

# CHAPTER-I

## INTRODUCTION

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Global energy supply is based mainly on fossil fuels which have many disadvantages, besides their fast depletion. It is widely agreed that more sustainable alternative energy source needs to be developed in the near distant future. One potentially promising option consists of biofuel, which are renewable in nature and do not contribute to the green house effect and help in reductions of particulate matter (PM), carbon mono-oxide (CO), unburned hydrocarbons and SO<sub>2</sub> emissions which are essential for the improvement of air quality. In Europe and United States of America, biodiesel is produced predominantly from edible oils, which are cultivated on good soils. About 130 million hectares of eroded wasteland exist in India, which is currently unsuitable for food production. The recognition that *Jatropha* oil can yield an exceptional biodiesel has led to a surge of interest in *Jatropha* across the globe, more so in view of the potential for avoiding the dilemma of “food vs. fuel”. *Jatropha* is a shrub which thrives on different soil conditions with very low requirement for irrigation and crop management. Hence, *Jatropha* is an ideal plant for wasteland and its non-edible oil a prime choice for biodiesel production. Besides biodiesel, *Jatropha* cultivation can make considerable impact in promoting organic farming through use of deoiled seed cake. The fruit shell having a calorific value equivalent to coal can be made into briquettes and used in solid fired boilers and other applications as a substitute for fossil fuel.

The genus *Jatropha* has 172 species from the family Euphorbiaceae, native to South America and widely distributed in South, Central America and now distributed throughout the world (Fairless, 2007). Among them, 12 species i.e *Jatropha curcas*, *Jatropha gossypifolia*, *Jatropha integerrima*, *Jatropha multifida*, *Jatropha podagrica*, *Jatropha glandulifera*, *Jatropha neynei*, *Jatropha maheshwarii*, *Jatropha tanjorensis*, *Jatropha villosa*, *Jatropha nava*, *Jatropha hastate* are recorded in India and found growing on degraded and marginal lands making it suitable to utilize wastelands without competing conventional

crops for land. Out of these, *J. curcas* also called physic nut is one of the best candidates for future biodiesel production. Biodiesel prepared from *J. curcas* has been successfully tested in both mobile and stationary engines without modification in any of the engine parts. Now there is a surge of interest in *J. curcas* as a biodiesel “miracle tree” to help alleviate the energy crisis and generate income in rural areas of developing countries (Francis et al., 2005).

*J. curcas* is thought to be native to South America and Mexico where it occurs naturally in the forests of coastal regions. However, *J. curcas* is almost pan tropical now, and although toxic, it is widely planted as a biofuel plant. Due to the presence of several toxic substances such as lectin (curcin), phorbol esters, saponins, protease inhibitors and phytates, seed, press cake and oil of *J. curcas* cannot be used for human or animal nutrition (Makkar et al., 1998). Increased production of *J. curcas* as a fuel source will increase the production of toxic by-product, which cannot be utilized as feed. In spite of best nutritional composition, seed cake obtained from the toxic *J. curcas* remains un-utilized as an animal feed due to its toxic nature and no successful attempts were made till now for eliminating toxic principle (Makkar and Becker, 1997; Makkar et al., 1998). Globally *J. curcas* is promoted for large acreage cultivation in a big way for biodiesel production (Sujatha et al., 2005; Ghosh et al., 2007) and selective cultivation of non-toxic cultivar reported from Mexico, whose innocuous nature was established (Makkar and Becker, 1997; Makkar et al., 1998) will add value to the crop through utilization of deoiled seed cake as a safe animal feed.

*J. curcas* is a deciduous shrub with spreading branches and stubby twigs that grows up to 20 feet high under favorable conditions. The seeds contain 30-35% viscous and non-edible oil that has attracted the attention of world as alternate biofuel (Mandpe et al., 2005). Besides biofuel, *J. curcas* is also used in medicine (Duke and Wain, 1981). Seed cake obtained after expelling the oil is rich in proteins (19%), carbohydrates (17%), Nitrogen (6%), Phosphorus (2.75%) and Potassium (0.94%).

The species is primarily propagated through seeds, and thus the yield and oil content vary significantly. The seed viability and rate of germination are low, and quality seed screening is another laborious task thus, seed propagation

cannot provide quality planting material for sustainable use. Propagation can be also carried out without losing the traits by stem cutting but the limitation is generation of large scale quality planting material. Thus, conventional propagation through seeds are not reliable and vegetative cuttings are inadequate to meet the demand of large scale quality planting material. Therefore, *Jatropha* improvement programme by modern methods of agrobiotechnology are of interest worldwide. This has increased the importance of developing tissue culture methods to facilitate large scale production of true to type plants and for the improvement of the species using genetic engineering techniques.

Significant variability in seed yield and oil content in natural population was observed during the survey work conducted by Central Salt & Marine Chemicals Research Institute, India. Some genotypes have been identified which are high yielding as well as of high quality and require biotechnological improvement through homogenous tissues like leaf and petiole explant because heterogeneous tissues like cotyledon explant would result in genetic segregation and loss of the desirable character of high yield and good quality. Regeneration from leaf explant is maximally preferred because: (i) leaf explant is homogenous. (ii) leaf explant has chloroplast DNA that has extremely high copy number and thus the level of ex-pression can be amplified by several folds if leaves are used during genetic manipulations. (iii) leaf offer larger surface area for application of any genetic manipulation techniques. (iv) leaf provides an abundant supply of starting material. (v) using leaf as an explant will not hamper the general well being and growth of the plant. Despite its multifarious potentiality *in vitro* regeneration techniques offer a powerful tool for germplasm conservation, mass-multiplication and genetic transformation.

*J. curcas* is susceptible to insects such as beetles, hoppers and leaf minor larvae feeding (*Spodoptera litura*), Lepidoptera larvae, die-back of branches/ cushion scale (*Pinnaspis strachani*) blue bugs sucking on fruits (*Calidea dregei*) locust feeding on leaves of seedlings (*Oedaleus senegalansis*), green stink bug sucking on fruits (*Nezraviridula*) and Cercospora leaf spot (*Jatropha curcas*) and pests such as Bark eater (*Indarbela spp*) and capsule

borer are the two major pests affecting the plants. Minor diseases such as *Clitocybe tabescens* (root rot), *Colletotrichum gloeosporioides* (leaf spot) and *Phakopsora jatrophiicola* (rust) were reported in *J. curcas* (Paramathma et al., 2007). Conventional plant breeding techniques have limitations to control these insects and pests. Recently, major efforts have been directed towards the introduction of new agricultural traits through genetic transformation. *Agrobacterium*-mediated transformation is the preferred method of gene transfer and more so when somatic tissue like leaf explant of a target plant is used. The use of tissue like cotyledonary explants chosen from seeds can result in genetic variability and loss of characteristics of the target plants. There is only a single report on callus mediated genetic transformation in *J. curcas* (Li et al., 2008). Efficient plant regeneration protocol is a prerequisite for the application of *in vitro* genetic manipulation techniques, such as variant selection and transformation, for economically more desirable characters. Despite the availability of a genotype with variability for various agronomic characters, there are no known sources of insect pest /diseases, salt resistant and high oil yielding germplasm in *J. curcas*. Biotechnological crop improvement thus appears to be the only effective and alternative approach wherein transgenic production will be the most important in achieving the above parameters.

Despite the research efforts in *J. curcas* tissue culture, attempts have been made to regenerate *J. curcas* using different explant (Sujatha and Mukta, 1996; Wei et al., 2004; Sujatha et al., 2005; Rajore and Batra, 2005; Jha et al., 2007; Deore and Johnson, 2008). All the above studies reported were either through callus mediated regeneration or direct shoot morphogenesis with interspersed callus from hypocotyl, leaf, and petiole. Despite sufficient regeneration systems achieved from different explant of *J. curcas*, the presence of intermediary callus or callus-mediated regeneration is least desired for the production of true to type plants. It is also reported that regeneration in *J. curcas* is highly genotype dependent (da Camara Machado et al., 1997). From the literature, it is evident that there is no genotype independent regeneration protocol available for *J. curcas*. Leaf and petiole are somatic tissues hence plants raised from the same are comparatively more resistant to genetic variation (Pierik, 1991). No protocol

for transformation has been developed from leaf explant so far. In view of its economic importance and the limited research work done in the area of tissue culture and genetic transformation, present thesis work is proposed to develop an efficient protocol for regeneration and genetic transformation in *J. curcas*. The objectives of the thesis are therefore aimed at fulfilling these pre-requisites so as to produce *J. curcas* with agronomically desirable traits.

The objectives were:

- Screening of selected *J. curcas* genotype for regeneration potential.
- Development of reproducible regeneration protocol from leaf and petiole explant.
- Attempts to genetically transform via *Agrobacterium tumefaciens* mediated transformation.
- Histochemical GUS assay and molecular characterization of the transgenic micro-shoots.