

An overview of the genetics and cytogenetics of *Jatropha curcas* L. for its improvement

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Received: 16 July 2020
Accepted: 21 August 2020
Published: 30 September 2020

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Jatropha curcas, an ingenious source for providing biodiesel is becoming a god given crop to sustain the energy requirements in the years to come. Biodiesel, an alternative diesel fuel, is made from renewable biological sources such as vegetable oil and animal fats. Though many non edible oilseeds are available, *Jatropha curcas* L. is recognized as the most potential species for biodiesel production since its seed contains high oil (30 to 38%) and could be grown under different land use situations. India is fifth in the world in terms of fossil fuel consumption which amounts to 40 million tones of diesel and hence there is imperative need for identifying and utilizing plant species that are capable of yielding biodiesel. Centre of excellence in biofuels at TNAU concentrates on the breeding, agronomic and systematic evaluation of seed/ hybrid clones for oil yield maximization. An earnest attempt is made to summarize available information on *Jatropha* genetics, cytogenetics, genetic variability, heritability, genetic advance, association of traits and molecular approaches, interspecific hybridization programmes and prospects of tissue culture, mutation and transgenic breeding for further crop improvement to provide comprehensive understanding on complete exploitation of *Jatropha*.

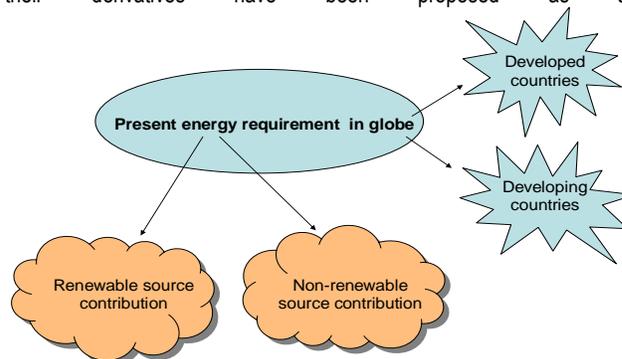
Key words: biofuel, cytogenetics, energy, genetics, jatropha

INTRODUCTION

Energy explicitly drives everything in the Universe. Energy is the major and significant component in economic development of any nation. The demand for oil as an energy source for both human and vehicles is increasing but the production is not commiserating with that resulting in a mismatch between demand and supply of both edible and non-edible oils (Paramathma et al., 2006a). In recent times, efforts are being made to explore plant based resources as a substitute for fossil fuels which are renewable and environmentally safe. The source for biofuels is the forests which is the storehouse of more than 100 tree borne oilseeds. Amongst them, *Jatropha curcas*, an excellent shrub having natural spread across the globe, is one of the promising biofuel crops ideally suitable for growing in the wastelands of the country (Paramathma et al., 2006b). Greater potential exists in India for bringing millions of hectares of wasteland under extensive plantation of *Jatropha*, virtually converting unproductive lands into green oil fields. The oil from *Jatropha curcas* can be used as bio diesel which can be blended upto 20 per cent as targeted by 2020 in India.

Current status of energy

Energy demand in India is increasing at the rate of 6% annually compared to 2% for developed countries. Current import of petroleum crude is about 70% of the energy requirement. Vegetable oils, fats and their derivatives have been proposed as an



alternate renewable and eco-friendly energy source. The bio-diesel production from vegetable oils during 2004–2005 was estimated 2.36 million tonnes globally. Of this, EU countries accounted for about 82% and USA about 6% (Divakara et al., 2010). Shortage of edible oil for human consumption in developing countries does not favour its use for bio-diesel production. India consumes approximately 40 million tonnes of diesel and ranked fifth in the world after the US, China, Russia and Japan in terms of fossil fuel consumption.

Ideal plant for diesel

The total number of oil-bearing species range from 100 to 300 and of them, 63 belonging to 30 plant families hold promise for bio-diesel production (Sunita et al., 2005 and Krawczyk, 1996). Many developed countries are using edible oil-seed crops such as soybean, rapeseed, groundnut, coconut, palm oil and sunflower for production of bio-diesel. As India imports more than 40% of its edible oil requirement, it has to necessarily depend on non-edible oils for biodiesel. Hence various non-edible tree-borne oil crops such as neem, mahua, jatropha and pongamia and paradise tree, available in the country are of great value to the Indian researchers in particular (Paramathma et al., 2005). In India, more than 100 species of forest plants – Tree Borne Oilseeds (TBOs) have been identified as a source of fatty oils with an estimated potential of 11.3 lakh tonnes. Among the many oil yielding species, *Jatropha* (*Jatropha curcas* L.), an exotic non-edible oilseed bearing shrub and traditionally grown hedge plant (Heller, 1996) is widely considered as a potential feed stock crop for bio-diesel production because of its shorter gestation period, smaller canopy suitable for high-density planting and convenient seed collection, higher seed oil content and wide adaptability compared to most other non-edible oilseed yielding species (Martin and Mayeux, 1985; Openshaw, 2000; Basha and Sujatha, 2007). Varied agroclimatic condition along with vulnerable situation in many of the dryland fields that are presently with oilseeds annual could marginally yield up to *Jatropha* in near future provided its productivity is essentially felt. Siphoning the full potential of *Jatropha curcas* for further crop improvement is essential. Several factors such as technical, social, economical and institutional constraints interfere in the way of unveiling *Jatropha curcas*. Understanding its biology, bio ecology, plant and floral morphology, cytological and variability studies could guide in dissecting the genetic behaviour of the species. Since, many of under utilized species of *Jatropha* have the potential value to broaden the genetic base, an attempt is made to review available literature about *Jatropha* from various journals, articles, reports, conference and research proceedings to comprehend an extensive array of information and for easy its availability.

BOTANICAL DESCRIPTION OF JATROPHA

Origin

Jatropha is commonly known as physic nut (English), Ratanjot or Jangaliarandi (Hindi) and Kadalamanakku or Kattamanakku (Tamil). The word *Jatropha* is derived from the Greek words '*Jatros*' means 'Doctor' and '*trophe*' means 'Nutrition'. It is a morphologically diverse genus comprising of 176 species and it is generally grown as live fence in almost all parts of India (Fairless, 2007). Though *Jatropha sp.* are not really cultivated for any direct consumption, one of its acclimatized species namely *Jatropha curcas* is found throughout the tropics and is native to Mexico and Central America. *J. curcas* was probably distributed by Portuguese seafarers via the Cape Verde Islands and Guinea Bissau to other countries in Africa and Asia (Heller, 1992). It is known by nearly 200 different names, indicating its availability in various countries. In

India, 12 species have been recorded so far and they showed wide variation for vegetative, floral characters and oil content (Paramathma et al., 2004). *J. podagrica* is a multipurpose shrub commonly found in Africa, Asia and Latin America (Olapeju et al., 2007). *J. tanjorensis* Ellis & Saroja, reported to be native to India appears in only few districts of Tamil Nadu and is generally grown as a hedge plant originating as natural inter-specific hybrid between *J. curcas* L. and *J. gossypifolia* L. (Sujatha, 1999). *J. multifida* found naturally in Mexico is a popular landscape plant in South Florida (Yotam et al., 2000). *J. gossypifolia* also called as bellyache bush is a major weed in Australia (Taofeeq et al., 2005). *J. integerrima*, *J. multifida*, and *J. podagrica* are important ornamental plants, whereas, *J. curcas*, *J. glandulifera* have medicinal value (Ramchandani and Jolly, 1988).

Plant morphology of Jatropha sp.

The *Jatropha* plant is a small tree or large shrub which can reach a height up to 6 meters. The branches contain latex. Normally, five roots are formed from seeds, one central (tap root) and four peripheral. Cuttings, when planted, do not form a tap root. The plant is monoecious and flowers are unisexual. Pollination is generally by insects. The leaves are smooth, 4–6 lobed and 10–15cm in length and 5–8 cm in width. The genus *Jatropha* exhibits wide and marked variation for both morphological and floral characters and species are distinguishable by the key characters such as plant habit, leaf shape, hairiness, colour of young leaf, height of plant etc. *J. villosa* var. *villosa*, *J. villosa* var. *ramnadensis* and *J. glandulifera* have highly branching and rhizomatous roots. Hence, these species have the capacity to withstand the prolonged drought (Paramathma et al., 2007). Three species viz., *J. curcas*, *J. multifida* and *J. tanjorensis* have tree like habit with 5–7 branches while the rest of species are profuse branching types. The species such as *J. villosa* var. *villosa*, *J. villosa* var. *ramnadensis* and *J. glandulifera* have 5–6 branches in general. *J. podagrica*, *J. integerrima* and *J. multifida* species are of ornamental value (Airy Shaw, 1972). *J. podagrica* was nearly distinct without branches and gout stem. Gout is a special feature of water storing capacity at the base of the stem (Sunil et al., 2008).

Floral morphology of Jatropha sp.

The extent of variation in respect of floral traits was also critically investigated in the study. The role of reproductive efficiency is on a comparative basis among the taxa studied. The striking differences perhaps are in the number of anthers in each flower, being 8 or 10. In *J. integerrima*, *J. curcas* with ten anthers and the rest of species with eight anthers (Prakash et al., 2007). The capsule also varies distinctly among the species. *J. curcas* and *J. multifida* have non-dehiscent capsules with yellow colour (Singh, 1970). *J. multifida* has very big size capsule than other species. Cytological and molecular studies in this species would help in transferring the region governing the bigger capsule size to *Jatropha curcas* species. Other species are with brown colour capsules and dehiscent in nature.

Female to male flowers ratio

An interesting and important feature in flowers is the relative abundance of male flowers in relation to the number of female flowers. The ratio of female to male flowers ranges from 1:13 to 1:29 (Raju and Ezradanam, 2002 and Tewari, 2007) and decreases with the age of the plant (Prakash et al., 2007). Ratio of female to male flowers ranged from 1: 6 (*J. glandulifera*) to 1: 20 (*J. multifida*) which is the highest ratio among all the species studied (Fig 1). Female to male ratio ranged from 1:6 to 1: 10 in *J. villosa* var. *villosa* and *J. villosa* var. *ramnadensis* species have

Table 1. Cytological observations of the different species

S. No.	Species	No. of Bivalents during Metaphase I	Total 2n number	Time of flower bud fixation	Pollen fertility per cent	References
1	<i>Jatropha villosa</i> var <i>villosa</i>	10 ^{II}	20	7.10 AM	85.50	Sasikala and Paramathma, 2010; Sasikala <i>et al</i> , 2009
2	<i>Jatropha villosa</i> var <i>ramnadensis</i>	10 ^{II} and disjunction of chromosomes in anaphase I	20	7.10 AM	86.12	Sasikala and Paramathma, 2010; Sasikala <i>et al</i> , 2009
3	<i>Jatropha multifida</i>	11 ^{II} (7 ring and 4 rod bivalents)	22	7.30 AM	93.52	Sasikala and Paramathma, 2010; Dehgan, 1984
4	<i>Jatropha podagrica</i>	11 ^{II}	22	8.00 AM	88.69	Sasikala and Paramathma, 2010; Sarkar, 1989; Krishnappa and Rashme, 1980
5.	<i>Jatropha maheswarii</i>	11 ^{II}	22	7.30 AM	90.58	Sasikala and Paramathma, 2010; Kothari <i>et al</i> , 1980
6.	<i>Jatropha glandulifera</i>	11 ^{II}	22	7.00 AM	96.81	Sasikala and Paramathma, 2010; Navaneetham <i>et al</i> , 1983
7.	<i>Jatropha gossypifolia</i>	11 ^{II}	22	8.30 AM	90.82	Trivedi and Trivedi, 1982; Gill <i>et al</i> , 1981; and Krishnappa and Rashme, 1980.
8	<i>Jatropha tanjorensis</i>	No proper counting of chromosomes and sporads of unequal size	-	6.30 AM	0.16	Sasikala and Paramathma, 2010; Prabakaran and Sujatha, 1999
9	<i>Jatropha integerrima</i>	11 ^{II} (6 ring and 5 rod bivalents)	22	7.30 AM	93.11	Sasikala and Paramathma, 2010; Soontornchainasaeng and Jenjittikul, 2003
10	<i>Jatropha curcas</i>	11 ^{II} (7 ring and 4 rod bivalents)	22	6.30 AM	92.10	Sasikala and Paramathma, 2010; Soontornchainasaeng and Jenjittikul, 2003

more number of female flowers per inflorescence. Bhattacharya (2005) suggested that this particular character could be utilized for further breeding purpose to increase the number of female flower composition in the commercially cultivated *Jatropha* species.

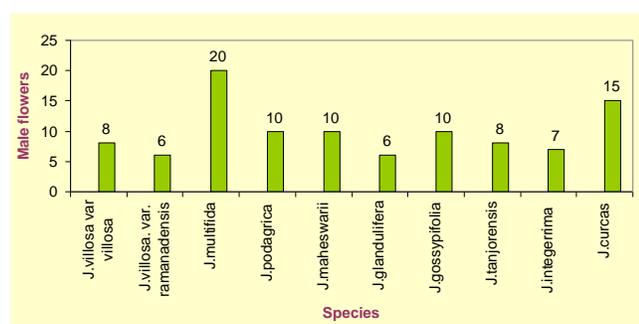


Figure 1. Ratio of male flowers to one female flower in *Jatropha* species

The flowers are unisexual and hence much of the energy is needed for the production and maintenance of male flowers, an aspect that need to be avoided in selecting efficient germplasm for capsule and seed yield. Being capable of having vegetatively reproduction an assured approach in *Jatropha*, the poor reproductive efficiency may not be of immediate significance. However, number of inflorescence produced per plant will decide ultimate number of female flowers and progeny size. A critical estimation of pollen production ability or potential need to be made in future studies (Qing *et al.*, 2007).

CYTOGENETIC STUDIES IN JATROPHA

Fertility status of *Jatropha* species

The fertility status of different species of the genus *Jatropha* such as *J. integerrima*, *J. curcas*, *J. multifida*, *J. podagrica*, *J. gossypifolia* and *J. glandulifera* are fertiles. *J. integerrima* Rosea was partially sterile and *J. tanjorensis* is a fully sterile species in the genus *Jatropha* (Sujatha and Prabakaran (1997). Chromosome counts in the hybrid of *J. curcas* and *J. integerrima* were made from young leaves (Baldwin, 1939). According to the report, young leaves of hybrid had a chromosome number of $2n=22$. Prabakaran and Sujatha (1999) indicated that *Jatropha tanjorensis* (Ellis and Ramnad Saroja) is found abundantly in Tanjore, Pudukottai, Trichirapalli and Ramnad districts of Tamil Nadu. Meiotic studies revealed abnormal divisions with formation of tri and uni valents at metaphase I and an unequal anaphase separation leading to the formation of laggards and sporads of unequal size.

Ploidy status

Cytological studies such as chromosome numbers, their pairing behaviour in prophase I of meiosis and their morphological characters throw light on the genetic behaviour of the species. *Jatropha* is a highly cross pollinated genus. Crossing between the species present in the genus *Jatropha* is a potential breeding tool to transfer of agronomically important character from underutilized species to cultivated species. (Carvalho *et al.*, 2008). The course of meiosis was studied upto tetrads

stage and formation of the bivalents in all species such as *J. multifida*, *J. podagrica*, *J. villosa* var. *villosa*, *J. villosa* var. *ramnadensis*, *J. maheswarii*, *J. glandulifera*, *J. integerrima* and *J. curcas* were observed. The bivalent formation and regular equal separation of chromosomes indicated that the species investigated are essentially diploids. However distinct differences were noticed in respect of number of bivalents at metaphase I. All the species including hybrid studied had 11 bivalents and thus $2n = 22$ and $x = 11$ (Sootornchainaksaeng & Jenjittikul, 2003). But in *J. villosa* var. *villosa* and *J. villosa* var. *ramnadensis*, they showed only 10 bivalents and hence, their $2n$ number is 20 with haploid number of $x = 10$ (Table 1). The existence of diploid number of $2n = 22$ and $2n = 20$ in *Jatropha* is of significance bringing out the fact that two kind of diploids, in which eight species with $2n = 22$ and two species have $2n = 20$ (Sasikala et al., 2010). This study helped to conclude the occurrence of two kinds of haploid chromosome number of $n = 10$ and $n = 11$. It can be postulated that $n = 10$ has arisen from the basic chromosome number of $x = 5$ and the $n = 11$ in turn evolved from dibasic chromosome numbers of $x = 5$ and $x = 6$, thereby indicating that the present day diploid species with $2n = 20$ ($n = 10$) and $2n = 22$ ($n = 11$) are secondary diploid species. It is yet another cytological evolutionary step to understand i.e. to how the basic chromosome numbers of $x = 5$ and 6 have evolved. It may be either by increase in number from $x = 5$ to $x = 6$ or by decrease of chromosome numbers and $x = 5$ resulting from $x = 6$. In the karyotype evolution, it is known that basic (x) chromosome numbers evolve as a result of addition or loss of chromosomes by structural rearrangements. The study of chromosome association in the F_1 s of $2n:20 \times 2n:22$ species alone can be the critical step to get precise understanding of the existence of genome homeology, genetical similarities and probable basic chromosome number and scope to generate recombinants.

GENETIC VARIABILITY, HERITABILITY AND GENETIC ADVANCE

Genetic variability

Trees harbour greater variability and hence intensive studies on variation within a species are necessary for tree improvement. The major job of a tree breeder is to recognize the variability, isolate it and pack it in a desired individual (Zobel and Talbert, 1984). For any tree improvement programme to be successful, studies on variation are of prime importance. Such studies are lacking in *Jatropha* (Antonovics, 1971; Nienstaedt, 1975; Elridge et al., 1993). The best gains are made for characteristics that have a wide range of variability and are strongly under genetic control (Zobel, 1971; Lacaze, 1978; Zobel and van Buijtenen, 1989). Studies on variability do not indicate the extent of heritable portion of variation in the population. The variation is expressed by estimates of genetic parameters, each of which is a numerical quantity which specifies a population in respect to some characteristics (Allard, 1960). Hence, quantification of the extent of existing variability is the preamble for initiating any tree breeding programme. Traditionally, morphological observations and progeny tests have been used as descriptors of genetic diversity; however, they failed to reveal the exact taxonomic relationships because most of the morphological characters are plastic and influenced by environmental factors (Heller, 1996). Production of new ornamental *Jatropha* hybrids through interspecific hybridization (Sujatha and Prabakaran, 2003) is successful from two economically important species of *Jatropha*, viz., *J. curcas* and *J. integerrima*. The interspecific hybrids exhibited morphological intermediary for various vegetative characteristics but produced flowers with three distinct colours. Backcrossing of the F_1 hybrids resulted variation in flower colour ranging from dark pink through green to white thereby enhancing the ornamental value of the genus. The genetic variability on plant characters such as early maturity, 100 seed weight

and number of branches have improved by irradiating the plant with gamma rays (Ita Dwimahaya and Ishak, 2004, Ginwal et al., (2004). Variations in growth traits were observed at nursery stage viz., shoot length, root length, dry weight and vigour index. This was found evident among seed sources of *J. curcas* (Kumar et al., 2004). The assessment of variability in *Jatropha curcas* L. germplasm was carried out from four ecogeographic zones of India and it was observed that the variability present in the plant height, collar thickness, number of fruits per cluster and oil content contributed to yield superior lines and also observed that the oil content of 162 accessions ranged from 22% to 42% (Sunil et al., 2007 and Gohil and Pandya (2008)). The germplasm accessions of *Jatropha curcas* L. were studied for the presence of wider variability for both hundred seed weight and oil content by Mukta et al. (2009), Kaushik et al. (2007), Wani et al. (2006) and Rao et al. (2008). Sixteen *Jatropha curcas* genotypes collected from four states grown and studied for 12 characters showed significant differences in most component traits and seed yield, excepting primary branches/plant, fruits/bunch and seeds/fruit (Das et al., 2010). Significant variation was found among types collected from zones and among different provenances within zones for all traits of seed & seedlings (Ghosh et al., 2011 and Parthiban et al., 2011).

Heritability and Genetic advance

The proportion of total variation which is heritable is termed as broad sense heritability (Lush, 1937). Heritability estimate has an important place in the tree breeding as it provides an index of the relative strength of inheritance against the environment. As estimation of heritability helps in assessing the heritable portion of variation and would help in selection (Zobel, 1971), it is worthwhile to determine the heritable components. However, detailed studies are either insufficient or lacking in *Jatropha* and its related species in these areas. For manipulating the quantitative characters, the tree breeder must have an understanding on the nature, magnitude and inter-relationship of heritable and non-heritable variations which could help in exploiting that relationship.

ASSOCIATION OF MORPHOMETRIC TRAITS

For any tree improvement programme, it is essential to understand the magnitude and type of association between growth and its component characters. In the integrated structure of plant, path coefficient analysis delineates the direct influence of one variable upon another and permits the separation of correlation coefficients into components of direct and indirect effects (Wright, 1921). Knowledge on association among components of economic important traits can help in proving the information for efficient selection. Besides, the genetic interaction studies in this crop are inevitable for the improvement of this crop (Paramathma, 2020).

Correlation between quantitative traits

Correlation analysis was carried out among oil yield and its component characters. Significant correlation for growth characters were observed in *Jatropha curcas* L. collected from various locations of Central India (Ginwal et al., 2004). It was also observed that growth traits viz., plant height, collar diameter, leaf area and field survival were found to have significant inter-correlation with each other. Significant correlation between seed characters and germination was observed in *Jatropha curcas* L. (Ginwal et al., 2005, Rao et al., 2008 and Prasanthi et al., 2009). These studies indicated that seed kernel weight, seed oil content and kernel oil content were highly significant and had positive correlation with whole seed weight. Ranwah et al. (2009) suggested that the kernel content and the ratio of male to female flowers showed negative

correlation with oil content and he also reported that hundred seed weight registered positive correlation with oil content. The plant height and number of branches were found to be positively correlated with yield but the male to female flower ratio does not (Rao, 2009).

Path coefficient analysis

The path co-efficient analysis, which apportions the correlation co-efficient into direct and indirect effects and measures the relative importance of the causal factors involved (Dewey and Lu, 1959) is more useful. Path coefficient analysis is being extensively utilized for understanding the complex traits in breeding programmes (Frakes *et al.*, 1961; Ramanujam and Rai, 1963; Paroda *et al.*, 1975; Tewari and Singhania, 1984) in different crop plants. If the correlation between dependent and independent character is due to the direct effect it reflects the true relationship between them and thus selection can be practiced for such characters in order to improve the dependent trait. If the correlation is due to indirect effect through another component trait, then the selection has to be done for the latter trait. Hence a thorough study on the static dynamic relationship between various traits to be investigated would finally reflect on the dependent characters (Sekar, 2003, Ashok Kumar *et al.*, 1998 and Nesamani, 2005) that would help in improving selection.

MOLECULAR APPROACHES FOR CROP IMPROVEMENT

Molecular markers based on differences in DNA sequence between individuals generally detect more polymorphism than morphological and protein based markers (Botstein *et al.*, 1980; Tanksley *et al.*, 1989) as their availability is fairly high compared to the latter. DNA markers provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers (Soller and Beckmann, 1983). Because of their heritable nature, they were found to act as versatile tools in the fields like taxonomy, physiology, embryology, genetic engineering etc. Major applications of these DNA markers in the field of genetics and plant breeding are in (i) diversity analysis and phylogenetic studies (ii) mapping genes and (iii) marker assisted selection (MAS). Genomics-based breeding strategy is one of the ways for *J. curcas* improvement. However, the molecular genetic map of *J. curcas* is not yet available and very limited information is available with respect to molecular markers (Wen *et al.*, 2010). A large number of polymorphic markers are required to measure genetic relationships and genetic diversity in a reliable manner. Molecular genetic markers would aid the long term objective of identifying diverse parental lines to generate segregating populations for tagging important traits such as gene(s) for high content of specific fatty acids like oleic, linolenic, etc. (Gupta *et al.*, 2008). Single marker analysis albeit not completely reliable can be used to detect the association between the marker classes and phenotypic traits. The identification of gene loci governing important traits can be useful in marker-assisted selection. The threat of genetic erosion led to a significant interest in the assessment of genetic diversity in germplasm collections (Manifesto *et al.*, 2001).

Molecular diversity analysis in *Jatropha curcas* L.

Bakliwal *et al.* (2007) reported broad genetic diversity among the *Jatropha* cultivars of Arawali hills using RAPD analysis with 19 primers. DNA finger printing studies using AFLP / RAPD reveal significant differences among the *Jatropha* germplasm as reported by Reddy *et al.* (2007). With AFLP 8-10 per cent polymorphism and with RAPD, 14-16 per cent was found among the 20 elite accessions studied. The genetic diversity studies in 12 *Jatropha* species based on RAPD analysis carried

out using 26 random primers resulted in 18 reproducible bands accounting for 80.2% polymorphism. The primer OPA 4, OPF 11 and OPD 14 generated 100% polymorphism. UPGMA cluster analysis indicated three distinct clusters of different species of *Jatropha*. (Ganesh Ram *et al.*, 2007). The assessment of genetic diversity was carried out among the accessions of *Jatropha curcas* L using two Single Primer Amplification Reaction methods (SPAR). The UPGMA tree analysis showed the separation of several accessions clearly. (Shirish Ranade *et al.*, 2007). The genetic relationships of 58 *J. curcas* accessions were assessed based on Simple Sequence Repeat (SSR) and Amplified Fragment Length Polymorphism (AFLP) analyses. Seventeen microsatellite markers were developed using the FIASCO (Fast Isolation by AFLP of Sequences Containing repeats) protocol; only one SSR primer was polymorphic with two alleles as reported by Qi Bao *et al.* (2008). The extent of genetic variability and phylogenetic relationship among *Jatropha curcas* L, *J. glandulifera*, *J. gossypifolia*, *J. integerrima*, *J. multifida*, *J. podagrica* and *J. tanjorensis* were investigated using RAPD and AFLP markers. The percentage of loci that are polymorphic among the species was found to be 97.74% RAPD and 97.25% AFLP. The mean percentage of polymorphism (PP) was found to be 68.48 for RAPD and 71.33 for AFLP. The phylogram generated with RAPD and AFLP data showed maximum similarity. Both RAPD and AFLP techniques were found to be comparable in deducing divergence of *Jatropha* species. (Sudheer Pamidiyarri *et al.*, 2008). Genetic diversity and genetic relationships among germplasm collection of *Jatropha curcas* L. was assessed using Random Amplified Polymorphic DNA (RAPD) technique by Kumar *et al.* (2009). Out of 55 decamer primers tested, 26 primers produced good amplification products. A total of 6,011 amplification products were scored from which only 1,859 bands (30.92 per cent) were found to be polymorphic. UPGMA cluster analysis revealed clear genetic difference among *J. curcas* germplasm

BREEDING PROGRAMMES

Hybridization broadly refers to any cross mating of two genetically different individuals that leads to hybrid progeny (Rieger *et al.*, 1968). The production of a hybrid between carnation (*Dianthus caryophyllus*) and sweet William (*Dianthus babratus*) by Thomas Fairchild in 1717 was the first authentic record of distant hybridization for crop improvement. The use of hybrids is very common for agricultural crops and in forestry; hybrid trees have long been known (Tanaka, 1882).

Interspecific hybridization

Interspecific hybridization is an important and significant part of successful tree improvement programmes. Interspecific hybridization followed by backcross breeding is an important method to transgress the desirable genome to the cultivar with varieties to have more seed yield and oil content. Such backcross breeding is limited in the genus *Jatropha*.

Jatropha curcas x *Jatropha integerrima*

Sujatha and Prabakaran (2003) reported that interspecific hybridization was successful between two economically important species of *Jatropha*, viz., *Jatropha curcas* and *Jatropha integerrima*. The interspecific hybrids exhibited morphological intermediary for various vegetative characteristics but produced flowers with three distinct colours. Backcrossing of the F₁ hybrids resulted in a number of flower colours varying from dark pink through green to white enhancing the ornamental value of the genus. Extensive trials on interspecific hybridization were

conducted to study the phylogenetic affinities in *Jatropha*. The study revealed that all hybrids from the cross between *J. curcas* and *J. integerrima* were more vigorous than the parental species. The study further concluded that in all those crosses that were successful, *J. curcas* was used as the maternal parent (Dehgan, 1984). An attempt has been made earlier by Earlene et al. (1970) and a vigorous interspecific self-sterile hybrid has been obtained from cross pollinations between two tropical woody species of *Jatropha*, *Jatropha curcas* and *J. integerrima*. Successful backcrossing with *J. integerrima* exhibited segregation for leaf characters.

Jatropha curcas x *Jatropha tanjorensis*

Interspecific hybrids of *Jatropha* are available in India through natural hybridization as well as artificial hybridization. A new species of *Jatropha* identified by the Botanical Survey of India viz., *Jatropha tanjorensis* (Ellis and Saroja, 1961) was proved to be an interspecific hybrid between *Jatropha curcas* and *J. gossypifolia* (Sujata & Prabakaran, 1997). *Jatropha* plantation through F₁ hybrids can be carried out either through macro or micro propagation because these hybrids are from open pollinated interspecific origin. It is difficult to produce large quantity of vegetative propagules in a shorter time due to urgency of establishing 2.0-11.20 million ha of *Jatropha* before 2017 (Planning commission, 2003). But the plantation of seed origin can cover the required area in time.

FUTURE PROSPECTS

Success in plant breeding primarily depends on the nature and magnitude of variation present in the population. The conservation of the crop (Paramathma et al., 2020), assessment of heritable and non-heritable components in the total variability observed is of immense value for selecting suitable breeding programmes. Such studies are still in infancy stage in *Jatropha*. Genetic improvement through conventional breeding approaches is under progressed in many of centers. If these conventional approaches are assisted by molecular breeding, it will reduce the time and increase the efficiency of breeding programme and by pass the risk of maintenance of too much population. Karyotyping and sequencing studies will give exact picture on the potentiality of *Jatropha*. Emphasis should be given to do tissue culturing to develop somoclonal variants and doubled haploids. Mutation studies will help to develop toxic free *Jatropha* types for extensive cultivation. Genetic transformation and gene transfer studies can aid for improving available form to as ideal form.

CONCLUSION

Recent wave of interest to grow *Jatropha* indicates many reasons particularly in the forthcoming context of the energy challenge in both developed and developing countries. The rapid depletion of fossil fuel reserves and high prices has prompted a search for alternative fuels in the world. Among the most tree borne oilseeds namely Karanja, Neem, Jojoba, *Jatropha curcas* L. is prominent one because of its wider distribution with considerable genetic variation, high oil content, and its suitability to grow in all kinds of soil. In order to meet out the energy challenge, it is better if the variation present in *Jatropha* is exploited through conventional, molecular breeding approaches and its combination. Inter and intraspecific hybridization between *Jatropha* related genera and germplasm lines followed by selection of elite genotypes with respect to oil content, resistance to pest and diseases and which could overcome stress can help in achieving progress in

Jatropha breeding. Transgenic breeding can also create variation required for further crop improvement.

AUTHOR CONTRIBUTIONS

Paramathma – written the article. Sasikala, Manivanan, Parthiba & Kiruba – Read and approved the article.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ETHICS APPROVAL

Not applicable.

REFERENCES

- Aiyelaagbe, O. O., Adesogan, K., Ekundayo, O., & Gloer, J. B. (2007). Antibacterial diterpenoids from *Jatropha podagrica* Hook. *Phytochemistry*, 68(19), 2420-2425.
- Allard, R. W., & Wiley, J. (1960). Principles of Plant Breeding.
- Antonovics, J. (1971). The effects of a heterogeneous environment on the genetics of natural populations: The realization that environments differ has had a profound effect on our views of the origin and role of genetic variability in populations. *American Scientist*, 59(5), 593-599.
- Ashoke, B., Kalyani, D., & Datta, S. K. (2005). Floral biology, floral resource constraints and pollination limitation in *Jatropha curcas* L. *Pakistan Journal of Biological Sciences*, 8(3), 456-460.
- Bajpai, A., Srivastava, N., Rajan, S., & Chandra, R. (2008). Genetic diversity and discrimination of mango accessions using RAPD and ISSR markers. *Indian Journal of Horticulture*, 65(4), 377-382.
- Bakliwal, N., Joshi, A., Mehta, S.L., Khandelwal, S. K. (2007) Molecular characterization of *Jatropha* cultivar of Arawali hill of Mewar region. *Int J Trop Agric*, 25 (3), 591-598.
- Baldwin, J. J. (1939). Chromosomes from leaves. *Science*, 90(2332), 240-240.
- Basha, S. D., & Sujatha, M. (2007). Inter and intra-population variability of *Jatropha curcas* (L.) characterized by RAPD and ISSR markers and development of population-specific SCAR markers. *Euphytica*, 156(3), 375-386.
- Bhatt, D., & Reddy, T. P. (1981). Correlations and path analysis in castor (*Ricinus communis*). *Canadian Journal of Genetics and Cytology*, 23(3), 525-531.
- Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American journal of human genetics*, 32(3), 314.
- Campbell, R. K. (1979). Geneecology of Douglas-fir in a watershed in the Oregon Cascades. *Ecology*, 60(5), 1036-1050.

- Carvalho, C. R., Clarindo, W. R., Praça, M. M., Araújo, F. S., & Carels, N. (2008). Genome size, base composition and karyotype of *Jatropha curcas* L., an important biofuel plant. *Plant Science*, 174(6), 613-617.
- Das, S., Misra, R. C., Mahapatra, A. K., Gantayat, B. P., & Pattnaik, R. K. (2010). Genetic variability, character association and path analysis in *Jatropha curcas*. *World Applied Sciences Journal*, 8(11), 1304-1308.
- Dehgan, B. (1984). Phylogenetic significance of interspecific hybridization in *Jatropha* (Euphorbiaceae). *Systematic Botany*, 467-478.
- Dewey, D. R., & Lu, K. (1959). A Correlation and Path-Coefficient Analysis of Components of Crested Wheatgrass Seed Production 1. *Agronomy journal*, 51(9), 515-518.
- Dhillon, R. S., Bisla, S. S., & Bangarwa, K. S. (1992). Correlation and Path Coefficient Studies in Morphological Characters of Shisham (*Dalbergia Sissoo* Roxb.). *MYFOREST*, 28, 349-349.
- Divakara, B. N., Upadhyaya, H. D., Wani, S. P., & Gowda, C. L. (2010). Biology and genetic improvement of *Jatropha curcas* L.: a review. *Applied Energy*, 87(3), 732-742.
- Dwimahyani, I. (2004). Induced Mutation on *Jatropha* (*Jatropha Curcas* L.) for Improvement of Agronomic Characters Variability. *Atom Indonesia*, 30(2), 53-60.
- Eldridge, K., Davidson, J., Harwood, C., & Wyk, G. V. (1994). *Eucalypt domestication and breeding*. Clarendon Press.
- Ellis, J. L. (1961). A new species of *Jatropha* from South India. *Journal of the Bombay Natural History Society*, 58, 834-836.
- Fairless, D. (2007). Biofuel: the little shrub that could-maybe. *Nature News*, 449(7163), 652-655.
- Frakes, R. V., Davis, R. L., & Patterson, F. L. (1961). The Breeding behavior of Yield and Related Variables in Alfalfa. II. Associations Between Characters 1. *Crop Science*, 1(3), 207-209.
- Franklin, E. C. (1979). Model relating levels of genetic variance to stand development of four North American conifers. *Silvae genetica*, 28(5-6), 207-212.
- Ghosh, L., & Singh, L. (2011). Variation in seed and seedling characters of *Jatropha curcas* L. with varying zones and provenances. *Tropical Ecology*, 52(1), 113-122.
- Ginwal, H. S., Phartyal, S. S., Rawat, P. S., & Srivastava, R. L. (2005). Seed source variation in morphology, germination and seedling growth of *Jatropha curcas* Linn. in central India. *Silvae genetica*, 54(1-6), 76-80.
- Ginwal, H. S., Rawat, P. S., & Srivastava, R. L. (2004). Seed source variation in growth performance and oil yield of *Jatropha curcas* Linn. in central India. *Silvae Genetica*, 53(1-6), 186-192.
- Gohil, R. H., & Pandya, J. B. (2012). Genetic diversity assessment in physic nut (*Jatropha curcas* L.). *International Journal of Plant Production*, 2(4), 321-326.
- Gupta, S., Srivastava, M., ... & Singh, R. (2008). Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different *Jatropha curcas* genotypes. *African Journal of Biotechnology*, 7(23).
- Gupta, S., Srivastava, M., Mishra, G. P., ... & Singh, R. (2008). Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different *Jatropha curcas* genotypes. *African Journal of Biotechnology*, 7(23).
- Heller, J. (1992). Studies on genotypic characteristics and propagation and cultivation methods for physic nuts (*Jatropha curcas* L.).
- Heller, J. (1996). *Physic nut, Jatropha curcas* L (Vol. 1). Bioersity international.
- Kaushik, N., Kumar, K., Kumar, S., Kaushik, N., & Roy, S. (2007). Genetic variability and divergence studies in seed traits and oil content of *Jatropha* (*Jatropha curcas* L.) accessions. *Biomass and Bioenergy*, 31(7), 497-502.
- Kedharnath, S., & Vakshasya, R. K. (1978). Estimates of components of variance, heritability and correlations among some growth parameters in *Eucalyptus tereticornis*. In 3. *World Consultation on Forest Tree Breeding, Canberra (Australia)*, 21 Mar 1977. CSIRO.
- Kochhar, S., Kochhar, V. K.,...& Pushpangadan, P. (2005). Differential rooting and sprouting behaviour of two *Jatropha* species and associated physiological and biochemical changes. *Current science*, 89(6), 936-939.
- Krawczyk, T. (1996). Biodiesel-alternative fuel makes inroads but hurdles remain. *inform*, 7, 801-815.
- Kumar, A., & Gurumurthi, K. (1998). Genetic assessment of clonal material of *Casuarina equisetifolia*. *Indian forester*, 124(3), 237-242.
- Kumar, R. S., Parthiban, K. T., & Rao, M. G. (2009). Molecular characterization of *Jatropha* genetic resources through inter-simple sequence repeat (ISSR) markers. *Molecular biology reports*, 36(7), 1951-1956.
- Kumar, R. V., Tripathi, Y. K., Shukla, P., Ahlawat, S. P., & Gupta, V. K. (2009). Genetic diversity and relationships among germplasm of *Jatropha curcas* L. revealed by RAPDs. *Trees*, 23(5), 1075-1079.
- Kumar, S., Parimallam, R., Arjunan, M. C., & Vijayachandran, S. N. (2003, August). Variation in *Jatropha curcas* seed characteristics and germination. In *Proceeding of the national workshop on Jatropha and other perennial oilseed species. Pune, India* (pp. 63-6).
- Lacaze, J. F. (1978). Advances in species and provenance selection. *Unasylva*.
- Levin, Y., Sherer, Y., Bibi, H., Schlesinger, M., & Hay, E. (2000). Rare *Jatropha multifida* intoxication in two children. *The Journal of emergency medicine*, 19(2), 173-175.
- Lush, J.L. (1973). Animal breeding plan. Iowa state college press Ames, Iowa. 47.

- Manifesto, M. M., Schlatter, A. R., Hopp, H. E., Suárez, E. Y., & Dubcovsky, J. (2001). Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. *Crop science*, 41(3), 682-690.
- Martin, G., & Mayeux, A. (1985). Curcas oil (*Jatropha curcas* L.): a possible fuel. *Agric. Trop*, 9, 73-75.
- Mignouna, H. D., Ikea, J., Thottapilly, G., & Ng, N. Q. (1998). Genetic diversity in cowpea as revealed by random amplified polymorphic DNA [RAPD-*Vigna unguiculata* (L.) Walp]. *Journal of Genetics & Breeding (Italy)*.
- Mukta, N., Murthy, I. Y. L. N., & Nagaraj, G. (2009). Collection and identification of potential tree borne oilseeds germplasm in Andhra Pradesh.
- Namkoong, G., & Conkle, M. T. (1976). Time trends in genetic control of height growth in ponderosa pine. *Forest Science*, 22(1), 2-12.
- Namkoong, G., Usanis, R. A., & Silen, R. R. (1972). Age-related variation in genetic control of height growth in Douglas-fir. *Theoretical and applied Genetics*, 42(4), 151-159.
- Nesamani K. (2005). Studies on seed sources, micropropagation and molecular characterization of sex specificity in *Simarouba glauca* DC; M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore.
- Nienstaedt, H. (1976). Adaptive variation--manifestations in tree species and uses in forest management and tree improvement. *USDA Forest Service General Technical Report NC*.
- Oduola, T., Adeosun, G. O., Oduola, T. A., Awioro, G. O., & Oyeniyi, M. A. (2005). Mechanism of action of *Jatropha gossypifolia* stem latex as a haemostatic agent.
- Openshaw, K. (2000). A review of *Jatropha curcas*: an oil plant of unfulfilled promise. *Biomass and bioenergy*, 19(1), 1-15.
- Pamidiyarri, D. S., Pandya, N., Reddy, M. P., & Radhakrishnan, T. (2009). Comparative study of interspecific genetic divergence and phylogenetic analysis of genus *Jatropha* by RAPD and AFLP. *Molecular biology reports*, 36(5), 901-907.
- Panse, V. G., & Sukhatme, P. V. (1957). Genetics of quantitative characters in relation to plant breeding. *Indian J. Genet*, 17(2), 318-328.
- Paramathma, M. (1992). *Studies On Genetic Inheritance In Interspecific Crosses Of Eucalyptus* (Doctoral dissertation, Tamil Nadu Agricultural University, Coimbatore).
- Paramathma M. (2020). Genetic interaction of physiological traits in *Eucalyptus* species. *Journal of Innovative Agriculture*, 7(1), 5-8.
- M. Paramathma, P. Jayamani, K.T. Parthiban, & M. Kiruba. (2020). Conservation and management in genetic resources of biofuel crops. *Journal of Innovative Agriculture*, 7(2), 1-4.
- Paramathma M, Reeja S, Parthiban KT, Malarvizhi D. Development of interspecific hybrids in *Jatropha*. *Biodiesel Conference Towards Energy Independence-Focus on Jatropha*. 2006b; 136-142.
- Paramathma M, Venkatachalam P, Sampathrajan A, Vairavan K, Jude Sudhagar R, Parthiban KT, Subramanian P, Kulanthaisamy S. Cultivation of *Jatropha* and Biodiesel Production.; Centre of excellence in Biofuels, Tamil Nadu Agricultural University, Coimbatore, 2006a.
- Paramathma M, Venkatachalam P, Sampathrajan A. *Jatropha* improvement, management and production of biodiesel. 2007. Pub: Centre of Excellence in Biofuels, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. 170.
- Paramathma, M., Parthiban, K.T, Neelakantan, K.S.. 2004. *Jatropha curcas*, Bharat press, Mettupalayam, Tamil Nadu, 1-45.
- Paroda, R.S., Dangi, O.P. & Grewal, R.P.S. (1975). Correlation and path analysis in forage sorghum. *Sorghum Newsletter*, 27: 16-17.
- Parthiban, K. T., Kirubashankkar, R., Paramathma, M., Subbulakshmi, V., Thiyagarajan, P., Vennila, S., ... & Durairasu, P. (2011). Genetic association studies among growth attributes of *Jatropha* hybrid genetic resources. *International Journal of plant breeding and Genetics*, 5(2), 159-167.
- Patil, B. R., Rudraradhya, M., Vijayakumar, C. H. M., Basappa, H., & Kulkarni, R. S. (1996). Correlation and path analysis in sunflower. *Journal of oilseeds research*, 13, 162-166.
- Prabakaran, A. J., & Sujatha, M. (1999). *Jatropha tanjorensis* Ellis & Soroja, a natural interspecific hybrid occurring in Tamil Nadu, India. *Genetic Resources and Crop Evolution*, 46(3), 213-218.
- Prakash, A. R., Patolia, J. S., Chikara, J., & Boricha, G. N. (2007, March). Floral biology and flowering behaviour of *Jatropha curcas*. In *Expert seminar on Jatropha curcas L. Agronomy and genetics* (pp. 26-28).
- Prasanthi, L., Maheswara, P., Reddy, P. S., Sudhakar, B., Babu, B., & Reddy, R. (2009). Genetic variability and path analysis for yield and yield attributes in *Jatropha* (*Jatropha curcas* L.). *Journal of Oilseeds Research*, 26, 230-233.
- Qing Y, Ping PD, Biao DZ, Liang WZ, Xiang SQ, Study on pollination biology of *Jatropha curcas* (Euphorbiaceae). *J South China Agricult Univ* 2007; 28(3): 62-6.
- Raju, A. S., & Ezradanam, V. (2002). Pollination ecology and fruiting behaviour in a monoecious species, *Jatropha curcas* L. (Euphorbiaceae). *Current science*, 1395-1398.
- Ram, S. G., Parthiban, K. T., Kumar, R. S., Thiruvengadam, V., & Paramathma, M. (2008). Genetic diversity among *Jatropha* species as revealed by RAPD markers. *Genetic Resources and Crop Evolution*, 55(6), 803-809.
- Ramanujam, S., & Rai, B. (1963). Analysis of yield components in *Brassica campestris* var. yellow sarson. *Indian J. Genet*, 23, 121-143.

- Ramchandani, M., & Jolly, C. I. (1988). Pharmacognostical and phytochemical studies on *phyllanthus fraternus* webster and *Jatropha glandulifera* ROXB. *Indian J. of Pharm.I Sci.*, 50(5), 276.
- Ranade, S. A., Srivastava, A. P., Rana, T. S., Srivastava, J., & Tuli, R. (2008). Easy assessment of diversity in *Jatropha curcas* L. plants using two single-primer amplification reaction (SPAR) methods. *Biomass and Bioenergy*, 32(6), 533-540.
- Ranwah, B.R., Sinha, S.S., Shah, M.A., Lakshyadeep & Bochalya, T.C. (2009). *In situ* variability in Physic nut. *J. curcas L, J Oilseeds Res*, 26, 237-239.
- Rao, G. R., Korwar, G. R., Shanker, A. K., & Ramakrishna, Y. S. (2008). Genetic associations, variability and diversity in seed characters, growth, reproductive phenology and yield in *Jatropha curcas* (L.) accessions. *Trees*, 22(5), 697-709.
- Rao, G.R. (2009). Genetic associations and variability in seed characters and growth of *Jatropha curcas* L accessions. *J Oil seeds Res*, 26, 242-245
- Reddy, M. P., Chikara, J., Patolia, J. S., & Ghosh, A. (2007, March). Genetic improvement of *Jatropha curcas* adaptability and oil yield. In *Expert seminar on Jatropha curcas L. Agronomy and genetics* (pp. 26-28).
- Rieger, R., Michaelis, A., & Green, M. M. (2012). *Glossary of genetics and cytogenetics: classical and molecular*. Springer Science & Business Media.
- Rupert, E. A., Dehgan, B., & Webster, G. L. (1970). Experimental Studies of Relationships in the Genus *Jatropha*. *IJ curcas x integerrima. Bulletin of the Torrey Botanical Club*, 321-325.
- Sasikala, R., & Paramathma, M. (2010). Ploidy Level and Basic Chromosome Number in Some Species of *Jatropha* L. *International Journal of Agriculture, Environment and Biotechnology*, 3(1), 67-70.
- Sekar, I. (2003). *Seed storage, seed source variations, molecular characterization and in vitro propagation in Simarouba glauca* DC (Doctoral dissertation, Ph. D. Thesis, Tamil Nadu Agricultural University, Coimbatore, India).
- Shaw, H. A. (1972). The Euphorbiaceae of Siam. *Kew Bulletin*, 191-363.
- Singh, R. P. (1970). Structure and development of seeds in Euphorbiaceae: *Jatropha* species. *Beitrag zur Biologie der Pflanzen*.
- Soller, M., & Beckmann, J. S. (1983). Genetic polymorphism in varietal identification and genetic improvement. *Theoretical and Applied Genetics*, 67(1), 25-33.
- Soontornchainaksaeng, P., & Jenjittikul, T. (2003). Karyology of *Jatropha* (Euphorbiaceae) in Thailand. *Thai Forest Bulletin (Botany)*, (31), 105-112.
- Sujatha, M. (1996). Genetic and tissue culture studies studies in castor (*Ricinus communis* L.) and related genera. Osmania University, Hyderabad, India, 295.
- Sujatha, M. & Prabakaran, A.J. (1997). Characterization and utilization of Indian *Jatropha*. *Indian J PI Genet Resources*, 10(1), 123-128.
- Sujatha, M., & Prabakaran, A. J. (2003). New ornamental *Jatropha* hybrids through interspecific hybridization. *Genetic Resources and Crop Evolution*, 50(1), 75-82.
- Sujatha, M., & Prabakaran, A. J. (2003). New ornamental *Jatropha* hybrids through interspecific hybridization. *Genetic Resources and Crop Evolution*, 50(1), 75-82.
- Sun, Q. B., Li, L. F., Li, Y., Wu, G. J., & Ge, X. J. (2008). SSR and AFLP markers reveal low genetic diversity in the biofuel plant *Jatropha curcas* in China. *Crop Science*, 48(5), 1865-1871.
- Sunil, N., Varaprasad, K. S., Sivaraj, N., Kumar, T. S., Abraham, B., & Prasad, R. B. N. (2008). Assessing *Jatropha curcas* L. germplasm in situ—a case study. *Biomass and bioenergy*, 32(3), 198-202.
- Tanaka, Z. (1882). A variation of pine. *Bull. Japanese For Assoc.*, 7, 251-252.
- Tanksley, S. D., Young, N. D., Paterson, A. H., & Bonierbale, M. W. (1989). RFLP mapping in plant breeding: new tools for an old science. *Bio/technology*, 7(3), 257-264.
- Tewari, D.N. (2007). *Jatropha and biodiesel*. 1st ed. New Delhi: Ocean books Ltd., p. 228.
- Tewari, S.K. & Singhania, D.L. (1984). Character association and path analysis in grain sorghum. *Sorghum Newsletter*, 27: 16-17.
- Wani, S. P., Osman, M., D'Silva, E., & Sreedevi, T. K. (2006). Improved livelihoods and environmental protection through biodiesel plantations in Asia. *Asian Biotechnology Development Review*, 8(2), 11-29.
- Wen, M., Wang, H., Xia, Z., Zou, M., Lu, C., & Wang, W. (2010). Development of EST-SSR and genomic-SSR markers to assess genetic diversity in *Jatropha Curcas* L. *BMC research notes*, 3(1), 1-8.
- Winter, P., & Kahl, G. (1995). Molecular marker technologies for plant improvement. *World Journal of Microbiology and Biotechnology*, 11(4), 438-448.
- Wright, S. (1921). Correlation and causation. *J. agric. Res.*, 20, 557-580.
- Yang, Q., Peng, D., Duan, Z., Wang, Z., & Sun, Q. (2007). Study on pollination biology of *Jatropha curcas* (Euphorbiaceae). *Journal of South China Agricultural University*, 28(3), 62-66.
- Zobel, B. J. (1971). The genetic improvement of southern pines. *Scientific American*, 225(5), 94-103.
- Zobel, B. J., & Van Buijtenen, J. P. (2012). *Wood variation: its causes and control*. Springer Science & Business Media.
- Zobel, B., & Talbert, J. (1984). *Applied forest tree improvement*. John Wiley & Sons.