# Introduction is a perennial shrub or a

Nutrition

IntroductionToday’srising demand for fuels has diverted the attention towards biofuels due togradual exhaustion of fossil fuels and increased pollution, causing globalwarming. Thus, an alternate source of energy is needed which is sustainable andeco-friendly. Biofuel such as bioethanol and biodiesel has already been used in addition to the fossilfueland have advantage in terms of renewability and environment friendly. Biodiesel form plants and algae are being considered as most promising sourcesof biofuels. 1.

Biodieselfrom plants, is an excellent substitute for fossil fuels as it is non-toxic, biodegradable and emits lower amount of carbon monoxides and hydrocarbons thanpetro-diesel. An important component of plant seed oils is triacylglycerolswhich are highly similar to fossil fuels thus, an excellent precursor forproducing biodiesel. Transesterification of triacylglycerols in plantseed oils with methanol in presence of an alkali or acid resulting in formationof biodiesel chemically known as fatty acid methyl esters (FAMEs)2. The efficiency of biodieseldepends upon the composition of fatty acids blend in the seed oil. Five typesof fatty acids are present in plant oils which are Palmitic acid (16: 0), stearic acid (18: 0), oleic acid (18: 1), linoleic acid (18: 2) and linolenic acid(18: 3). Earlier, edible crop plants were used for biodiesel production whichcaused the scarcity for overall food supply and agricultural lands. Thus, forsustainable biodiesel production, non-edible crops have gained importance dueto non-competition with food security and soil with food crops. Ideal biodieselcrop can be grown in wastelands thus no competition for agricultural land for foodcrops 3.

Ofmany energy plants, Jatropha (Jatrophacurcas L) has emerged as a potential bioenergy plant due to its high seedoil content (45-50%). Jatropha is a perennial shrub or a small tree whichbelongs to family euphorbiaceae. It can grow upto a height 6 m and have 40-60 years of lifeexpectancy. Oil can be extracted from Jatropha seeds after 2-5 years dependingupon the climatic conditions. Jatropha is a monoecious plant i.

e. male and female flowers grow on the same plant. Flowers are unisexual orhermaphrodite and are pollinated by moths and bees (Raju and Ezradanam, 2002; Dehgan and Schutzman, 1994). Morphologicallydiverse genus Jatropha comprised of more than 200 species which are dispersed primarilyin dry tropical areas of America. Jatropha, primarily originating from CentralAmerica, has been recently introduced into many tropical and subtropicalcountries in Asia and Africa. Now Jatropha is cultivated globally as abiodiesel crop (Akbar et al. 2009).

Itis introduced in India in 16th century by Portuguese settlers. About18 species of Jatropha are found in India and are scattered in various statesof the country (Ginwal et al 2005). Jatropha can easily grow in extreme conditionssuch as in tropical savannah and monsoon climates, temperate and semi-aridclimates without any requirement of special nutritive regime (Maes et al. 2009). Other factor for Jatropha oil popularity is the higher content ofunsaturated fatty acids and high oil content (50%) and a non-edible crop, thusno competition with food security (Table 1. 1). Jatropha is listed as a fuel andfuel additive with the world environmental protection agency (WEPA) 7. Jatrophagained prominence over other oil seed plants because of its added features likeexcellent adaptability to various habitats, rapid growth, easy propagation, wide adaptability, larger fruits and seeds, drought hardiness, soilconservation capabilities, small gestation period, thriving well as live fenceand can easily be grown in wastelands.

Jatropha seeds are toxic due to the presence of phorbolesters and curcin. Even though it's a potent biodiesel crop and toxic innature, it has a medicinal value. Most of the parts of Jatropha is used asindustrial raw material for making insecticides, soaps, cosmetics etc and asource of green manure (Gubitz et al., 1998; Lin et al., 2003).

Though numerous efforts havebeen made to develop Jatrophaas an industrial crop, the scant information onits agronomic practices and lack of improved genotypes and cultivars are themajor bottlenecks in its full exploitation as a potential bioenergy crop Taxonomicclassification of Jatropha curcas L. Kingdom Plantae Subkingdom Tracheobionta Division Magnoliophyta Sub division Spermatophytina Class Magnoliopsida Subclass Rosidae Order Euphorbiales Family Euphorbiaceae Genus Jatropha L. Species Jatropha curcas L. Table1. 1 Fattyacid composition and oil content of major oil plants Fatty acids (%) Jatropha Castor bean Sunflower Soybean Palmitic acid 10 3 10 10 Stearic acid 10 2 5 5 Oleic acid 45 10 30 35 Linoleic acid 35 10 50 45 Linolenic acid 1 – 5 5 Ricinoleic acid – 75 – – Total oil content (%) 25-50 40-45 25-35 20-25 Jatrophaisan economically important plant to produce good quality biodiesel. Due tovarious constraints like low seed yield, unreliable flowering and fruiting, non-availability of sufficient feedstock, limited availability of wasteland, high plantation maintenance cost and susceptibility to biotic and abioticstresses limits its commercialization of this plant as a source of biodiesel. Jatrophafeedstock is highly affected by seed oil content, number of branches per plant, number of bunches per branch, number of fruits per bunch, number of seeds perfruit and seed weight/size etc.

The seed yield of Jatropha majorly depends onnumber of female flowers per inflorescence.  At each inflorescence, 10-12 female flowersare formed out of ~300 present at each inflorescence. This results in only 8-10ovoid fruits which is quite low when compared to the total number of flowerspresent at the inflorescence. Thus, increasing the female flower number bygenetic intervention can be targeted to increase the overall yield of Jatropha. Floraldevelopment of Jatropha is a complex process where female flower is present onthe top whereas sub-branches may produce either female or male 2. Study on floral development showed nosexual differentiation till the sixth phase.

When sexual differentiation occurs, the top of female elongates whereas no such development occurs in male flowers. Female flowers are present in a bisexual stage till sixth phase of development. As sexual differentiation begins, abortion of male occurs in female flowers andthere traces aborted stamens could be found in mature females. However, maleflower development is unisexual right from the beginning and no traces of femalesare present. When abortion of male tissues does not occur in female flowersthen they develop as males at the female flowering site. Such inflorescence iscalled as middle type inflorescence with either female/male flowers at aninflorescence.

These middle type infloresncnces showed variation in total numberof female flowers at each inflorescence. Thus, these might play an importantrole in increasing the female to male flower ratio. Apart from male/females, hermaphroditic flowers were also reported in Jatropha 2, 4-5. They are similar to female flowers in structure but have8-10 stamens like those in male flowers.

(Lourdes 2016). Manystudies have been done to understand the molecular factors for female floweringby various approaches like genomics, transcriptomics and bioinformatics. Transcriptomeprofiling and microarray analysis of Jatropha inflorescences identifiedflowering genes and meristem identity genes however, information on molecularmechanism of sex determination in very limited. Even though transcriptionanalysis of staminate and instaminate flowers of Jatropha was done. Theirresults did shed light on role of hormones in development of floral organsduring their different developmentals stages. However, genetic factors i.

e. sexrelated genes which causes differentiation of male and female organs were notstudied. Through these studies it was found that flowering genes such as CUC1 GI, LFY, and SOC1Iwere found to activate floweringsignals ( ). GASA4, CLV1 and AMP-activated protein kinase were identified for their rolein stamen differentiation. A recentstudy on sex differentiation of Jatropha identified that cytokinins activates the formation of female floral primordia. After this phenomenon other phytohormones such as BR signaling, JA signaling, ABA signaling and GA signaling promotes the female floral development alongwith ABCDE genes. Thus, identification of key genes associated with sexdetermination and abortion of male tissues which can be targeted for geneticengineering in Jatropha for enhanced yield. Increase in number of flowers (females) withsubsequent increase in number of fruits have been achieved by exogenousapplication of growth hormones such as cytokinins, brassinosteroids andgibberellic acids, etc.

(Pan et al., 2011; Gayakavad et al., 2014). Of all thephytohormones, Cytokinins have proven to be the most promising growth regulatorfor improving the number of female flowers and seed yield.

Benzyl adenine (BA)and thidiazuron treatment resulted in a drastic increase in number of flowersand female flowers along with induced bisexual flowers in Jatropha (Pan et al. 2011; Pan et al. 2016).

However, the fruiting rate was relatively low i. e. 2-3 folds as comparedto increased female flowers with upto 9-10 folds. Also, there was a reductionin 100 seed weight in fruits formed after cytokinin treatment (Pan et al.

2011; Chen et al. 2014). This reduction in seed weight led to compromised seed yield evenafter the application of phytohormones such as cytokinins. Apart from Jatropha, reduction in seed yield after cytokinin application has also been reported inother plant species such as soybean, jojoba and lupin (Ma et al. 1998; Nagel et al. 2001; Prat et al. 2008). Differentialtranscriptional profiling of Jatropha inflorescence after cytokinin treatment, revealed the molecular cues for increased flower number.

However, noinformation exists on molecular signals associated with compromised seed yieldfollowing exogenous hormonal application in Jatropha. We hypothesize that the compromised seed yield could be aconsequence of the inability of the photosynthetic source to fully support sinki. e. seed development in spite of having significantly increased number offlowers.

The reduction in final seed formation is also due toabortion of floral buds in cytokinin treated plants at later stages ofdevelopment because all female flowers do not transit to formation of fruitsdue to higher flower abortion rate, which may be due to non-fulfillment ofincreased requirement of sink (Pan et al. 2016; Yashima et al. 2005). Limitedsupply of photo assimilates to increased flowers is due to increasedcompetition among leaves, stems, nodules and reproductive organs which might bethe possible reasons for flower abortion (Brun and Betts 1984; Antos andWiebold 1984). The negative correlation between fruiting rate and seedformation with the number of female and bisexual flowers per inflorescence onplants treated with cytokinins, may be a result of either the shortage ofphotosynthetic products or reduced source to sink strength (Pan et al. 2011; Pan et al.

2016). To understand the molecular cues of sexdifferentiation as well as female flower transitions affecting the overallratio of female to male flowers were identified through comparative genomics. Toidentify genes associated with high female flowering, expression analysis of 42floral genes known to be associated with sex differentiation in other plantspecies were identified in Jatropha genome. Their expression analysis wascarried out at different floral developmental stages in high female to maleratio accession (IC561235) of Jatropha. Theratio of female to male flowers may vary wth respect to season, climate, andnutrition among different genotypes in Jatropha 2. Thus, by studying the expressionstatus of key genes in Jatropha genotypes with low female flower ratio growing insame environmental conditions as the high ratio genotype would reflect theinherent genetic differences for higher female flowering.

Tounravel transcriptional regulation, the promoter regions of key genes showing associationwith female flowering were analyzed to identify the regulatory elements. Furthermore, to understand molecular mechanisms underlying the compromised seed yield aftercytokinin treatment, transcriptome analysis was done. Transcriptomes ofJatropha inflorescence meristems treated with Benzyl adenine (BA) at different time intervalswas performed, following reference based genome mapping approach.  This provided the molecular insights on howcytokinin treatment affects carbon fixation, carbon availability and nitrogenmetabolism thereby, altering C/N ratio, which might be affecting biomassproduction, thus fruit/seed yield in Jatropha. This analysis provided the repertoire of genes associated withcarbon fixation and flux in response to cytokinin treatment after fifteen daysand then decreased after thirty days of cytokinin treatment.  Keepingin view, the lack of information on genetic factors contributing to differencesin oil content among oil contrasting genotypes and our partial knowledgetowards understanding of molecular mechanisms associated with disease responseand disease resistance, the present study was carried out with followingobjectives: 1) Relative expression of FA and TAGbiosynthesis pathway genes in high versus low oil content genotypes of Jatropha curcas 2) Deciphering molecular components of aviral disease response in Jatropha curcas