Jatropha Biodiesel Source

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Environmental pollution is one of the most pressing challenges in today's world. The main cause of this pollution is fuel emissions from automobiles and other sources. As industrialization progresses, people will be unable to compromise on the use of energy to power heavy machines and will be forced to seek out the best options. As a consequence, utilizing green fuel, such as biodiesel derived from natural sources, is a realistic option. *Jatropha curcas* L. (Euphorbiaceae) is recognized as the greatest feedstock for biodiesel production throughout the world, and it has gained a huge market value in the recent years. Conventional cultivation alone will not be sufficient to meet the global need for the plant's biomass for the production of biodiesel. Adoption of plant tissue culture techniques that improve the biomass availability is an immediate need.

Jatropha curcas | biodiesel | natural resource | micropropagation | plant genetic transformation

1. Introduction

Jatropha curcas L. is a non-edible, oil-bearing, and zero waste perennial shrub or small tree belonging to the Euphorbiaceae family. The plant is commonly known by several names, such as Barbados nut, termite plant, fig nut, black vomit nut, curcas bean, physic nut, and purge nut. The plant grows well in tropical and sub-tropical climates with a variety of medicinal properties in its oil [1]. Its rapid growth, hardness, and easy propagation make it grow under a wide range of rainfall regimes and harsh climatic conditions; thus, this species has spread far beyond its original distribution. The height of the plant can range from three to five meters, and under favorable conditions it can grow up to 10 m. The plant exhibits articulated growth, a straight trunk, and thick greenish-bronze-colored branches with a soft wood [2]. The plant has a well-established taproot system with four shallow lateral roots. This type of root system aids in the prevention of soil erosion [3]. The leaves are 10–15 cm long, five-lobed and heart-shaped, with simple, smooth margins that are cordate at the base and acute at the apex [4][5]. Jatropha is a monoecious plant with male and female flower ratio of approximately 29:1 [6]. The flowers are yellowish-green, arranged in axillary clusters. The inflorescence is complex, with the main and co-fluorescence formed terminally on branches. The fruits are 3–4 cm long, ellipsoidal, and trilocular. Each fruit contains three black, oblong, large seeds [1][5] weighing approximately 0.53 to 0.86 g. The seed kernel is composed of 57–63% of lipids and 22–27% of protein [1]. Approximately 4–40% of the viscous curcas oil can be extracted from its seeds [7]. The life span of the plant is found to be about 50 years [2].

Oil has played an important role in recent advancements and economic development, as it is the most useful source of essential energy. It plays a key role in the progress of industry, agriculture, and transportation. With the rise in population, the need for petroleum products has increased, and thus the production rate has elevated, leading to the depletion of the world petroleum reserves and heightened environmental concerns [8]. The search for alternate sources for petroleum-based fuel has been triggered due to these reasons. Usage of biodiesel as an alternative form of energy has increased recently. According to Energy Information Administration (EIA) reports, the US consumed 43 million barrels of biodiesel, which is expected to increase in the coming decades (https://www.eia.gov/, accessed on 13 March 2022). Biodiesel based on biomass is one of the most appealing strategies, in which fatty acid methyl esters from vegetable oil can be considered to be the best substitute for diesel [9]. Biodiesel extracted from Jatropha, a non-edible vegetable oil source, is a cheap and also the best renewable alternative to conventional diesel. By 2030, it is expected that Mexico alone will be equipped to produce 255.75 metric tons of jatropha biomass for biodiesel production (https://www.statista.com/, accessed on 13 March 2022).

The oil obtained from *Jatropha curcas* was used as lamp oil and in the production of soap for centuries in Portugal [10]. The oil contains approximately 97.6% neutral lipids, 0.95% glycolipids, and 1.45% phospholipids. The unsaturated fatty acids are predominantly higher than saturated fatty acids. This oil can also be used as a substitute for fossil fuel, as it has 41.5 to 48.8% of oleic acid, 34.6 to 44.4% of linoleic acid, 10.5 to 13% of palmitic acid, 2.3 to 2.8% of stearic acid, along with cis-11-eicosadienoic and cis-11,14-eicosadienoic acids as the main fatty acids. This oil, when used as fossil fuel, could also help to decrease the emissions of greenhouse gases [11]. Apart from its use as fossil fuel, this oil is also used in the manufacture of candles, soaps, and cosmetics. After the extraction of oil from the seeds, the seed cake can be detoxified and can be used as animal feed, as it is highly nutritious and can supplement protein [12]. The by-products after the oil extraction can also be used for the production of cellulosic methanol [13].

Along with their economic value, jatropha species are also regarded as rich sources of phytochemicals such as terpenes, cyclic peptides, alkaloids, and lignans. Diterpenes (phorbol esters, dinorditerpenes, deoxypreussomerins, and pimarane, lathyrane and rhamnofolane); sesquiterpenoids and triterpenes (taraxasterol, β-amyrin, and β-sitosterol, (Z)-3-O-coumaroyloleanolic, stigmasterol and daucasterol, friedelin); alkaloids (pyrrolidine (5-hydroxypyrrolidin-2-one) and pyrimidine-2,4-dione (uracil), imidazole, diamide (curcamide)); flavonoids (flavonoid glycoside I and flavonoid glycoside II, nobiletin, tomentin); phenolics (3-hydroxy-4-methoxybenzaldehyde and 3-methoxy-4-hydroxybenzoate acid, caffeoylaldehyde and syringaldehyde); lignans, neolignans, coumarins, coumarino-lignoids, and phytosterols are some of the most studied phytochemicals of the plant [2]. There are many medicinal and pharmacological effects exhibited by the plant due to the presence of these varieties of phytochemicals. Some of the popular and well-studied pharmacological activities include anti-inflammatory effects, antimicrobial properties, anti-oxidant, anti-cancer, antiviral, anti-diabetic, analgesic activity, hepatoprotective activity, wound healing activity, anticoagulant, and procoagulant activity [2].

Jatropha can be sexually propagated via seeds and vegetatively propagated through stem cuttings. Stem cuttings are usually preferred over seeds for propagation. In nurseries, cuttings are prepared from one-year-old terminal branches inoculated with mycorrhizal fungi to improve symbiosis in field conditions. Under tropical, humid

conditions, fruiting lasts for four months per year and can be harvested thrice. Some of the major factors that affect seed production and oil yield are reduced branching, low female flower count, inadequate pollination, and poor soil quality. [1]. Vegetative propagation of jatropha is reported to show a low seed set. It was also observed that vegetative propagation cannot form deep-rooted plants that can be easily uprooted [7]. Thus, numerous studies on tissue culture of jatropha, along with genetic manipulation, have been extensively done due to their many beneficial applications. The present entry focuses on the tissue culture aspects for in-vitro propagation of *J. curcas* through direct and indirect organogenesis and somatic embryogenesis.

2. Genetic Transformation Studies of *Jatropha curcas* L.

Plant genetic transformation is a widely used tool for the generation of transgenic plants with a required specific trait. Gene transformation permits the introduction of a useful gene from one organism into another, with the subsequent stable integration and expression of the introduced foreign gene. This plays a significant role in plant breeding programs by producing novel genetically diverse plant materials. The transformation or gene delivery methods include: electroporation, lipofection, microinjection, sonication, particle bombardment, silicon carbide mediate transformation, laser beam mediated transformation, agrobacterium-mediated method, and virus-based methods [14]. *Agrobacterium* mediated transformation is widely used and is preferred over other methods due to its simplicity, cost-effectiveness, lesser rearrangements of the transgene, and most importantly, its ability to transfer relatively larger DNA segments and integration of foreign genes into transcriptionally active regions [15].

Li et al., (2008) was the first to perform agrobacterium-mediated transformation using cotyledonary disc as explant. The transformation was performed using the LBA4404 strain, and phosphinothricin was used for selection. They observed that approximately 55% of the cotyledonary explants produced phosphinothricin-resistant callus on the MS medium. The transformants were detected by β-glucuronidase activity and confirmed using PCR and southern hybridization analysis. Of the total inoculated explants, 13% were found to produce transgenic plants after four months [16]. Kumar et al., (2010) studied various factors that would influence agrobacterium-mediated transformation of *J. curcas* using leaf explants. The LBA4404 strain of agrobacterium harbouring binary vector pCAMBIA 1304 with sense-dehydration responsive element binding (S-DREB2A), β-glucuronidase (gus), and hygromycin-phosphotransferase (hpt) genes were transformed into jatropha. The highest stable transformation efficiency of 29% was achieved when four- day precultured, non-wounded explants were infected with the agrobacterium culture and co-cultivation with acetosyringone on MS medium. The transformation was confirmed using GUS histochemical analysis. The presence of the transgene was confirmed using PCR and DNA gel blot hybridization [17].

As discussed earlier, development of elite germplasm is a major goal of gene transformation studies. Salinity can impact the growth and yield of jatropha. Jha et al., (2013) developed transgenic jatropha plants with the SbNHX1 gene (*Salicornia brachiate* vacuolar Na+/H+ antiporter gene) using microprojectile bombardment mediated transformation. They confirmed the transgene integration by PCR and RT-PCR methods. Real-time qPCR was used to determine the copy number. The developed transgenic lines were reported to show salt tolerance up to 200 mM NaCl, which was better in comparison with the wild type [18]. The plant breeding program has significantly

enhanced agricultural productivity through the development of high-yielding crop varieties. Many traits could be targeted for the improvement of *J. curcas*, such as increasing the flower and fruit production, seed quality (size, oil content, and oil component), etc. [19][20]. The genome size of *J. curcas* is relatively small and is organized within 22 chromosomes (2n) [21]. Ha et al. reported that the whole genome size of *J. curcas* using PacBio and Illumina platforms was approximately 339 Mbps [22]. The smaller genome size of *J. curcas* has many advantages, including easy genetic transformation and short generation duration. Jatropha has become one of the most attractive model plants for wood energy and genome analysis among the family Euphorbiaceae [21].

The effect of endogenous cytokinins treatment on the flower development in *J. curcas* was studied by Ming et al., (2020) through transgenic expression of cytokinin biosynthetic gene AtIPT4 under the control of JcTM6 (*J. curcas* tomato mads box gene 6) promoter that is mostly active in flowers. They found an increase in the number of flowers in a single inflorescence, but both the male and the bisexual flowers were infertile due to the continuous expression of the transgene. The transformation was performed using *A. tumefaciens* EHA105 [23].

Transgenic jatropha producing enlarged seeds were successfully developed by transformation methods. Chacuttayapong et al., (2021) transformed jatropha using genes for the larger seed size found via the rice FOX-hunting system, identified as the genes LOC_Os03g49180 (Os03), LOC_Os04g43210 (Os04), LOC_Os08g41910 (Os08), and LOC_Os10g40934 (Os10). Rice FOX-hunting system was established by the introduction of rice full-length cDNA into Arabidopsis plants by *A. tumefaciens* mediated transformation. The LOC_Os03g49180 gene encodes for ceramidase enzyme that hydrolyses ceramide into sphingosine and fatty acids. Sphingolipids are reported to be important in the kernel development of sunflower seeds. In the study conducted by Chacuttayapong et al., (2021), two types of overexpressing constructs were developed using LOC_Os10g40934.3 and LOC_Os10g40934.11. Transgenic jatropha was produced from excised shoots by using auxins for promoting root formation (kept under dark) and delaying the timing of antibiotic selection in cultivation media [20].

The main component of the jatropha seed storage oil is triacylglycerol (TAG). TAGs contain C16 or C18 fatty acid chains that are covalently linked to glycerol. This makes TAGs a high energy source for seed germination, seedling growth, and development. TAGs are synthesized through the Kennedy pathway. Diacylglycerol acyltransferase (DGAT) and phospholipid: diacylglycerol acyltransferase (PDAT) are the key enzymes involved in TAG biosynthesis in Arabidopsis. TAG biosynthesis could be upregulated by overexpression of the DGAT1 gene [22][24]. Maravi et al., (2016) developed transgenic jatropha by ectopically expressing *Arabidopsis DGAT1* gene (*AtDGAT1*) via *Agrobacterium*-mediated transformation, and it was found to have increased oil content (TAG and Diacylglycerols (DAG)) by 20–30% in seeds and 1.5 to 2.0-fold increase in leaves. They also observed an increase in the plant height, seeds per tree, seed length and breadth, and average seed weight of the transgenic plant in comparison with the wild type [24]. Gene expression and homology modelling studies revealed that PDAT homolog Jatcu.04g000545 has higher expression levels at all stages than DGAT homolog Jatcu.04g000511, indicating that TAG biosynthesis in jatropha is mainly catalyzed by PDAT [72[p]. Studies carried out by Arockiasamy et al., (2021) made information available for both phenotype and genotype of jatropha, assisting in identification of quantitative trait locus (QTLs). They also established a genetic transformation approach using cotyledonary leaves, with a

transformation rate of 10–12% and molecular characterization of 70 transgenic events confirming the incorporation of the kanamycin selection marker gene. [25].

3. Haploid and Double Haploid Production of Jatropha

The use of gametes containing haploid chromosome number (n) for the development of the entire plantlet results in the production of haploid plants. Haploid production is essential for the generation of hybrids with high yield and oil along with disease resistance. Haploid production aids in the development of genetically homozygous plants from heterozygous parent plants and, in return, can serve as parents in crossbreeding. Gametic embryogenesis can be done to produce homozygous lines within a short duration when compared to conventional breeding methods that involve the selfing of several generations. Culturing of male gametophyte (androgenesis) and female gametophyte (gynogenesis) results in haploid embryo development. Double haploids can be generated by genome duplication either through spontaneous duplication or by using microtubule depolymerizing agents, such as colchicine and trifluralin [26].

The first successful anther culture of jatropha was reported by Madan et al., (2019). They induced callus from anthers of immature buds of jatropha. The anthers cultured on MS medium supplemented with 1.0 mg/L BA and picloram induced 77% callus. The obtained callus showed regeneration of plants on medium supplemented with 2.0 mg/L BA, 0.5 mg/L KN, and 0.5 mg/L NAA. Approximately 90% of the elongated shoots showed rooting on half-strength MS medium with 2.0 mg/L IBA. All the in-vitro plants derived from anther showed 100% success in primary hardening (grown under greenhouse conditions) and 85% in secondary hardening (field conditions). The embryogenic callus analyzed using molecular and flow cytometry showed that 5.7% of plantlets were haploid and 3% of the plantlets were double haploids [26].

For the development of haploid and double haploid plants, microspore culture is most preferred, especially in jatropha, as it contains more male flowers than female flowers. Shrivastava et al., (2021) reported microspore gametic embryogenesis for the first time in jatropha. They observed that when tetrads, early, mid-un-vacuolated, and vacuolated late-stage uninucleate microspores inoculated on modified MS medium supplemented with 2.0 mg/L 2,4-D, 0.1 mg/L KN, 300 mg/L casein hydrolysate, 1.0 g/L glutamine, 0.5 mg/L folic acid, 0.05 mg/L biotin, and 5% sucrose resulted in induction and formation of embryo-like structures (ELS). The cultures were incubated at 4 °C for seven days followed by incubation at 25 °C for 15 days and then under 15 °C for 10 days. The different developmental stages of microspore embryogenesis were confirmed by microscopic analysis. The established calli and ELSs were verified to be haploid by flow cytometric analysis [27]. Although the number of female flowers in jatropha is low, Lopez-Puc et al., (2021) developed homozygous lines of *J. curcas* by gynogenesis. They established a protocol for the development of in-vitro plants from unfertilized ovules of *J. curcas*. They reported that green friable gynogenic calli developed on MS medium supplemented with 6.66 µM BAP and 4.9 µM IBA when transferred to MS medium with 22.09 µM BAP and 3.40 µM paclobutrazol (PBZ) resulted in the formation of gynogenic embryo. These generated embryos were cultured on MS medium containing 2.22 µM BAP and 0.28 µM IAA for the shoot development. Root development occurred on half-strength MS medium supplemented with 18.65 µM IBA [28].

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