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REVIEW PAPER

Potential of *Jatropha curcas* as a source of renewable oil and animal feed

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Abstract

***Jatropha curcas* (L.) is a perennial plant of the spurge family (Euphorbiaceae). Recently, it has received much attention as a potential source of vegetable oil as a replacement for petroleum, and, in particular, the production of biodiesel. Despite the interest that is being shown in the large-scale cultivation of *J. curcas*, genetic resources remain poorly characterized and conserved and there has been very little plant breeding for improved traits. At present, the varieties being used to establish plantations in Africa and Asia are inedible. The meal obtained after the extraction of oil cannot, therefore, be used as a source of animal feed. Naturally existing edible varieties are, however, known to occur in Mexico. The toxic components of *J. curcas* seeds, the potential for plant breeding to generate improved varieties, and the suitability of *J. curcas* oil as a feedstock for biodiesel production are discussed.**

Key words: Biodiesel, curcin, *Jatropha curcas*, oilseed, phorbal ester, ribosome inactivating protein, seed meal.

Introduction

Plant oils, in addition to being a food commodity, are important as a renewable resource for both the fuel and chemical industries (Dyer and Mullen, 2008). Demand for vegetable oils as a source of biodiesel in particular has increased recently due to a number of factors, including increased prices of petroleum, the desire to reduce CO_2 emissions, and fuel security. At the same time, there are increased demands on land, water, and other resources used in the production of food for a growing world population.

Most of the crops grown today, including oilseeds, are annuals. Perennial crops have deeper root systems, which help store more carbon, maintain soil quality, and manage water and nutrients more conservatively. They have therefore been advocated as potentially more efficient ways of farming, especially on marginal soils (Cox *et al.*, 2006; Glover *et al.*, 2007). For the production of plant oils, *Jatropha curcas* is one species that has received much attention recently (Gubitz *et al.*, 1999; Achten *et al.*, 2007; Fairless, 2007). It is a tropical species, although it has a broader geographical range than oil palm. Figure 1 shows the approximate geographic distribution of the species. Much of the interest in *J. curcas* has arisen due to its ability to grow on 'marginal land'. Although this term is poorly defined, it is generally used to describe land that is of poor quality in terms of agricultural use and typically not used for crop cultivation. Using marginal land for *J. curcas* cultivation is therefore attractive since it would not displace food-producing crops. Current estimates suggest that there are now 2.5 million hectares of *J. curcas* planted in India and China alone, with plans for an additional 23 million acres by 2010 (Fairless, 2007). Interest in the cultivation of *J. curcas* is coming from both the public and private sectors, and a number of public companies are now involved in *J. curcas* cultivation including D1 Oils plc (www.d1plc.com), Viridas plc (www.viridasplc.com), and Energem Resources Inc (www.energem.com).

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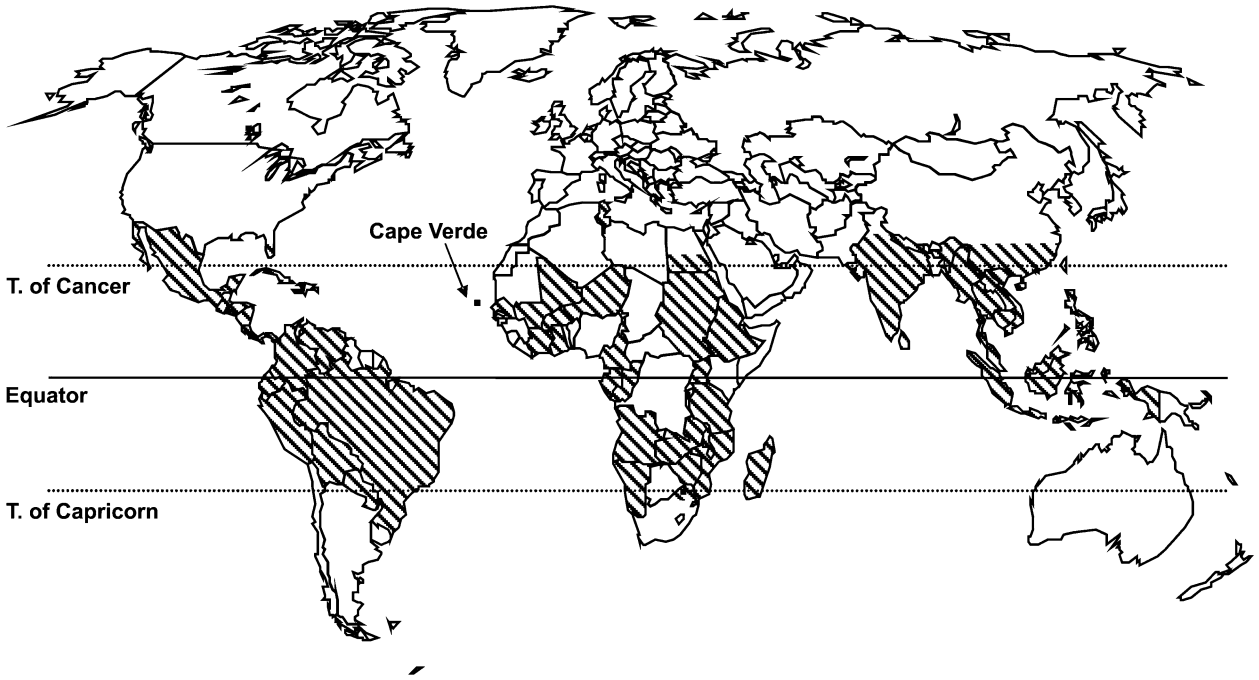

Fig. 1. Approximate global distribution of *J. curcas*. Shaded regions indicate areas in which *J. curcas* is found. Data obtained from Heller (1996) and from a search of the Missouri Botanical Garden databases (http://www.mobot.org/MOBOT/Research/herbarium.shtml).

Despite the interest in *J. curcas*, the available yield performance data for this species are limited and somewhat uncertain. A summary of reported yields was compiled by Heller (1996). Most of the available yield information has been obtained from plants which have not yet reached maturity (<5 years old). Two publications which present yield data for mature plantations (Matsuno *et al.*, 1984; Foidl *et al.*, 1996) suggest yields of 4 and 5 tonnes of seed per hectare, respectively. This would equate to approximately 1.5 tonnes of oil per hectare. However, the supporting data provided in Matsuno *et al.* (1984) are limited, and the data presented in Foidl *et al.* (1996) are projections based on field trials that were not included in the publication. More yield data are likely to become available over the next few years, as recently established plantations begin to reach maturity.

Unlike the major oilseed crops, there are currently no agronomically improved varieties available for *J. curcas*. In addition, the seed meal obtained after extraction of the oil cannot be used as an animal feed due to its toxicity. Despite the toxicity of *J. curcas*, edible varieties are known to exist in Mexico which are not currently being exploited. The potential of these edible varieties and the potential of plant science to create agronomically improved varieties is discussed. We also discuss the suitability of *J. curcas* oil for biodiesel production.

Toxicity of *J. curcas* seeds and the potential value of seed meal

The seeds of *J. curcas* form within seed pods (Fig. 2). Each seed pod typically contains three seeds (Fig. 2C). The typical mass and composition of the seeds is detailed in Table 1. In addition to being a valuable source of oil, the seeds are also rich in protein. The protein composition of *J. curcas* seed meal has been analysed, and it has been shown to compare favourably with soybean meal (Makkar *et al.*, 1998*a*, *b*), containing a good balance of essential amino acids, with the exception of lysine. The seeds of most tested varieties of *J. curcas* are inedible, and remain so after the heat-inactivation treatments used in seed-meal processing (Heller, 1996). As a consequence, the protein rich seed meal of *J. curcas* is not used as animal feed. The prices of seed oil and meal fluctuate depending on supply (harvest) and demand. The prices of seed oil and meal for soybean, canola, and sunflower are shown in Fig. 3. Although oil is more valuable than meal, the seed meal is potentially a valuable commodity. The ability to use *J. curcas* meal as animal feed not only improves the economics of *J. curcas* production, but also means the crop would produce both fuel and feed. Although the seeds of *J. curcas* contains a range of toxins and antinutrients, the toxicity of *J. curcas* seeds has been attributed to the presence of a protein (curcin) and phorbol-esters (diterpenoids).

Curcin

Curcin is often classified as a lectin and described as being similar to ricin from castor bean with the implication that it has similar toxicity. These descriptions are inaccurate. Both curcin and ricin are ribosome inactivating proteins (RIPs), which depurinate rRNA, thus arresting protein synthesis. Curcin is a type-I RIP (Barbieri *et al.*, 1993; Juan *et al.*, 2003; Qin *et al.*, 2005) whereas ricin is a type-II RIP. Type II RIPs contain both a catalytic A-chain (RIP)

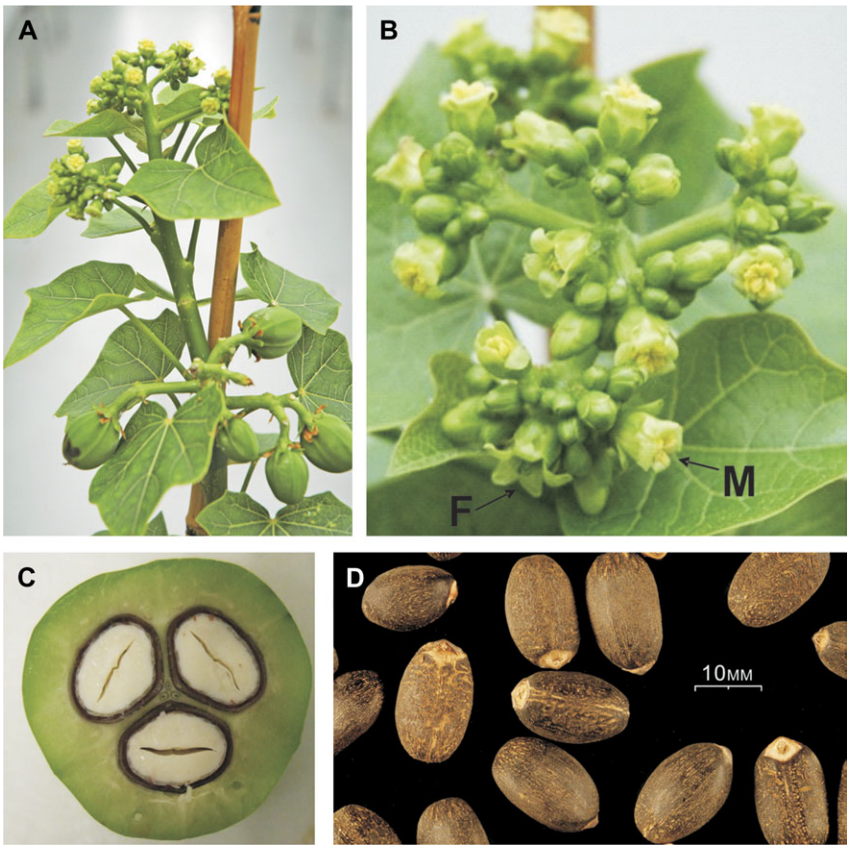

Fig. 2. Images of *J. curcas*. (A) Young *J. curcas* plant with both flowers and developing seed pods. (B) *J. curcas* inflorescence containing both male staminate flowers (M) and female pistillate flowers (F). (C) Cross-section of a *J. curcas* seed pod containing three developing seeds. (D) Mature seeds of *J. curcas*.

Table 1. Range of average seed mass, oil content, and protein content reported for *J. curcas* seeds:(Makkar *et al*., 1998*a, b*; Martinez-Herrera *et al*., 2004, 2006; Rao *et al*., 2008).

	Reported ranges
Average seed mass	450–860 mg
Testa (shell) (%)	30–40%
Kernel (%)	60–70%
Average oil content	
Whole seed	39–37%
Kernel	44–62%
Protein content	
Kernel	22–35%
Seed meal after oil extraction	48–64%

and a carbohydrate binding lectin B-chain, which are encoded by the same gene (Hartley and Lord, 2004). Type I RIPs such as curcin lack this lectin domain. The toxicity of type-II RIPs such as ricin is partly attributed to the ability of the lectin domain to bind to cell surfaces and mediate the entry of the RIP into the cell (Olsnes *et al.*, 1974). Due to the lack the lectin domain, the LD_{50} values of type I RIPs are typically over 1000-fold higher than those observed for type-II RIPs in whole animal (mouse) models (Barbieri *et al.*, 1993). In addition, Type-I RIPs are found in many edible plant materials including many cereal grains such as wheat and barley (Motto and Lupotto, 2004), beetroot, spinach leaves, and asparagus (Barbieri *et al.*, 2006). The presence of curcin in the seeds of *J. curcas* is therefore unlikely to present a significant barrier to the processing of *J. curcas* seed meal into animal feed.

Phorbol-esters of J. curcas

Despite the toxicity of the *J. curcas* seeds, edible varieties exist in Mexico. These are often consumed by the local population after cooking. These varieties are known to occur in the Yucatan peninsula (Schmook and Serralta-Peraza, 1997), and in the Totonacapan in the Mexican states of Puebla and Veracruz (J Cuevas, personal observations). A comparative analysis of edible and non-edible seed varieties revealed that edible seeds lacked phorbol-esters (Makkar *et al.*, 1998*a*, *b*). The heat-treated meal of an edible *J. curcas* variety has been tested as a foodstuff for carp and rats (Makkar and Becker, 1999). No adverse effects were reported. Although more studies are required on the suitability of Jatropha meal as an animal feed, this initial study is promising.

The presence of phorbol-esters in *J. curcas* seeds has been known for some time (Adolf *et al.*, 1984; Hirota

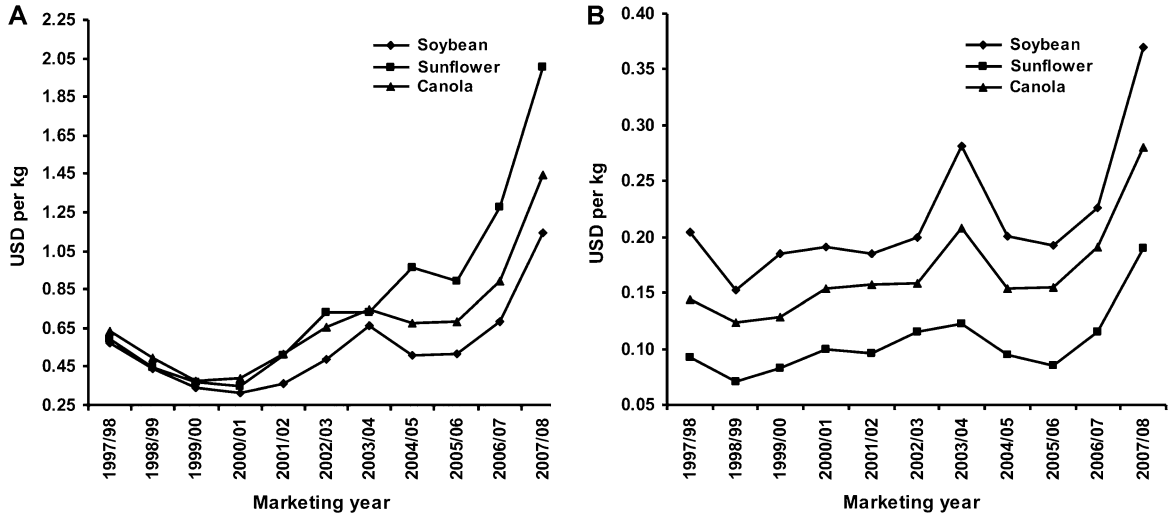

Fig. 3. Average annual prices of (A) vegetable oil and (B) seed meal of soybean, sunflower, and canola over the past decade. Data obtained from USDA (http://www.ers.usda.gov/Briefing/SoybeansOilCrops/data.htm). Data updated on 12/11/2008. Figures for the 2007/2008 marketing year are preliminary.

et al., 1988) and the structure of six phorbol-esters has now been determined using NMR (Haas *et al.*, 2002). The structure of one of the *J. curcas* phorbol-esters and 12-hydroxy-16-deoxyphorbol is shown in Fig. 4. Phorbol-esters are produced by a number of other plant species within the Euphorbiaceae, especially species belonging to the Crotonoideae and Euphorbioideae subfamilies (Beutler *et al.*, 1989). These are usually found either in the seeds or the latex, which is exuded from these plants after wounding. The phorbol-esters are analogues of diacylglycerol, an activator of many isoforms of protein kinase C (PKC). (Zhang *et al.*, 1995). PKCs act as regulators of many cellular processes. As diacylglycerol has a short biological half-life in the cell, the activation of PKC is usually only transient. Activation of PKC by phorbol-esters, however, is much more prolonged (Griner and Kazanietz, 2007), and this leads to a number of biological activities. The phorbol-esters are acutely toxic, and oils containing phorbol-esters are known purgatives (Gandhi *et al.*, 1995). The phorbol-esters are also skin-irritants. Of more concern is that the phorbol-esters may contribute to the formation of various cancers by at least two mechanisms. Phorbol-esters act as co-carcinogens or 'tumour promoters'. They do not cause tumour formation alone, but can lead to the increased risk of tumour formation when there is co-exposure to a chemical carcinogen (Griner and Kazanietz, 2007). Although most studies on tumour-promoting effects of phorbol-esters have used PMA (phorbol 13-myristate 12-acetate) from *Croton tiglium*, the tumour-promoting effect for both *J. curcas* oil and one of its phorbol-esters has been demonstrated in mice (Horiuchi *et al.*, 1987; Hirota *et al.*, 1988). Phorbol-esters are also known to activate the lytic cycle of the latent Epstein–Barr virus (MacNeil *et al.*, 2003). It has been proposed that exposure to the latex of the plant *Euphorbia tirucalli*, a phorbol-ester-producing plant of the Euphorbioideae subfamily, is a co-factor for the development of Burkitt's lymphoma in EBV carriers (MacNeil *et al.*, 2003). It is difficult to quantify the risk associated with handling *J. curcas* material, but presumably extensive prolonged exposure to crushed seeds or oil would increase the risk. Measures should therefore be taken to protect those who have an occupational exposure to *J. curcas* (Gminski and Hecker, 1998). Adoption of varieties lacking phorbol-esters, in addition to providing a potential source of

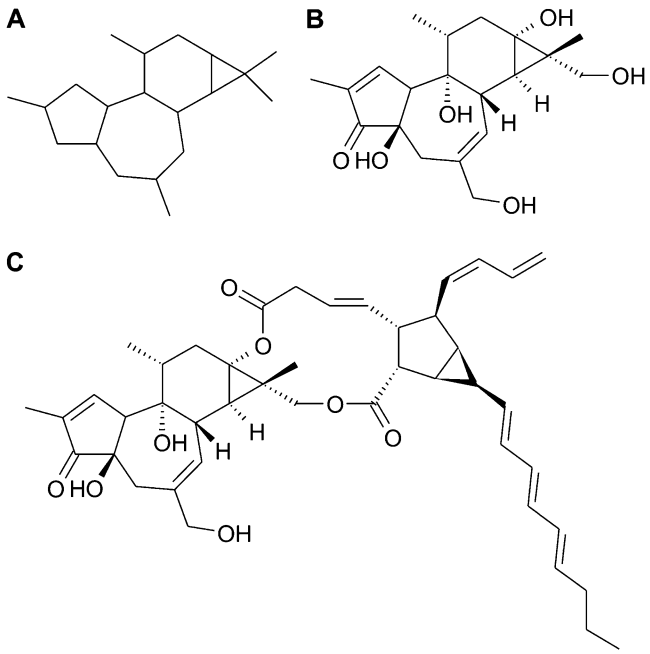

Fig. 4. (A) 5-7-6-3 tigliane ring structure common to all phorbols. (B) 12-hydroxy-16-deoxylphorbol structure common to all phorbol-esters from *J. curcas* and (C) *J. curcas* factor C_1, one of the six phorbol-esters identified in the seeds of *J. curcas* (Haas *et al.*, 2002).

income from animal feed, would also eliminate any potential risks associated with prolonged exposure to phorbol-esters.

As current plantations have been established using phorbol-ester-containing varieties, a number of attempts have been made to detoxify the meal of *J. curcas*. Although quantitative extraction of phorbol-esters from ground seeds can easily be achieved in the laboratory, solvent extraction processes used on an industrial scale are unlikely to remove all of the phorbol-esters. A range of methods have been used to try and detoxify defatted seed meal, including extraction with polar organic solvents, and combined heat/$NaHCO_3$ treatments Using a combination of both solvent extraction and heat/$NaHCO_3$ treatment, a 48-fold reduction in phorbol-ester content of seed-meal was obtained (Martinez-Herrera *et al.*, 2006). It is not clear at present whether an economically feasible method of eliminating the phorbol-esters from *J. curcas* meal could be developed.

An alternative use that has been suggested for *J. curcas* seed meal is organic fertilizer (Heller, 1996). It has been proposed that meal from inedible phorbol-ester-containing varieties in particular could be used for this purpose. However, research into the fate of phorbol-esters in the environment, and their impact on soil ecology is required before the suitability of *J. curcas* meal as a fertilizer can be more thoroughly evaluated.

Fatty acid composition of *J. curcas* oil, and its suitability as a feedstock for biodiesel production

Much of the current interest in *J. curcas* oil is for its potential as a feedstock for biodiesel production. A number of factors determine the suitability of a particular feedstock for the production of biodiesel and many countries have defined specification standards. Although it may be preferable to produce and use the biodiesel locally, biodiesel specifications are not available for many of the countries in which *J. curcas* is being grown, so the suitability of *J. curcas* oil for the production of biodiesel meeting European Union and United States of America standards is discussed here. Standards for the production of biodiesel are described in EN14214 for the European Union and in ATSM D6751 for the United States of America. The ability of a biodiesel to meet the specified criteria for both these standards is largely determined by the fatty acid composition. In particular, cetane number (CN), cold-flow and cloud point properties, kinetic viscosity, and oxidative stability are all influenced by the fatty acid composition. The production of biodiesel involves the transesterification of the vegetable oil with methanol to produce fatty acid methyl esters (FAMES). This reduces the viscosity of the oil. Unfortunately, no one single FAME is ideal when matched against all these parameters, but oils containing high levels of oleate and palmitoleate are desirable (Knothe, 2008).

We have recently undertaken a study on the fatty acid composition of the seeds obtained from 23 sites in Madagascar (Table 2). Interestingly, AFLP analysis revealed very little genetic variation in the seed samples collected at these sites, indicating that the variation in the fatty acid composition of these seeds were largely due to environmental differences (data not shown). The most significant variation between the different samples was between the relative amounts of oleate (18:1) and linoleate (18:2). It is worth noting that many of the oleate-rich samples were obtained from sites close to sea level, where mean annual temperatures would be higher than mountainous regions. Temperature is known to have an affect on the fatty acid composition of other oilseed crops (Harris *et al.*, 1978; Wolf *et al.*, 1982).

The CN value is perhaps the most important factor for biodiesel. The CN value is a measure of delay in the combustion of the fuel from ignition. Diesel in the United States and Europe must meet minimum criteria. ASTM D6751 requires a minimum CN of 47, whereas EN 14214 specifies a minimum of 51. Above a CN value of 54.5, there are no performance improvements in engine performance (Içingür and Altiparmak, 2003). A formula has recently been published to estimate the cetane value from the fatty acid profile of oils, which is reported to give 88% accuracy (Bamgboye and Hansen, 2008). This formula has been applied to our analysis of seed samples collected from Madagascar (Table 1). Based on these calculations, all of the oil samples would be suitable for producing biodiesel (FAMES) meeting the stricter EU requirement, but many of the linoleate-rich samples, in particular, gave a calculated cetane of 51. The actual cetane values of *J. curcas* biodiesel has been determined by a number of groups, and has so far been within the range of 50–57 (Foidl *et al.*, 1996; Senthil Kumar *et al.*, 2003; Sarin *et al.*, 2007). As biodiesel production from *J. curcas* increases, more information should become available on the cetane values associated with oils producing different fatty acid compositions, but preliminary evidence suggests that the ASTM D6751 standard is likely to be met, and in most instances, the EN 14214 standard. The cetane number can also be increased through use of ethyl esters rather than methyl-esters, or by adding cetane enhancers (Foidl *et al.*, 1996; Knothe, 2008). The cold-flow and cloud point of biodiesels is determined largely by the concentration of saturated FAMES present in the blend (Imahara *et al.*, 2006). The saturated fatty acid content of *J. curcas* oils typically includes 14–16% palmitate (16:0), 5–8% stearate (18:0), and a trace of longer chain saturates (Foidl *et al.*, 1996). The cloud point of *Jatropha* FAMES has been determined as 4–8 °C (Sarin *et al.*, 2007; Krishnakumar *et al.*, 2008). This temperature range is too high for use during winter in temperate climates as 100% biodiesel (B100), but most biodiesel is currently sold as blends (e.g. B5). The kinetic viscosity of biodiesel has also been specified by both ASTM D6751 and EN 14214, being 1.9–6.0, and 3.5–5.0 $mm^2\ s^{-1}$ at 40 °C, respectively. The kinetic viscosity of FAMES produced from *Jatropha* and most other plant oils (i.e. those comprising mainly C16 and C18 fatty acids) falls within these values (Sarin *et al.*, 2007; Knothe, 2008; Krishnakumar *et al.*, 2008).

Table 2. Fatty acid composition of *J. curcas* seeds collected from different sites in Madagascar

Fatty acids abbreviations; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; 20:0, arachidic acid. Cetane number (CN) has been estimated according to Bamgboye and Hansen (2008). Fatty acid composition was determined via acid-catalysed transmethylation of ground seed kernel followed by analysis using GC-FID (Larson and Graham, 2001).

Site	Elevation (m)	% fatty acid composition								Calculated CN
		16:0	16:1	18:0	18:1	18:2	18:3	20:0	Others	
Marovoay, Mahajanga	5	14.3	0.7	7.5	51.7	25.3	0.3	0.3	<0.1	55
Ampitolova, Mahajanga	26	14.8	0.8	7.6	48.5	27.8	0.2	0.2	<0.1	55
Site 1, Antalaha	ND	14.1	0.7	7.9	49.1	27.7	0.3	0.3	<0.1	55
Andranovory, Toliary	ND	14.5	0.9	6.8	45.4	31.9	0.2	0.2	<0.1	54
Lokomby, Vatovavy Fito Vinany	20	14.7	0.8	7.5	43.5	33.0	0.2	0.2	<0.1	54
Site 2, Antalaha	ND	15.4	0.8	6.9	43.3	33.1	0.2	0.2	<0.1	54
Befoly, Toliary	460	14.5	0.9	6.6	42.3	35.3	0.2	0.2	<0.1	54
Rivière Sakay, Moyen Ouest	852	16.0	1.1	6.2	37.2	39.1	0.3	0.2	<0.1	53
Kianjavato, Vatovavy Fito Vinany	61	15.5	0.9	6.0	37.4	39.8	0.2	0.2	<0.1	52
Ankaramena, Ambalavoa	776	14.2	0.9	5.9	39.8	38.8	0.2	0.2	<0.1	52
Ambaiboho, Lac Alaotra	754	15.3	1.1	5.6	37.1	40.4	0.3	0.2	<0.1	52
Andalatanosy, Androy	460	14.1	0.9	6.0	38.8	39.8	0.2	0.2	<0.1	52
Ambohikambana, Moyen Ouest	903	14.9	1.0	5.5	38.1	40.1	0.2	0.2	<0.1	52
Amparaky, Moyen Ouest	1145	13.4	0.8	6.6	38.6	40.2	0.2	0.2	<0.1	52
Mahasolo, Moyen Ouest	903	14.1	0.9	5.9	37.9	40.7	0.2	0.2	<0.1	52
Andasy, Soavina	1084	15.4	1.0	5.4	36.2	41.5	0.2	0.2	<0.1	52
Manganoro, Lac Alaotra	772	14.4	0.8	6.9	34.6	42.8	0.2	0.2	<0.1	52
Kelilalina, Vatovavy Fito Vinany	620	12.7	0.6	6.1	39.7	40.4	0.2	0.2	<0.1	51
Ambatofolaka, Moyen Ouest	974	14.2	0.8	5.8	37.0	41.8	0.2	0.2	<0.1	51
Ankasina, Lac Alaotra	833	15.2	1.0	5.3	35.2	42.8	0.2	0.2	<0.1	51
Trajavona, Ambalavoa	957	15.0	1.0	5.3	34.9	43.3	0.2	0.2	<0.1	51
Fitamatsina, Soavina	1104	15.0	1.0	5.3	35.0	43.3	0.3	0.2	<0.1	51
Mahavanona, Ambalavoa	964	15.5	1.1	5.3	33.8	43.9	0.2	0.2	<0.1	51

Genetics of *J. curcas* and target traits for crop improvement

J. curcas is a diploid species with a 2n chromosome number of 22 (Dehgan, 1984). A recent study has estimated the genome size (1C) to be 416 Mbp (Carvalho *et al.*, 2008). This is relatively small for a plant genome (Zonneveld *et al.*, 2005), and could make *J. curcas* an attractive candidate for genome sequencing. In addition, the castor genome project, which is currently available as four-times draft (http://castorbean.jcvi.org), could serve as a useful resource for the identification of genes by homology, or assisting in the creation of genetic and physical maps if sufficient synteny exists between the species.

The development of high-yielding crop varieties through plant breeding has significantly increased agricultural productivity, especially in the latter half of the 20th century (Evenson and Gollin, 2003). There are a number of traits which could be targeted for improvement in *J. curcas* including seed yield, oil content, and seed toxicity (phorbol-ester content). Improvements in seed yield could be achieved in a number of ways. *J. curcas* is monoecious, and has a male:female flower ratio of around 29:1. The plant is insect pollinated, although self-pollination is possible via geitonogamy (Raju and Ezradanam, 2002). Figure 2B shows the differing appearance of the male and female flowers. Increasing the ratio of female flowers may lead to increases in the seed yield. One recent report highlighted a correlation between male:female flower ratio and yield, and also noted that this trait was highly heritable (Rao *et al.*, 2008). Yield increases in a number of plant species have also been obtained through the modification of plant architecture (Sakamoto and Matsuoka, 2004). Increasing the number of branches on *J. curcas* may lead to an increased number of inflorescences, and, ultimately, the number of seeds produced per plant. Much progress has been made recently in understanding the mechanisms involved in the control of branching in both crop and model species including *Arabidopsis* and rice (Sakamoto and Matsuoka, 2004; Ongaro and Leyser, 2007).

Increasing the oil content in seeds can be achieved by altering the expression levels of enzymes in the triacylglycerol 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). The regulation of seed development and triacylglycerol biosynthesis in seeds have been studied in some depth (Santos-Mendoza *et al.*, 2008). 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 factors *GmDOF4* and *GmDOF11* in *Arabidopsis* has also been shown to result in increased oil content (Wang *et al.*, 2007).

Although varieties of *J. curcas* lacking phorbol-esters already exist, it is possible in the future that it will be desirable to introduce this trait into other elite varieties with improved traits (e.g. high yields). A molecular marker for seed toxicity would be useful in allowing the rapid introgression of 'non-toxicity' into other varieties. Alternatively, non-toxic (edible) varieties could be developed by disruption of genes in the phorbol-ester biosynthetic pathway. Although there is no direct biochemical evidence available at present for the steps involved in phorbol-ester biosynthesis, the first committed step is likely to involve conversion of geranylgeranyl pyrophosphate (GGPP) into a tigliane-diterpene (Fig. 4A). Disruption of the putative diterpene synthase involved in this step should result in a plant lacking phorbol-esters.

Other target traits for improvement of *J. curcas* include synchronous flowering, which may facilitate mechanical harvesting and reducing the thickness of the testa may allow oil to be extracted from the seeds more efficiently.

Techniques for the breeding of new *J. curcas* varieties

Today there are a number of strategies available for the creation of new plant varieties, including conventional breeding, the creation of interspecific hybrids, mutation breeding, and genetic engineering. Before conventional plant breeding can be used, it is useful to have some understanding of the natural genetic variation that is present within a species. The precise origin of *J. curcas* is somewhat disputed, but it is thought to be native to the Americas, and to have been transported to Asia and Africa by the Portuguese (Heller, 1996). If this is correct, then one can assume that there will be more genetic variation in the Americas than in Africa or Asia. The level of genetic variation present in Africa and Asia will be determined largely by which material was exported from the Americas. The export of material from the Americas could have been a single event. Recent studies have come to the conclusion that the genetic variability within *J. curcas* in Asia is very low (Basha and Sujatha, 2007; Sudheer Pamidimarri *et al.*, 2008; Sun *et al.*, 2008). Further assessment of the available genetic variation within the species, especially material from the Americas, will therefore be a useful approach to identify distinct varieties for use in breeding programmes. It is not currently known whether potential yield increases could be achieved through heterosis and the performance of F_1 hybrids needs to be tested. Once genetically distinct varieties have been identified, these will serve as a useful resource for identifying varieties suitable for different climates and the creation of new varieties through breeding. The speed and precision of this breeding will be greatly facilitated by the production of molecular markers and a robust genetic map.

The *Jatropha* genus contains around 170 known species (Dehgan and Webster, 1979). Currently there is only one published study available analysing the potential for the creation of interspecific hybrids within the *Jatropha* genus, but it appears that this approach may be feasible. Some of the interspecific crosses were successful. The most successful hybridization obtained was between *J. curcas* and *J. macrorhiza*, where 21 F_1 seeds were obtained from pollination of seven flowers, all of which germinated. The F_1 plants, however, had low fertility when selfed (three seeds from 17 pollinations) (Dehgan, 1984). No data were provided for backcrosses but it is possible that subsequent backcrossing would have improved fertility.

Mutation breeding is a technique which has been applied to the improvement of many crops species. In excess of 2000 plant varieties have been released, including rice, wheat, sunflower, and rapeseed (Ahloowalia *et al.*, 2004). Although phenotypic screening of mutants is a lengthy process, the selection of plants containing mutations in specific genes can now be achieved using heteroduplex mapping (Henikoff *et al.*, 2004). This approach is most useful where it can be envisaged that disruption of a gene may give rise to a desirable phenotype. A potential target for this approach for increased yield in oil crops would be the homeobox gene *GLABRA2* (Shen *et al.*, 2006).

Plant transformation is another valuable method for the development of improved plant varieties. The transformation of *J. curcas* cotyledon discs using *Agrobacterium tumefaciens* was recently reported (Li *et al.*, 2008). This recent development means that the full range of plant breeding techniques are now available for the development of *J. curcas* as a robust commercially viable crop.

Outlook

As the scale of *J. curcas* cultivation increases, it becomes more important to develop new varieties that produce the maximum amount of oil and by-products with the minimum amount of input. Although the oil extracted from *J. curcas* appears suitable for biodiesel production, little value can be captured from the meal. Development of varieties which lack phorbol-esters is therefore a desirable goal. This would allow the seed meal to be converted to animal feed, and eliminate risks to human health associated with the phorbol-esters. The identification of a molecular marker associated with the absence of phorbol-esters would facilitate breeding programmes. A thorough evaluation of the existing germplasm, with particular emphasis on material from the Americas is also required in order to assess the range of genetic variation available for plant breeding programmes, and for use in the creation of mapping populations. In addition to the natural genetic variation available within the species, mutation breeding or the creation of interspecific hybrids could also allow a wider range of agronomically beneficial traits to be incorporated into *J. curcas*. The ability to transform this species offers another route to the creation of new varieties. Although there is still much fundamental research to be

conducted into *J. curcas*, advances in the available techniques in molecular marker analysis such as high-throughput sequencing and genotyping technologies will greatly assist these tasks. These advances, together with investment in well-designed field trialling, should ensure relatively rapid development of new improved varieties.

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