Sujatha Mulpuri · Nicolas Carels
Bir Bahadur *Editors*

Jatropha, Challenges for a New Energy Crop

Volume 3: A Sustainable Multipurpose Crop

Springer

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Foreword – I

Despite its proven potential as a cost-efficient bioenergy feedstock, *Jatropha curcas* L. (Jatropha) remains to be a domesticated plant in terms of various traits. These include processes such as its reproductive biology, which is critical in gaining an insight into breeding for synchronous flowering. That insight would accrue through a molecular understanding of the process, for which genomic information on the plant is required.

Nearly more than a decade and a half of systematic, international, collaborative studies has been conducted on understanding the morphological and genetic diversity of Jatropha in order to guide parent selection for cross-pollination. Numerous genetic markers have been developed for the purposes of uncovering genetic diversity to assist in the breeding efforts. Hence, the first wave of Jatropha improvement is coming from traditional breeding approaches that utilize natural variations within *J. curcas* and in other genetically compatible species. There have also been efforts to introduce more variation through mutagenesis. Commercially relevant traits of shorter stature, higher number of female flowers, better self-branching, increased seed oil content, and decreased input requirements are being targeted. While efforts through traditional breeding are beginning to show initial successes in these target traits, there are many problem areas where traditional breeding-based solutions have limitations. These include Jatropha plant diseases, oilseed toxicity, and its less than ideal fatty acid profile.

Modern plant science-based approaches provide a valuable avenue in further improvement of Jatropha, similar to crops such as cotton, canola, soybean, and maize. Such approaches include the biotechnology-mediated possibility of introducing some well-characterized genes from other organisms to test for effects in Jatropha. Fine-tuning the seed's fatty acid profile and its biosynthesis and that of other metabolites such as the seed oil toxins is a primary target. A precondition to Jatropha biotechnology is the availability of efficient and robust regeneration systems and transformation systems, preferably through *Agrobacterium*. There have been several reports on regeneration and genetic transformation of Jatropha, and it remains to be tested if there is an efficient and robust protocol across laboratories.

With good transformation system, it is also important to have promoters that can drive spatiotemporal-specific gene expression to suit various objectives. The primary source of such promoters would be known from promoters of other plants, but a systematic understanding based identification of endogenous promoters is also required. Similarly, a good structural, functional, and comparative understanding of the Jatropha genes, genomes, and genotypes will help in achieving the goal of manipulating its secondary metabolism. A recent report of seed-specific enhancement of oleic acid in Jatropha illustrates the beginning of utilizing biotechnology in Jatropha improvement. Also noteworthy are the use of one endogenous promoter and the deletion of antibiotic selection marker gene in the final transgenic Jatropha. The use of CRISPR technologies, absence of foreign genes, and the fact that humans will not be directly exposed to the transgenic Jatropha products will all help in regulatory approvals for commercialization. With such possibilities in understanding and manipulating the most desirable traits of better biotic and abiotic stress tolerance, and quality and quantity improvement in seed oil, the doors will open to addressing traits that impact a whole range of products, including oils and bio-plastics, along value addition, in establishing Jatropha plant as a natural bioreactor. Thus the "explosion" of scientific activity on Jatropha since 2008 leading to the availability of a genome sequence and a lot of upstream research within 4 years since, coming up to successes with regeneration and transformation, is already establishing Jatropha as a model non-edible oilseed crop, including as a first crop to be purposely domesticated in modern times. Availability of records on such a process would be very useful in comparative evolution.

Publication of this book intends to produce a synthesis of what has been achieved and to help scientists move forward in understanding and utilizing Jatropha. It serves as a milestone but also showcases how quickly we can achieve a critical understanding on a novel bioenergy plant with the help of accumulated knowledge on model food crops and with the advanced scientific and research tools and technologies. The editors of the book, Drs. Mulpuri, Carels, and Bahadur, are the doyens in Jatropha research and development and have made significant contributions in our understanding of the potential and uses of this plant. It is most appreciated that they work closely and tirelessly to bring together information to update and guide the Jatropha research into the future through the present volume on *Jatropha, Challenges for a New Energy Crop*: *Volume 3 – A Sustainable Multipurpose Crop*. Their efforts and results, and those of all the scientists, presented in this volume and as a body of research outputs, will hopefully contribute to an increased funding from public and private sector to further support Jatropha research and development, which will further expedite the process of developing Jatropha as a new energy crop.

Platform Leader-Strategic Innovation, Ajay Kohli
International Rice Research Institute,
Los Baños, Philippines

Foreword – II

During his tenure as Director General, Indian Council of Agricultural Research, Dr. R.S. Paroda made several efforts to significantly boost oil seed productivity in the country.

I am indeed happy that the learned Editors have decided to bring out the present volume on *Jatropha curcas*, considered as a potential bioenergy feedstock plant. This is the third volume which speaks of their dedication, diligence, and sheer determination to popularize this oil seed plant.

The present volume covers several areas like *physiology and plant production, selective breeding and genetic diversity, feeding use, coproducts, processing, and socioeconomic sustainability*.

Undeniably, the plant has been around for the past several decades, though it is only recently that attempts are being made to understand its reproductive features and genome information. Initially, improvement was based on traditional breeding that utilizes natural variants existing within *J. curcas* and in other genetically compatible species. With a view to raising desired hybrids, suitable parents with desired traits, based on morphological and genetic diversity, need to be identified for crossing. Consequently several genetic markers have been developed with a view to unravel the genetic diversity. With a focus on several desirable traits, e.g., increasing oil productivity and decreasing input, better self-branching, shorter stature, higher number of female flowers, and higher oil content are being developed, and time-tested techniques like mutagenesis and transgenesis have been exploited.

Unarguably, traditional breeding poses several "roadblocks," for instance, *Jatropha* pathogens, toxicity of oil seeds, and its less than ideal desired fatty acid profile. In this context, biotechnological techniques provide valuable alternative to traditional methods as demonstrated in several cash crops including oilseeds. I am happy to state that the umpteen impressive publications on Jatropha of the editors of the present volume, in high-impact journals, need special mention.

In my view, the present volume provides a deep glimpse of our revered experts in their respective fields of specialization and explores all conceivable horizons in the field.

I am of the considered opinion that it offers a broad perspective on the current status on the economic and sustainable aspects of this important bioenergy plant.

I congratulate the eminent editors for timely bringing out this publication which will enthuse the growers to domesticate this important oilseed species.

Former Director, Life Sciences,
and Advisor, Jaipur National University,
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C. P. Malik

Preface

We initiated the Jatropha books project about 9 years back, and the first volume, entitled *Jatropha, Challenges for a New Energy Crop: Farming, Economics and Biofuel* (30 chapters), was published in 2012 followed by the second volume *Genetic Improvement and Biotechnology* (31 chapters) in 2013 both published by Springer New York. At that time, physic nut (*Jatropha curcas* L.) was emerging as an oilseed option to expand biodiesel production especially in marginal land poorly suitable for the cultivation of crops producing edible oil. These books gave a comprehensive account of the research going on internationally with the purpose of stimulating future development in this area. Five years went by since the publication of the first two volumes, and an update is now proposed with a third volume. Considering its potential as an alternative to fossil fuel, there have been considerable works on various technological aspects of physic nut, but it must still be considered as a semi-wild species. Further efforts considering selective breeding are necessary to increase yield and improve agricultural features to bring physic nut to the status of an industrial crop, which is needed for a commodity such as oil for biodiesel, given that selling prices can only be low. Volume 3 (25 chapters) intends to give a positive global picture on physic nut, a crop that has suffered from the fact that it is not yet fully domesticated despite its promising agronomical and economical features. Physic nut has the benefit of a high potential productivity larger than 7 tons of seeds per hectare (but actually commonly less than 2 tons per hectare) associated to proper oil composition for biodiesel conversion. In addition, physic nut is easy to subculture in vitro and to manipulate in laboratory as well as to transform in vitro genetically. Furthermore, it has a significant genetic diversity in its center of origin (Central America) and is relatively easy to cross-hybridize within the various species of the genus. Because of these promising features, physic nut is definitively on the rise as a crop, and some companies have already successfully understood how to handle it up. Thus, it is our concerted duty to sustain the efforts at domestication of this crop in order to provide additional solutions to the still too few industrial oilseed crops available for biofuel production.

The Editors have the expertise in agronomy, botany, selective breeding, biotechnology, molecular biology, genomics, and bioinformatics, which enabled them to gather worthy and sounding contributions. Actually, at this stage of physic nut journey as an industrial crop, the understanding of its physiology and its selective breeding remain the main bottlenecks to improve its economic status. In addition, we dedicated some chapters to the discussion on (i) how its return can be improved by the exploration of by-products such as animal feed, biomass, and chemicals for health and medicinal aspects, (ii) how its oil can be better processed into biofuel, and (iii) what are the objectives to be reached to warrant its sustainability in the future. Thus, Volume 3 should interest biologists, biotechnologists, agronomists, breeders, decision-makers, and investors of the biodiesel chain.

By publishing this book, we aimed at supporting the people in developing a crop that should help populations from marginal areas to gain access to a biofuel that may boost their economy. In that respect, we believe that the book will be seen as helpful by the interested communities as has already been proven by the success of the two previous volumes.

We wish to express our gratitude to all the contributing authors from all over the world for readily accepting our invitation not only for sharing their knowledge but also for admirably integrating their expertise to the vast information from diverse sources and enduring editorial suggestions to finally produce this venture. We also acknowledge the huge support received from many colleagues in the preparation of the manuscripts as well as to our family members and relatives for bearing with our commitment to the book. We wish to express our appreciation for the help given by Dr. Mamta Kapila (Senior Editor, Springer Nature India, New Delhi), Mr. Daniel Ignatius Jagadisan, Project Coordinator (Books) for Springer Nature, and their team for the excellent cooperation being extended and many valuable suggestions. We wish to thank Dr. Kenneth Teng from Springer New York, USA, where the book proposal for Volume 3 was submitted and approved prior to be subsequently transferred to Springer India.

We wish a pleasant reading of Volume 3 to scientists and students around the world who are interested in the subject of physic nut as a multipurpose crop.

Finally, we would like to apologize for any omissions or mistakes, or failures that may subsist in the book.

Hyderabad, India — Sujatha Mulpuri
Rio de Janeiro, Brazil — Nicolas Carels
Warangal, India — Bir Bahadur

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Editors and Contributors

About the Editors

Sujatha Mulpuri graduated in Plant Sciences from the University of Hyderabad (UoH), India. She has a Ph.D. in Genetics from Osmania University (OU), Hyderabad, and has worked on intergeneric and interspecific affinities between Ricinus and Jatropha. Dr. Sujatha is a versatile researcher, adopting conventional and modern tools for the improvement of oilseed crops encompassing the areas of genetics, tissue culture, and biotechnology. Her achievements include the development of stable male sterile lines in safflower, sunflower, and niger, optimization of tissue culture and genetic transformation protocols, development of transgenic events in castor for foliage feeders and sunflower for resistance to necrosis disease, and use of molecular markers in diversity analysis and tagging of desirable traits in sunflower (downy mildew, fertility restoration) and Jatropha (non-toxicity). Dr. Sujatha has also carried out pioneering work on Jatropha with regard to tissue culture, genetic diversity analysis of native and world collections, and interspecific hybridization, which have provided valuable leads for genetic enhancement of *J. curcas*.

Nicolas Carels graduated in Agronomy in Belgium and completed a Ph.D. in Plant Pathology (FUSAGx, Gembloux) prior to working as a scientist on the elaboration of the first genetic map of sugar beet at the end of the 1980s (ICIseed-SES, Belgium). He then moved to Paris (IJM, CNRS, France) where he completed a second Ph.D. on the genome organization in plants. He continued his work on genomics in Italy (SZN, Naples) and Spain (INTA-CAB, Madrid, Torrejon de Ardoz) before moving to Brazil (Bahia, Ilhéus, UESC), where he contributed to the application of bioinformatics and genomics to the improvement of cocoa and rubber tree for resistance to fungal diseases. His initial investigations on Jatropha covered the measurement of the genome size by flow cytometry and the application of reverse genetics to detect QTLs for oil production with the purpose of breeding Jatropha for this trait. He is now a Federal Officer of Fiocruz (Rio de Janeiro, Brazil) and is interested in the exploration of genomics, system modeling, bioinformatics, computational biology, and natural products for the benefit of human health, with a particular focus on therapeutics for cancer.

Bir Bahadur former Professor, Chairman and Head of the Department, and Dean of the Faculty of Science at Kakatiya University, Warangal, India, has also taught at Osmania University, Hyderabad, India. He obtained his Ph.D. in Plant Genetics from Osmania University and was closely associated with Prof. J.B.S. Haldane, F.R.S, a renowned British geneticist. He made significant contributions in several areas of plant biology, especially heteromorphic incompatibility, genetics, mutagenesis, plant tissue culture morphogenesis, biotechnology, plant asymmetry and handedness, ethnobotany, application of SEM pollen and seeds in relation to systematics, medicinal plants, and Jatropha and Castor. He has mentored thousands of graduates and postgraduate students and taught genetics, biotechnology, plant molecular biology, plant reproduction, and related subjects for over 45 years and has accumulated 50 years of research experience in these areas. He has been the recipient of numerous awards, fellowships, and honors, including the Prof. Vishwambhar Puri Gold Medal, Bharath Jyoti Award, and Royal Society Bursary & Honorary Fellow of Birmingham University (UK).

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Part I
Selective Breeding and Genetic Diversity

Chapter 1
Genetic Improvement of Edible and Non-edible Jatropha for Marginal Environments in Sub-Saharan Africa

Matthias Martin, Brigitte Bohlinger, Elisa Senger, Euloge Dongmeza, Zafitsara Tantely Andrianirina, and Juan M. Montes

Abstract Jatropha has been grown in the past mainly for producing oil for biofuels preferably in marginal environments in sub-Saharan Africa, yet many projects collapsed due to overestimated yields and underestimated costs. The cultivation of jatropha genotypes that produce seeds lacking toxic phorbol esters ("non-toxic jatropha," "edible jatropha") currently receives increasing attention for animal feeding and food production. We give an overview of the challenges of jatropha cultivation in sub-Saharan Africa, discuss results from field trials in marginal environments from that region, and propose strategies for jatropha genetic improvement. Average seed yields obtained from selected hybrids at marginal places in Cameroon and Madagascar over 4 years demonstrated superiority of hybrids (2.2–8.3 t/ha) over wild germplasm, considerable extent of midparent heterosis (~400%), and potential to select for stably performing hybrids exhibiting less genotype-by-environment interaction. Cultivation of edible and non-edible jatropha hybrids had positive contribution margins per hectare and year (124–665 €/ha) in contrast to negative contribution margins of wild germplasm. The main breeding objective for edible and non-edible jatropha is to increase seed yield and stability across years and environments. Breeding objectives for seed quality parameters differ depending on the market segment. New hybrid varieties adapted to different climates have now become available. Jatropha companies and institutions providing

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solutions for superior genetics and technical guidance will lead to a new start in jatropha cultivation to turn future projects into success stories.

Keywords Breeding · Edible jatropha · Genetic improvement · Marginal environments · Non-toxic jatropha · Sub-Saharan Africa

1.1 Introduction

Jatropha curcas L. (jatropha) is considered a promising crop for the production of feedstock for biofuel production, preferably on wastelands and in areas where food production is hardly economical (marginal environments). Due to enduring low petrol prices however, the production of jatropha oil for biofuels has become less attractive, and alternative uses of jatropha are being investigated. Jatropha has traditionally been used as a medicinal plant in Africa (Sabandar et al. 2013), and nearly all plant parts (leaves, fruits, seeds, stem bark, latex, and roots) have been attributed effects. For instance, dried latex is used to stimulate wound healing, and recently, research activities have focused on the antitumor effects of curcin, a Type I ribosome inactivating protein in jatropha seeds (Luo et al. 2007; Prasad et al. 2012). Currently, the cultivation of jatropha genotypes that produce seeds lacking toxic phorbol esters ("non-toxic jatropha," "edible jatropha") receives increasing attention for animal feeding or food production purposes (Makkar and Becker 2009; Senger et al. 2017; Martinez Herrera et al. 2017). Prior to being used as food, a heat treatment of press cake, kernels, or kernel fragments for reduction of antinutrients is necessary. Additionally, the growth of resilient jatropha varieties for beneficial environmental effects, such as prevention of desertification, carbon capture, and afforestation of degraded land, has been proposed by various authors (Becker et al. 2013; Diédhiou et al. 2017; Noulèkoun et al. 2017).

Jatropha oil production has high potentials in semiarid and arid regions in sub-Saharan Africa (Wicke et al. 2011). Benefits of jatropha cultivation include its potential as a driver of rural economic and social development, potential developmental benefits (local production and supply of energy, creating additional markets for agricultural products, income generation for rural populations), as well as potential environmental benefits (improving soil conditions, increasing soil carbon storage, reducing soil erosion, and increasing agricultural productivity). Most of the jatropha projects that were established during the last 15 years, however, collapsed due to overestimations of yields coupled with underestimations of costs (von Maltitz et al. 2014).

Sub-Saharan Africa, i.e., that area of the African continent south of the Sahara, is one of the major jatropha-producing zones in the world (Jingura 2012). The regional agroecological zones in this vast area have been further classified by FAO (1994) as warm arid and semiarid tropics, warm subhumid tropics, warm humid tropics, and cool tropics, and many sub-Saharan African countries share mixes of these zones.

The lack of genetically improved jatropha varieties specifically selected for marginal environments in sub-Saharan Africa has been a major constraint for the economic success of jatropha projects in this region. In this chapter, our objectives were (1) to give an overview of the challenges of jatropha cultivation in sub-Saharan African countries, (2) discuss results from jatropha field trials in marginal environments from that region, and (3) propose strategies for genetic improvement of jatropha.

1.2 Challenges of Jatropha Cultivation in Sub-Saharan African Countries

During the global jatropha hype in the first decade of the twenty-first century, many jatropha projects were initiated especially in sub-Saharan Africa. The majority of these projects however failed to demonstrate viability due to a number of challenges. Gasparatos et al. (2015) identified energy security, poverty alleviation, and economic development as major policy imperatives that were the rationale behind the promotion of large-scale cultivation of jatropha as a bioenergy crop. The various projects had significant and country-specific environmental and socioeconomic impacts (positive and negative), namely, on greenhouse gas emissions, water availability and pollution, deforestation, biodiversity loss, poverty alleviation, energy security, loss of access to land, and food security. However, agronomic, institutional, and market failure were the main barriers for viable and sustainable biofuel investments at that time. Currently, the development of biofuels is hampered by absence of a clear vision of stakeholders and a lack of coordination between public actors in many African countries. Public actors have to establish institutional frameworks to facilitate sustainable jatropha production (Gatete and Dabat 2017). Policies promoting the cultivation of jatropha in sub-Saharan Africa need to be properly informed by empirical evidence, as pointed out by Jingura and Kamusoko (2018). These authors stressed the need to support the development of elite planting germplasm of jatropha to realize optimal seed yields, thereby providing opportunities for rural people to monetize the substantial labor inputs needed in jatropha cultivation and post-harvest processing. They concluded that only best practices will lead to achieving the acclaimed attributes of jatropha (reclaiming marginal soils and wastelands, drought tolerance, water and nutrient use efficiency, resistance to pests and diseases, low labor requirements, noncompetition with food production), which needs proper support by suitable policies.

In the past, jatropha cultivation attracted a large number of investors and farmers, who were promised high yields despite claims of low nutrient requirements and little need for care in combination with resilience of the plant to arid climates. This raised high expectations regarding its revenue potential for straight vegetable oil (van Eijck et al. 2014). Once it has been purified (filtering, neutralization, degumming, bleaching), straight vegetable oil can be used to fuel diesel, flex-fuel, or plant oil engines, as well as combined heat and power plants. The oil productivity of jatropha plantations in the past, however, has often been very limited due to cultivation of undomesticated wild germplasm (Achten et al. 2008). The seed yield in mature stands was often not viable, and knowledge of fertilization requirements and plant management was scarce (GTZ 2009). Most jatropha projects collapsed because of numerous reasons ranging from overestimated business plans, to low yield, underestimated costs, lack of market, and lack of policy framework to regulate the biofuel sector (von Maltitz et al. 2014; Gasparatos et al. 2015). One recurring and perhaps the most important reason for failure, however, is that jatropha plantations were unable to realize viable seed yields. In fact, in several instances in the past, seed yields had been far overestimated or could not be realized (Terren et al. 2012; Almeida et al. 2014; Muys et al. 2014; Romijn et al. 2014; Slingerland and Schut 2014; von Maltitz et al. 2014; Ahmed et al. 2017). Numerous examples from different sub-Saharan countries demonstrated the dependency of economic viability of jatropha plantations on seed yields.

Terren et al. (2012), for instance, reported a huge gap between expected and realized seed yields (5.25 t/ha vs. 0.5 t/ha in the 4th year) in a pilot plantation on marginal land in Senegal, although the best-known production techniques had been applied. The authors identified susceptibility to soilborne pathogens and a short vegetation period of the used plant material as main reasons for the low seed yields.

In a techno-economic assessment of the jatropha value chain for production of straight vegetable oil from jatropha to feed fuel generators for local electrification in Mali, Bouffaron et al. (2012) found that electrification projects had a high sensitivity especially to seed yields, petrol prices, characteristics of geographic locations, and labor costs. They reported that the highest financial risk was carried by the farming sector, and the most competitive jatropha projects are characterized by higher seed yields and low to medium investment costs.

Similarly, Somorin et al. (2017) reported that, based on a life cycle assessment of self-generated energy in Nigeria, replacing diesel fuel by jatropha biodiesel in electricity generators can reduce greenhouse gas emissions by 76%. The benefits however would depend on seed yields, material inputs, and environmental status of fossil diesel.

Ghana has seen a multifaceted development of jatropha-based biofuel projects (Nygaard and Bolwig 2017). High capital investments and low oil production volumes combined with market risks contributed to the collapse of the jatropha sector. Considering the technical and management perspective, discontinuation of jatropha projects was caused by a reduced access to information due to low levels of learning and knowledge-sharing and weak public research and development support. Osei et al. (2016) developed techno-economic models for optimized jatropha utilization under an outgrower scheme for Ghana. They found variability in the profitability of the models studied for farmers and processors. Key parameters influencing net present value and internal rate of return were (i) variation in prices of jatropha seeds as well as of oil and by-products, (ii) the assumed discount rate, and (iii) variation in jatropha seed yields. Acheampong and Campion (2014) reported that contrary to the expectation of growing jatropha on marginal lands, biofuel companies in Ghana had been given fertile land by local chiefs to grow jatropha. As a consequence, local farmers were forced to move to less productive land – a policy often leading to violent confrontation between parties.

On the other hand, Bosch and Zeller (2013) reported a wide acceptance and appreciation of a jatropha plantation established on marginal land in central Madagascar. They found significant positive effects on incomes and food security for the local population due to additional income generated by the labor-intensive jatropha production on the plantation.

Interviews with stakeholders involved in jatropha cultivation made it clear why many projects failed to meet initial expectations in Kenya (Hunsberger 2016). Commonly mentioned reasons, why jatropha activities and optimism had diminished, were lack of performance, i.e., low seed yields, susceptibility to pests and diseases, as well as high labor intensity.

Jingura (2011) emphasized the need to supply elite planting materials for optimization of seed yield and quality in Zimbabwe and considered the use of improved jatropha germplasm over wild germplasm, an objective for successful plantation establishment. Furthermore, the author suggested increasing the efficiency of jatropha cultivation through the development of improved plant varieties with a better response to fertilization.

In Tanzania, first commercial jatropha projects started in 2005. Van Eijck et al. (2014) compared two jatropha production systems: a smallholder system with a central oil processing facility (Diligent Tanzania Ltd.) and a plantation system (BioShape Tanzania Ltd.). Both models only showed marginal profitability at the time due to low yields and revenues, preventing sustained positive societal impacts. However, the authors expected that jatropha breeding would lead to more reliable varieties with higher yields consequently increasing financial feasibility. A profitability boost was expected by improved valorization of jatropha by-products, in particular by the use of the press cake for animal feeding.

Romijn et al. (2014) studied jatropha projects in Mali, Mozambique, and Tanzania. They found weak business cases and economically inviability of plantations due to a combination of reasons (investment costs, low yields caused by slow and unreliable crop maturation, inefficient oil pressing, inadequate utilization of by-products, and competitive prices of fossil diesel and palm oil). For smallholders on the other hand, jatropha only had limited value as a hedge crop in disadvantaged areas. Western support organizations phasing out their support for jatropha, based on the somewhat premature conclusion of general inviability of jatropha cultivation, further aggravated the situation. The authors concluded that the prospects would improve with more reliable and better yielding jatropha varieties due to plant breeding efforts.

To fully exploit the potential of jatropha as an energy source for the rural areas of Sudan, Abdelraheem et al. (2013) considered genetically improved cultivars, adequate agronomy and environments, as well as presence of insect pollinators as necessary conditions. Furthermore, the authors identified the complete utilization of by-products (e.g., press cake) as a key aspect for optimizing the economics of this industry.

Reasons for rise and fall of so many jatropha biofuel projects in Southern Africa were manifold. As outstanding positive examples on the other hand, von Maltitz et al. (2014) compared a smallholder project in Malawi (BERL) and a large-scale plantation in Mozambique (Niqel), which had in common to have based their operations on relatively modest seed yield assumptions (≤3 t/ha). Despite promising frame conditions (climatic suitability, low yield expectations, sound economic planning, good management, sensitivity to local issues) shown by these projects, von Maltitz et al. (2014) stressed that viability at the time was still not guaranteed and jatropha needed to be considered as a high-risk crop until proven otherwise. Furthermore, the authors assumed that jatropha would remain a low-value crop due to high labor requirements and in particular due to the low value of the press cake of toxic jatropha varieties, which reduces overall profitability by limiting its utilization as valuable by-product. They concluded that plant breeding may partly resolve the problem of low seed yields by developing high-yielding varieties.

In the past, jatropha projects in sub-Saharan Africa often have been risky undertakings due to socioeconomic, market, cost, and agronomic uncertainties. From an agronomic point of view, realized seed yield is the most crucial factor for economic viability. Seed yield is determined by a number of factors, such as edaphic and climatic suitability of the cultivation site, crop management practices, and choice of variety, just to mention a few of them. Many of the aforementioned studies demonstrated that selective breeding and development of adapted varieties of jatropha is a crucial element for its successful cultivation in sub-Saharan Africa. Therefore, new jatropha varieties will be characterized by high seed yield potential and stability in the edapho-climatic conditions of this region. In addition, new varieties will contribute to the reduction of management costs (resource use efficiencies, mechanization potential) and to the increase of return opportunities based on a larger exploitation of seeds (oil content, non-toxicity) and their potential by-products (press cake, animal feeding, biomass). In the next section, we will present results from our breeding program focusing on jatropha performance in marginal areas of sub-Saharan Africa.

1.3 Performance of Jatropha in Marginal Environments of Cameroon and Madagascar

Perennial and multi-site field testing is necessary for selection of superior jatropha genotypes. After a 3-year screening of a global jatropha germplasm collection in multi-site field trials (Martin and Montes 2015), a set of superior and promising edible and non-edible jatropha accessions was available. Using molecular marker information, Montes et al. (2013) had shown that these accessions had a high degree of homozygosity and formed distinct clusters. Subsequently, we selected crossing partners from both clusters based on complementary characteristics as well as based on genetic distance estimates with the goal of exploiting heterosis, taking advantage of its increase with genetic distance (Reif et al. 2005). The selected accessions were further self-fertilized, and finally controlled crossings were conducted to produce testcross hybrid seeds.

Within the framework of our global field trial network, our goal was to evaluate the performance of edible and non-edible jatropha hybrids and conduct quality analyses on seeds at the end of the harvest season. Seeds of 44 non-edible and 11 edible testcross genotypes as well as seeds of 2 non-edible check lines and 1 edible check line were sown in nurseries at two sites in Cameroon (Batchenga, Garoua) and at one site in Madagascar (Ihosy). Seedlings were raised for an average period of 3.8 months and then transplanted to the field in 4 m by 2 m spacing. At all locations, the genotypes were evaluated in plots comprising four plants in an alpha lattice field trial design with three replications. Agronomic management followed standard practices.

The two locations in Cameroon have been described in detail by Senger et al. (2016). Cameroon has been termed *Afrique en miniature* (which means *Africa in miniature*) due to its wide range of climatic conditions from the humid south to the arid north of the country. The same is true for soil quality, as one can find fertile and marginal land in this country. The growing number of regions characterized by marginal land and the expanding desertification are major threats for agriculture in sub-Saharan Africa. Here, we refer to *marginal environments* as lower quality agricultural land, where food production is less productive (Shortall 2013) and/or unfavorable tropical climates particularly characterized by limited water availability. The trial in the south of Cameroon (Batchenga) is located in a humid environment with high temperatures throughout the year and a growing season of 9.5 months with high amounts of rainfall (1342 mm/year, on average over the trial period). Based on the regional agroecological zones (AEZ) framework of the FAO (1994), it falls into AEZ4 (warm humid tropics). Its sandy loam soil has low pH values and low amounts of available phosphorus. The trial site in the north of Cameroon (Garoua) is located in an arid environment with a short growing season of 5 months, moderate amounts of rainfall (716 mm/year, on average over the trial period), and high temperatures throughout the year. It falls into AEZ1 (warm arid and semiarid tropics). Its loamy sand soil is characterized by low to medium pH values, limited availability of phosphorus, low content of organic matter, and limited water holding capacity. Agricultural production in Madagascar, Earth's fourth largest island, frequently has to cope with extreme weather events, particularly with cyclones leading to temporary flooding of arable land, as well as with droughts. The trial site in central Madagascar (Ihosy) is characterized by a short growing season of 5 months and a moderate amount of rainfall (770 mm/year, on average over the trial period). It falls into AEZ1 (warm arid and semiarid tropics). The soil quality of the loamy sand at this location is characterized by acidity, limited availability of phosphorus, and very low cation exchange capacity.

1.3.1 *Performance of Edible and Non-edible Jatropha in Perennial Field Experiments*

Average seed yields at Batchenga, Garoua, and Ihosy increased with each growing season (Fig. 1.1). In the past, non-edible jatropha hybrids and lines have shown a better and more stable performance than edible jatropha genotypes. Correspondingly, average seed yields in the non-edible genotypes were superior to those of the edible genotypes. At the time of finalizing this manuscript, the harvest in 4th year was just completed at Garoua and Batchenga, where the best performing jatropha hybrids yielded, on average, 8334 kg/ha and 2153 kg/ha, respectively. At Ihosy, the first 2 years of growth were characterized by negligible seed yields. The best performing non-edible hybrid at Ihosy yielded on average 2161 kg/ha in the 3rd year. Results from the 4th year are not presented, because harvest was still ongoing at the time of finalizing this manuscript. Edible jatropha genotypes are known to be more susceptible to environmental constraints such as drought, frost, or excess of water. Nevertheless, it was possible to identify certain edible hybrids that significantly outperformed less performing non-edible genotypes at all three testing locations. Despite very high temperatures and low amounts of rainfall at Garoua, edible jatropha performed unprecedentedly well. The best performing edible jatropha hybrid yielded on average 4935 kg/ha at Garoua in the 4th year. On the other hand, the performance of the edible genotypes at Batchenga stagnated in the 3rd and 4th year on a lower level compared to non-edible hybrids. The difference between edible and non-edible jatropha in terms of seed yield was less pronounced at Ihosy. In the 3rd year, the best performing edible jatropha hybrid yielded on average 2020 kg/ha.

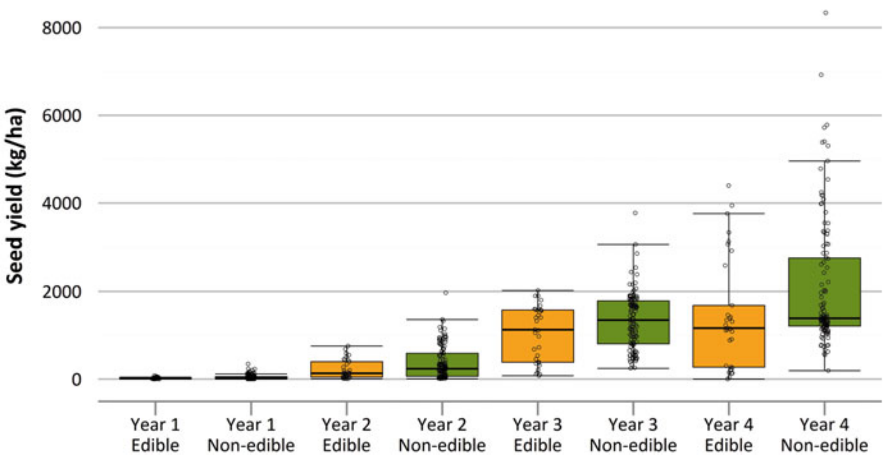

Fig. 1.1 Boxplots displaying the distribution of the population-wise mean seed yields of edible and non-edible jatropha lines and hybrids across two locations in Cameroon and one in Madagascar. Genotype means (circles) are based on three replications

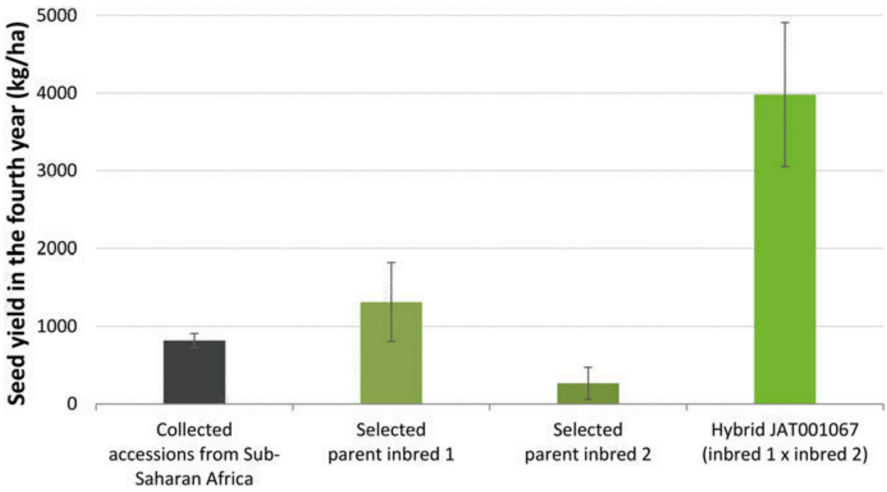

Fig. 1.2 Comparison of mean seed yields (kg/ha) in the fourth growing year at the semiarid location in Cameroon (Garoua) for 35 accessions collected in Cameroon, Chad, Gambia, Madagascar, and Tanzania, as well as selected inbred parents and their corresponding hybrid progeny

Many jatropha projects in sub-Saharan Africa in the past failed due to the cultivation of unselected jatropha germplasm, which was locally available at the time of project launch. Usually, such material had not undergone scientific evaluation in multi-year or multi-site field experiments and has a limited seed yield potential. Figure 1.2 shows the realized mean seed yield of 35 accessions from 5 different sub-Saharan African countries compared to that of a selected non-edible hybrid and its corresponding inbred parents randomly grown at the same location (Garoua). The hybrid genotype showed a significantly better performance than the wild-type germplasm. Interestingly, compared to the wild-type population, the inbred parent lines had only slightly higher or even less seed yields, respectively, which might be due to inbreeding depression. The hybrid progeny on the other hand was superior to both the wild-type population and its parents, exhibiting considerable midparent heterosis (~400%). Such an extent of heterosis ("hybrid vigor") as shown by this example is comparable to what is known from corn and shows that the prospects of hybrid breeding for jatropha are very promising.

Jatropha seeds are a rich source of oil. The average oil content in the seeds measured across 3 years was significantly higher at Ihosy (40.5%) than at Batchenga (36.2%) and Garoua (34.9%). While there was no significant difference at Ihosy between edible and non-edible jatropha, the edible hybrids had significantly higher oil contents than non-edible hybrids, namely, on average more than 1% at Batchenga and more than 2% at Garoua (Fig. 1.3a). Additionally, seeds of edible jatropha represent a valuable source of high-quality protein for animal feeding or food purposes (Senger et al. 2017). The protein content was measured in the 3rd year in the kernels of edible jatropha, i.e., after removing the seed shell, which has no nutritional value. The average protein contents in the kernels from Batchenga