the introduction of accessions with broad geographical background and creation of additional genetic variation through induced mutations and wide hybridization. The sequence information of *J. curcas* genome can be used to develop SNP markers and exploit them in various applications of molecular biology in *J. curcas.* The genome sequence of castor is also deciphered and can be used for synteny analysis and comparative genomics.

The future of *J. curcas* as a sustainable energy crop will largely depend on successful integration of molecular breeding approaches coupled with metabolic engineering. The efforts of the authors in identifying abiotic stress tolerance governing factors led to major discovery of transcriptional elements and genes in the stress tolerance pathway. The characterized genes and outcome of the results can be successfully utilized in molecular breeding of stress resistant *J. curcas.* As a newly emerged energy crop, *J. curcas* is still in the initial developmental stage and might need some more gestation period before it can be exploited as a commercially successful crop.

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