

2.1 General Overview

Jatropha curcas Linn. belonging to the family *Euphorbiaceae* is a native species of tropical America, cultivated throughout the tropics and is sub-spontaneous in Mauritius and Seychelles (Becker 1877). It is a very prominent and widely acclaimed species with wide variety of uses. It is a small tree or large shrub, which can reach a height of up to 5 m and has high potential for greening and eco-rehabilitation of wastelands as well as for bio-aesthetic reasons (Heller 1996). In India, it is found in semi-wild conditions in the vicinity of villages and is one of the most promising drought tolerant perennial plants adapted to various kinds of soil conditions. The plant is widely distributed and fits easily into agricultural systems in the form of hedges, windbreak, anti-erosion barrier or source of firewood (Srivastava, 1999).

In the recent years energy conservation and its production has acquired significant importance in the wake of the world energy crisis. A number of options for production of liquid fuel as an alternative source have been considered in many countries. Oil seed crops are potential renewable sources of fuels and in this category *J. curcas* has won over the interest of various developmental agencies. The *J. curcas* oil has been identified as an efficient substitute to be used as fuel for diesel engines (Bhasabatra and Sutiponpeibun, 1982a; Bhasabatra and Sutiponpeibun 1982b; Raina 1985). The engine performance and fuel consumption with *J. curcas* oil has been compared favourably with normal diesel oil (Takeda 1982b; Takeda and Minoru 1981; Bhasabutra and Sutiponpeibun 1982a, b; Jarray 1984; Martin and Mayezix 1984; Munch and Keifer 1989). Being a naturalized species with a wide range of distribution, *J. curcas* embraces a considerable scope of variation, which suggests a high potential of this genus in India. The screening of existing populations for growth and oil yield could be utilized profitably for selection of best sources for afforestation and productions of oil. Since the oil crisis of the 1970s and recognition of the

Limitations of world oil resources, most of the oil importing countries including India has been highly motivated to develop alternative sources of energy to meet their domestic needs from natural resources. *J. curcas* has been found highly promising species which can yield oilseed as a source of energy in the form of bio-diesel owing to its short gestation period, hardy nature, high quality oil content etc. The oil can also be used in soap and candle industries and its by-product glycerin can be used in the pharmaceutical industry. Considering vast semi-wild distribution of *J. curcas* in different parts of India, it would be expected to have considerable genetic variation. Sufficient information on such aspect is lacking in this species in spite of its many uses.

Karp and Edwards (1998) had stated that, the knowledge of genetic relationship and variation in the species is a pre-requisite in any breeding program as it permits the organization of germplasm, including elite lines, and provide for more efficient parental selection. But, though the production of fuel from oilseed plants is one of the recent hot topic in this 21st century, the major constraints of achieving higher quality oil yield from such crops are the lack of information regarding its genetic variability, oil composition and absence of suitable ideotype for different cropping systems (Singh *et al.*, 2010). Environmental factors in combination with genetic and physiological factors play important role in determination of plant potential for seed quality. These characters appear to be under strong genetic control (Roy *et al.* 2002). Depending on the species, germination responses of seed vary according to geographical and environmental factors, *viz.* latitude, elevation, soil moisture, soil nutrient, temperature, kind and density of plant cover, degree of habitat disturbance of the site where the seed matures.

2.2 Genetic Diversity Assessment by Traditional Approaches:

To study the relationship and variation among the forest trees a wide variety of techniques had been used till date. Many researchers had conducted different studies to identify the genetic variations in the populations, provenances and clones of *J. curcas* through traditional morphometric and biochemical marker techniques (Singh *et al.*, 2010). So far, only few records of provenance trials of the *J. curcas* exist where an attempt was made to examine the genetic variation (Heller, 1996; Ginwal *et al.* 2004).

Sukarin *et al.* (1987) did not observe any differences in vegetative development and first seed yields among 42 clones in the planted crop field though they were originated from different locations of Thailand. Heller (1996) stated his findings of significant differences in vegetative development among 13 provenances in multi-location field trials. He also stated that however the provenances were very uniform in morphological characters such as leaf shape. A significant correlation between the oil contents and other morpho-physiological characters *viz.* seed weight, stem diameter and total leaf area was described by Ginwal *et al.* (2004). A significant positive effect of altitude on various oil yield components, including branch no. per tree, fruits per tree, seeds per tree but a significant reduction in kernel oil content was described by Pant *et al.* (2006) in the range from 100m to 400m of elevation.

On the other hand, Rao *et al.* (2008) evaluated the genetic association and variability in seed and growth characters among the 32 candidate plus trees (CPTs) of *J. curcas* from different locations of India and found significant differences in all the seed characters such as seed morphology and oil contents as well as growth characters like height of the plant, ratio of male and female flowers, seed yield *etc.* Sunil *et al.* (2008) stated significant differences found among the accessions they studied in an in-situ experiment for the phenotypic characters *viz.* plant height, number of fruits,

oil contents and its composition. However, they did not undertaken any steps for genetic characterization of the accessions, thus the reason for the variability was not clear.

Only a limited number of studies involving biochemical markers for assessment of genetic diversity in *J. curcas* have been reported which mainly involved isozyme markers (Singh *et al.*, 2010). Most of the studies to evaluate germplasm have been done utilizing materials collected from CPTs of different regions, different aged plants (3-20 years) and propagated through seeds or vegetative cuttings (Anonymous, 2006). Heller (1996) stated that the comparison of yield-contributing traits based on such accessions results in erroneous conclusions about the superiority of the identified clone as it is strongly influenced by the mode of propagation, soil type, climatic conditions, age of the plant and plant density.

2.3 Genetic Diversity Assessment by Molecular Markers:

To characterize the genotypes and to measure the genetic relationship among the accessions or provenances more incisively, the DNA markers provided a great opportunity than other markers (Soller and Beckmann, 1983). Polymerase chain reaction (PCR) technology had led to the development of many simple and quick techniques by producing several markers like randomly amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP) etc., which are very useful in characterization of genetic diversity in forest tree species. To assess the genetic diversity among the *J. curcas* species or accessions or provenances, along with some novel approaches most of these markers have been extensively utilized too (Montes *et al.*, 2009; Popluechai *et al.*, 2009). In Table.2.1 a list of different research work carried out by several researchers

regarding the assessment of genetic diversity of *J. curcas* by using different molecular markers is showing including their findings (Source: Singh *et al.*, 2010).

It has been stated by Van der Linden *et al.* (2004) that, genetic variability was low in African and Indian *J. curcas* accessions, but they found high genetic variability in Guatemalan and other Latin American accessions. Thus these studies had proved that molecular markers provide an efficient and quick tool in characterization of genetic diversity among the clones, accessions and populations of *J. curcas* (Singh *et al.*, 2010).

Besides the assessment of genetic diversity, efforts were also made by many researchers (*viz.* Prabakaran and Sujatha, 1999; Ganesh Ram *et al.*, 2008; Pamidiamarri *et al.*, 2009a,b; Senthil Kumar *et al.*, 2009; Basha and Sujatha, 2009 *etc.*) to establish the phylogenetic relationship of *J. curcas* with other related *Jatropha* species and natural hybrids (eg. *J. tanjorensis* J. L. Ellis et Saroja.) by using the markers like RAPD, AFLP, ISSR and other microsatellite markers in addition to some novel molecular techniques. The results of these studies also revealed the significant closeness between the different species of the genus *Jatropha*. Moreover, the RAPD and ISSR markers also confirmed the *J. tanjorensis* as a natural hybrid between *J. gossypifolia* and *J. curcas* (Singh *et al.*, 2010).

Powell *et al.* (1996) stated that in comparison to multilocus markers like RAPD and AFLP, microsatellites have advantages like locus specificity, codominant nature, high reproducibility and substantial size polymorphism. Thus microsatellite provides better tools to assess the amount and distribution of molecular diversity and for population genetic studies. Pamidiamarri *et al.* (2009c) had isolated 12 microsatellites from *J. curcas* and characterized them in 32 accessions collected from a natural population in Junagadh Gir forest region, Gujarat, India.

Table 2.1: Molecular markers used for assessment of genetic diversity in *Jc* accessions & *Jatropha* spp.

Species/Accessions	Marker used	% of polymorphism	Genetic diversity	References
42 accessions	RAPD ISSR	42.0 33.5	Moderate	Basha & Sujatha,, 2007
23 provenances	RAPD AFLP	15.0 9.0	Low	Reddfy <i>et al.</i> , 2007
12 species	RAPD	80.2	High	Ganesh ram <i>et al.</i> , 2008
13 accessions	RAPD ISSR	84.26 76.54	High	Gupta <i>et al.</i> , 2008
58 accessions	AFLP	14.3	Low	Sun <i>et al.</i> , 2008
7 species	RAPD AFLP	68.48 71.33	Moderate	Pamidiamarri <i>et al.</i> , 2009a
8 spp. and 3 accessions	ISSR	98.14	High	Senthil Kumar <i>et al.</i> , 2009
72 accessions from 13 countries	RAPD ISSR	61.5 35.5	Low to high	Basha <i>et al.</i> , 2009
28 accessions	RAPD	38.0	Moderate	Singh <i>et al.</i> , 2009
48 accessions	AFLP	88.0	High	Tatikonda <i>et al.</i> , 2009

Source: Singh *et al.* (2010).

Popluechai *et al.* (2009) also assessed the genetic variation in 38 *J. curcas* accessions collected from 13 different countries of 3 continents by using RAPD, AFLP and combinatorial tubulin based polymorphism (cTBP) and they revealed the narrow genetic diversity among the accessions, while six *Jatropha* species from India showed pronounced genetic diversity indicating higher possibilities of improving *J. curcas* by interspecific breeding. The cTBP method which uses variation in the length of the first and second intron of members of the plant β -tubulin gene family was

earlier successfully used to detect the intra and inter- species polymorphism in diverse plants including oilseed plants- rapeseed and peanuts as stated by Breviario *et al.* (2007 & 2009).

Very recently Saikia *et al.* (2015) assessed the genetic variation among the 31 accessions of *J. curcas* collected from different agroclimatic regions of India including all the North-Eastern states of India by using cTBP, hTBP and DNA methylation methods. The results of all the methods revealed absence of any polymorphisms in their genetic make-up. They stated that, though morphologically, physiologically and even biochemically the performances of these accessions are significantly different but there is no variation found at their molecular level.