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Review paper

Conservation and management in genetic resources of biofuel crops

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Biomass is emerging as one of the promising environmentally friendly renewable energy options if the major conventional energy sources like petroleum oil, coal and gas become depleted. Biomass can be converted into liquid and gaseous fuels through thermochemical and biological methods. Fuels produced from these technologies are referred to as biofuels. It is generally held that biofuels offer many benefits over conventional petroleum fuels, including availability from locally available biomass sources, reduction of greenhouse gas emission, biodegradability, and contributing to sustainability. However, Biofuels contain oxygen levels of 10–45% by mass while petroleum has essentially none. This makes the chemical properties of biofuel more favorable for complete combustion. In addition, biofuels from all sources have very low sulphur content and many have a low nitrogen level which make them more eco-friendly. As a consequence, biodiesel is widely used as an alternative fuel for diesel engines, whereas ethanol is used to replace gasoline.

Key words: conservation, biofuel, biomass, energy, genetic resources

INTRODUCTION

Demand for energy is increasing every day due to the rapid growth of population and urbanization. In general, biodiesels are fuel obtained through the esterification of oil derived from plants or animal fat. While the biodiesel properties are comparable to regular petrodiesel, the primary difference is they are not derived from petroleum sources such as crude oil. Currently, the sources of biodiesel under investigation include soybean oil (Goodrum and Geller, 2005), sunflower oil, corn oil, used fried oil, olive oil, rapeseed oil (Terry, 2005), castor oil, lesquerella oil, milkweed seed oil (Holser, and Harry-o'kuru, 2006.), *Jatropha curcas*, *Pongamia glabra* (karanja), *Madhuca indica* (mahua) (Kaul, 2007) and palm oil (Raadnui and Meenak, 2003). These are usually produced from edible oil feedstock and are known as first generation biodiesels (Rashid and Anwar, 2008). The most contentious issue affecting the production of first generation biodiesel is the use of agricultural land for biodiesel production. This issue is commonly referred to as the "Food verses Fuel" debate, in which the main two issues are the use of edible crops for biodiesel production, and the amount of land space devoted to growing inedible crops. Therefore an alternative must be considered which eliminates the disadvantages of first generation biodiesels. Research is currently taking place on second generation biodiesels which are targeted at addressing the "Food verses Fuel" debate (Bradshe, 2008). However, the current production of the above mentioned feedstock does not come close to a value representative of replacing fossil fuel use. This is more prevalent when land use and potential yields are considered, which eventually affects the feasibility of biodiesel production on an industrial scale.

CHEMISTRY OF BIOFUELS

The major components of vegetable oils and animal fats are triglycerides. Chemically, these are esters of fatty acids (FA) with glycerol. Vegetable oils and animal fats typically contain several different FA. Thus, different FA can be attached to one glycerol backbone. To obtain biodiesel, the vegetable oil or animal fat is subjected to a chemical reaction termed *trans-esterification*. In that reaction, the vegetable oil or animal fat is reacted in the presence of a catalyst (usually a base) with an alcohol (usually methanol) to give the corresponding alkyl esters (or for methanol, the methyl esters) of the FA mixture that is found in the parent vegetable oil or animal fat. Biodiesel has better properties than that of petroleum diesel such as renewable, biodegradable, non-toxic, and essentially free of sulfur and aromatics.

POTENTIAL OF BIOFUELS

Biodiesel fuel has the potential to reduce the level of pollutants and the level of potential or probable carcinogens stated that Biodiesel has become more attractive recently because of its environmental benefits and fact that it is made from renewable resource. However, the bottleneck to produce biodiesel in commercial scale is the high cost of edible virgin oil, in which account for more than 70% of the overall biodiesel production cost. In addition, using edible virgin oil such as rapeseed, sun flower, soybean and palm oil in biodiesel production has raised the concern of food versus fuel debate. Thus, recent biodiesel development has shifted to use non-edible and waste oil as a new and sustainable feedstock for long term production. It is strongly believed that using these oils will help in improving economical feasibility of biodiesel and minimize the hurdle of food versus fuel phenomena.

JATROPHA GENETIC RESOURCES

The genus Jatropha has 175 known species of the plant belonging to the family Euphorbiaceae (Dehgan 1984). Originating in the Caribbean, Jatropha was spread as a valuable hedge plant to Africa and Asia by Portuguese traders. The plants are drought-resistant succulent shrubs or trees, and are recognized as potential biofuel crop (Jones and Miller 1991; Openshaw 2000). *J. curcas, J. integerrima,* and *J. glandulifera*are native to South America. Among the various *Jatropha* species *Jatropha curcas, J. glandulifera, J. gossypifolia, J. integerrima, J. multifida, J. nana, J. podagrica,* and *J. tanjorensis*are widely cultivated, naturalised and distributed in India. The term “Jatropha” is usually and commonly used to refer to the species *J. curcas*. This plant is a deciduous perennial shrub distributed all over the world. The name is derived from Greek words *iatros* meaning physician and *trophe* meaning nutrition. So the plant is commonly known as physic nut. *J. cuneata*stems are often used for basket making in Mexico. *J. nana* is a small bushy ephemeral and is endemic to Western India. It shows absence of glandular hairs. Seed oil from *J. nana* is used for energy. In traditional medicine it is used as anti-irritant in opthalmia (Ambasta 1992; Das and Venkataiah 2000). *Jatropha curcas* L. (Euphorbiaceae), a deciduous perennial shrub with Central America origin, is now widely cultivated in tropics and subtropics worldwide (Deore and Johonson2008). Seed oil content of this plant is about 40%, higher than the typical oil crops such as soybean and rape (Gubitz et al. 1999; Deore and Johonson 2008). The oil can be used in diesel engines after simple processing because it is similar to diesel oil in characteristics, being a potential substitute for fossil fuel and a renewable energy (Berchmans and Hirata2008; Deore and Johonson 2008). Thus, *J. curcas* has been considered as a strategic plant resource in many countries (Carvalho et al., 2008).The oil content in the whole seed is around 27-30%, de-coated seeds ranges from 35-40% and the kernels 55-60%. The Jatropha seed oil has a fatty acid composition similar to that of edible oils (Gubitz et al., 1999). Also, the seed oil can be used as a diesel engine fuel as it has characteristics close to those of fossil fuel, diesel. The composition and content of the Jatropha seeds. The plant and its seeds are toxic and so is not edible either by animals or humans. Jatropha plants contain several toxic compounds, including lectin, saponin, carcinogenic phorbol, and a trypsin inhibitor.

CONSERVATION STRATEGIES AND CONSTRAINS OF JATROPHA

The application of molecular techniques in plant diversity conservation becoming increasingly popular, the isolation of impact, high-molecular mass genomic DNA becomes an important pre-requisite (Dhakshanamoorthy and Selvaraj, 2009). However, species of *Jatropha* contain polysaccharides and polyphenols posing a major problem in the isolation of high quality DNA. Although several protocols are used for isolation of genomic DNA in *Jatropha* species, all of them use expensive and toxic chemical liquid nitrogen. A poor seed germination, scanty and delayed rooting of seedlings and vegetative cuttings (Heller 1996; Openshaw 2000; Purkayastha et al., 2010) paved way for the necessity of micropropagation of *Jatropha* through embryo or embryo derived explants cultures with promising method for disease free and in vitro culture manipulations (Purkayastha et al., 2010), a means for conservation of elite germplasm. The development of an efficient regeneration system amenable to genetic transformation is a prerequisite for plant genetic engineering (Misra and Misra 1993; Misra et al., 1994). Genetic interactions studies in the *J. curcas* also important for the improvement of this crop (Paramatham, 2020) to increase the yield potential. Efforts for the last two decades have failed to provide a reliable protocol of *invitro* plant regeneration of *J. curcas* (Jha et al., 2007; Deore and Johnson, 2008). However, in recent years, plant regeneration in *J. curcas* has been accomplished through organogenesis from various explants, including mature leaf (Sujatha et al., 2005; Deore and Johnson, 2008), petiole and hypocotyls (Sujatha and Mukta, 1996), axillary node (Sujatha et al., 2005; Shrivastava and Banerjee, 2008) and via somatic embryogenesis from mature leaf explants (Jha et al., 2007). Seed-derived explants are, in general, known to be more responsive to rapid regeneration (Tiwari and Tuli, 2009) and Agrobacterium-mediated transformation (Patnaik et al., 2006; Paz, 2009). Purkayastha et al. (2010) recently developed a method for rapid and efficient plant regeneration from shoot apices, and generation of transgenic plants by direct DNA delivery to mature seed-derived shoot apices of *Jatropha*. Mazumdar et al. (2010) studied the effects of age and orientation of the explant on callus induction and *denovo* shoot regeneration from cotyledonary leaf segments of *J. curcas*. Highest regeneration response was reported in the young explants, derived from the cotyledonary leaf of germinating seed compared to the leaves from one- and two-week-old seedlings. This gradient with age of the explant was observed in callus induction (%), shoot organogenesis (%) from callus. The explants cultured with their abaxial side in medium showed significantly higher regeneration response. The youngest explant was found to be most amenable to Agrobacterium- mediated transformationas compared to older explants giving rise to stable transgenic plants in J. curcas. Agrobacterium-mediated transformation of Jatropha using cotyledonary leaf explants was reported by Li et al. (2008). However, this was inefficient and difficult to reproduce. But, in a consorted effort to develop efficient protocol for genetic transformation of *Jatropha,* Mazumdar et al. (2010) and Kumar et al. (2010) simultaneously reported an efficient Agrobacterium-mediated genetic transformation and plant regeneration protocol for *J. curcas* using leaf explants. Kumar et al. (2010) reported highest transformation efficiency of 4-day pre-cultured, non-wounded leaf explants infected with Agrobacterium and co-cultured on Murashige and Skoog (MS) medium supplemented with thidiazuron (TDZ) for regeneration of shoot buds, and proliferation of shoots in MS medium containing kinetin (Kn), 6-benzylaminopurine (BA), and naphthalene acetic acid (NAA). Subsequent growth of the shoots was promoted in MS medium supplemented with BA and IAA, and rooting in 0.5N MS medium with IBA, IAA, NAA, and activated charcoal. GUS expression anlysis, PCR and DNA gel blot hybridization confirm the expression and presence of transgene. A transformation efficiency of 29% was achieved for leaf explants using this protocol Kumar et al. (2010).These protocols have the potential to facilitate the genetic modification and subsequent true to-type in vitro multiplication of *J. curcas* cultivars. Development of this technology for *Jatropha* can lead to a better understanding and improvement of the biofuel species (Mazumdar et al., 2010).

Due to pressure on edible oils like groundnut, rapeseed, mustard and soybean etc. non-edible oil of *Jatropha curcas* and karanja (*Pongamia pinnata*) are evaluated as diesel fuel extender. *Pongamia pinnata* is a species of family Leguminasae, native in tropical and temperate Asia including part of India, China, Japan, Malaysia, Australia. Commonly it is called as karanja (in MS), pongam (in Gujarat), dalkaramch (in Tamil Nadu). Karanja is drought resistant, semi-deciduous, nitrogen fixing leguminous tree. It grows about 15-20 meters in height with a large canopy which spreads equally wide. The leaves are soft, shiny burgundy in early summer and mature to a glossy, deep green as the season progresses. Cropping of pods and single almond sized seeds can occur by 4-6 years and yields 9-90 kg's of seed. The yield potential per hectare is 900 to 9000 Kg/Hectare. As per statics available pongamia oil has got a potential of 135000 million tonnes per annum and only 6% is being utilized. The tree is well suited to intense heat and sunlight and its dense network of lateral roots and its thick long tap roots make it drought tolerant.

CONCLUSION

The natural genetic variation available within the species can be exploited to create interspecific hybrids for incorporating a wide range of beneficial traits to be incorporated into *Jatropha* species for improvement of the crop. Tree based oilseeds offers scope for rehabilitating degraded lands and improve livelihoods in developing countries. Green cover over barren and unproductive lands will reverse the process of degradation caused mostly by water erosion. The fertility of these marginal lands will improve through recycling of nutrients from the deeper layers, addition of leaf litter, nitrogen fixation (*Pongamia*) and carbon will be sequestered unlike fossil fuels. Studies have shown that *Jatropha* returns 19 kg N ha^{-1} $year^{-1}$ through litter fall from the third year. In conclusion, the development of biodiesel plantation provides win-win situation and needs targeted investments towards research and development aspects for ensuring the supply of raw material.

AUTHOR CONTRIBUTIONS

Paramathma – written the article. Jayamani, Parthiban, and Kiruba **–** Read and approved the article.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ETHICS APPROVAL

Not applicable.

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