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REVIEW [REVISIÓN]

EUPHORBIACEAE - A CRITICAL REVIEW ON PLANT TISSUE CULTURE

[EUPHORBIACEAE – UNA REVISIÓN CRÍTICA SOBRE CULTIVO DE TEJIDOS]

Rajesh Kondamudi[1], K. Sri Rama Murthy[1]* and T. Pullaiah[2]

[1]School of Conservation Biology and Plant Biotechnology, Department of Biotechnology, Montessori Mahila Kalasala,Vijayawada - 520 010, Andhra Pradesh, India,
[2]Department of Botany, Sri Krishnadevaraya University, Anantapur – 515 203, Andhra Pradesh, India. E-mail: drksrmurthy@yahoo.com
**Corresponding author*

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.

RESUMEN

Los miembros de la familia Euphorbiaceae son una fuente valiosa de diversos productos valiosos como tintes, tuberculos 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 díficiles 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 decadas.

Palabras clave: Euphorbiacea; cultivo de tejidos vegetales; micropropagación.

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, Excoecaria, Givotia, Glochidion, Hevea, Jatropha, Mallotus, Manihot, Phyllanthus, Putranjiva, Ricinus, Sapium* and *Uapaca.*

Major components of *Euphorbia* latex are sterols, terpenoids vitamins and insecticides and anti cancer drugs (Stohs and Rosenberg, 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 (Tidemann and Hawker, 1982). Cracked or fermented latex can be used as fuel (Nielsen *et al.,* 1977; Calvin, 1980; Depeyre *et al.,* 1994). Depletion of petroleum resources is creating an opportunity for exploitation of vegetable oils as bio-diesel. All over the world major sources of diesel include rape seed (USA), sun flower (Italy and South France), soybean (USA and Brazil), oil palm (Malaysia), lin seed (Spain), cotton seed (Greece), beef tallow (Ireland) and *Jatropha* (Nicaragua and South Africa) (Jayasingh, 2004).

Jatropha oil is a real bio product, CO_2 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, CO_2 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 *Ephorbia 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 (Desmukh 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). Antiviral effects against hepatitis-B virus and possibly against the reverse transcriptase of retroviruses have also been reported (Thyagarajan *et al.,* 1988; Shead *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 phyllemblin, 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 (Ueno *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 B_5 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* (Khanna 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 curcus* and *Jatropha elliptica* respectively. Chitra and Madhusoodanan (2005) studied the influence of auxins in direct *in vitro* morphogenesis of *Euphorbia nivulia.* 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.

Taxa	Type of culture	Source of Explant	Result	Reference
Baliospermum montanum (Willd.) Mull-Arg	Node	Node, *in vitro* shoots	Shoot proliferation, root initiation.	Johnson and Manickam, 2003
Cleistanthus collinus Roxb.	Shoot bud	Nodal explants	Apical and axillary shoot proliferation, rooted *in vitro,*	Quraishi and Mishra, 1998
Codiaeum variegatum L.	Shoot tip.	Shoots	Shooting, roots induced.	Asma Nasib *et al.,* 2008
Codiaeum variegatum L.	Leaf	Leaf explants	Somatic embryos, adventitious buds, Shoot multiplication, rooted *in vitro.*	Marconi and Radice, 1997
Codiaeum variegatum L.	Shoot	Shoots	Axillary shoot proliferation (increased when shoot tips were removed and inverted).	Orlikowska *et al.,* 2000.
Croton sublyratus Kurz.	Shoot tip	Shoots	Axillary shoot proliferation, shoot regeneration.	Shibata *et al.,* 1996
Croton urucurana Baill.	Callus	Leaf segments	Callus induced	Lima *et al.,* 2008
Emblica officinalis Gaertn.	Callus	Mature endosperm	Shoots, embryo like bodies, plantlets.	Sehgal and Khurana., 1985
Emblica officinalis Gaertn.	Callus	Mature embryo	Embryos, multiple shoots, rooted *in vitro.*	Tyagi and Govil,1999
Euphorbia antisyphilitica Zucc.	Shoot	Shoot explant	Shoot proliferation	Jakobek *et al.,* 1986
Euphorbia characias L.	Callus	Callus	Deferent levels of triterpinols	Ferriera *et al.,* 1992
Euphorbia esula L.	Callus and rhizobacteria	Callus	Cell alterations noticed.	Souissi *et al.,* 1996
Euphorbia esula L.	Hypocotyl segment	Hypocotyls / all the parts.	Studied the effect of auxins, cytokinins,	Davis and Olson, 1993

Taxa	Type of culture	Source of Explant	Result	Reference
Euphorbia esula L	Cell suspension	Stem callus, hypocotyls, Seedling root segments,	Organogenesis, plant lets, shoots, rooted *in vitro*	Davis *et al.,* 1988
Euphorbia lagascae Spreng	Bud, shoot	Apical shoot, axillary shoot	Rooting	Torres, 2004
Euphorbia lathyris L.	Shoot tips	Shoot tip	Adventitious shoot and organogenesis etiolated micro shoot rooting.	Ripley and Preece, 1986
Euphorbia nivulia Buch.-Ham.	Mesophyll	Mesophyll cells	Somatic embryos, multiple shoots, rooted *in vitro.*	Martin *et al.,* 2005
Euphorbia pulcherrima Willd.	Node	Node	Somatic embryogenesis	Jasrai *et al.,* 2003
Euphorbia pugniformis	Shoot	Tubercle	Shoot proliferation Crestate shoot proliferation	Balotis and Papafotiou, 2003
Euphorbia tirucalli L.	Axillary bud	Inter node	Adventitious bud proliferation, shoot regeneration	Uchida *et al.,* 2004
Excoecaria agallocha L.	Node	Node	Axillary sprouting	Rao *et al.,* 1998
Givotia rottleriformis Griff.	Embryo	Zygotic embryo	Callus, seedlings, shoots, rooted *in vitro*	Rambabu *et al.,* 2006
Givotia rottleriformis Griff.	Seed	Seeds	Seed germination	Rambabu *et al.,* 2005
Glochidion multiloculaire Mull-Arg.	Callus, shoot tip	Shoot apical meristem, nodes	Multiple shoots, rooted *in vitro.*	Yamuna *et al.,* 1995
Hevea brasiliensis Müll.Arg.	Callus	Callus	Embryogenesis	Ferriere *et al.,* 1992
Hevea brasiliensis Müll.Arg.	Anther	Immature anther	Somatic embryogenesis, callus induction, plant regeneration.	Jayasree *et al.,* 1999
Hevea brasiliensis Müll.Arg.	Immersion culture	Embryogenic callus	Somatic embryogenesis	Etienne *et al.,* 1997
Hevea brasiliensis Müll.Arg.	Callus	Tissue	$CaCl_2$ effect, embryogenic calli, friable calli.	Montoro *et al.,* 1995
Hevea brasiliensis Müll.Arg.	Somatic embryos	Somatic embryos	Observed the effects of desiccation, osmolarity of the medium and ABA on maturation.	Etienne *et al.,* 1993
Jatropha curcas L.	Node	Node	Axillary shoot proliferation	Datta *et al.,* 2007
Jatropha curcas L.	Axillary bud	Leaf segments, axillary buds, nodes	Adventitious shoots, multiple shoots.	Sujatha *et al.,* 2005
Jatropha curcas L.	Hypocoty, petiole and leaf bits.	Hypocotyls, petiole and leaf bits	Regeneration of adventitious shoots, rooted *in vitro.*	Sujatha and Mukta, 1996

Taxa	Type of culture	Source of Explant	Result	Reference
Jatropha curcas L.	Shoot tip	Shoot tip	Shooting rooted *in vitro*	Rajore and Batra, 2005
Jatropha elliptica (Pohl) Müll.Arg.	Nodal multiplicatio n	Nodes	Shooting, rooting, acclimatization	Campos *et al.,* 2007
Mallotus repandus (Willd.) Müll.Arg	Node, inter node	Node, inter node	Shoot induction, Shoot elongation, organogenesis.	Prathanturarug *et al.,* 2007
Mallotus repandus (Willd.) Müll. Arg	Node	Nodal, shoots	Shoot formation, rooted *in vitro*,	Kaewsuwan *et al.,* 2005
Manihot esculenta Crantz	Somatic embryo	Somatic embryo	Shoot organogenesis, somatic embryogenesis	Ma, 1998.
Manihot esculenta Crantz	Leaf	Young leaf lobes	Induction of somatic embryos, germination of somatic embryos shoot development	Raemakers *et al.,* 1993 b
Manihot esculenta Crantz	Apical meristem	Apical meristem	Virus free plants	Kartha *et al.,* 1974
Manihot esculenta Crantz	Leaf	Leaf explant	Direct and indirect somatic embryogenesis	Raemakers *et al.,* 2000
Manihot esculenta Crantz	Somatic embryo culture	Somatic embryo	Somatic embryo culture Differentiated, developed and germinated.	Groll *et al.,* 2002
Manihot esculenta Crantz	Root	Primary root	Observed the ability of primary root storage.	Ricardo *et al.,* 2007
Manihot esculenta Crantz	Axillary bud	Mononodal micro cutting	Plants were recovered.	Rommanee *et al.,* 2003
Manihot esculenta Crantz	Protoplast	Leaf	Protoplast division was observed.	Anthony *et al.,* 1995
Manihot esculenta Crantz	Cryopreserv ed shoot tips	*In vitro* grown shoot tips	Regeneration of shoots	Escobar *et al.,* 1997
Manihot esculenta Crantz	Primary embryo culture	Leaf lobes	Production of embryos	Raemakers *et al*., 1993b
Manihot esculenta Crantz	Axillary bud culture	Node, axillary buds, meristems	Formation of shoots and multiple shoots.	Konan *et al.,* 1997
Manihot esculenta Crantz	Node	Node	Multiple shoots	Bhagwat *et al.,* 1996
Manihot esculenta Crantz	Cotyledon	Cotyledons	Somatic embryogenesis	Konan *et al.,* 1994
Manihot esculenta Crantz	Callus	Floral tissue	Somatic embryogenesis	Woodward and Kaerlas., 2001
Manihot esculenta Crantz	Leaf lobes	Young leaf lobes	Somatic embryos, shoot and root axis.	Mathews *et al.,* 1993
Manihot esculenta Crantz	Node, Shoot apices	Shoot apices, nodes	Callus formation, shoot formation.	Villaluz, 2006

Taxa	Type of culture	Source of Explant	Result	Reference
Manihot esculenta Crantz	Meristems	Meristem	*In vitro* flowering	Tang *et al.*, 1983
Manihot esculenta Crantz	Leaf	Immature leaves	Adventitious Shoot, somatic embryogenesis	Ma and Xu, 2002
Phyllanthus amarus Schum. & Thonn.	Shoot, node and inter node ulture.	Shoot tip, node, inter node.	Multiple shoots, rooted *in vitro*	Ghanti *et al.*, 2004
Phyllanthus amarus Schum. & Thonn.	Synthetic seed	Shoot tip (alginate encapsulate)	Growth reduced plantlet formed	Singh *et al.*, 2006
Phyllanthus amarus Schum. & Thonn.	Shoot tip	Shoot tip	Multiple shoots, rooted *in vitro*	Bhattacharyya and Bhattacharya., 2001
Phyllanthus beddomii (Gamble) Mohanan	Seed germination	Seeds	Plantlet	Maridass and Thangavel, 2008.
Phyllanthus coroliniensis Walter	Axillary shoot	Nodal segments	Multiple shoots rooting initiated.	Catapan *et al.*, 2000
Phyllanthus fraternus Webster	Shoot tip, nodal	Shoot tips, nodal parts	Multiple shoots, axillary bud sprouting	Rajasubramaniam and Saradhi, 1997
Phyllanthus niruri L.	Node	Nodal segments	Shoots, multiple shoots, flowers and fruiting	Liang and Keng, 2006
Phyllanthus stipulatus Raf.	Axillary bud	Nodal segment	Callus induction, micro shoots, multiple shoots, rooted *in vitro, ex-vitro* flowering, regeneration.	Catapan *et al.*, 2001
Phyllanthus urinaria L.	Node and Callus	Node	Callus induction, multiple shoots, rooted *in vitro*	Catapan *et al.*, 2002
Ricinus communis L.	Cotyledon culture	Cotyledon	Shoot proliferation, multiple shoots, callus, *in vitro* rooted,	Kumari *et al.*, 2008
Ricinus communis L.	Bud culture	Apical and axillary buds	Shoot regeneration, Rooted *in vitro,* elongated.	Molina and Schobert, 1995
Ricinus communis L.	Shoot tip	Meristematic explant	Deferential cytokinin effects were studied.	Sujatha and Reddy, 1998

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-Diclorophenoxy acetic acid (2,4-D) on seed germination of the *Euphorbia esula*. In *Givotia rottleriformis* Rambabu *et al*., (2005) studied the effect of Gibberellic acid (GA_3) on enhancement of *in vivo* seed germination. Thapliyal (2004) reported twin seedlings in *Putranjuva roxburghii.* The transmission of juvenile rooting ability from seedlings to adults of *Hevea brasiliensis* was studied by Muzik and Cruzado (1958).

Cotyledonary nodes and hypocotyl tissue

Ricinus communis cotyledons, cultured on the medium with Murashige and Skoog's medium salts (MS) + B_5 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*. Cotyledinory explants of *Manihot esculenta* were first treated with 2,4-D (4mg/l^{-1}) then placed on MS + NAA (1-naphthaleneacetic acid) + BA + GA_3, 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^{-1}) 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 Sudarshanam (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-furfurylamino 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 Bhattacharya, 2001). Ghanti *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 $CaCl_2$, H_2O. 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)+ GA_3(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 utilissima*. Cryopreserved and thawed apices of *Manihot esculenta* were cultured on a 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 GA_3, 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 (2.3-46.5µM), BA (2.2-44.4µM) and TDZ (2.3-45.4µM) individually, the leaf segments were cultured on MS+ BA (8.9µM) + IBA (2.5µM) and got adventitious shoots (Sujatha *et*

al., 2005). Datta *et al.,* (2007) reported *in vitro* clonal propagation of biodiesel plant *Jatropha curcas*.

The shoot tips of *Glochidion multiloculaire* 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 antisyphilitica* 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 pugniformis* were cultured on the MS + sucrose 2% and NAA 0.1 mgl^{-1} and BA, gave cristate shoots of both forms. While 20% gave normal shoots. The rooting combination for cristate shoots was MS+ IBA ($0.5mgl^{-1}$), 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, 2003). Effects of several factors on rooting of Poinsettias was recorded by Sochacki and Chimid (1994). Acclimatization systems for micro cuttings of *Euphorbia fulgens* was developed by Zhang and Stoltz (1989). *In vitro* production of shoot buds in *Euphorbia pulcherrima* was carried out by Nataraja *et al.,* 1973.

Shibata *et al*. (1996) developed a micropropagation protocol for *Croton sublyratus* the results demonstrate that the feasibility of applying the shoot tip culture technique for enhancing production of plaunotol by cultivating uniform populations of *Croton sublyratus* with higher plaunotol level. The morphogenetic studies on cultured endosperm of *Croton banplandianum* was carried out by Bhojwani and Johri (1971).

Shoots of *Codiaeum variegatum* cultured on MS + BAP (4.4µM) and GA_3 (5.8µM) doubled the number of axillary shoots in comparison to non defoliated controls. An additional increase in shoot number was achieved when the explant shoots were placed on the medium horizontally or vertically in an inverted position with shoot tip down. Exogenous IAA decreased shoots number and 2,3,5-Triiodo benzoic acid did neither replace defoliation nor diminish the size of callus formed at the base of the shoots. Almost all the shoots obtained were able to root on the medium with IBA (1mg/l) and acclimatized (Orlikowska *et al*., 2000). Asma Nasib *et al*., (2008) grew the shoot tips of *Codiaeum variegatum* 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). Gunitilleke and Samaranayake (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) + $CuSO_4$ (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 the *Cleistanthus collinus* were cultured on + Citric acid (CA, 104.1µM) and Polyvinylpyrrolidone

(PVP) 40 (12.5 or 25μM) + BA (0.44μM) resulted in shooting. In general, the explants from basal sprouts were more suitable than terminal twig explants for the micropropagation (Qurashi and Mishra, 1998). The nodes of *Excoecaria agallocha* were cultured on MS + BA (13.3μM) + Zeatin (4.65μM) + IBA (1.23μM) 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 repandus* 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 (Kaewsuwan *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 embryoids. The embryoids 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 repandus* 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 (Prathanturarug *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 callus 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 balustinum* 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 Gresshaff and Doy's basal medium supplemented with auxins results in indirect somatic embryogenesis. Depending on BA concentration, plants can be transferred either directly to green house 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_2SO_4 for 10 days. The zygotic embryos of *Givotia rottleriformis* 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 1m 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 axillary 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 Veisseire *et al*. (1994b). Effect of conditioned media on the somatic embryogenesis of *H. brasiliensis* was reported by Veisseire *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 Thulaseedharan, (2001). Kumarijayasree *et al*., (2001a), optimized the parameters effecting somatic embryogenesis and long term somatic embryos were initiated and maintained by Kumarijayasree and Thulaseedharan (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 embryoids 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 nivulia*, Kn 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. GA_3 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 and tissue and organ cultures of *Hevea brasiliensis*

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 Kn (0.5mg/l) resulted in the induction of the callus. By culturing, the callus on MS + Kn (0.7mg/l) and NAA (0.2mg/l) produced maximum number of somatic embryoids. 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 panduraefolia* (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, GA_3, 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^{-1}) and the plantlets were acclimatized. Hadrami and Auzac (1992) studied the effect of growth regulators polyamine content and peroxidase activity in *Hevea brasiliensis* callus. $CaCl_2$ at a concentration between 0-9 mM was used in the callogenesis medium of *Hevea brasiliensis,* low concentrations induced the embryogeny, at high concentrations(12 mM $CaCl_2$) induced the friable callus. They observed that the $CaCl_2$ 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 CO_2 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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