Chapter 5
The Importance of Jatropha for Brazil

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Introduction

In this chapter, we first present the Brazilian potential for biofuel production and the National Program for Production and Use of Biodiesel. We then review the raw materials currently used for biodiesel production in the country and their limitations. At the same time we present the characteristics that make some alternative oilseed species interesting as sources of vegetable oil for biodiesel production, highlighting why physic nut (*Jatropha curcas* L.) has been seen as one of the best alternatives, perhaps the best one. We then effectively demonstrate the importance of physic nut for Brazil describing the dense research program funded by the Brazilian Government, which is currently underway to try to solve most of the challenges (also reviewed) in making this crop effectively a biofuel crop.

Brazilian Potential for Biofuels Production

Fossil fuels currently supply most of the world's energy, even though it represents a finite resource (Vermerris 2008). Aiming at reducing the fossil fuels dependency, alternative sources of energy have been pursued in the last few years. In view of the urgent need to develop new technologies that may enable the widespread use of environment friendly forms of energy, biofuels in general and biodiesel in particular

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(La Rovere et al. 2011) are receiving considerable attention throughout the world and especially in Brazil (Laviola et al. 2012b; Laviola and Dias 2008; Laviola et al. 2010; Rosado et al. 2010).

In this context, it must be emphasized that Brazil is a country with a huge potential for production not only of biofuels, but also of other derivatives from vegetable oil. Such enormous capacity can potentially supply part of the national and international market demand on biofuels. The country possesses a privileged location in the tropical region of the world with high incidence of solar energy and adequate rainfall regime in most regions. Moreover, Brazil possesses a large amount of available land making possible the adequate planning of the land use in a sustainable basis, i.e., without compromising biome composition and structure. Current estimates indicate that in Brazil there are near 90 million hectares of available lands for agriculture expansion, without considering the 210 million hectares of degraded pastures (BAP 2006), from which some level of technology can be recovered and used for the production of food and biofuels. Nevertheless, there also exists in the country more than 200 oilseed species with different potentialities and different adaptation to the various natural occurring environmental conditions (Beltrão 2005). Some of such species can be incorporated in production systems and used for biofuel production and/or for other ends with higher aggregated value. The Brazil´s challenge is, then, to take maximum advantage of its biodiversity, of its regional potentialities to concomitantly enhance biofuel production and obtain greater social gains from the biodiesel production. This can be undoubtedly done by applying technology (agricultural, processing, etc.) not only on traditional crops, but also on undomesticated (to be explored) oilseed species (Laviola and Alves 2011). To achieve such goal, the Brazilian Federal Government has recently launched the National Program for Production and Use of Biodiesel (or in Portuguese: Programa Nacional de Produção e Uso de Biodiesel—PNPB).

The PNPB and Raw Materials Currently Used for Biodiesel Production

In 2005, the Brazilian Federal Government launched the National Program for Production and Use of Biodiesel grounded in the law nº 11.097 from January 13, 2005. The PNBP was elaborated to equate fundamental questions to the country, such as (i) reduction of foreign petroleum dependency, (ii) generation of jobs and income, (iii) social inclusion and reduction of pollutant emission and (iv) regional disparities in terms of development, by addressing social, strategic, economic and environmental aspects of the biodiesel production chain. It is determined that research should seek the substitution of part of the fossil diesel with biodiesel. It also established the need to diversify the sources of oilseeds used for biodiesel production, since today, near 87% of the biodiesel produced in the country is produced from soybean oil (animal fat represents the second main source of fatty acids for biodiesel production, followed by cotton). All other oilseed species if grouped

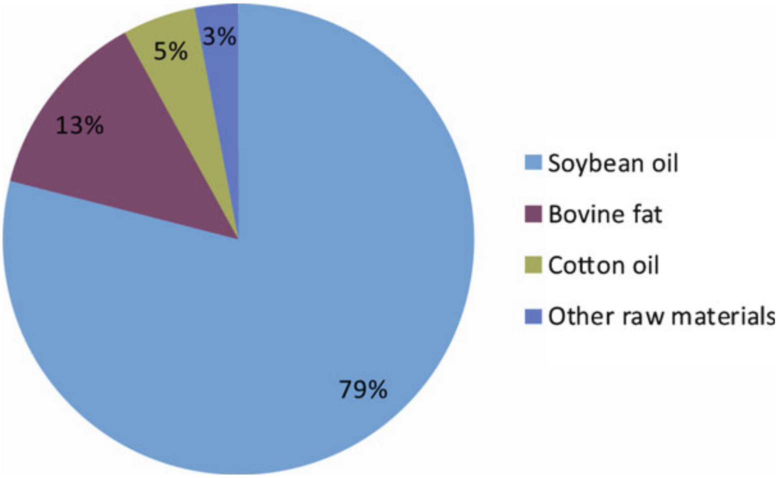


Fig. 5.1 Relative contributions of raw materials to the biodiesel matrix in Brazil (Source: Brazilian National Agency of Petroleum, Natural Gas and Biofuels –March 2011)

represent less than 1% of the biodiesel production chain (Fig. 5.1). The challenges and strategies to be met by the Brazilian biodiesel program involve technical-scientific bottlenecks in the raw material production, industrial processing and integration with existing productive chains.

Aiming at gradual substitution of petroleum diesel by biodiesel, the PNPB established as its initial goal to fix 2% (B2) as the minimum percentage of biodiesel addition to the petroleum diesel in any part of the national territory by 2008 and as 5% (B5) by 2013. However, with the rapid expansion of soybean production and industrial processing units, the government decided to anticipate its goals and officially introduced B5 by 2010. Nowadays, the capacity of biodiesel production is 2 times higher than the compulsory demand for biofuel, being then possible to immediately regulate B10 when the plant oil offer will be sufficient to meet the demand. However, to progress in the regulation of new mixtures, other questions need to be weighted, such as the raw material availability and diversification.

Generally two distinct groups of species are recognized: (i) the traditional raw materials, for which technological aspects of its production have already been addressed and (ii) the potential alternative raw materials, for which research is still required. Oil extracted from species like canola (oilseed rape; *Brassica napus* L.), soybeans (*Glycine max* L.), castor beans (*Ricinus communis* L.) and sunflower (*Helianthus annuus* L.) have been traditionally used for biodiesel production (Vermerris 2008; Yuan et al. 2008). Current estimates indicate that 600 kg ha^{-1} is the actual oil productivity of these traditional species (Table 5.1). In Brazil, besides being an oilseed species with low energetic productivity, soybean is the only crop among those species considered to be traditional raw materials for biodiesel production that totally meets the basic parameters of a program with the dimensions of the PNPB (Laviola and Alves 2011). These parameters belong to the (i) technological

Table 5.1 Main raw materials currently used for biodiesel production in Brazil, the oil content in seeds, seed and oil yield per hectare

Raw material	Oil (%)	Seed yield (kg ha^{-1})	Oil yield (kg ha^{-1})
Soybean	18	3,000	540
Cotton	20	1,900	360
Sunflower	42	1,500	630
Peanut	45	1,800	800
Oil palm	20	20,000	4,000
Castor beans	47	1,500	705
Canola	40	1,300	500

Source: Laviola and Alves (2011)

Table 5.2 Main potential raw materials for biodiesel production in Brazil. The yield is given in percentage of oil in seeds, seed productivity and oil production per hectare

Raw material	Oil (%)	Seed or fruit yield (kg ha^{-1})	Oil yield (kg ha^{-1})
Physic nut	35	4,500	1,500
Macaw palm	20	20,000	4,000
Inajá	20	17,500	3,500
Tucumã	20	12,000	2,400
Babaçu[a]	5	10,000	500

[a] Mainly used for energy cogeneration. (Source: Laviola and Alves 2011)

domain—Brazil is one of worldwide leaders in soybean research and development (what allowed for example its cultivation with minimum dependency on nitrogen fertilization); (ii) production scale—today less than 20% of the whole soybean production is sufficient to supply the current demand of the PNPB (other oilseed species such as cotton, sunflower and castor beans are not productive enough to support B2); and (iii) logistics—soybean is the only species among those considered to be traditional raw materials for biodiesel production that can be grown and produced in all regions of the country and with a whole distribution system to drain the production already installed. Even though all these characteristics contribute to make soybean the main raw material in the context of the PNPB nowadays, it is important to highlight that the continuous development of other oilseed species with higher energetic productivity is necessary, especially when considering questions related to diversification and regionalization of raw materials. Actually, the energy segment is too much important to assume the risk of its dependency on only one or few crop(s) in the long term. The importance of raw material diversification can be easily understood from the larger potential productivity of many alternative crops as presented in Tables 5.1 and 5.2. Moreover, it is necessary to remember that as much as 40–60% of biodiesel production costs are related to feedstock costs (BAP 2006). Thus, increasing the average oil productivity should prove to be an effective approach to increase the competitiveness of biodiesel when compared to gasoline and ethanol.

In this last context, the use of non-edible species is highly desirable as it offers the advantage of not competing with food crops for area and resources. However, such competition between food and fuel crops is not expected in the short term in Brazil, because Brazil is a country with continental dimensions and millions of hectares of land still available for agriculture expansion. But this does not necessarily apply to other countries, where food agriculture occupies almost the entire agricultural available lands. In such countries, the cultivation of non-edible species will almost certainly compete with food crops for land and resources. It is noteworthy that research should aim at improving the production system and also at incorporating new un-domesticated energetic species to the list of biofuel crops, e.g., physic nut and some tropical palms such as Macaw palm also known as Macaúba or *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. (Moura et al. 2010; Rosado et al. 2010).

Alternative Feedstocks for Biodiesel Production

The PNPB established that research should seek new sources of raw materials with higher energetic productivity than soybean in order to enhance oil yield up to 4,000–6,000 kg ha^{-1}. The PNPB also indicate that research should aim at identifying new oil species so as to maximize biodiesel production according to region potentialities and biodiesel requirement.

Because Brazil holds ~20% worldwide biodiversity, a number of alternative oilseed species are available for biodiesel production. A number of species can also be used as source of biomass for cogeneration process. For example, Macaw palm is an alternative for the expansion of biofuel production in the Brazilian savannah, since its estimated oil yield is extremely high (Table 5.2). If one considers that the Macaw palm produces pretty much the same yield as oil palm and that oil palm has been improved via traditional breeding over decades, it is evident that the Macaw palm is an excellent choice as an alternative oilseed species. The macaw palm can be used in sustainable production systems (i.e., by extractivism or exploring the native individuals in their natural environment). Another possibility is to produce the Macaw palm in consortium with other crops species, which warrant farmers with incomes even in the first years after palm plantation. Other highly productive palms, such as Inajá (*Maximiliana maripa* (Aublet) Drude) and Tucumã (*Astrocaryum aculeatum* G. Meyer) (Table 5.2), grow spontaneously in the Northern region of Brazil and can potentially be explored in a variety of sustainable forms. By contrast, Babaçu palm (*Orbignya phalerata*, Mart.) is well adapted to Northeast Brazil and has been over the years used to produce a highly calorific charcoal. Recently its potential for biodiesel production has also been demonstrated even though its oil yield is not comparable to other potential palms (Table 5.2). Along with these palms, physic nut has been considered one of the best alternative species for sustainable oil production due to its large geographic extension covering almost every region of the country and other characteristics which are reviewed.

Why Physic Nut?

As mentioned above, physic nut has been considered as one of the best alternative oilseed species. There are a number of reasons for that, such as its (i) hardiness, (ii) easy propagation, (iii) drought tolerance, (iv) high oil content, (v) short life cycle, (vi) rapid growth, (vii) adaptation to a wide range of agro-climatic conditions, (viii) bushy/shrubby nature, and (ix) multiple uses of different plant parts (Achten et al. 2010). In addition, physic nut can be grown on wastelands or degraded lands provided pH, fertility and water deficit correction; consequently, it does not compete for land with food crops.

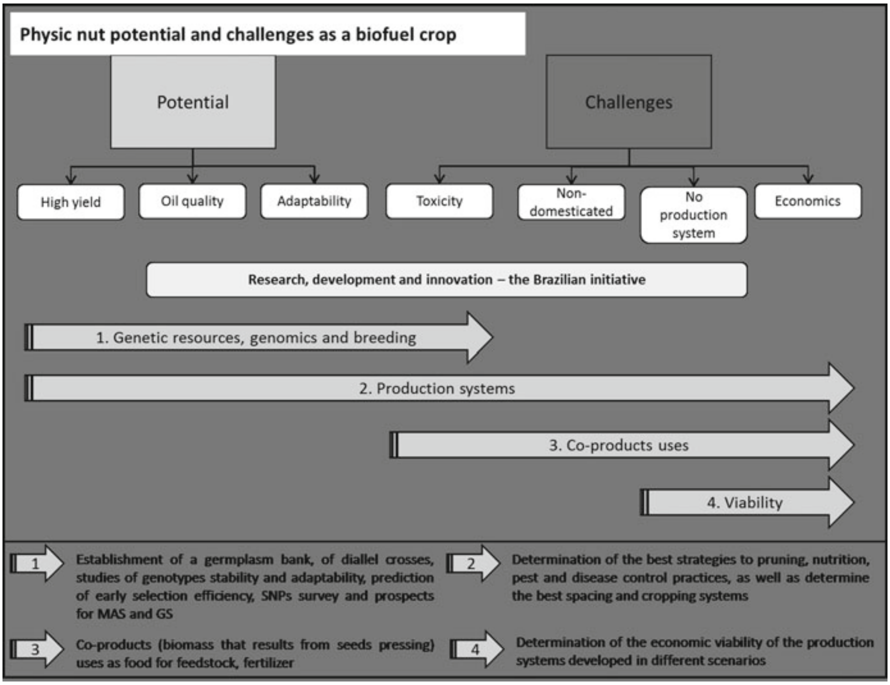
A single physic nut plant from non-elite material can currently produce as high as 2.5 kg of seeds (Drumond et al. 2009), with oil content varying between 30% and 40% (Ginwal et al. 2004; Rao et al. 2008; Sunil et al. 2008). In such a scenario, a single plant can produce 0.75–1.0 kg of oil. Since 1,250 plants can be cultivated in one hectare with 4×2 m spacing, physic nut has nowadays the potential to produce between 930 and 1,250 kg of oil per ha. Such estimate however will change radically as soon as its selective breeding will be undertaken. Other desirable characteristics are high oil quality (Figure 5.2), low cost of oil conversion into biodiesel either by chemical (Berchmans and Hirata 2008) or biological transesterification (Modi et al. 2007) and satisfaction of technical requirements of Brazilian, US and European standards for biodiesel.

The popular claims concerning drought tolerance, low nutrient requirement, pest and disease resistance increased the expectations on this species to unreachable levels (Achten et al. 2010) even though most of these claims are yet to be supported by scientific evidence (Achten et al. 2010). The truth is that, besides the advantageous characteristics mentioned above, physic nut cultivation is challenging as it is a quasi-undomesticated species requiring substantial investment in research and development (R&D) (Fig. 5.2). We describe the main challenges that need to be addressed in order to make physic nut a viable biofuel crop in the near future.

Challenges in Making Physic Nut a Viable Biofuel Crop

Physic nut can be considered as a quasi-undomesticated species because neither elite cultivars nor defined production systems are yet available (Carels 2009). In Brazil, current commercial plantations are in the initial phase of implantation and less than 4 years old (Laviola et al. 2010). In order to boost this installation processes, basic knowledge on seed production techniques is paramount as well as information on propagation systems, planting density, pruning systems, mineral nutrition and fertilization and perhaps most important correct management practices (Fig. 5.2). Along with such limitations, one has also to considerer the regulation on cultivar and pesticides or fungicides registration, which is eventually not available.

Physic nut is also susceptible to a wide range of diseases and pests. One of the most important diseases that currently affect physic nut plantations in Brazil is powdery mildew caused by the fungus *Oidium heveae* B.A. Steinm (Ramakris and Radhakri 1963). Mildew occurs on seedlings and on trees in the field. The main sign of the infection is the presence of a whitish mycelium over the leaves and shoots



Establishment of a germplasm bank, of diallel crosses, studies of genotypes stability and adaptability, prediction of early selection efficiency, SNPs survey and prospects for MAS and GS

Determination of the best strategies to pruning, nutrition, pest and disease control practices, as well as determine the best spacing and cropping systems

Co-products (biomass that results from seeds pressing) uses as food for feedstock, fertilizer

Determination of the economic viability of the production systems developed in different scenarios

Fig. 5.2 Physic nut potential and challenges. Clear boxes indicate the physic nut characteristics that makes it a potential biofuel crop as well as those that currently hamper its wide adoption as a biofuel crop. Arrows indicate the main research fields and their description (*bottom*) that are included in the Embrapa's network for physic nut research (Source: Durães et al. 2011)

(Ramakris and Radhakri 1963). Even though the infection can be mitigated with the application of fungicides, the use of resistant plants is seen as a best strategy in the mid to long-term. However, virtually nothing is known concerning genetics and molecular aspects of the physic nut-*Oidium heveae* interaction and dedicated studies are necessary to obtain such information.

Another important aspect of physic nut is its toxicity. The most problematic toxic components are probably a number of phorbol esters found in high concentrations mainly in the seeds (Devappa et al. 2010b, 2011; Goel et al. 2007). As phorbol esters are compounds known to cause severe biological effects, including inflammation and tumor promotion (Devappa et al. 2010b), removal of such compounds is imperative in order to make use of the press cake, perhaps the physic nut co-product with the highest potential as animal feed. Physic nut seeds also contain a trypsin inhibitor, lectins and phytates that are antinutritional factors limiting the potential use of the press cake for animal feeding (Devappa et al. 2011; Goel et al. 2007). However, both trypsin inhibitor and lectins can be completely denatured and destroyed by exposure to elevated temperature as occurs before and/or during the oil extraction process technology (Devappa et al. 2011). The addition of synthetic phytases might mitigate phytate problem. Alternatively, press cake can be returned to soil and used as a crop fertilizer (Devappa et al. 2010a).

Finally, fruit maturation is quite uneven and discontinuous during the production period (which last between 4 and 5 months in the Brazilian conditions). Because of these characteristics, fruits must be harvested in four or five occasions, thus increasing the production cost.

Besides these technical and biological aspects of physic nut cultivation, there are other challenges, most of which are related to lack of reliable statistics pertaining to crop production. Currently there is no conclusive information about seed and oil productivity under commercial planting conditions also. In experimental evaluations, oil content was estimated to vary between 20% and 40% (Ginwal et al. 2004; Rao et al. 2008; Sunil et al. 2008). However, seed yield and oil content are expected to be heavily affected by (i) abiotic factors, such as nutrition, drought, management practices, and (ii) biotic factors, such as pests, diseases, age. With regard to this last concern, it has been initially shown that the production stability occurs after the fourth or fifth year (Dias et al. 2007) and that plants can produce as high as 2.5 kg of seeds (Drumond et al. 2009). As this information is pivotal to determine the economic viability of the crop, comprehensive investigations of genetic factors affecting yield are urgently needed.

In agreement with previous considerations, it is evident that physic nut cultivation is not as viable as it could be because of the innumerous questions that must yet be answered. However, we believe that a vibrant and focused research program may successfully make physic nut a viable feedstock for biodiesel production in couple of years. Below we review the Embrapa research program on physic nut that is supposed to address all the concerns mentioned above.

The Embrapa Research Initiative

The Embrapa research initiative in physic nut was built on the idea that an integrated program is necessary for making physic nut a viable biofuel crop in a short to mid-term period. Fourteen areas were identified as priority, i.e., selective breeding, seeds and seedlings production, pruning, spacing, fertilization, irrigation, weeds, pests, diseases, harvest, post-harvest, biodiesel, co-products, transversal studies and zoning. An action plan named BRJATOPHA (Research, Development and Innovation in physic nut for Biodiesel Production) was elaborated encompassing more than 130 research actions and 22 institutions (Fig. 5.3) and granted by FINEP (a Brazilian agency for the promotion and financing of innovation through scientific and applied research in private and public sectors).

This integrated program effectively began in 2008 and was just evaluated through an inquiry covering 39 research fields that encompasses all the 14 major areas discussed above and identified as priority. Researchers were asked upon the current stage of development in each field based on their experience according to a zero to five scale: (0) Without knowledge, i.e., researcher does not have enough knowledge to express his opinion, (1) No information, i.e., there is no information or research results available, (2) Common sense, i.e., there is no information available, but practices from other crops may be adopted, (3) Low knowledge, i.e., initial research

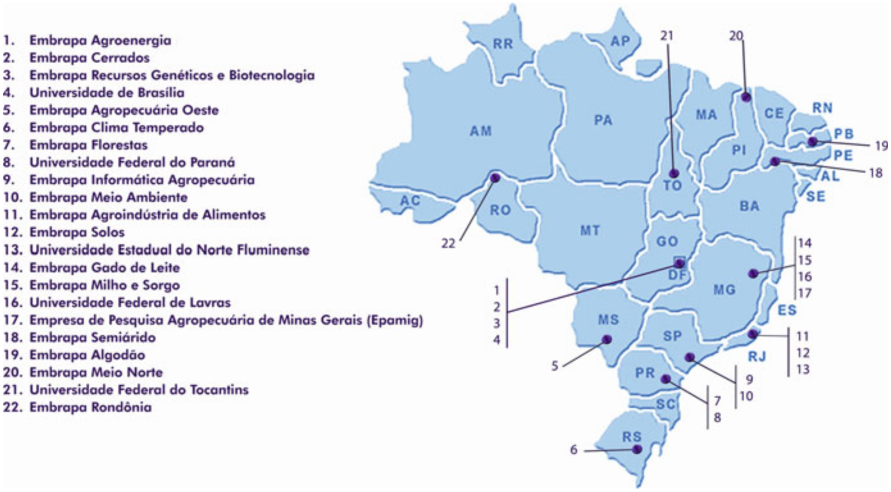


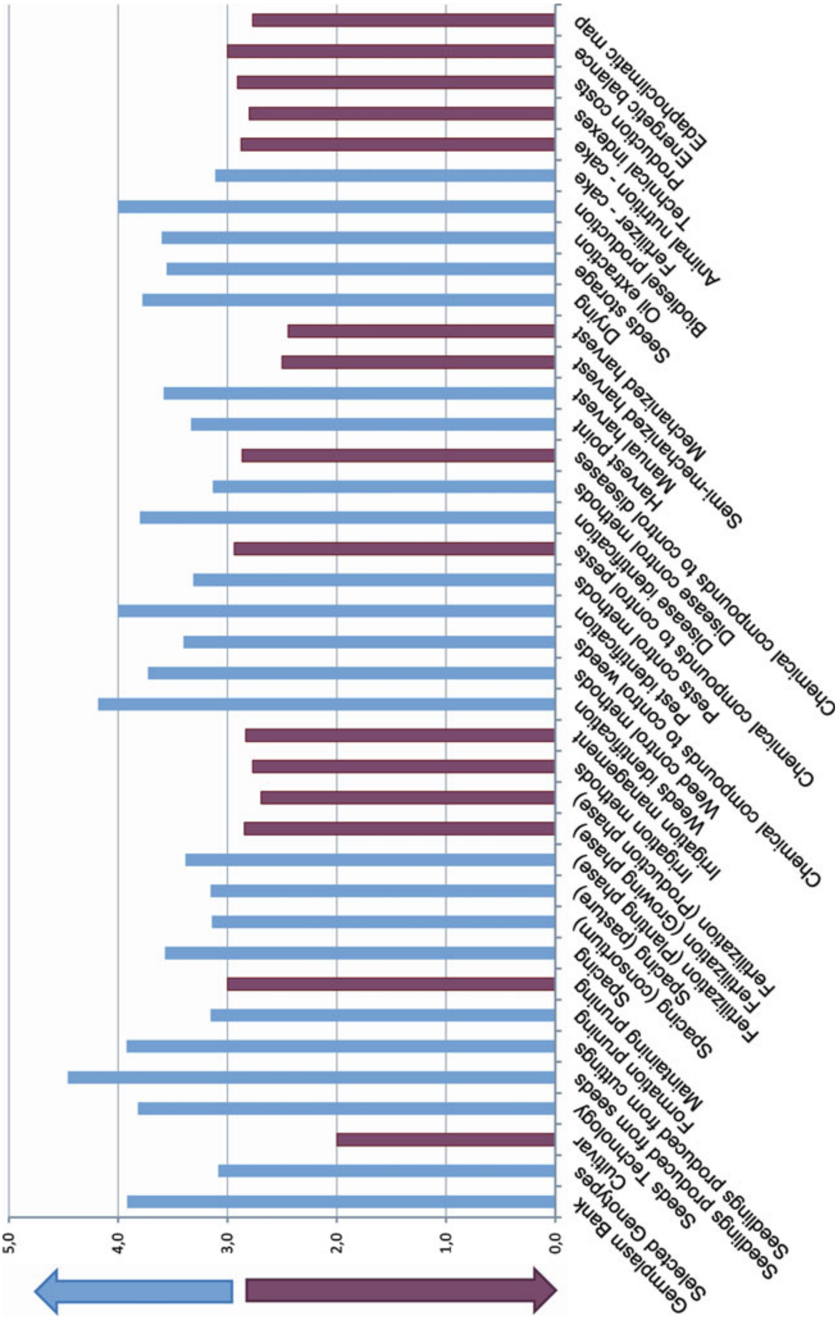
Fig. 5.3 The Embrapa's network for physic nut research involves 22 institutions distributed across the country and more than 130 research programs

results does not support recommendations for commercial applications, (4) Medium knowledge, i.e., partial research results support recommendations for commercial applications with restrictions, (5) Elevated knowledge, i.e., conclusive results supporting recommendations for commercial applications.

The result of this inquiry (Fig. 5.4) indicated that, in the opinion of the researchers, there are some fields, e.g., seeds technology in which research has had tremendous advances since 2008 and in which some technology transfer actions can now be implemented. It also indicated that some research fields still require attention in order to reach a stage where technology transfer actions can begin, e.g., selective breeding and cultivar development.

Genetic Resources, Genomics and Selective Breeding

Despite the lack of some basic and technical information, *J. curcas* is being disseminated in various Brazilian regions and recent analyses indicate that more than 40,000 ha of physic nut are already planted (Rosado et al. 2010) even though no elite (improved) cultivars are available (Carels 2009). As a result, much of the research effort is concentrated on building resources that may allow the genetic improvement of the species (Achten et al. 2010; Divakara et al. 2010; Rosado et al. 2010). In the perspective of future breeding efforts, Embrapa assembled and characterized a germplasm bank (Fig. 5.5) with about 200 accessions (Laviola et al. 2010; Rosado et al. 2010). This germplasm bank represents most of the genetic variability of the species in Brazil (Rosado et al. 2010). The initial characterization


Germplasm Bank
Selected Genotypes
Seeds Technology
Cultivar
Seedlings produced from seeds
Seedlings produced from cuttings
Formation pruning
Maintaining pruning
Spacing
Spacing (pasture)
Spacing (consortium)
Fertilization (Planting phase)
Fertilization (Growing phase)
Fertilization (Producing phase)
Irrigation methods
Weeds management
Weeds identification
Weed control methods
Chemical compounds to control weeds
Pest identification
Pests control methods
Chemical compounds to control pests
Disease identification
Diseases control methods
Chemical compounds to control diseases
Harvest point
Manual harvest
Semi-mechanized harvest
Mechanized harvest
Drying
Seeds storage
Oil extraction
Biodiesel production
Fertilizer production
Animal nutrition - cake
Technical indexes
Production costs
Energetic balance
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3,0
4,0
5,0

Fig. 5.4 Current knowledge in different physic nut research fields (*x axis*) and their respective knowledge score (*y axis*). The scores are obtained according to the following rating: (*0*) Without knowledge, i.e., researcher does not have enough knowledge to express his opinion, (*1*) No information, i.e., there is no information or research result available, (*2*) Judgment, i.e., there is no information, but practices from other crops may be adopted, (*3*) Low knowledge, i.e., initial research results (does not support recommendations of commercial applications), (*4*) Medium knowledge, i.e., partial research results (supports recommendations of commercial applications with restrictions), (*5*) Elevated knowledge, i.e., conclusive results (support recommendations of commercial applications). *Blue bars* indicate a specific field for which a considerable amount of information has already been gathered. By contrast, *red bars* indicate a specific field for which considerable amount of research still needs to be done. The blue arrow indicates that there is an urgent need of more refined research and of technology transfer actions. The *red arrow* indicates that there is an urgent need to scale up research in order to get enough information for the fields marked in *red*

Fig. 5.5 Physic nut germplasm bank established by Embrapa in Planaltina (Distrito Federal, Brazil). The germplasm bank contains near 230 accessions and it is now being enriched with seeds collected in the center of origin of the species (i.e., Mexico)

of such bank, using phenotypic (Laviola et al. 2010) and molecular data (Rosado et al. 2010), indicated that although limited, there is enough genetic variability available for breeding purposes (Laviola et al. 2012b). Sources of variability for *Oidium* spp. resistance, seed toxicity and rate of male/female flowers, for example, have been characterized in this bank.

It seems that although limited, the genetic variability is sufficient to ensure that elevated genetic gains can be obtained with selective breeding for superior individuals and families. It is important to note that genetic diversity estimation based on neutral molecular markers not always mimic the genetic variability for the traits of interest, since markers can be located in genes not directly involved in the genetic control of the trait, but also in non-coding regions. Therefore, we do believe that the accessions represented in the Brazilian germplasm constitute a population that can be used as a starting material for breeding purposes. Of course, since the variability is limited, the addition of accessions collected at the centers of origin and diversification of the species is highly desirable and necessary to ensure that genetic gains can still be obtained in the long term.

Two main strategies are considered for increasing physic nut seed yield: the plant management and the selection of superior genotypes (Openshaw 2000). Pallet and Sale (2006) observed an additive relationship between these factors due to the expression of superior genotypes in favorable environmental conditions. Based on these considerations, Embrapa initiated a breeding program (Table 5.3) with the purpose to select materials of physic nut with high seed yield and/or oil production by recurrent selection. Genetic improvements for grain yield will rely on intra-population recurrent selection, manipulation of genetic variance and vegetative

Table 5.3 Embrapa's program of physic nut improvement

Activity	Year 1		Year 2		Year 3		Year 4		Year 5		Year 6		Year 7	
	1° S	2° S	1° S	2° S	1° S	2° S	1° S	2° S	1° S	2° S	1° S	2° S	1° S	2° S
Collection	▨													
AGB[a] implantation		▨												
AGB enrichment					▨		▨		▨		▨			
Agronomic characterization			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨		
Genotypic characterization				▨		▨		▨		▨		▨		
Crosses					▨		▨		▨		▨		▨	
Mass and recurrent selection					▨		▨		▨		▨		▨	
Evaluation of GxE interaction						▨		▨		▨		▨		▨
Elite cultivars and clones														▨

[a] "AGB" is for active germplasm bank. "S" is for semester

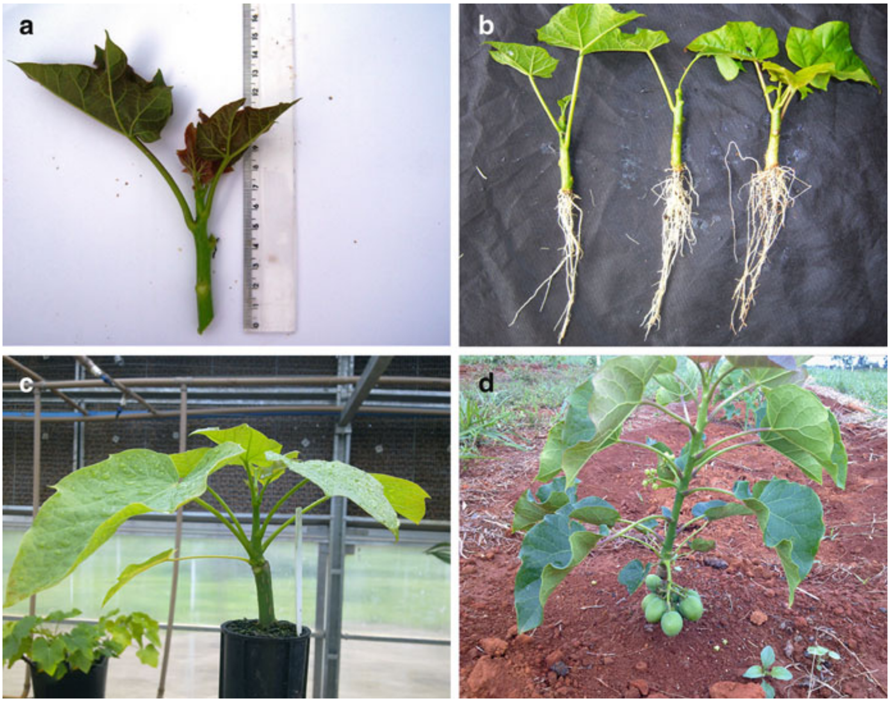

Fig. 5.6 Evaluation of a cloning methodology by mini-cutting (**a**) of physic nut to propagate elite genotypes (**b**, **c**, **d**) for the Embrapa's breeding program

propagation of superior genotypes. Asexual propagation allows breeders to maximize the genetic variation that is transmitted to the next generation. Vegetative propagation allows breeders to shorten the time required to release improved materials to the market by selecting superior genotypes in heterogeneous populations. Since physic nut is an allogamous species, the use of clones may also be an alternative to generate more uniform plantations. In this context, the use of mini-cuttings has been considered promising (Fig. 5.6). Field validations are still needed in order to evaluate the development of the seedlings produced by this technology.

In terms of breeding for seed production, it must be stressed that several traits, such as seed size, seed oil content, seed yield and plant branching seems to determine the final oil production in physic nut (Heller 1996). However, refined evaluations of these oil yield components showed that more than 90% of the oil yield variability is related to the seed yield variability, indicating that biomass production is the major determinant in oil production (Spinelli et al. 2010). In this context, it should be mentioned that despite some optimistic prognosis of seed yields over 3 $t\,ha^{-1}$, only small seed yields (1 $t\,ha^{-1}$ or less) have been reported in different environmental conditions (Costa et al. 2010; Ginwal et al. 2004; Raiger et al. 2011), indicating that much can potentially be gained with breeding efforts. The genetic basis of physiological stress induced by water deficit or pests and diseases affecting the production of physic nut are being investigated.

The intra-population recurrent selection is expected to increase the frequency of favorable alleles for traits of interest, including seed yield. An initial study of the breeding potential of the Brazilian material suggested that three consecutive measurements are necessary to predict accurately and efficiently the true breeding value of an individual. Genetic correlations among the numerous traits of interest were also obtained providing starting points for indirect selection of the best individuals and to better design management practices (Laviola et al. 2012b).

However, as no elite cultivars are currently available and because breeding approaches based on recurrent selection for elite genotypes are only in their initial stages, rapid development and release of improved physic nut cultivars is highly desirable. It is well known that the productivity is generally a polygenic trait, based on quantitative gene expression, with low heritability and, thus, strongly affected by environment factors. In Physic nut however, genetic studies were carried out to assess the variability and inheritance of this trait. These studies showed that both individual heritability and heritability of progenies are elevated (~70%), providing evidence that selection for genotypes with higher yield is possible (Laviola et al. 2010, 2012a). Different strategies have been used for the early selection of superior genotypes and Drumond et al. (2010) selected genotypes in irrigated conditions with a yield of 2.12 kg plant^{-1} 12 months after planting. With regard to other environments, Laviola et al. (2010) observed that seed yield ranged from 0 to 0.18 kg plant^{-1} 12 months after planting in the semi-arid conditions of the Brazilian savannah. Rocha et al. (2011) observed a genetic progress of 99% when selective breeding according to mass selection was applied to the ten more productive genotypes, which corresponds to an estimated grain yield of 1.23 kg plant^{-1} or 2.25 t ha^{-1}. Another study conducted in Brazil (Laviola et al. 2012b) showed that (i) early selection of physic nut is possible, (ii) it can promote high genetic gains on the initial stages of the breeding program, and (iii) genotypes with increased productivity can be potentially selected and released as improved cultivars. Moreover, it showed that genetic improvement of physic nut can be performed by early recurrent selection as an alternative to the slower, but more accurate recurrent selection with three consecutive measurements (Laviola et al. 2012b).

In addition to breeding methods, such as (i) assessment of the variation in wild sources, or germplasm collections, (ii) selection of superior or elite genotypes, (iii) recurrent selection of naturally breeding populations, the physic nut improvement should also benefit from the application of genomic breeding strategies, e.g., the use of molecular markers (Gomes et al. 2010; Johnson et al. 2011; Liu et al. 2011; Mastan et al. 2012) in marker assisted selection (MAS) (Grattapaglia and Kirst 2008) and genomic selection (GS) (Grattapaglia and Resende 2011; Resende et al. 2012a, 2012b). The rationale is that the physic nut small genome size (~400 Mb) is a favorable feature for the use of such tools (Carvalho et al. 2008). In depth studies are needed to test the efficiency in integrating genomic breeding tools in physic nut improvement. By adopting the strategy to sequence and align cDNA from various physic nut individuals, a large SNP (Single Nucleotide Polymorphism) discovery initiative is currently underway in Brazil (Gomes et al. 2010; Silva-Junior et al. 2011). Initial results indicated that thousands of SNPs were discovered and additional validation of such SNPs could prove to be efficient to build a chip, with hundreds to thousands genotypable SNPs. DArT

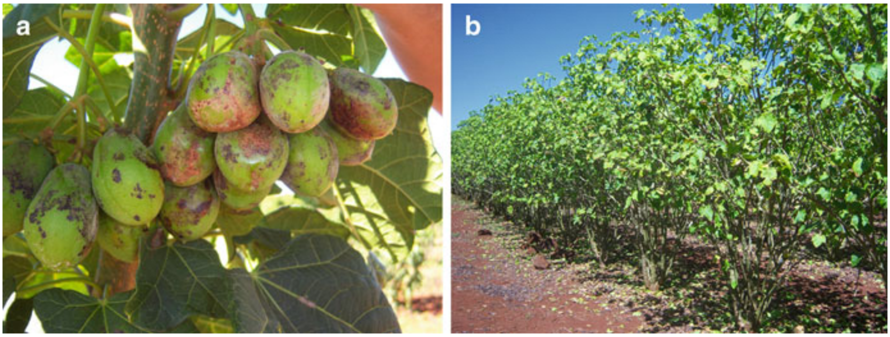

Fig. 5.7 Occurrence of *Oidium* in physic nut fruits (**a**), "Green leafhopper" (*Empoasca kraemeri*) causing severe defoliation in a physic nut plantation (Mato Grosso do Sul, Brazil) (**b**)

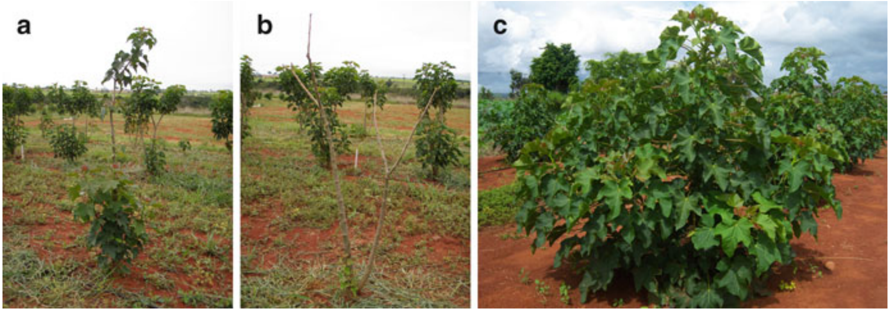

Fig. 5.8 Comparative growing vigor in non-toxic genotypes (**a** and **b**) and toxic genotype (**c**), 12 months after planting. The toxic accessions are much more vigorous than the non-toxic genotypes

(Diversity Array Technology) markers are also being developed in collaboration with Diversity Arrays Technology Pty Ltd. Such resources (SNPs and DArTs chip) could potentially be used to assist breeding efforts, either by MAS strategies (MARS—marker assisted recurrent selection or GS—genomic selection) in the near future. Powdery mildew resistance, pest resistance (Fig. 5.7) and non-toxicity (Fig. 5.8) are among the traits that could be the initial targets for MAS, and productivity as well as oil content among the traits that are to be targeted by GS. It is interesting to note that the development of commercial non-toxic cultivars could contribute to aggregate value to the press cake that results of the oil extraction, also contributing to reduction of the environmental passive (nowadays it is necessary to detoxify the press cake). However, since genotypes that do not produce phorbol esters are less vigorous and produce less oil than the toxic cultivars (Fig. 5.8), it is probable that MAS will have to be implemented in backcrossing schemes. It is also being considered to cross non-toxic genotypes with high yielding individuals followed by selection of high yielding and vigorous non-toxic plants.

Since Synthetic Genomics Inc. (SGI), Asiatic Centre for Genome Technology (ACGT) and Kazusa DNA Research Institute have completed the physic nut genome project recently (Sato et al. 2010), the annotation of 95% of the physic nut genes are available freely by online access (http://www.kazusa.or.jp/jatropha/). The genome information by Kazusa is expected to boost the discovery of new molecular markers. As tissue culture protocols are available for regeneration of physic nut genotypes (Jha et al. 2007), genetic transformation and gene transfer, can perhaps be an alternative to modify and, or introduce genes for important traits, especially for those for which variability is rather limited.

Production Systems

One of the most popular claims about physic nut is that it tolerates low technological level in its cropping conditions. However, in such conditions the fact is that its productivity is low, making it economically unviable (Laviola and Dias 2008) Although physic nut is indeed a species considered rustic and adaptable to different environmental conditions, it is now clear that physic nut needs cultivation technologies (fertilizer application, pest and disease control, management practices, etc.) to sustain economic levels of oil production.

In order to establish a production system, an important issue that we are considering is the plant phenology. All management practices depend upon this information. Although the phenological phases are not rigid, the duration of each phase is likely to be influenced by environmental conditions that occur in a specific region or year. Considering this, we have attempted to study the plant´s phenology in one of the main Brazilian vegetation, the savannah (also known as cerrado). The results of such effort indicate that initial establishment of the plants is probably one of the most important phases as any stress that occurs in this phase can compromise the plants architecture and hence, the plants yield. It is now clear that, following the second year, the phenological phases occur in repeated cycles. During the raining period, with the rise of the temperature and humidity, the plant's vegetative growth reaches its maximum, with the formation of new shoots and inflorescences. In the Brazilian conditions, fruiting occurs on average 3 months after the start of the growing season, mostly in the terminal region of the shoots recently grown. In that respect, it is noteworthy that the production of new inflorescences is highly dependent on continuous vegetative growth. This indicates that in this period it is important to manage the plants properly, i.e., by avoiding the occurrence of biotic and abiotic stresses so as to allow the plant to express all its potential. At the end of the growing season, with the fall of temperature and humidity, the deciduous stage begins. In this period physic nut loses its leaves and remains in rest conditions until the beginning of the next vegetative growth phase.

As outlined above, the phenological data support perhaps all decisions on management practices to be performed. Aiming at providing the plants an ideal environment for the vegetative growth stadium, the control of invading seeds, pests and diseases is imperative. According to Dias et al. (2007), a number of pests and diseases from other plant species may affect physic nut. Currently there are no specific pesticides and fungicides recommended for the culture and because of that situation,

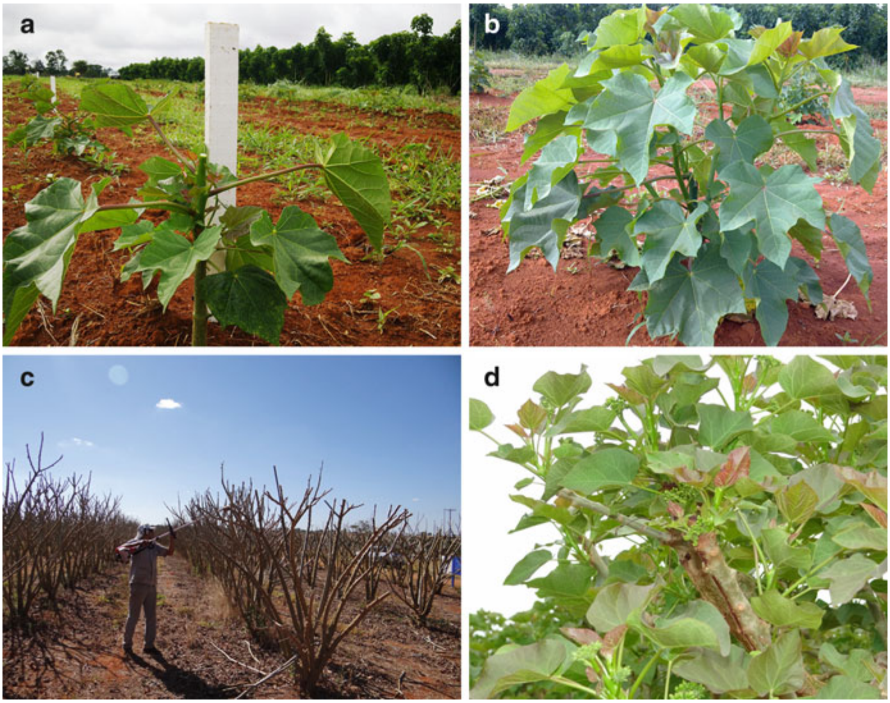

Fig. 5.9 Pruning in physic nut. The formation pruning is to be performed 45 days after planting (**a**–**c**). The maintenance pruning is carried out when plants are 2 m high and is seen as a way to standardize the plant`s architecture and production (**d**)

an integrated management approach is been pursued. Such an approach considers the adoption of cultural, chemical and biological strategy concurrently to control the undesirable biotic interactions. In that context, the development of cultivars with enhanced resistance/tolerance to such stresses is seen as one of the best approaches. Initial results are encouraging in that respect, since resistant genotypes for many of these stresses were already identified in the Brazilian germplasm bank (unpublished data).

While trying to develop a production system devoted to physic nut cultivation, it is important to determine the plant's responses to mineral nutrition. Initial results gathered among many Brazilian culture conditions indicate that physic nut responds well to fertilizer applications and a correct balance of nutrients is required to warrant plants with satisfactory fruit yield (Laviola and Dias 2008). Thus, annual fertilization is necessary to supply enough amounts of nutrients to support the plant's vegetative growth and reproductive development.

Another issue that has been a matter of study in the context of the Embrapa research initiative on physic nut is the effect of pruning. Two types of pruning are being tested, i.e., formation pruning and production pruning (Dias et al. 2007) (Fig. 5.9). The objective of the formation pruning is to standardize the plants (in

Fig. 5.10 Mechanical harvesting systems in physic nut from front (**a**) and lateral (**b**) (photos taken in Bioauto Farm, Nova Mutum, Mato Grosso, Brazil)

terms of number of basal branches and height) and to favor the basal shoot ramification by breaking the apical dormancy (the apical meristem is actually removed). On the other hand, the objective of production pruning is to renew the productive ramifications as well as to reduce the plant size, which facilitates cultural practices and fruit harvesting. Even though the effects of these techniques are still being evaluated, it seems to be very promising as it contributes to standardize fruit production (unpublished data).

Considering fruit production, the most important aspect that is currently being studied is the maturation process. In general, maturation is quite uneven during the production period (which last between 4 and 5 months in the Brazilian conditions) and progressive with a mix of immature and mature fruits on the same raceme. Because of these characteristics, fruits must be harvested in four or five occasions, burdening the production cost (discussed below). Two approaches are currently being investigated to understand the determinants of maturation process in physic nut. The first is to gather genetic individuals that display fruit maturation concentrated in discontinuous and short periods (as few as possible). Such approach depends on the existence of genetic variability for this trait in the germplasm bank and on its inheritance. The second approach relies on the application of an exogenous chemical that will interfere with the plant physiology and allow the standardization of fruit maturation. Both approaches are in initial stages and no conclusive results are yet available.

Nowadays physic nut fruits are harvested manually. Mechanized (Fig. 5.10) and semi-mechanized harvest systems are currently being developed in Brazil by taking advantage on the knowledge gathered with other perennial cultures, such as coffee, for example. Correct dimensioning of such systems however depends on the size of the genetic material to be harvested and on the plants density, which ultimately depends on planting spacing. These issues are still being studied and correct dimensioning of the harvest systems will be possible. These mechanized and semi-mechanized systems are expected to improve the harvest efficiency besides lowering significantly the costs of physic nut production.

By-Products

The cake that results from pressing the physic nut seeds may potentially constitute an excellent organic fertilizer since it is rich in nitrogen, phosphorous and potassium (Achten et al. 2008). Since the press cake is also rich in protein (43–63% depending on the method used for oil extraction), it could theoretically be used along with the fruits shell as a high protein supplement in ruminant feeds, however, such use is currently impossible given the high toxicity level of the press cake. As previously mentioned, a number of phorbol esters occur in high concentrations in the seeds. Removal of phorbol esters during processing is achievable (Rakshit et al. 2008), however the possible presence of toxic degradation products from phorbol esters after treatment cannot be ruled out (Achten et al. 2010). Aiming at solving such a problem, we are currently focusing on two different, yet complementary approaches to completely remove phorbol esters from physic nut seeds.

The first approach relies on the identification of phorbol ester free genotypes in our germplasm bank. The current data indicate that as toxicity, in terms of phorbol esters presence seems to be expressed qualitatively (Sujatha et al. 2005) and may be regulated by only one or a few genes (see Sato et al. 2010; Gomes et al. 2010, for a discussion). It has also been suggested that the trait is subjected to maternal inheritance, i.e., the phenotype of the maternal parent is passed on to the progeny. Such mode of inheritance was suggested earlier by Sujatha et al. (2005), however, it is still to be proven in the next couple of years. In that respect, non-toxic genotypes are common in the Mexican germplasm (Mastan et al. 2012) and we are now confirming the genetic basis of such trait in our genetic material through the generation of large segregating populations and genetic mapping. The intention is to map the locus (loci) responsible for (non) toxicity with molecular markers that are currently in development to assist selective breeding for non-toxic genotypes. If the non-toxicity is indeed inherited from the mother to the progeny, this can have a profound effect on breeding programs worldwide, as the main traits subjected to improvement generally does not follow such mode of inheritance. The incorporation of trait subjected to maternal inheritance in the breeding programs will force breeders to consider this information for cross designs.

The second approach relies on the application of the following treatments: (i) thermoplastic extrusion process associated with chemical additives, (ii) solvent washing and (iii) biotransformation (by means of fungus, such as yeast). The main focus here is to detoxify the press cake, either through the removal of phorbol esters or modification of phorbol ester molecules so that the new form loses their toxic activity. The absence of phorbol esters guarantee that no soil (Gressel 2008), animal or human contamination occurs. *Phorbol zero* cultivars would add tremendous value to the culture especially for small farmers (the majority of Brazilian physic nut growers), since it would allow the press cake recycling through animal feed.

Perspectives of Physic Nut Cultivation According to Different Scenarios

The determination of the economic viability of physic nut cultivation is an important issue. Therefore, it is necessary to consider that physic nut cultivation can occur in different scenarios: (i) large production systems with high technological level, (ii) small production systems with mid-technological level and (iii) small production systems with low technological level associated or not with livestock or/and other intercrops, such as oleaginous species, grasses or even food crops.

Considering the first scenario, it is clear that improved genotypes are necessary. Such improved genotypes must have large seed and oil productivity, since physic nut is the main product. The genotypes are also expected to be highly responsive to improved environmental conditions, since fertilization and irrigation are likely to be the default situation in this scenario. Genotypes highly tolerant to biotic and abiotic stresses, such as the ability to grow in marginal areas are the ones that most likely will support large physic nut plantations (in order to avoid food crop displacement). Such improved genotypes are expected to be commercialized as mixed populations of elite vegetative clones to maximize the genotypic potential of the individuals whose features are optimized to growing conditions. Mechanization of both cultural practices, such as (i) weeds, pest and disease control and (ii) fruit crops will be a *sine qua non* condition, since the costs of manual operation of these services is too expensive to make the business viable. All these operations are undoubtedly bound to increase costs. However, with the use of elite individuals, fertilization, irrigation and mechanized practices, the marginal return of physic nut planting is expected to be maximized and the gains to be a function of the venture scale. Moreover, the commercialization of co-products, such as press cake should generate extra profit when non-toxic genotypes will be available and contribute to increasing the viability of physic nut plantation.

It can be argued that growing physic nut on an industrial scale with high inputs (irrigation, fertilizers, and pesticides) would lead to the failure of many principles of sustainability of biofuel production and, thus, expose this new crop to negative pressures from environmentalists that could claim other oilseed species eventually better matching their considerations. However, this concern can be ruled out, since PNBP stimulates planting of a diverse array of oilseed species and encourages the diversification of oilseed materials in order to fit local conditions to the most possible extent. In addition, Brazil fortunately has a huge amount of land available for agriculture and in our opinion physic nut can be grown together with other oilseed species without competing risks between food and fuel crops, since oilseed species are thought to be regulated on a regional basis.

Keeping the second and the third scenarios in view, it is clear that genotypes will have to be improved considering that adequate management practices will not always be available. In this way, genotypes with robust features are desirable as such genotypes maintain their productivity potential even if the environmental conditions are not at the optimum. In these last scenarios, the press cake will probably assume another level of importance, since it can be used to feed livestock created in the same farms. In order to keep the implantation costs as low as possible, improved genotypes will probably be commercialized by means of their seeds. Enhanced seed and oil productivity will also be important, but another important issue could be to keep the wild type of continuous fruit maturation. This characteristic is important for small farmers, since it minimizes the period without production and fruit harvesting can rely on family labour, reducing the impact of its costs. A last issue that needs to be considered is that, in such cases, profit can be both direct and indirect. Direct profit comes from de-commercialization of seed, oil or press cake while indirect profit comes from the use of the press cake as an organic fertilizer or as feed for livestock. Indirect profit can also be obtained by the valorization of intercrops. In that respect, the intercrop production may indeed be an important factor to make small production systems viable. In all cases, economic studies are underway to determine the exact conditions needed to make physic nut cultivation a viable business.

Conclusions

The report presents the importance of physic nut for the Brazilian economy considering its potentials, challenges and the concerns covered by the research that is underway in the country. We intentionally avoid focusing on the economic aspects of physic nut cultivation because we believe that it can only be considered after the availability of improved cultivars and suitable production systems for the different Brazilian regions. As discussed above, some more years will be necessary to develop such improved cultivars and to determine suitable production systems even with worldwide initiative.

However, our cautious, but yet optimistic vision is that the Brazilian research network may successfully contribute to make physic nut a viable feedstock for biodiesel production in the near future. The increasing demand for biodiesel and the potential demand for jet fuel (biokerosene) in the near future is a guarantee that the biofuel market will grow continuously in the upcoming years and also provides stimulus to scale up physic nut research.

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