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REVIEW: PLANT TISSUE CULTURE OF *JATROPHA CURCAS* L.

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Abstract

Plant tissue culture is the culture of plant cells or tissues in a synthetic culture medium under controlled aseptic conditions to produce millions of identical plants. Now it has direct commercial applications as well as value in basic research into cell biology, genetics and biochemistry. This review paper outlines the work done on *Jatropha curcas* L. family Euphorbiaceae. *Jatropha curcas* L. a multipurpose, drought resistant, perennial plant belonging to Euphorbiaceae family is gaining lot of importance for the production of biodiesel. It is a tropical plant that can be grown in low to high rainfall areas either in the farms as a commercial crop or on the boundaries as a hedge to protect fields from grazing animals and to prevent erosion. Before exploiting any plant for industrial application, it is imperative to have complete information about its biology, chemistry, and all other applications so that the potential of plant could be utilized maximally. The taxonomy, botanical description of the plant, its distribution and ecological requirement are discussed in this paper. Various propagation methods including tissue culture to get large diseased resistant plantlets of *Jatropha* are reviewed.

Keywords: *Jatropha curcas*, Euphorbiaceae, Taxonomy, Propagation.

1. Introduction

1.1 Introduction to plant tissue culture

Tissue culture is the culture and maintenance of plant cells or organs in sterile, nutritionally and environmentally supportive conditions (*in vitro*). It has applications in research and commerce. In commercial settings, tissue culture

is primarily used for plant propagation and is often referred to as Micropropagation.[1]Another way to define the Plant tissue culture is the culture of plant cells or plant tissues in a synthetic culture medium under controlled aseptic conditions is called "Tissue culture". The controlled conditions give the culture a suitable microenvironment for the successful growth.[2]

Plant tissue culture now has direct commercial applications as well as value in basic research into cell biology, genetics and biochemistry. The techniques include culture of cells, anthers, ovules and embryos, protoplast isolation and fusion, cell selection, merited and bud culture on experimental to industrial scales. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation which include

1. The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits, to produce mature plants and multiples of plants in the absence of seeds or necessary pollinators to produce seeds.
2. Regeneration of whole plants from plant cells that have been genetically modified.
3. Production of plants from seeds that have very low chances of germinating and growing, i.e.: orchids and nepenthes. Other applications like germplasm maintenance, hybrid productions for incompatible species, production of haploid plants etc.[3,4]

There are different types of plant tissue culture: Callus culture, Cell-suspension culture,[5] Protoplast culture, *Explant culture,* Microspore culture**,** Embryo Culture ,Ovary Culture**,** Root cultures, Shoot tip and meristem culture, Anther and pollen culture,[6] Mass cell culture, Organ Culture,[7] Nucleus culture.[8] Media used to grow tissue culture mainly include the Murashige-skoog(MS) media, Orchid plant culture media, Micro biochemical media and Other plant culture media.[9]

1.1.1 Techniques for plant tissue culture

Modern plant tissue culture is performed under aseptic conditions under filtered air. Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilization of starting materials (explants) in chemical solutions (usually alcohol or bleach) is required.[10] Mercuric

chloride is seldom used as a plant sterilant today, as it is dangerous to use, and is difficult to dispose of. Explants are then usually placed on the surface of a solid culture medium, but are sometimes placed directly into a liquid medium, particularly when cell suspension cultures are desired. Solid and liquid media are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones. Solid media are prepared from liquid media with the addition of a gelling agent, usually purified agar. The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effects on the morphology of the tissues that grow from the initial explant. For example, an excess of auxin (Indoleacetic acid, napthaleneacetic acid) by stimulating cell expansion, particularly cell elongation promote the proliferation of roots, while an excess of cytokinin (zeatin, kinetin) may yield shoots by producing two immediate effects on undifferentiated cells: the stimulation of DNA synthesis and increased cell division.[11] A balance of both auxin and cytokinin will often produce an unorganised growth of cells, or callus, because both cell division and cell expansion occur in actively dividing tissue, but the morphology of the outgrowth will depend on the plant species as well as the medium composition. As cultures grow, pieces are typically sliced off and transferred to new media (sub-cultured) to allow for growth or to alter the morphology of the culture. As shoots emerge from a culture, they may be sliced off and rooted with auxin to produce plantlets which, when mature, can be transferred to potting soil for further growth in the greenhouse as normal plants.[12]

1.2 Introduction to plant

1.2.1 Botanical description of *Jatropha curcas* L

Jatropha curcas L. or physic nut, is a bush or small tree (up to 5 m height) and belongs to the Euphorbia family and contains approximately 170 known species. The genus name *Jatropha* derives from the Greek jatrós (doctor), trophé (food), which implies medicinal uses. *curcas* is the common name for physic nut in Malabar, India. The plant is planted as a hedge (living fence) by farmers all over the world, because it is not browsed by animals.[13] *Jatropha curcas* L., or physic nut, has thick glorious branchlets. The tree has a straight trunk and grey or reddish bark, masked by large white patches. It has green leaves with a length and width of 6 to 15 cm, with 5 to 7 shallow lobes. The leaves are arranged alternately. The branches contain whitish latex, which causes brown stains, which are very difficult to remove. Normally, five roots are formed from seeds: one tap root and others are lateral roots.[14] Plants from cuttings develop only lateral roots. Inflorescences are formed terminally on branches. The plant is monoecious and flowers are unisexual.[15] Pollination is by insects. After pollination, a trilocular ellipsoidal fruit is formed. The exocarp remains fleshy until the seeds are mature. The seeds are black and in the average 18 mm long and 10 mm wide ripe Jatropha fruits. The seed weight (per 1000) is about 727 gm.[16]

1.2.2 Distribution

Jatropha curcas L. originates from Central America. From the Caribbean, *Jatropha curcas* was probably distributed by Portuguese seafarers via the Cape Verde Islands and former Portuguese Guinea (now Guinea Bissau) to other countries in Africa and Asia. Today it is cultivated in almost all tropical and subtropical countries.[17-18]

1.2.3 Phytochemistry of *Jatropha curcas* L.

Chemicals isolated from different parts of plant.

Various parts	Chemical composition
Aerial parts	Organic acids (*o* and *p*-coumaric acid, *p*-OH-benzoic acid, protocatechuic acid, resorsilic acid), saponins and tannins[19]
Stembark	Amyrin, sitosterol and taraxerol[20]
Leaves	Cyclic triterpenes stigmasterol, stigmast-5-en-3, 7 diol, stigmast-5-en-3,7 diol, cholest-5-en-3,7 diol, campesterol, sitosterol, 7-keto--sitosterol as well as the d-glucoside of sitosterol. Flavonoids apigenin, vitexin, isovitexin. Leaves

	also contain the dimer of a triterpene alcohol (C63H117O9) and two flavonoidal glycosides[21,22]
Latex	Curcacycline A, a cyclic octapeptide Curcain (a protease)[23]
Seeds	Curcin, a lectin Phorbolesters Esterases (JEA) and Lipase (JEB)
Kernal and press cake	Phytates, saponins and a trypsine inhibitor[24]
Roots	Sitosterol and its d-glucoside, marmesin, propacin, the curculathyranes A and B and the curcusones A–D. diterpenoids jatrophol and jatropholone A and B, the coumarin tomentin, the coumarino-lignan jatrophin as well as taraxerol[25]

1.2.4 Uses of *Jatropha curcas* L. plant

Jatropha curcas L. is planted in the form of hedges around gardens or fields to protect the crops against roaming animals like cattle or goats and to reduce erosion caused by water and/or wind.[26]

The superior quality oil can be extracted from the seeds. The oil can be used as a mixed fuel for diesel/gasoline engines.[27,28]

The *Jatropha curcas* L. plant is used as a medicinal plant such as seeds against constipation; sap for wound healing; leaves as tea against malaria etc.

In Comore islands, in Papua New Guinea and in Uganda *Jatropha curcas L.* plants are used as a support plant for vanilla plants; as a source of shade for coffee plants in Cuba.[29]

Uses of different parts of *J. curcas* L. in medicines[14]

Plant part used	**Diseases**
Seeds	To treat arthritis, gout and jaundice
Tender twig/stem	Toothache, gum inflammation, gum bleeding, pyorrhea
Plant sap	Dermatomucosal diseases
Plant extract	Allergies, burns, cuts and wounds, inflammation, leprosy, leucoderma, scabies and small pox
Water extract of branches	HIV, tumor
Plant extract	Wound healing

2. Plant tissue culture of *jatropha curcas* l.

Researchers had tried to encounter the problem of yield of specific phyto-constituents which are pharmacologically useful in *Jatropha curcas* L.. Some of which researches had been reviewed are as follows:-

2.1 *In vitro* clonal propagation of biodiesel plant (*Jatropha curcas* L.)[30]

Datta *et al.* had reported the *in vitro* clonal propagation of biodiesel plant (*Jatropha curcas* L.). In this investigation, *in vitro* clonal propagation of seven-month-old *Jatropha curcas* L. was achieved employing nodal explants**.** Axillary shoot bud proliferation was best initiated on Murashige and Skoog's (MS)[31] basal medium supplemented with 22.2 μM N6-benzyladenine (BA) and 55.6 μM adenine sulphate, in which cultures produced 6.2 ± 0.56 shoots per nodal explants with 2.0 ± 0.18 cm average length after 4–6 weeks. The rate of shoot multiplication was significantly enhanced after transfer to MS basal medium supplemented with 2.3 μM 6-furfuryl amino purine (Kn), 0.5 μM indole- 3-butyric acid (IBA) and 27.8 μM adenine sulphate for 4 weeks. Both shoot number (30.8 ± 5.48) and average shoot length (4.8 ± 0.43 cm) were found to increase significantly.[32]About 52% of root induction occurred in MS basal medium supplemented with 1.0 μM IBA in 2–3 weeks. Further elongation of roots with average length of 8.7 ± 1.35 cm was obtained in unsupplemented MS basal medium for 2–3 weeks. The plantlets (12–16-week-old) were successfully acclimatized in soil with 87% survival frequency.[33]

2.2 Establishment of an *agrobacteriuim*-mediated cotyledon disc transformation method for *Jatropha curcas* L.[34]

Li *et al.* had reported the establishment of an *agrobacteriuim*-mediated cotyledon disc transformation method for *Jatropha curcas* L.. Seeds of *Jatropha curcas* L. were collected and a transformation procedure for *J. curcas* L. has been established for the first time via *Agrobacterium tumefaciens* infection of cotyledon disc explants. The results indicated that the efficiency of transformation using the strain LBA4404 and phosphinothricin for selection was an improvement over that with the strain EHA105 and hygromycin. About 55% of the cotyledon explants produced phosphinothricin- resistant calluses on Murashige and Skoog (MS) medium supplemented with 1.5 mg l^{-1} benzyladenine (BA), 0.05 mg l^{-1} 3–indolebutyric acid (IBA), 1 mg l^{-1} phosphinothricin and 500 mg l^{-1} cefotaxime after 4 weeks.[35] Shoots were regenerated following transfer of the resistant calli to shoot induction medium containing 1.5 mg l^{-1} BA, 0.05 mg l^{-1} IBA, 0.5 mg l^{-1} gibberellic acid (GA3), 1 mg l^{-1} phosphinothricin and 250 mg l^{-1} cefotaxime, and about 33% of the resistant calli differentiated into shoots.[36] Finally, the resistant shoots were rooted on 1/2 MS media supplemented with 0.3 mg l-1 IBA at a rate of 78%. The transgenic nature of the transformants was demonstrated by the detection of b-glucuronidase activity in the primary transformants and by PCR and Southern hybridization analysis. 13% of the total inoculated explants produced transgenic plants after approximately 4 months. This procedure useful for both, the introduction of desired genes into *J. curcas* L. and the molecular analysis of gene function.[37]

2.3 Establishment of callus and suspension culture in *Jatropha curcas* L..[38]

Soomro *et al.* had been reported the establishment of callus and suspension culture in *Jatropha curcas* L.. Callus cultures were initiated from leaf and hypocotyl explants isolated from 4 days old seedling of *Jatropha curcas* L., on Murashige & Skoog (1962)[31] basal medium supplemented with different growth regulator formulations including 2,4-D, BA, GA3, and coconut milk. Excellent growth of callus was obtained in medium supplemented with 0.5mg/L 2, 4-D alone and with 2% v/v coconut milk in hypocotyl explants, Callus produced from hypocotyl explants grew faster during 7 to 30 days of culture then stabilized at a low growth rate. Calli cultured on this medium showed 8

fold increase in fresh weight by the fourth week of incubation. Callus was soft, friable, globular, lush green in color.[39,40] Hypocotyl explant and 0.5mg/L 2, 4-D proved to be most effective in inducing of callus on a large scale in short period of time. The friable green callus was then used for establishment of homogeneous and chlorophyllous suspension culture. Maximum growth of suspension culture was achieved in medium supplemented with 0.5mg/L 2, 4-D, with initial inoculum cell density of 1%.[41] The growth rates of cells were initially slow but as the cultures proceeded, the growth increased significantly and accumulated a great amount of fresh weight (5fold) over a period of 21 days then the growth of cells was stable for 30 days. The fresh weight was balanced in terms of dry weight which almost corresponded to fresh weight. Total chlorophyll content in cell culture varied between 50.7 to 75.7ug/g FW with in growth cycle of these cultures.[42]

2.4 *In vitro* multiple shoot induction of physic nut (*Jatropha curcas* L.) [43]

Thepsamran *et al*. had been reported the *in vitro* multiple shoot induction of physic nut .The appropriate *in vitro* multiple shoot induction medium for *Jatropha curcas* L. from axillary bud-derived shoots, about 0.7 cm., was established.[44] Shoot proliferation was assessed on Murashige and Skoog (MS) medium supplemented with singly different concentrations of N^6 benzyladenine (BA) and in combinations with indole-3-butyric acid (IBA). MS medium with 2.22 μM BA and 0.049 μM IBA provided the best shoot multiplication about 5.9 shoots from an axillary bud-derived shoot for 6 weeks.[45] Regenerated shoots were rooted on MS medium with 2.46 μM IBA after 5 weeks, then transferred to MS medium without plant growth regulator.[46]

2.5 A simple regeneration protocol from stem explants of *Jatropha curcas* L. [47]

Singh *et al*. had been reported the simple regeneration protocol from stem explants of *Jatropha curcas* L.- a biodiesel plant. A simple, rapid and cost effective protocol has been developed for high frequency regeneration using stem segments of elite genotypes (CSMCRI-I, CSMCRI-II and CSMCRI-III) of *Jatropha curcas* L.. Shoot bud induction (10–15 buds per explant) was achieved on Murashige and Skoog's (MS) medium supplemented with 1.0 ml^{-1} benzylaminopurine (BAP) in combination with 1.0mgl^{-1} 6-furfurylamino purine (KN). Stem explant of CSMCRI-II showed highest response (65.3%) followed by CSMCRI-I and CSMCRI-III. These shoot buds

developed into shoots when subcultured on MS medium supplemented with 0.5mgl^{-1} BAP and 1.0mgl^{-1} IAA (indole-3-acetic acid). Shoots of 4.0–5.0cm length were harvested and cultured on MS medium containing different concentrations of indole-3-butyric acid (IBA) and 40% rooting was achieved in 0.1mgl^{-1} IBA after 5 weeks in all the genotypes used. For direct rooting, shoots of 4.0–5.0cm length were used and rooting was achieved by dipping the base of shoots in MS medium supplemented with 0.1mgl^{-1} IBA and 3.5% sodium alginate matrix and subsequently dropping in polymerization medium containing 2.0% calcium chloride. Encapsulated shoots were transferred in polybags filled with sterile soil wetted with sterile distilled water containing 0.5% broad-spectrum fungicide (Bavistine). Rooting could be achieved in 62% of shoots within 3 weeks. Rooted plantlets were successfully hardened and transferred to green house with 92% establishment.[48]

3. Conclusion and future prospects

Although the results from this review are quite promising for the use of *Jatropha curcas* L. as a multi-purpose medicinal agent. All kinds of reports on differentiation of shoots, embryos from callus or regeneration of shoot tips, establishment of an agrobacteriuim-mediated cotyledon disc transformation have been included in the absence of even formation of complete plant leave alone their *ex vitro* growth in soil. In vitro protocol could be very useful for multiplication of those endangered species whose propagation through conventional means is difficult. It should be useful in increasing the production of secondary metabolites even for resistance plant to adverse weather, pathogenic agent and pest.

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