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REVIEW [REVISIÓN]

EUPHORBIACEAE - A CRITICAL REVIEW ON PLANT TISSUE CULTURE

[EUPHORBIACEAE – UNA REVISIÓN CRÍTICA SOBRE CULTIVO DE TEJIDOS]

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SUMMARY

The members of Euphorbiaceae are valuable source of different kinds of useful products like dyes, edible tubers, oil crops, furniture, agricultural implements, ornamental plants, pharmacological products, rubber, timber and aesthetic items. Micropropagation is an alternative mean of propagation that can be employed in conservation of the flora in relatively shorter time. Tissue culture is useful for multiplying and conserving the species, which are difficult to regenerate by conservation methods and save them from extinction. Cryopreservation of germplasm would help in maintaining the genetic diversity of the endangered population. Improved cell and tissue culture technologies would help in producing the active compounds in vitro with better productivities without cutting down the natural resources. There is sufficient progress at research level to suggest that the tissue culture of Euphorbiaceae can and should be further developed. This review emphasizes the in vitro manipulation and remarkable achievements with biotechnology in this family made during the last six decades.

Key words: Euphorbiaceae; plant tissue culture; micropropagation.

INTRODUCTION

Researchers are constantly exploring new evidence for natural resources. A lot of economical factors are based on our resources which makes a transition from one element to another very difficult. The family Euphorbiaceae comprises nearly 322 genera and 8910 species (Bingtao Li et al., 2008) many of which have their own economic value and hence contribute to the floristic wealth of tropical and subtropical countries of the world. The family comprises a number of endemic and endangered taxa. However the in vitro studies are confined only to a few genera of aesthetic, medicinal, timber yielding, rubber yielding, dye yielding, cottage industries, ornamental and food crops like Acalypha, Baliospermum, Codiaeum, Cleistanthus, Croton, Euphorbia, Emblica, Eryngium, Exoceaaria, Givota, Glochidion, Hevea, Jatropha, Mallotus, Manihot, Phyllanthus, Putranjiva, Ricinus, Sapium and Uapaca.

RESUMEN

Los miembros de la familia Euphorbiaceae son una fuente valiosa de diversos productos valiosos como tintes, tubérculos comestibles, aceites, implementos agrícolas, plantas ornamentales, productos farmacológicos, lates, madera y productos estéticos. La micropropagación es una herramienta que puede ser empleada como una herramienta para la conservación de la flora en un lapso de tiempo menor. El cultivo de tejidos es útil para multiplicar y conservar especies, las cuales son difíciles de regenerar con otros métodos de conservación y salvarlas así de la extinción. La criopreservación del germoplasma ayudaría a mantener la diversidad genética de población en peligro. Tecnologías mejoradas para el cultivo de células y tejidos ayudaría a producir compuestos activos in vitro con una mejor productividad y sin afectar los recursos naturales. Existe suficiente progreso en materia de investigación que sugiere que el cultivo de tejidos de Euphorbiaceae puede y debe ser desarrollada. Esta revisión enfatiza la manipulación in vitro y los logros que la biotecnología ha logrado en esta familia en las últimas seis décadas.

Palabras clave: Euphorbiaceae; cultivo de tejidos vegetales; micropropagación.
Major components of *Euphorbia* latex are sterols, terpenoids, vitamins, and insecticides and anti-cancer drugs (Stohs and Rosenbarg, 1975; Heftmann, 1975; Deshmukh and Borle 1975; Biesboer and Mahlberg,1979; Yamamoto et al., 1981; Saigo and Saigo, 1983, Itokawa et al., 1989; Wu et al., 1991; Rani et al., 2003). Abel-Fattah and Rozk (1987) published on chemical constituents and economic important plants of Euphorbiaceae. The family is also well reputed for the production of valuable secondary metabolites like alkaloids and flavonoids in nature (Puebla et al., 2003; Maciel et al., 1998; Martin et al., 2002; Rani et al., 2002). Several of *Phyllanthus* species produce useful secondary metabolites, which have been extracted from whole plants (Unander, 1996). The species of *Phyllanthus* contain alkaloids, tannins, flavonoids, lignans, phenols, terpenes and show antinociceptive action in mice and other therapeutic activities (Filho et al., 1996). The members of the family are rich in reduced hydrocarbon materials that can be extracted and converted to petroleum like compounds (Tideman and Hawker, 1982). Cracked or fermented latex can be used as fuel (Nielsen et al., 1977; Calvin, 1980; Deperey et al., 1994). Depletion of petroleum resources is creating an opportunity for exploitation of vegetable oils as biodiesel. All over the world major sources of diesel include rape seed (USA), sunflower (Italy and South France), soybean (USA and Brazil), oil palm (Malaysia), linseed (Spain), cotton seed (Greece), beef tallow (Ireland) and *Jatropha* (Nicaragua and South Africa) (Jayasinghe, 2004).

*Jatropha* oil is a real bio product, CO₂ neutral, non toxic, bio degradable, free of sulphur and chlorine, caloric equivalently to mineral oil; *Jatropha* oil is non consumable, 100% pure biomass and as a result, CO₂ neutral (green) energy production. Global castor seed production is around 1 million tons per year. Leading producing areas are India (with over 60% of the global yield), China and Brazil. (Arifin et al., 2008)

The latex of *Euphorbia pulcherrima* has been reported to be poisonous to livestock (Anonymous, 1978) however, in veterinary medicine it is used to kill Maggots in the wounds of livestock. *Euphorbia lagascae* is a spurge which is present wildly at South Eastern Spain, it produces 50% of seed oil with 60% of cis 12, 13-epoxy oleic or Vernolic acid (Kleiman et al., 1964). Present objective for breeding in *Euphorbia lagascae* would be the reduction of skin irritant compounds in the latex (Turley et al., 2000). The leaf extracts of this family are reported to have many medicinal properties including purgative, sedative, antifungal, antiamoebic and anti-cancerous activities (Deshmukh and Borle, 1975; Kupchan et al., 1976). *Jatropha curcas*, a multipurpose plant, is valued not only for its medicinal properties and resistance to various stresses but also for its use as an oil (curcas) seed crop (Heller, 1996; Openshaw, 2000). The seeds press cake and oil of *Jatropha curcas* cannot be used for human or animal consumption but can be used as organic fertilizer due to the presence of several toxic substances including a lectin (curcin) phorbol esters, saponins, protease inhibitors and phytates (Makkar et al., 1998). Anti viral effects against hepatitis-B virus and possibly against the reverse transcriptase of retroviruses have also been reported (Thyagarajan et al., 1988; Sheed et al., 1992). Pharmacological studies carried out with callus extracts of *Phyllanthus niruri*, *P. tenellus* and *P. urinaria* have shown antinociceptive properties and the main compounds identified in the extracts were flavonoids, tannins and phenols (Santos et al., 1994). In Brazil, infusion of leaves, stems and roots of *Phyllanthus* species are used in folk medicine for treating intestinal infections, diabetes, Hepatitis-B and disturbances of kidney and urinary bladder (Calixto et al., 1998). Additional studies on callus and root extracts of these different species have shown the presence of phylembin, tannin that has antimicrobial activity. Hydrolysable tannins which inhibited DNA polymerase and reverse transcriptase of geraniin and its derivatives which showed high activity in the inhibition of Human Immunodeficiency Virus (HIV) reverse transcriptase and angiotensin converting enzyme involved in diabetic complications (Uno et al., 1988; Ogata et al., 1992; Unander., 1996). One of the main impediments in tissue culture studies in this family is the presence of latex.

### IN VITRO MANIPULATIONS

Murashige and Skoog (1962) medium was mostly used to initiate and improve the response in *in vitro* cultures, White’s basal medium, Woody plant medium and B5 media (Gomborg’s medium) have also been employed in some of the cases. Now a day, the transgenic plants produced through tissue culture methods showed superior abiotic and biotic stress tolerance (Ganesan and Jayabalan, 2006). Roy and Jinnah (2001) studied the *in vitro* propagation of poinsettias (*Euphorbia pulcherrima*). The hormonal control of triterpinols synthesis in *Euphorbia characias* calli was studied by Ferriera et al. (1992). Successful *in vitro* vegetative propagation has been reported for *Euphorbia* species (Lang he et al., 1974; Lee et al., 1982; Jakobek et al., 1986; Zhang et al., 1987; Nielsen et al., 2003). Few studies available on the tissue culture of *Phyllanthus* species are on callus culture of *P. emblica*, *P. urinaria*, *P. amarus*, *P. abnormis*, *P. caroliniensis*, *P. tenellus*, *P. niruri* and on transformed root cultures of *P. niruri* (Khanha and Nag, 1973; Unander, 1991; Ishimaru et al., 1992; Santos et al., 1994). *Croton* was chosen for micropropagation due to its rare success in conventional breeding and very little data is available.
for its in vitro production (Shibata et al., 1996; Orlikowska et al., 1995; Orlikowska et al., 2000).

Regarding castor bean regeneration, few reports were available for whole plant regeneration through shoot tip and embryonal axis culture (Sujatha and Reddy, 1998) and genetic transformation through direct regeneration methods (Sujatha and Sailaja, 2005). Carron and Enjalric (1983), Sompong and Muangkaewangam (1992) and Asokan et al., (1988) gave protocols for micropropagation of *Hevea brasiliensis* and Ferriere et al., (1992) focused his research on origin and ontogenesis of somatic embryos. Qin et al. (2006); Compos et al. (2007) studied the tissue culture and plant regeneration of *Jatropha curcas* and *Jatropha elliptica* respectively. Chitra and Madhusoodanan (2005) studied the influence of auxins in direct in vitro morphogenesis of *Euphorbia nivalia*. Rout et al., (2006) reviewed critically on present scenario and future prospects of tissue culture of some Euphorbiaceae members. In Table 1 a summary of tissue culture research is presented.

### Table 1. Tissue culture studies in Euphorbiaceae.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Type of culture</th>
<th>Source of Explant</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Codiaeum variegatum</em> L.</td>
<td>Leaf</td>
<td>Leaf explants</td>
<td>Somatic embryos, adventitious buds, Shoot multiplication, rooted <em>in vitro</em>.</td>
<td>Marconi and Radice, 1997</td>
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<td><em>Codiaeum variegatum</em> L.</td>
<td>Shoot</td>
<td>Shoots</td>
<td>Axillary shoot proliferation (increased when shoot tips were removed and inverted).</td>
<td>Orlikowska <em>et al.</em>, 2000</td>
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<td><em>Croton sublyratus</em> Kurz.</td>
<td>Shoot tip</td>
<td>Shoots</td>
<td>Axillary shoot proliferation, shoot shoot proliferation, shoot regeneration.</td>
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<td><em>Croton urucurana</em> Baill.</td>
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<td>Callus induced</td>
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<td><em>Euphorbia antisyphilica</em> Zucc.</td>
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<td><em>Euphorbia characias</em> L.</td>
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<td><em>Euphorbia esula</em> L.</td>
<td>Callus and rhizobacteria</td>
<td>Callus</td>
<td>Cell alterations noticed.</td>
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<tr>
<td><em>Euphorbia esula</em> L.</td>
<td>Hypocotyl segment</td>
<td>Hypocotyl / all the parts</td>
<td>Studied the effect of auxins, cytokinins,</td>
<td>Davis and Olson, 1993</td>
</tr>
<tr>
<td>Taxa</td>
<td>Type of culture</td>
<td>Source of Explant</td>
<td>Result</td>
<td>Reference</td>
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<tr>
<td><em>Euphorbia esula</em> L</td>
<td>Cell suspension</td>
<td>Stem callus, hypocotyls, Seedling root segments, Apical shoot, axillary shoot</td>
<td>Organogenesis, plantlets, shoots, rooted <em>in vitro</em></td>
<td>Davis <em>et al.</em>, 1988</td>
</tr>
<tr>
<td><em>Euphorbia lagascae</em> Spreng</td>
<td>Bud, shoot</td>
<td>Apical shoot, axillary shoot</td>
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<td>Torres, 2004</td>
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<td><em>Euphorbia lathyris</em> L</td>
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<td>Shoot tip</td>
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<td><em>Euphorbia nivulia</em> Buch.-Ham.</td>
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<td><em>Givotia rottleriformis</em> Griff.</td>
<td>Embryo</td>
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<td><em>Hevea brasiliensis</em> Müll.Arg.</td>
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<td>Somatic embryos</td>
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<td>Node</td>
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<td><em>Jatropha curcas</em> L.</td>
<td>Axillary bud</td>
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<td>Sujatha <em>et al.</em>, 2005</td>
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<tr>
<td><em>Jatropha curcas</em> L.</td>
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<td>Hypocotyls, petiole and leaf bits</td>
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<td>Taxa</td>
<td>Type of culture</td>
<td>Source of Explant</td>
<td>Result</td>
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<td>Jatropha curcas L.</td>
<td>Shoot tip</td>
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<td>Shooting rooted in vitro</td>
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<td>Jatropha elliptica (Pohl) Müll.Arg.</td>
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<td>Induction of somatic embryos, germination of somatic embryos, shoot development</td>
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<td>Manihot esculenta Crantz</td>
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<td>Leaf explant</td>
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<td>Somatic embryo culture Differentiated, developed and germinated.</td>
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<td>Manihot esculenta Crantz</td>
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<td>Leaf</td>
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<td>Cryopreserved shoot tips</td>
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<td>Manihot esculenta Crantz</td>
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<td>Node</td>
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<td>Cotyledons</td>
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<td>Taxa</td>
<td>Type of culture</td>
<td>Source of Explant</td>
<td>Result</td>
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<td><em>Phyllanthus amarus</em> Schum. &amp; Thonn.</td>
<td>Synthetic seed</td>
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<td>Growth reduced plantlet formed</td>
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<td><em>Phyllanthus amarus</em> Schum. &amp; Thonn.</td>
<td>Shoot tip</td>
<td>Shoot tip</td>
<td>Multiple shoots, rooted <em>in vitro</em></td>
<td>Bhattacharyya and Bhattacharyya, 2001</td>
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<td><em>Phyllanthus coroloinensis</em> Walter</td>
<td>Axillary shoot</td>
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<td>Multiple shoots rooting initiated.</td>
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<td><em>Phyllanthus fraternus</em> Webster</td>
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<td>Shoot tips, nodal parts</td>
<td>Multiple shoots, axillary bud sprouting</td>
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<td><em>Phyllanthus niruri</em> L.</td>
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<td>Shoots, multiple shoots, flowers and fruiting</td>
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<td><em>Phyllanthus urinaria</em> L.</td>
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<td><em>Ricinus communis</em> L.</td>
<td>Cotyledon culture</td>
<td>Cotyledon</td>
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<td><em>Ricinus communis</em> L.</td>
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<tr>
<td><em>Ricinus communis</em> L.</td>
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<td>Meristematic explant</td>
<td>Deferential cytokinin effects were studied.</td>
<td>Sujatha and Reddy, 1998</td>
</tr>
</tbody>
</table>

**Seed germination**

Sterilization is must for the seeds prior to inoculation in order to avoid the contamination. Jamal *et al.* (1993) studied the negative effect of 2,4-Dichlorophenoxy acetic acid (2,4-D) on seed germination of the *Euphorbia esula*. In *Givota rotteriformis* Rambabu *et al.*, (2005) studied the effect of Gibberellic acid (GA₃) on enhancement of *in vivo* seed germination. Thapiyil (2004) reported twin seedlings in *Putranjiva roxburghii*. The transmission of juvenile rooting ability from seedlings to adults of *Hevea brasiliensis* was studied by Muzik and Cruzado (1958).

**Cotyledary nodes and hypocotyl tissue**

*Ricinus communis* cotyledons, cultured on the medium with Murashige and Skoog’s medium salts (MS) + B₃, Vitamins, resulted in the formation of multiple shoots, callusing, roots and the plantlets were later acclimatized (Kumari *et al.*, 2008). Hypocotyl tissue from zygotic embryo axis of *Ricinus communis* produced adventitious shoots when treated with either...
Thidiazuron (TDZ) or BA (N6-Benzyladenine). While Indole butyric acid (IBA) induced the rooting very effectively and plants established in the soil (Ahn et al., 2007). Molina and Schobert,(1995) studied the micropropagation of Ricinus communis. Cotyledonary explants of Manihot esculenta were first treated with 2,4-D (4mg/l) then placed on MS + NAA (1-naphthaleneacetic acid) + BA + GA3, MS medium or 4 × MS micro salts were efficient for the formation of somatic embryos. 6% Sucrose was optimal concentration for the development of somatic embryos after an induction treatment using 2% sucrose, ABA (0.52 mg/l) induced good number of somatic embryos production (Konan et al., 1994). Seneviratne (1991) studied the micropropagation of juvenile and mature Hevea brasiliensis.

**Shoot and root regeneration**

Viruses makes plants to grow less by their effects like necrosis, curling of leaves, decreased yield and plant death (Quak, 1997). Shoot apical meristems and first set of primordial leaves in an elongating shoot are generally not connected to the vascular system of the plant and therefore are not contaminated by viruses as that travel through the vascular system. The confusion in the true shoot meristem consists only of the isolated apical dome without visible primordial leaves attached. The nodal segments of Baliospermum montanum were cultured on MS + BAP (3.10µM). In vitro raised shoots were rooted on half MS medium fortified with various concentrations and combinations of auxins (Johnson and Manickam, 2003). In vitro micropropagation of Baliospermum axillare was recorded by Kamalendra and Sudarshanan (2003). For the micropropagation of Hevea brasiliensis the shoot tips were used (Gunatilleke and Samaranayake 1988). Another important medicinal plant Phyllanthus amarus apical meristems were cultured on MS + Kn (6-furfuryl-amino purine (Kinetin))/BAP singly or in combination with Indole acetic acid (IAA). Growth regulators at lower range (0.1-1.0 mg L⁻¹) stimulated direct regeneration of shoots. Kn is superior to BAP and Kn + IAA combination was more suitable than Kn alone. The micropropagated plants were successfully established in the soil with high survival rate of 80% (Bhattacharyya and Bhattacharyya, 2001). Ghanzi et al., (2004) reported high frequency shoot regeneration from shoot tip, nodal and internodal segments of Phyllanthus amarus and studied the effect of different concentrations of BAP, Kn and coconut milk on shoot tips, axillary and internodal segments. Hardening was done in the liquid MS medium and the survival rate of the acclimatized plants was 85%. Singh et al. (2006), encapsulated the shoot tips of Phyllanthus amarus with 3% sodium alginate and 75 mM CaCl₂, H₂O. They were grown in vitro and 90 % of shoots were regenerated. There are so many factors effecting the growth of the shoot tips like, MS salts in the sodium alginate beads, concentration of sodium alginate and storage duration etc.

In the genus Phyllanthus the nodal culture, callus culture and rooting were observed in many species. viz., Phyllanthus caroliniensis (Catapan et al., 2000); Phyllanthus fraternus (Rajasubramaniam and Saradhi, 1997) Phyllanthus stipulatus (Catapan et al., 2001) Phyllanthus urinaria (Catapan et al., 2002).

The nodal explants of Manihot esculenta were cultured on two different media, liquid MS + TDZ (0.11-0.22µM) where internodal elongation was observed, followed by solidified MS+BA (2.2 µM)+ GA₃ (1.6µM) generated clusters of buds and fasciated stems which developed into shoots (Bhagwat et al., 1996). The nodes of Manihot esculenta were cultured on medium containing BAP (10mg / l) to get enlarged axillary buds, they gave multiple shoots on shifting to the fresh medium (Konan et al., 1997). Kartha et al., (1974) produced viral free plants from the Apical meristems of Manihot utilisissima. Cryopreserved and thawed apices of Manihot esculenta were cultured on an osmoprotected medium. Successfully vitrified apices showed growth within a week and developed into shoots. The average recovery rate was about 70% (Rommanee et al., 2003). Escobar et al., (1997) presented a protocol for recovering Manihot esculenta plants from shoot tips maintained in liquid nitrogen. The shoot tips responded as per the pre and post freezing steps. Finally, they have succeeded to produce 50-70% of plants. Villaluz (2006) developed a protocol for the rapid meristem development and mass propagation of Manihot esculenta and the explants were cultured on either liquid or solid medium containing GA₃, BAP, NAA at (0.25, 0.1 and 0.2 mg/l) respectively. MS medium with NAA induced both shoot and root development (Ricardo et al., 2007).

High frequency regeneration from various explants of Jatropha integerrima has been reported (Sujatha and Dhingra, 1993). Sujatha and Mukta.(1996) worked on Jatropha curcas to study the morphogenesis and plant regeneration from tissues. The shoot tips of the Jatropha curcas were cultured on the MS + BAP (2.0mg/l) and IAA (0.5mg/l) along with adenine sulphate, glutamine and activated charcoal, these shoots were rooted on the half MS + IBA (0.5-5.0mg/l) the high frequency rooting was on MS + IBA 3.0 mg/l. The survival rate of acclimatized plants was 60-70% (Rajore and Batra, 2005). Shoot buds from the axillary nodes and leaf segments of non-toxic Jatropha curcas were cultured on MS + Kn (2.3-4.5µM), BA (2.2-4.4µM) and TDZ (2.3-4.5µM) individually, the leaf segments were cultured on MS+ BA (8.9µM) + IBA (2.5µM) and got adventitious shoots (Sujatha et

The shoot tips of *Glochidion multiloculare* produced multiple shoots when cultured on MS + BA (1.0 mg/l) and IAA (1.0 mg/l) callus derived from the leaf and stem explants on a medium containing 2,4-D (0.5-2.0 mg/l) produced shoot buds when transferred to MS + BA (1.0-2.0 mg/l) + CM (Coconut milk, 10% v/v) the rooting combination was MS + IBA (1.0 mg/l) and were acclimatized (Yamuna et al., 1995). The shoots of *Euphorbia antisiphilitica* were cultured on modified MS medium + NAA (0.13µM) and BAP (4.44µM), the rooting combination was half MS+IBA (0.49µM and acclimatization was very simple (Jakobek et al., 1986). The axillary shoots of *Euphorbia lagascae* were dipped in IBA (50 mg/l) for 2 min. IBA increased the survival rate up to 80-100% (Torres, 2004). The tips of cristate lateral shoots of *Euphorbia pungniformis* were cultured on the MS + sucrose 2% and NAA 0.1 mg/l and BA, gave cristate shoots of both forms. While 20% gave normal shoots. The rooting combination for cristate shoots was MS+ IBA (0.5mg/l), 95-100% of rooted shoots were acclimatized *ex vitro*. Quite a lot of cristates reverted into the normal form. Effect of MS nitrogen salts had a considerable effect on the cristate form shoot reversion (Balotis and Papafotiou, 1993). Effects of several factors on rooting of Poinsettias was recorded by Balotis and Papafotiou (2003). Effects of several factors on rooting of Poinsettias was recorded by Balotis and Papafotiou (2003). Effect of MS nitrogen salts had a considerable effect on the cristate form shoot reversion.

Shoots of *Codiaeum variegatum* cultured on MS + BAP (0.5mg/l) + peptone (25mg/l) which enhanced the shoot formation and rooting was induced on MS + IBA (2.0mg/l) medium and they were acclimatized with 95% survival rate. Marconi and Radice (1997) studied organogenesis and somatic embryogenesis in *Codiaeum variegatum*. To study the laticiferous systems of the *Hevea*, Bouychou (1953) of the Institute Francia Caoutchouc started *in vitro* cultures first time and followed by Wilson and Street, (1975). Guntilleke and Samarayake (1988) used shoot tips for the micropropagation of *Hevea brasiliensis*.

**Leaf cultures**

The leaves of *Uapaca kirkiana*, *Uapaca nitida* and *Jatropha curcas* were co-cultured for the callus induction. The objective of this investigation was to evaluate the early signs of grafting compatibility of *Uapaca kirkiana* (Mng’omba et al., 2007). The propagation of *Uapaca kirkiana* using tissue culture techniques was carried out by Maliro, (1997) and the effect of cytokinins on *in vitro* propagation of *Uapaca kirkiana* was studied by Chishimba et al., (2000). A clonal propagation protocol was developed for *Uapaca kirkiana* by Mng’omba, (2007). Anthony et al., (1995) developed a protocol for the leaf protoplast culture of *Manihot esculenta*. The leaf lobes of *in vitro* grown *Manihot esculenta* were cultured on MS + 2,4-D (4 mg/l) + CuSO₄ (2µM) to improve the frequency of somatic embryogenesis. They were transferred to basal medium supplemented with activated charcoal. The desiccated bipolar somatic embryos showed 92% germination and 83% plants regeneration (Mathews et al., 1993). Immature leaves of *Manihot esculenta* were cultured on MS + 2,4-D or NAA to induce somatic embryogenesis and adventitious shoots. Results showed that auxin was a key factor for inducing embryogenic cells, BAP stimulated adventitious shoots. Histological examinations supported the conclusion (Ma and Xu, 2002). The leaf bits of *Croton urucurana* were cultured on woody plant medium + different concentrations of 2,4-D in combination with TDZ, BAP induced the callus, the highest callus fresh mass was observed when treated with 2,4-D (Lima et al., 2008).

**Nodes and internodes**

Nodal meristems are an important tissue source for micropropagation and plants raised from these are comparatively more resistant to genetic variation (Pierik, 1991). Konan et al., (1994) reported an efficient mass propagation system for Cassava (*Manihot esculenta*) based on nodal explants and axillary bud-derived meristems. The nodes and basal sprouts of *Cleistanthus collinus* were cultured on + Citric acid (CA, 104.1µM) and Polyvinylpyrrolidone.
(PVP) 40 (12.5 or 25µM) + BA (0.44µM) resulted in shoot proliferation and for rooting, IBA (0.23µM) was suitable. The regenerated plants were successfully acclimatized with 85% survival rate (Rao et al., 1998). Nodes and shoots of Mallotus repandas cultured on MS + BA, 2ip (6- (γ, γ-dimethylallylamine) purine), Kn and BA (4.44µM) induced shoots, where as the roots were induced with NAA (32.23µM), the regenerated plants were acclimatized properly and then analyzed chemically to compare with mother plants (Kaewsawman et al., 2005).

The nodal explants of Euphorbia pulcherrima, cultured on MS + NAA, 2,4-D, Kn and 2ip generated the embryogenic callus which on subculture to MS medium supplemented with 2ip (9.8µM) and NAA (0.54µM) gave somatic embryos. The embryos germinated successfully and were acclimatized with high survival rate (Jasrai et al., 2003). Euphorbia tirucalli inter node explants, cultured on Linsmaier and Skoog’s medium (LS) + TDZ (0.02 mg/l), resulted in the induction of the adventitious buds which on further culture on LS basal medium grew into shoots. The roots were induced by growing the shoots on the LS + NAA (0.02 mg/l) followed by half LS basal medium and were successful to obtain whole plantlets (Uchida et al., 2004). The nodes and internodes of Mallotus repandas were precultured on MS basal and then shifted to shoot induction medium comprising of BAP (4.44µM), the shoots were immediately shifted to shoot elongation medium. The results suggest that the explants need to acquire competence before shoot organogenesis. Rooting was obtained by incubating the regenerated shoots on half MS + NAA (10.74µM) and the plants were successfully transferred to soil (Prarthanturarag et al., 2007). Sujatha et al. (2005) reported the proliferation of shoot bud from nodes and leaf sections of non toxic Jatropha curcas. Nodal explants of Jatropha curcas were raised on MS + BA (22.2µM) and adenine sulphate (55.6µM) and thus shoots were multiplied on MS + Kn (2.3µM), IBA (0.5µM) and adenine sulphate (27.8µM). For rooting, MS + IBA (1.0µM) and subsequent transfer to MS basal and later acclimatized. (Datta et al., 2007). The effect of TDZ on nodal proliferation was recorded by Seneviratne and Flemingmann (1996).

**Callus initiation**

As first step in many tissue culture experiments, it is necessary to induce callus from the primary explant, callus is produced in response to injury. Callus was defined as tissue constituted by differentiated cells, which develop in response to a chemical or physical lesion, under determinate hormonal conditions. It can be obtained from a tissue fragment and only some of the callus cells exhibit the totipotency, i. e. the ability to differentiate into tissues, organs and even embryos, being able to regenerate whole plants (Pierik, 1990).

The callus cultures will be used to study protoplast isolation, cell type, cellular selection, somatic embryogenesis, organogenesis and secondary metabolite production. Oil body formation in Euphorbia tirucalli cell suspension culture was noted by Ohyama et al. (1984b) and protoplast isolation from Euphorbia tirucalli cell suspension cultures and sustained cell division was observed by Ohyama et al., (1984a). It was Chua, (1966), who studied the tissue culture of H. brasiliensis role of osmatic concentration, carbohydrate and pH value in induction of callus growth in plumule tissue from Hevea seedlings. Asokan et al. (2001), studied the isoenzyme markers for distinguishing embryogenic calli from non embryogenic during somatic embryogenesis in H. brasiliensis.

In Mallotus philippensis (Abbas, 1993) obtained a continuously growing callus on MS + 2,4-D (5.78µM) + Kn (2.5µM). These calli when sub cultured on MS + BA (13.3µM) + CH (Casein hydrolysate, 100mg/l) gave rise to four types of morphologically distinct cell lines. Among these four lines, only the green compact cell line was responsive for organogenic differentiation. Shoot regeneration occurred in this callus when sub cultured on MS + BA (13.3µM) + NAA (1.1µM). Euphorbia esula is a vigorous, highly competitive perennial weed of Eurasian origin that has spread rapidly over the Northern US and Canada and has become an economic pest in the past several years (Watson, 1985). Lee and Starratt (1972) were the first to establish cultures from roots of leafy spurge seedlings, using NAA. Nevertheless, they did not attempt to regenerate plants from their cultures.

**Suspension cultures and co-cultures**

The cell suspension obtained from the stem callus of Euphorbia esula on basal medium containing a reduced oxidized nitrogen ratio of 33.67 under the fluorescent lights. Roots and shoots formed and then acclimatized (Davis et al., 1988). Soussi et al., (1996) studied the interaction of Pseudomonas fluorescens isolate LS 102 and Flavobacterium baldusínum isolate 105 with Euphorbia esula callus. They concluded that the callus tissue might provide an excellent working model to investigate the mode and /or mechanism of potential bio-control agents on their host plants. The growth anatomy and morphogenetic potential of callus and cell suspension of Hevea brasiliensis was studied by Wilson and Street (1975).
Somatic embryogenesis and embryo culture

Studies utilizing immature zygote embryos help researcher gain greater insight into embryo development and seed maturation. Embryo rescue is a valuable tool for plant breeders to obtain hybrids from crosses that would otherwise abort on the plant. Multiplication of *Croton urucurana* can be achieved by micropropagation, either organogenesis or somatic embryogenesis (Grattapaglia and Machado, 1998). Tyagi and Govil (1999) worked on *Emblica officinalis* to observe somatic embryogenesis and micropropagation.

There have been several reports of cassava plant regeneration via somatic embryogenesis that are of reproducible (Stamp and Henshaw, 1982, 1987a; Stamp 1987; Szabados et al., 1987; Cabral et al., 1992) and secondary somatic embryogenesis (Stamp and Henshaw, 1987b). In *Manihot esculenta* the cyclic system of somatic embryogenesis was improved by using both liquid and solid media of which liquid medium was suitable and helped in the induction of multiple embryos (30) and they were converted into shoots (Raemakers et al., 1993a, b). Somatic embryo fragments of *Manihot esculenta* cultivar “Nanzhi 188” cultured on the media containing cytokinins like (BA and TDZ) induced the shoot organogenesis. While auxins stimulated somatic embryogenesis. The efficiency depends on the different cytokinins and their combinations with auxins (Ma, 1998). Raemakers et al., (1999) developed a protocol for the direct cyclic somatic embryogenesis of *Manihot esculenta* for mass production. The leaf callus derived cotyledons of primary somatic embryo of Cassava (*Manihot esculenta*), cultured on auxin supplemented MS medium (liquid / solid), resulted in the formation of the secondary somatic embryos which were cultured on the Gresshoff and Doy’s basal medium supplemented with auxins results in indirect somatic embryogenesis. Depending on BA concentration, plants can be transferred either directly to greenhouse or after using standard multiplication protocols (Raemakers et al., 2000). Woodward and Kaerlas (2001) studied the floral material embryogenic potential of *Manihot esculenta*.

Groll et al., (2002) studied the effects of quarter, half, full and double strength medium salt concentration on differentiation and maturation of somatic embryos of Cassava (*Manihot esculenta*) finally they suggested that half to full strength MS medium was quite suitable for the induction of proliferative nodular embryogenic callus after the desiccation in the saturated K$_2$SO$_4$ for 10 days. Thezygotic embryos of *Givotia rotterformis* were cultured on MS + 3% sucrose. On shifting, the seedling to medium containing tyrosine 100 mg l$^{-1}$ the survival rate of acclimatized seedlings was elevated to 60-70% (Rambabu et al., 2006).

Somatic embryos of *Hevea brasiliensis* were grown in the specialized conditions like desiccation, medium osmolarity, ABA concentration in the medium for the conversion into plantlet. Slow desiccation or maturation on 351 mol m$^{-3}$ sucrose supplemented with 1 mol m$^{-3}$ ABA strongly improved germination ability and conversion of embryos into plantlets indicated the increased level of vigor (Etienne et al., 1993). Etienne et al., (1997) developed a protocol for improvement of somatic embryogenesis in *Hevea brasiliensis* using the temporary immersion technique. For the last two decades, considerable progress has been made on *in vitro* techniques for multiplication and improvement of *Hevea*. The effect of thidiazuron on auxillary proliferation of *H. brasiliensis* was reported by Seneviratne and Flemingmann, (1996). Jha et al., (2007) reported somatic embryogenesis in *Jatropha curcas*. Somatic embryogenesis has been reported from anthers as well as inner integumental tissue (Wang et al., 1980; Nataraja,1975) noted the morphogenesis in embryonal calli of *Euphorbia pulcherrima*.

Effect of ABA and cytokinins on the somatic embryogenesis of *Hevea brasiliensis* was reported by Veisierre et al. (1994b). Effect of conditioned media on the somatic embryogenesis of *H. brasiliensis* was reported by Veisierre et al., (1994a). The influence of growth regulators and sucrose on somatic embryogenesis from immature inflorescences of *H. brasiliensis* was reported by Sushamakumari et al., (2000). In *H. brasiliensis* multiple shoots were induced from the somatic embryos (Sushamakumari et al., 1999). Effect of ABA and high concentration of polyethylene glycol on *H. brasiliensis* somatic embryo development was studied by Linossier et al., (1997) GA$_3$ regulated embryo induction and germination in *H. brasiliensis* was recorded by Kumarijayasree and Thulaseedhuran, (2001). Kumarijayasree et al., (2001a), optimized the parameters effecting somatic embryogenesis and long term somatic embryos were initiated and maintained by Kumarijayasree and Thulaseedhuran (2004) and Kumarijayasree et al., (2001b). Jayatillake (2007) studied the micrografting, female floret culture and somatic embryogenesis of *H. brasiliensis*. In vitro micro grafting of *H. brasiliensis* was studied by Kala et al., (2002). The water status of callus from *H. brasiliensis* during induction of somatic embryos was recorded by Etienne et al., (1991). Hadrami et al., (1989) and Carron et al., (1995) studied the effect of polyamines on the somatic embryogenesis. Carron and Enjarlic (1982) and Carron et al., (1985) developed protocols for vegetative propagation of *H. brasiliensis* by somatic embryogenesis and *in vitro* microcutting.
Organogenesis and morphogenesis

The organogenesis in *Euphorbia esula* was studied by Davis and Olson (1993) IAA induced the roots very effectively, high concentrations of IAA, NAA, 2,4-D promoted callusing. High or low concentrations of picloram reduced the efficiency of rooting. Martin et al., (2005) reported the influence of auxins in direct *in vitro* morphogenesis of mesophyll cells of *Euphorbia nivalia.* KNO3 reduced the rate of morphogenesis, where as BAP induced somatic embryogenesis. The combination of BA with NAA and IAA had positive effect on morphogenesis. IBA (13.3 µM) induced shooting and half MS + IBA (2.46µM) suitable for rooting; BA (4.44µM) +2,4-D (2.26µM) were optimum for somatic embryogenesis of proximal explant. GA3 supplementation to half MS medium resulted in the conversion of embryos into plantlets with survival rate of 90%.

Paranjothy and Gandimathi, (1975, 1976) reported on callus cultures and morphogenesis in *Phyllanthus niruri.* The organogenesis in *Euphorbia brasiliensis* was studied by Dewir et al., (1990) and Davis and Olson (1993) IAA induced the roots very effectively, high concentrations of IAA, NAA, 2,4-D promoted callusing. High or low concentrations of picloram reduced the efficiency of rooting. Martin et al., (2005) reported the influence of auxins in direct *in vitro* morphogenesis of mesophyll cells of *Euphorbia nivalia.* KNO3 reduced the rate of morphogenesis, where as BAP induced somatic embryogenesis. The combination of BA with NAA and IAA had positive effect on morphogenesis. IBA (13.3 µM) induced shooting and half MS + IBA (2.46µM) suitable for rooting; BA (4.44µM) +2,4-D (2.26µM) were optimum for somatic embryogenesis of proximal explant. GA3 supplementation to half MS medium resulted in the conversion of embryos into plantlets with survival rate of 90%.

Paranjothy and Gandimathi, (1975, 1976) reported on callus cultures and morphogenesis in *Phyllanthus niruri.*

Anther culture

The aim here is the production of haploid plants through the induction of androgenesis in the haploid cells of the immature pollen grain. Haploid plants are important for number of reasons. Because they possess single set of chromosomes, plant breeders interested in haploid plants because either spontaneous doubling of chromosome number (to 2n) or an application of the colchicine to double the chromosome number gives rise to homozygous plants (Razdan, 2003). Immature anthers of *Hevea brasiliensis* were cultured on the modified MS medium supplemented with 2,4-D (2.0 mg/l) and KNO3 (0.5mg/l) resulted in the induction of the callus. By culturing, the callus on MS + KNO3 (0.7mg/l) and NAA (0.2mg/l) produced maximum number of somatic embryos. They were further developed from embryos to plantlets on the hormone free medium and then acclimatized (Jayasree et al., 1999). Effect of altered temperature on plant regeneration frequencies in stamen culture of rubber trees was studied by Wang et al., (1998). Wang and Chen (1995) studied the effects of temperature on stamen culture and somatic plant regeneration frequencies in stamen culture of rubber tree. Shiji et al., (1990) prepared a report on anther cultures of *H. brasiliensis.* Propagation of calli from anthers of *H. brasiliensis* was done by Satchuthananthavale and Irugalbandara, (1972). Chen et al., (1979) prepared a protocol for obtaining pollen plants of *H. brasiliensis.* Chen et al., (1982) discussed the recent advances in anther culture of *H. brasiliensis.*

Endosperm culture

Morphogenesis from endosperm tissues has been reported in *Jatropha panduriformis* (Srivastava and Johri, 1974). Endosperm callus of *Emblica officinalis* sub cultured on MS + BAP (0.2mg/l) and IAA (0.1mg/l) resulted in the formation of shoots and embryo like structures in 50 and 8% cultures respectively. Regeneration of shoots was more frequent when both BAP (0.2mg/l) and IAA (0.1mg/l) were present than on BAP (0.2mg/l) alone. The embryo like structures produced plantlets (Sehgal and Khurana, 1985). Thomas and Chaturvedi (2008) focused on Endosperm cultures of Euphorbiaceae. The growth and metabolism of *Ricinus communis* endosperm in tissue culture was noted by Brown et al., (1970), Johri and Srivastava (1972). The organogenesis in endosperm tissue culture of *Codiaeum variegatum* was reported by Chikkannaiah and Gayatri (1974) and Gayatri (1978). Sehgal et al., (1981) reported the growth responses of mature endosperm of *Euphorbia genieulata.* Sehgal and Abbas (1996), induced triploid plants from the endosperm cultures of *Mallotus philippensis.* Srivastava (1973) reported the formation of triploid plantlets in endosperm cultures of *Putranjiva roxburghii.*

In *vitro* Flowering and fruiting

Flower heads had more number of meristematic initials, which facilitates the active proliferation of calli and respective organs. The nodes from the flower heads will give an excellent multiple shoots too. When we know the complete flowering mechanism, we can alter the growth and development patterns, or else we can revert the growth of the plant material. In case of *Euphorbia milli* the reversion of inflorescence development and its applications to large-scale micropropagation in airlift bioreactor was done by Dewir et al., (2005a). Tang et al., (1983) reported *in vitro* flowering in five genotypes of *Manihot esculenta* out of his 13 genotypes. It has given the recent trend towards selecting late flowering and non-branching cassava genotypes, *in vitro* flowering system may be the first step towards the possibility of recombining genetic material via *in vitro* fertilization in otherwise non-hybridizable lines. Liang and Keng (2006) reported the role of affecting factors (light, GA3, sucrose, number of subcultures) on *in vitro* flowering and fruiting while they are working on the micropropagation of valuable medicinal plant *Phyllanthus niruri.*
Factors affecting organogenesis and plant regeneration

This includes effect of temperature, light, pH, plant growth regulators and orientation of the explant on the medium. They have great effect on the explant response but very few workers concentrated on these aspects. The physical status of the plant and the genotype are significant. Continuous light period was found optimum for endosperm proliferation of *Ricinus communis* (Srivastava 1971a). The optimum temperature for the growth of endosperm reported as 25°C and the pH was 5.0 for *Ricinus communis* (Johri and Srivastava, 1973). For *Jatropha* and *Putranjiva* the optimum pH was 5.6 (Srivastava, 1971b). Ripley and Preece (1986) working on *Euphorbia lathyris* found that minimal wounding and vertical orientation of the explants during inoculation are must for the shoot tip rise for which BA is not required where as for callusing and adventitious shooting BA is must. The etiolated shoot tips were rooted well on MS + NAA (1.0 mg/l) and the plantlets were acclimatized. Hadrami and Auzac (1992) studied the effect of growth regulators polyamine content and peroxidase activity in *Hevea brasiliensis* callus. CaCl₂ at a concentration between 0-9 mM was used in the calllogenesis medium of *Hevea brasiliensis*. Low concentrations induced the embryony, at high concentrations(12 mM CaCl₂) induced the friable callus. They observed that the CaCl₂ has effective interactions with growth regulators and can decrease the rate of nitrogen metabolism, reduction in the water content (Montoro et al., 1995). Effect of strictly plant related factors on the response of *H. brasiliensis* was studied by Lardet et al., (1998). As per Matysiak and Nowak (1995), increased concentration of CO₂ also lessens water stress of microcuttings by closing the stomata. Unander et al., (1995) studied the factors effecting the germination and stand establishment of *Phyllanthus amarus*. Sochacki and Chimid, (1994) studied the effect of several factors on rooting of Poinsettias (*Euphorbia pulcherrima*). The effect of light emitting diode on growth and shoot proliferation and effect of paclobutrazole, light emitting diodes and sucrose on flowering of *Euphorbia milli* was studied by Dewir et al. (2005b, 2006).

A major focus of research during the past two decades has been the manipulation of growth media and growth conditions. Another is the testing of a variety of explant sources to obtain somatic embryogenesis, organogenesis and regeneration of plants. If we want to reduce the micropropagated seedling production cost and automation of the work we can use robots (Kozai et al., 1988; Kozai, 1991a, b). Rapid multiplication, germplasm conservation, pathogen elimination, genetic manipulations and for secondary metabolite production bioreactor technology may reduce the production cost provided proper precautions are taken to prevent contamination. Teixeira da Silva, (2003) reviewed on the usage of thin cell layer technology in ornamental plant micropropagation and biotechnology, which highlights organogenesis and somatic embryogenesis for plant regeneration and genetic transformation. Somatic embryogenesis facilitated cryopreservation, synthetic seed development, mutations and genetic transformation. Plant transformation methods and gene silencing technology can effectively used to evaluate and authenticate newly discovered endogenous genes to characterize their function in plants as well as genetically manipulate trait quality and productivity (Dandekar, 2003).

CONCLUSION

The review given here can be used for multiplication of the above said medicinal and economical plants commercially. Farmers, tribal people, pharmaceutical companies can utilize this information and can benefit economically. Active principles isolated from the cultures and from plants can be utilized as leads for further drug development by the pharmaceutical industry. Grafting compatibility studies are very helpful for the pathological studies.

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