Journal of
Bioremediation & Biodegradation
ISSN: 2155-6199

Martin, et al. J Bioremed Biodeg 2013, S4
DOI: 10.4172/2155-6199.S4-002

Review Article Open Access

Genetic Improvement, Sustainable Production and Scalable Small Microenterprise of *Jatropha* as a Biodiesel Feedstock

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Abstract

There is widespread interest in *Jatropha curcas* L. as a renewable source of biodiesel and a potential replacement for petroleum-based transportation fuels. As an oil-bearing feedstock it displays rapid growth and high seed yield across soils of varying fertility. Unfortunately, despite current optimism there exists little information on the agronomic management of this genus as a feedstock for biodiesel. Therefore, this article reviews research on Jatropha as a biofuels feedstock and explores the fast adaptation of Jatropha across India through a "lab-to-market" approach. An integrated approach to understanding the long-term viability of using Jatropha as a bioenergy crop is assessed. A variety of issues are discussed to understand and survey research that has been done on Jatropha and its applications in developing countries where it is being implemented. These issues include understanding the fundamentals of the biology, ecology, propagation, and cultivation of Jatropha, especially focusing on the thrust in developing countries to use wastelands and reclaimed lands for growing Jatropha as a bioenergy crop. This article also provides a status report with regard to germplasm collections and genomic resources for future accelerated crop improvement. A viable argument is made in favor of developing pilot energy farm enterprise clusters, which seems to be the best option for small farms in developing parts of the world. This thematic approach is discussed in detail under a "sun-to-satellite" regional model in which farmers can be engaged as stakeholders and participate in the decision making process. The potential impact of fossil-fuel replacement by Jatropha on climate change is also discussed.

Keywords: Bioenergy; Biomass; Biodiesel; Marginal lands; Productivity; Semiarid; Cooperative biofuels enterprise

Introduction

Growing concerns about rising CO_2 in the atmosphere caused by combustion of fossil fuels have prompted many countries to consider alternative and sustainable sources of energy. The need for research into and development of biofuels from renewable biofuels is well established. First-generation feedstocks such as corn, sugarcane, and sugar beet rely on the fermentation of sugars to produce ethanol, which can directly replace or supplement gasoline fuels. Second-generation lignocellulosic feedstocks for bioethanol (e.g., switchgrass, miscanthus, poplar, and willow) together with feedstocks suitable for the production of biodiesel (e.g., palm oil and Jatropha) are currently the subject of intensive investigation. Biodiesel is especially attractive in this regard for several reasons. Oils derived from seeds have shown great potential for development as diesel fuel or diesel fuel extenders [1-4]. Seed oils have been shown in general to have a higher energy content than other potential biofuels feedstocks [1,5,6] and compared to plant-based ethanol production, plant oils like Jatropha can be converted to biodiesel by processes that require low energy input [3,7]. Nevertheless, limitations associated with biodiesel keep demand for its utilization relatively low, such as poor performance in cold temperatures and increased emission of greenhouse gases [3].

Jatropha curcas L., known commonly as physic nut, is native or naturalized to tropical and subtropical parts of Asia, Africa, and Central/South America and has been identified as a multipurpose species with many attributes that give it considerable potential as a biodiesel crop in areas across the world [8]. Jatropha is a non-domesticated shrub (Figure 1) that can grow in well-drained soils with good aeration but can also adapt to marginal soils with low nutrient content as well as in shallow fields and rocky terrains. It has been shown that the seed kernel of this member of the Euphorbiaceae or spurge family contains 40–60% (w/w; weight of oil per weight of seed) oil deemed unsuitable for cooking due to the presence of toxic esters [9]. The seed oil of Jatropha was used as a diesel fuel substitute during World War II [10]. More recently the unmodified Jatropha oil and blends with diesel fuel [11,12], as well as trans-esterified oil esters, were tested as an alternative fuel for Thailand [13-15] following growing interest in Jatropha as a global biodiesel crop. In developing countries like India, the production of liquid biofuel,

Figure 1: Jatropha plant, fruits and seeds (photographs courtesy of Tree Oils India Ltd. and BayouDiesel.org).

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Received May 02, 2013; **Accepted** June 18, 2013; **Published** June 20, 2013

Citation: Martin MZ, Gunter LE, Jawdy SS, Wullschleger SD, Wheeler CS, et al. (2013) Genetic Improvement, Sustainable Production and Scalable Small Microenterprise of *Jatropha* as a Biodiesel Feedstock. J Bioremed Biodeg S4: 002. doi:10.4172/2155-6199.S4-002

particularly if small in scale, offers the possibility of reducing poverty by providing income-generating opportunities for farmers [16,17]. If used locally for cooking, heating, or power generation, liquid biofuels may also improve availability of and access to modern energy services in rural areas, where most households rely completely on firewood for their energy needs.

This article reviews research that has been performed on Jatropha as a biofuels crop and explores a small farm enterprise model approach for fast adaptation of Jatropha plantations across India through a "lab-to-market" pilot demonstration. The origin and domestication of *J. curcas*, its ecological and genetic diversity, genomic resources, and how those resources can be used or expanded to fit the needs of an accelerated domestication of Jatropha will be discussed. Specifically, this includes 1) sources of genetic diversity, 2) development of a genomics toolbox, 3) increasing production through innovative agronomic practices, and 4) developing a pilot "lab-to-market" approach through a biofuels farm cooperative. The purpose of a cooperative model is to share land and resources for equitable benefit and sustained growth for the future. There are benefits for both local growers and their entire community as the biodiesel production potential appears to be high. We will also review strategies for the ultimate utilization of Jatropha oils in engines and the potential impact on the environment.

Biology, ecology, propagation and cultivation

Jatropha is a diverse genus of the Euphorbiaceae comprised of ca. 175 species of monoecious or dioecious trees, shrubs, and annual or perennial herbs that grow in a narrow geographic range in seasonally dry regions of the tropics [18]. It has been classified into two subgenera, ten sections, and ten subsections [19] with centers of diversity in Central/ South America and Africa [18]. Subgenus *Jatropha* includes all the African, Indian, South American, Antillean, and a few Meso-American species. Subgenus *Curcas* is found primarily in Mexico and adjacent regions. Although the more distantly related taxa are genetically reproductively incompatible, and others are geographically isolated, many species are able to hybridize under experimental conditions [19]. *J. curcas* is considered to be the most primitive member of the genus based on morphological characteristics [19,20] as well as its ability to hybridize easily with species in both subgenera. This is an especially useful characteristic for breeding programs and for increasing genetic diversity in a crop with a relatively narrow genetic base [21].

Jatropha is currently widely grown as an oil crop in Mexico, Nicaragua, northeast Thailand, and parts of India and is now being promoted in southern Africa, Brazil, Mali, and Nepal [22]. The soil and climate condition varies depending on the particular species of Jatropha. However, it is known to grow over a wide climate range including areas that receive as low as 25 cm of annual rainfall to those that receive as much as 300 cm of annual rainfall. Additionally, it has been found growing at sea level and in areas as high as 1800 m in altitude. Efficient growth and management practices of Jatropha are poorly documented, contributing, in part, to the inability of growers to achieve optimum output from this crop. Fruit has the highest agricultural value. However, some information is available about silviculture and management of Jatropha. For example, it is known that Jatropha can be established from seed, seedlings, and cuttings in all soils except vertisols, though light sandy soils are preferred. Jatropha can tolerate high temperatures and generally grows very well under low fertility and moisture conditions. However, it is not a nitrogen-fixing species, so the soil in which it is grown needs to be supplemented with fertilizers such as nitrogen and phosphorus to maintain high biomass productivity. Fruit maturation generally takes 45-50 days and is typically noted by a change in color from green to yellow, at which point it is ready for manual harvest [23]. It has been confirmed that Jatropha forms an association with soil mycorrhizae which assist in the uptake of phosphorus and micro-elements found in the rhizosphere [24-28]. Jatropha plants that were inoculated with mycorrhizae showed a 30% increase in both biomass and seed production [29].

Jatropha is not known to be browsed by goats or cattle [30] due to its toxicity to mammals and most insects [31-34]. Despite this, it is not a disease- and pest-free crop. Insect problems in Jatropha cultivation include those caused by the scutellaria bug, *Scutellera Nobilis*, and the inflorescence and capsule-borer, *Pempeliamorasalis* [35]. Two additional insect pests found specifically in Nicaragua are *Pachycoris klugii Burmeister* and *Leptpglossus zonatus*. Both Nicaraguan species damage developing fruit, causing abortion and seed malformation. Yield loss can be as high as 18.5% even when these insects are present at low densities [36].

Aside from its potential as an alternative fuel source, Jatropha has several additional beneficial properties regarding its use as a crop plant. For example, it can be used to reclaim eroded land, sequester carbon, and serve as an alternative biofuel crop as well as one that reduces desertification. Jatropha cultivation has been observed to increase resistance to wind erosion and enhance macro and micro-aggregate stability to water erosion. As an additional benefit, stable micro-aggregates offer protection to soil organic carbon, thus increasing carbon sequestration rates. It should also be noted that Jatropha increases carbon sequestration rates by fixing atmospheric carbon and storing it in woody plant biomass. Finally, Jatropha has been used as a complementary crop in the form of a hedge or shelterbelt to deter animal damage, improve the microclimate, and provide humus to the soil. Several commercial crops may lend themselves to Jatropha fencing or shelterbelts, including coffee, market gardening, and tobacco [37].

Due to variation in the genetic properties of the crop and environmental conditions of planting sites, no reliable predictions of yield are available, but based on available data and modeling, expectations of seed yield in the range of ~5 t dry seed ha^{-1} yr^{-1} provide the most reasonable estimates to date [24].

Germplasm collections and genomic resources for crop improvement

Despite the growing interest in Jatropha as a biofuels plant, it lacks improved germplasm and, until recently, active breeding programs. Major germplasm collections for Jatropha are primarily found in India [38,39], Africa, and the Philippines. However, breeding programs have been on the rise as interest in developing this renewable energy resource has increased. The Lao Institute for Renewable Energy (lao-ire.org/programs/bio-energy) has initiated a crop improvement program where the emphasis in on germplasm collection and improving plant breeding skills of local agronomists. Similar activities are also underway by Chibas in Haiti (chibas-bioenergy.org) and by several members of the EU Consortium JATROPT. Most of the *J. curcas* evaluation and breeding programs are based on existing germplasm collections, with the exception of the collection of SG Biofuels, Inc., who claim to have the most extensive collection of Jatropha genotypes ever amassed as well as an intensive hybrid breeding program (sgbiofuels.com).

Information on genetic diversity in Jatropha is still limited because until recently most studies have surveyed accessions only from India and Africa where the shrub was brought by the Portuguese, and where it has naturalized. Reports using a variety of dominant markers (RAPD, ISSR, and SSR), etc., have for the most part suggested a relatively narrow genetic base for *J. curcas* in India of 60-80% similarity [40–42]; nevertheless, genetic markers were useful in distinguishing germplasm, suggesting that further development of markers is feasible for the domestication of Jatropha. Nonetheless, results comparing *J. curcas* to additional Jatropha species suggest that there is also considerable potential for genetic improvement of Jatropha through hybridization [43,44]. Given the narrow genetic base, it may be more feasible to obtain interspecific backcross hybrids to identify desirable traits and improve yields. *J. curcas* has been shown to hybridize readily with other Jatropha species [44,45]. Indeed, SG Biofuels has already established more than 12,000 genotypes from Southern Mexico into Central America and has also spent the last few years developing a proprietary cultivar (JMax™) that it claims has 250-900% greater seed yields than existing varieties (sgbiofuels.com).

In addition to conventional genetics, accelerated improvement via a transgenic approach may also allow Jatropha to be useful as an energy crop [46,47], but this will require genome sequencing and mapping to find genes of interest. The Jatropha genome is ca. 416 million base pairs, similar to the size of Arabidopsis and rice, with a base chromosome number of x=11. Most species are functional diploids, 2n=22, though a few tetraploids with 2n=44 have been reported [19]. Based on morphometric analysis, Jatropha is likely an autopolyploid [48], like tomato, rice, wheat, and soybean. In 2010, Synthetic Genomics (syntheticgenomics.com) in cooperation with Life Technologies Corporation (lifetechnologies.com), a global biotech company, announced a 100× coverage of the genome sequence of Jatropha and is now focusing on annotation to identify genes of interest to discovering genetic variations for use in marker-assisted breeding. These data have not been made publicly available. In 2011, Sato et al. [49] published a genome sequence covering a putative 95% of the gene-containing regions using a combination of platforms and established a database of 21,225 downloadable and searchable Jatropha unigenes (kazusa.or.jp/jatropha).

In addition to the genome sequence, several Expressed Sequence Tag (EST) libraries from Jatropha seeds, embryos, roots and other tissues have been developed for identifying genes related to lipid synthesis and degradation, salt stress, and developmental biology [50-54]. Several genes falling into three main categories including toxicity, oil production, and stress response have been cloned or studied with the intention of characterizing their function in *J. curcas*. Curcin 2, the compound produced by Jatropha thought to be responsible for its toxic effects, has been cloned and its expression pattern characterized by several groups [55-57]. Two genes thought to encode proteins involved in oil production have been studied including a β-ketoacyl-acyl carrier protein (ACP) III (KAS III) gene [58] and a stearoyl-acyl carrier protein desaturase (SAD) gene [57]. Lastly, several studies have been done on genes thought to control stress response including JcPIP2 and its involvement in drought response [59] and JcERF and its involvement in salt and freezing tolerance [60]. Sato et al. [49] annotated and described multiple classes of genes involved in triacylglycerol production, phorbol ester biosynthesis and other functions which could potentially be genetically manipulated to enhance oil production, reduce toxicity, and promote disease resistance.

Since the primary source of oil is from the seed, increasing seed size would be one of the primary objectives of breeding. The biosynthesis of five major fatty acids is responsible for the production of seed storage oils in plants. All of the genes involved in this biosynthesis process are known [4]. If we can apply the considerable amount of knowledge that has been gained from studies of wild and model crop plants to Jatropha, through genetic engineering we may be even better able to increase production of value-added petrochemical substitutes. As more is learned about specific Jatropha genes and their putative functions are characterized, it becomes increasingly important to have transformation techniques in place to expedite crop improvement. Efficient transformation techniques will be needed to quickly target and improve specific traits such as seed yield, oil content, and seed toxicity. Little work has been done in this area, but there has been some effort to establish how different factors such as tissue type and physiological state affect the efficiency of agrobacterium-mediated transformation techniques [47]. Several studies have also been done to test clonal propagation techniques of *J. curcas* with successful outcomes. As a result, both non-test tube and tissue culture propagation procedures have been established [61-63]. Promising results for *J. curcas* genotypes from both India and Nicaragua were also obtained by adventitious shoot formation from leaf discs [63,64].

Oil-producing characteristics of *Jatropha*

The fruit of Jatropha contain viscous oil that can be used for soap making, in the cosmetics industry, for medicinal and pharmaceutical purposes, and as a diesel/kerosene substitute or extender. However, for several reasons, both technical and economic, the full potential of Jatropha is far from being realized. Its growth and management are poorly documented, and there is little experience in marketing its products [22]. Diesel is a hydrocarbon with 8–10 carbon atoms per molecule, but Jatropha oil has 16–18. This makes the nut oil much more viscous than diesel and has a lower ignition quality (cetane number). Trans-esterification has been tried to reduce its viscosity and to increase the cetane number. Another important aspect is that removing the coat from the fruit increases its energy value by 20%. Detailed analysis has been reported of the costs and returns from Jatropha and its products which take into account the price of mineral oils including tallow and plant oils, the establishment and tending costs for *J. curcas*, and harvesting costs of fruit and wood [22]. This analysis led to calculation of the cost of Jatropha oil production and the price of competing products as a commercial bioenergy crop. A very important point about the calorific value of Jatropha is that the gross heat values of the seed, oil fraction, and hydrocarbon fraction indicate that it could be used as an intermediate energy source. Similarly the gross heat value of the hydrocarbon fraction is 9704.4 cal/g, which is higher than the heat value for anthracite coal and comparable with that of crude oil [30]. The calorific value of Jatropha is comparable to that of diesel oil. An added benefit is that the sulfur content in Jatropha oil is much less than that in diesel oil, indicating that this oil may have a less adverse impact on the environment [25]. Real-world experimental investigation was conducted [65] to explore the performance of Jatropha oil and its fuel blends with diesel in a direct-injection single cylinder diesel engine, and the results suggest the following conclusions. 1) Pure Jatropha, pure diesel, and blends of Jatropha and diesel oil exhibited similar performance and broadly similar emission levels under comparable operating conditions. 2) Introduction of Jatropha oil into diesel fuel appears to be effective in reducing the exhaust gas temperatures since the Jatropha oil could be considered to be emulsified as water was introduced into the milled Jatropha seed during the extraction process. 3) Jatropha oil can be used as an ignition-accelerator additive for poor diesel fuels when 2.6% by volume of Jatropha is introduced into pure diesel fuel. 4) Jatropha oil has substantial prospects as a long-term substitute for diesel fuels. The 97.4% diesel/2.6% Jatropha fuel blend compared favorably with diesel fuel and offers a substitute for pure diesel fuel. 5) Carbon dioxide emissions were similar to those of regular diesel, but there was an increase in brake thermal efficiency and brake power and a reduction in specific fuel consumption for Jatropha oil.

The Central Salt and Marine Chemicals Research Institute (CSMCRI) in Bhavnagar, Gujarat, in India started a large-scale cultivation with high seed productivity in 2006. The institute also demonstrated that high-quality Jatropha methyl ester conforming to EN 14214 specifications exhibits drastically reduced emission. By-products of the Jatropha seed capsule include the empty shell. This has high calorific value (3715 kcal/kg). Oil cake, soap, glycerol, and potassium sulfate are obtained as by-products of the biodiesel process that is used by this group, which includes starting from whole seed. The press cake (biomass left behind after oil has been extracted from the seeds can be used as fertilizer, and the organic waste products can be digested to produce biogas (CH_4) [8,29,66-68]. A primary motivation in cultivating Jatropha is the multi-purpose utility of the crop which can benefit small farmers in developing countries while minimizing waste [69].

Strategies for utilizing *Jatropha* oils in engines

As mentioned in the introduction, the seed oil of Jatropha was used as diesel fuel substitute during World War II. Interest in vegetable oils as fuels for internal combustion engines was reported in several countries during the 1920s and 1930s and later during World War II. Many European countries, such as Belgium, France [70], Italy, the United Kingdom, Portugal, Germany, and some South American countries, such as Brazil and Argentina, and also Japan and China in Asia were reported to have tested and used vegetable oils as diesel fuels during this time. Jatropha biodiesel was used in place of fossil diesel in Madagascar, Cape Verde, and Benin [10] during World War II. It was also reported that Jatropha was grown in Indonesia to produce oil for the Japanese war effort.

Two strategies have been discussed for utilizing Jatropha oils for fuel [8]. The first is adapting the engine to the fuel. This strategy can be accomplished by using specially developed diesel engines like the Elsbett engine. In this type of engine, the design attempts to limit the loss of energy as heat by avoiding the cooling of the engine block. This approach makes sense only when using neat vegetable oil in stationary engines, as the price of the engine is too costly based on low production numbers. The second strategy, adapting the fuel to the engine, may be more practical solutions for affordability in developing countries. In this approach different vegetable oils including *J. curcas* oil were evaluated in direct- and indirect-injection diesel engines; performance, fuel conversion efficiency, and specific consumption as well as exhaust gas emissions were compared [8,71]. The lowest exhaust gas emissions were obtained with coconut and Jatropha. Also a long-term durability test was conducted with *J. curcas* oil as fuel in a water pump driven by a modified direct-injection diesel engine. This 1000 h test indicated good behavior of the oil as fuel and no wear in the engine. At the same power output, specific fuel consumption was lower and efficiency was higher than those of diesel fuel. The economic evaluation has shown that the biodiesel production from *J. curcas* is very profitable provided the by-products of the biodiesel production can be sold as valuable products [8,72].

Developing pilot energy farm enterprise cluster

Developing countries face increasing local demand for energy in rural areas, but they must consider both economic and environmental pressures associated with agricultural land use. India has millions of hectares of area that is currently classified as marginal, waste, or degraded land (dolr.nic.in/iwdp1.htm. This includes land that was farmed in the past but has fallen out of production and is no longer suitable for growing arable crops. Much of this land is suitable for growing sustainable energy crops such as Jatropha since it can withstand the drought and hard soil. Rural poverty and unemployment are huge problems in the developing world, and energy crops like Jatropha will offer a promising solution by providing farming jobs and rejuvenating agriculture to the communities that depend upon it.

Energy agriculture can give farmers and their communities the opportunity to grow valuable cash crops and provide them with a sustainable livelihood. Countries will be able to produce biodiesel for their own transport and power generation needs, reducing dependence on expensive oil imports. Surplus production can be exported in volume to developed markets.

Growing energy crops holds great potential for some smallholder farmers and processors; however, a rural business model and a well-defined methodology for working together are lacking. Jatropha has long been an underutilized oil-bearing crop, hence an optimized agronomic practice and a systems approach for production, harvesting, processing, and related value chain are still needed in India. The nonpolluting green biodiesel from Jatropha provides an opportunity for good returns for rural economic development. However bringing together farmers for collective equity shareholding as an energy farm business model is a daunting task. Degraded and marginal lands are available at many parts of the world, but much of the land is wasted. Jatropha can be planted as both a wind breaker hedge and an energy crop on such land.

Jatropha's potential as a biofuel crop is now well recognized; however, the proven commercial pilot model for large regional adaptability is still missing. Need is to test the large grower cooperative as a commercial pilot due using a "sun-to-satellite" approach where farmers share the resources and can get equitable share of produce based on their land holding. Hence, the goal is to improve the existing genetic stocks, optimize agronomic practice, and develop farmers' enterprise clusters as a cooperative and efficient value chain for the Jatropha market access.

Jatropha demonstration and technology transfer approach

It is unlikely at present, that small-scale farmers will grow Jatropha for processing into biofuel unless they see a better economic possibility like, incentives from the government, guaranteed market of their produce by processors and availability of the credit to support production. A pilot demonstration needs to be developed as a cooperative of small and marginal farmers and cultivators. This demonstration can act as a business enterprise that oversees soil, water, and other natural resource systems as well as crop management and business enterprise cluster creation (Figure 2). This approach will allow the energy farm enterprise to explore different technology alternatives and identify the best cultivation methods for Jatropha plantation management. Such a demonstration can promote Jatropha farming and serve the energy needs of agricultural communities, as well as provide rural employment and alternative income. The economic and infrastructure support, mobile village extension services (dissemination of best management practices and awareness campaign about Jatropha) to a cooperative enterprise cluster (Jatropha producer association), partnership with processor, and value-added chain will maximize the ability of farmers make the transition to Jatropha (Figure 3). Knowledge and experience gained through the pilot demonstration will spur the adaptability and replicability of Jatropha to the various agro-climatic regions of India using a "field-to-market" approach.

The cultivated green biodiesel alternate fuel can propel engines, generators, and transport vehicles as well as bring electricity to rural households and support rural energy independence. The challenge of the Jatropha pilot is to produce high-quality biofuel that is cheaper over the long term than existing fuels without affecting operational economics.

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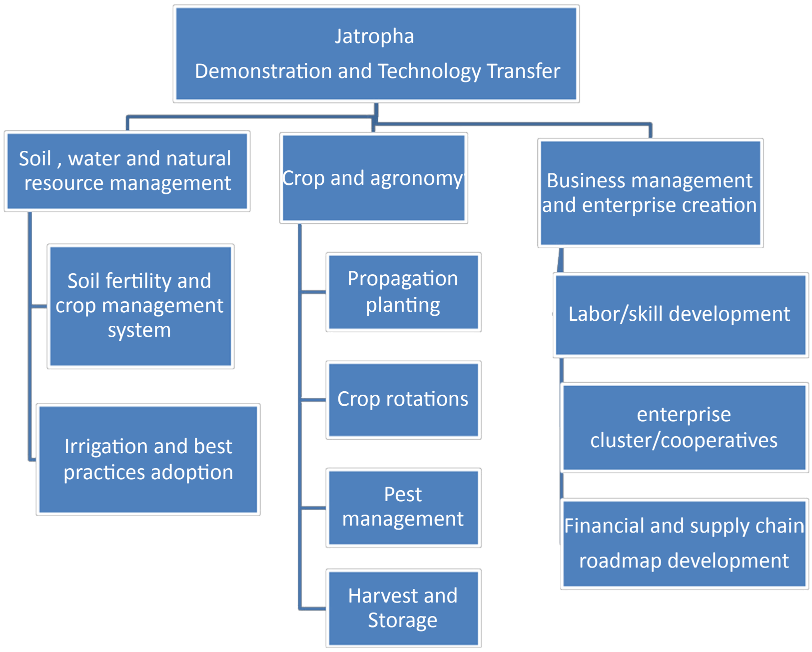

Figure 2: Sustainable skill and management practices improvement for Jatropha enterprise.

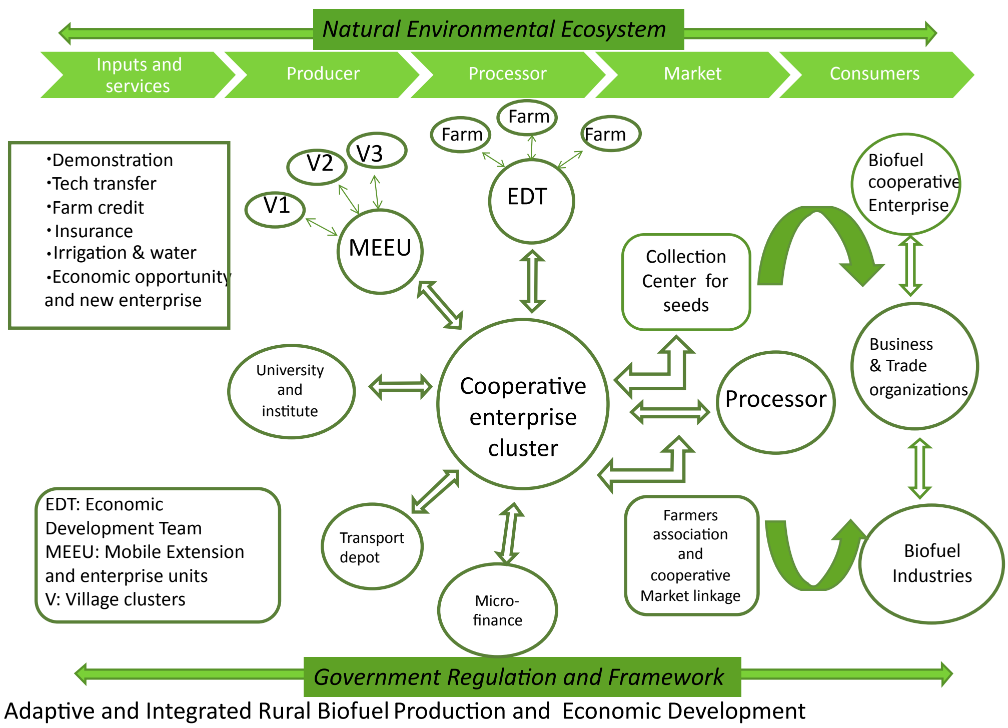

Figure 3: Adaptive and Integrated framework for Jatropha biofuel roadmap sustainability in India.

Sun-to-satellite regional enterprise cluster model

Appropriate agricultural technologies aimed at increasing productivity and providing income for energy crop producers need to be identified for technology transfer to be successful. Through the pilot cooperative farm, technical knowledge can be shared with farmers through education, research, and effective extension systems using both a bottom-up (Figure 4) and top-down approach [73]. In addition to these formal linking mechanisms, both researchers and extension personnel will be needed to maintain regular, informal contact with groups of energy farmers and stakeholders (non-government organizations, university professionals, private enterprises, and policy makers). The resilient energy farm enterprise (both individual and

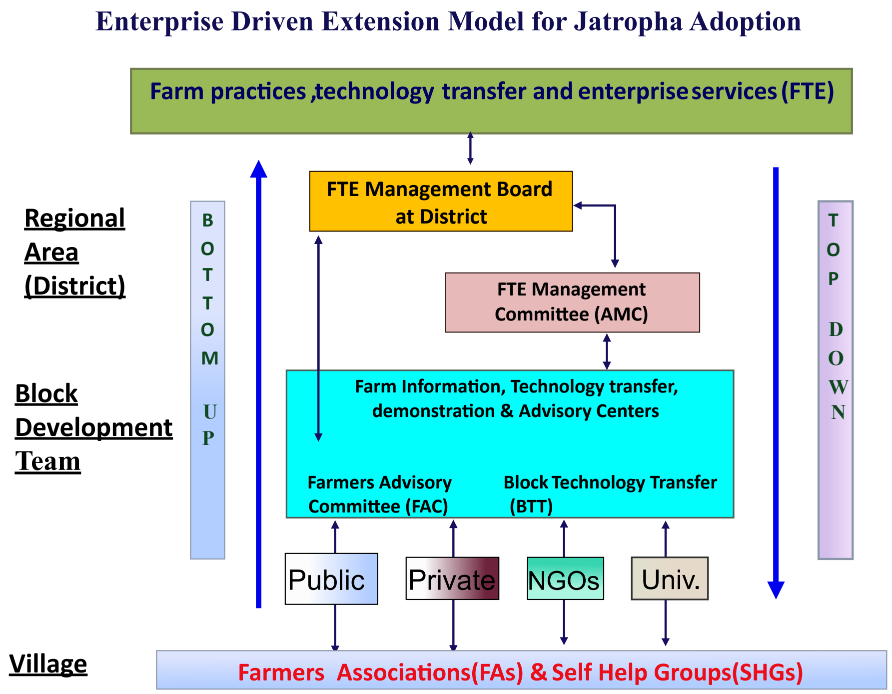

Figure 4: Technology transfer and energy farm cluster creation for Jatropha.

cooperative) will use a more tailor-made approach that can adapt to and evolve with regional farmer-led aspirations. A cooperative legal structure as an institution (rural energy farm enterprise associations) and Micro-Finance Institution (MFI) will provide a safety net of support and connection to the marketplace and will promote Jatropha as an attractive rural economic venture. Engagement of farmers, local governance, and technology providers will play a key role in sustaining the transition to rural energy farming.

Potential impact on climate change

Motivation behind the emergence of biofuels and an adoption of alternative fuels by local growers lies in the desire to secure a sustainable resource for the production of transportation fuels and, therein, energy security and mitigation of climate change. Achieving the latter objective hinges on obtaining a net reduction of eCO_2 emissions from the prospective feedstock compared to the fossil-fuel alternative, and doing so across the spectrum from raw material to end product. Bioethanol and biodiesel are, in general, both touted as carbon-neutral since the carbon emitted upon combustion was recently assimilated as atmospheric CO_2 in the biological process of photosynthesis. A more rigorous analysis of the energetic costs and benefits of biodiesel and ethanol biofuels can be achieved through life cycle assessment, in which energy requirements at all steps in the process are quantified to the extent possible [7]. Using such an approach, several investigations have documented that the production of Jatropha for biodiesel [74] or jet fuel [75] can result in a 55 to 72% savings in greenhouse gas emissions compared with conventional fuel use. Similarly, for Jatropha grown in West Africa and Thailand, the energy yield defined as the ratio of biodiesel energy output compared to the fossil energy input ranged from 4.7 to 6.03 [74,76]. Achten et al. [77] recently conducted a life cycle assessment for Jatropha in rural India and showed that the production and use of Jatropha biodiesel results in a 55% reduction in the Global Warming Potential (GWP) compared to the reference fossil-fuel-based system. This reduction in GWP is similar to that estimated for other bioenergy crops including oil palm, sunflower, rapeseed, soybean, corn stover, and switchgrass. Achten et al. [78] and Ndong et al. [74] both concluded that the cultivation step, specifically fertilization, was the largest contributor to observed greenhouse gas emissions. Other factors that contribute to current uncertainty in greenhouse gas emissions and energy yield from biofuels are harvestable yield, rates and magnitude of soil carbon sequestration, irrigation and other management inputs (e.g., pesticides), energy costs associated with raw material acquisition, and co-product allocation. In addition to the obvious benefits derived by improving the efficiency of nitrogen use by Jatropha, several of these factors also deserve consideration regarding how crop improvement strategies could help further enhance the overall energy benefits of biodiesel production. While many life cycle assessments have focused on GWP and greenhouse gas emissions associated with Jatropha crop production, other types of environmental impacts should not be ignored, including water usage, eutrophication, and terrestrial acidification [79].

Recommendations for Future Research

The implications of large-scale cultivation of Jatropha crop in developing countries to produce biodiesel for internal consumption and potential export need further study and would require research in accelerated domestication of Jatropha. The scientific focus would be to genetically evaluate the best candidate for disease-resistant and drought-resistant strains of this plant. Enhancement of the seed size is also an attractive goal for near-term Jatropha research. Therefore, there is an immediate need to create superior genotypes for successful implementation of *J. curcas* cultivation as a sustainable source of biodiesel which thrives with less water and low fertilizer use (although there is a great deal of controversy regarding the true water footprint of Jatropha, and this crop faces the same food v. fuel controversy as other biofuels). Metabolic analysis of the superior genotypic strain must also be included in new research in this area. The productivity of Jatropha from reclaimed lands, specifically in developing countries, is an additional factor that should be evaluated. Another area of research would be to evaluate plant performance under long-term agro-practices pertaining to plant spacing, irrigation scheduling, bio-fertilizers applications, and pruning protocols for optimizing tree architecture in order to determine crop productivity. More research on establishing various energy farm cooperatives models in rural parts of the world such as India will be needed in order to develop an integrated and sustainable model that promotes energy independence and provides opportunities to create multiple enterprise clusters using a "lab-to-market" approach.

Conclusion

This article has summarized the current state of research and implementation of Jatropha as a biodiesel crop in developing countries. In doing so, some very relevant contributions in the areas of germplasm collections and genomic resources have been made that would be needed for crop improvement. Since the primary source of oil is from the seed, increasing seed size and achieving stable yield under varying climatic conditions would be two of the primary objectives of a crop breeding program. It has been suggested that if the considerable amount of information that is known about wild and model crop plants can be applied to the accelerated domestication of Jatropha then we may be better able to increase production of value-added petrochemical substitutes. In addition, as more is learned about specific Jatropha genes and their putative functions are characterized, it becomes increasingly important to have transformation techniques in place to expedite crop improvement. The "sun-to-satellite" regional enterprise cluster pilot model has been proposed as the best approach to transitioning regional farmers to energy crops and providing them with a safety net of cooperative legal structure and MFI institution support connecting with enterprise associations and market.

Acknowledgments

This research was supported by the U.S. Department of Energy under the Office of the Biomass Program. This manuscript has been authored by UT-Battelle LLC under Contract No. DE-AC05-00OR22725 with the U.S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes.

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This article was originally published in a special issue, **Biofuels & their applications** handled by Editor(s). Dr. Kirill I Kostyanovskiy, Texas Agri Life Research & Extension Center, USA