REVIEW
OF
LITERATURE
Ayurvedic medicines are of plant origin. Either the whole plant or certain parts of leaf, flower, fruits, seeds, heartwood, stem bark, root, gum and resin are used for the treatment of diseases. The foremost aim of Ayurveda is to help people maintain health, treatment of diseases is only secondary. Indian medicine is based on the concept of a fundamental identity between man and nature (Khanna, 1987). Man is considered to be a microcosm of the macrocosm (Pal, 1989).

The Indian flora is very rich in medicinal plants. Over 7000 different species of plants found in the different ecosystems are said to be used for medicinal purposes in India. One of the earliest treatise of Indian medicine, the Charak Samhita (1000 B.C.), mentions the use of over 2000 herbs for medicinal purposes. Presently the Indian system of medicine uses about 1100 medicinal plants, of which five dozens are said to be in larger demand. Higher plants are still “the sleeping giants of drug development” (Fransworth & Morris, 1976), a virtually untapped reservoir of potentially useful sources of drugs (Fransworth, 1984), that will continue to serve mankind in the twenty first century as they have done since the dawn of history (Tyler, 1986).

Among the various samhitas Sushrut Samhita is well known to possess many formulations based on plants alone or in combination with animal products or organic substances and the salient features of plants are also described. Later, Nighantus as Bhavprakash, Dhanvantri, Raj Nighantu and Madanpal Nighantu have described the medicinal properties of different plant species. As a result of continued invasions by the foreign rulers in India work regarding Materia Medica was badly hampered. Credit goes to the workers who received the Indian Materia Medica and prominent among them are Rooplal
Vaish and Chandra Raj Bhandari, who contributed much in the field of Ayurveda. The Vanoushdhi Chandrodaya is the well-known work of Chandra Raj Bhandari.

In the 17th century most prolific writer was Bauhin (1623). "The Herbal" by Gerade in 1633 appeared on Indian medicinal plants. As early as in 1918, Basu compiled "Indian medicinal plants" which he revised with Kirtikar (1935) in 4 volumes. Between 1948 and 1966 came the "Wealth of India-Raw materials" by CSIR in several volumes by eminent Indian authors.

"The Indian sub continent represents, without doubt, one of the greatest emporia of ethnobotanical wealth," says Schultes. Many have ventured into this emporium to discover its wealth. Among them, the first names that come to mind are E.K. Janaki Ammel and S.K. Jain. Janaki Ammal initiated the ethobotanic study of subsistence food plants, particularly of the tribals, as an official program of the Botanical Survey of India. So she is considered as the pioneer in ethnobotany in India, while Jain, with his numerous books and papers on the subject is the leading researcher in the ethnobotany in India today.

In recent years, plant biotechnology has made an impressive progress as one of the frontiers of biotechnology of scientific and economic importance. Plant tissue culture involves the culture of all types of plant cells, tissues and organs under aseptic conditions. The definition also extends to the culture of excised embryos and to protoplast cultures (Smith & Drew, 1990). The origin of plant cell and tissue culture techniques lies back in the nineteenth century. The great German botanist Gottlieb Haberlandt (1854-1945) is now aptly regarded as the Father of Plant Tissue Culture. Haberlandt was the first person to culture isolated fully differentiated cells as early as 1898. He was able to isolate cells from higher plants and maintained in viable state for several weeks. After turn of the century, numerous researchers established growth and division of cells in culture.
Developments in the technology of plant tissue culture since its pioneering experiments by White (1934, 1937), Laibach (1925, 1929), Van Overbeck et al. (1941), Loo (1945), Gautheret (1934), Skoog (1944) and Murashige and Skoog (1962) have contributed in establishing a strong foundation for the applications of this versatile technology. Soon after working out the theoretical aspects of in vitro cultivars, plant scientists have made efforts to involve in the practical application of plant tissue culture i.e., micropropagation. Small amounts of tissue can be used to raise hundreds or thousands of plant in a continuous process. Ball (1946) successfully raised transplantable plantlets of *Lupinus* and *Tropaeolum* by culturing their shoot meristem. The utility of this technique was soon realized by Morel (1960) for rapid propagation of Orchids, *Cymbidium* and *Odontoglossum*. Murashige (USA) has developed standard methods of propagation of in vitro species ranging from ferns to foliage flowers and fruit plants. Chaturvedi et al. (1982) have reported that the explants eg. shoot apices, leaf segments, whole anthers and callus tissues of different plant species were used in studies regarding mass propagation. *Simmondsia chinensis* plants were raised in vitro by axillary shoot bud culture and grown in potted soil.

Tissue culture is a very useful system for the mass propagation of medicinal plants. Schieder (1985) has reported that plant, cell and tissue culture as well as genetic engineering, may be an alternative to conventional plant breeding for the improvement of medicinal plants. Cellarova et al. (1994) have reported that micropropagation of plants using tissue culture is valuable in plant breeding for provision of eg. disease resistant plant material. Nayak & Sen (1995) have reported that in vitro propagation of *Ornithogalum umbellatum* was carried out by direct organogenesis. Patnaik and Debata (1966) have reported a procedure for rapid in vitro propagation of aromatic and medicinal plants, *Hemidesmus indicus* (L) R. Br. from nodal explants. Debata (1996) has reported the micropropagation of *Bixa orelliana* Linn., popularly known as “annatto” an evergreen bush or small tree found native in tropical America. During the
past two decades, there has been a great interest and progress in *in vitro* propagation of medicinal plants using tissues, meristem, protoplast and organ culture. Tissue culture techniques have been established as a useful approach for *ex-situ* conservation of rare, endemic or threatened plant species.

*In vitro* morphogenesis from seedling explants and callus cultures of castor (*Ricinus communis*) was studied by Reddy *et al.* (1987). Morphogenesis was observed only from the hypocotyls and shoot tip explants and from shoot tip callus cultures. Kinetin proved superior to BAP for inducing shoot buds while NAA was superior to IAA for the induction of roots from the shoot tip callus.

Shoot regeneration from callus derived root cultures of *Plantago ovata* Forssk. (s.l. subgol) was reported by Barna & Wakhlu (1989). Adventitious shoots were induced from callus derived roots of *Plantago ovata* on MS medium containing IAA and Kn. Excised shoots produced roots on half strength MS medium supplemented with IBA.

*In vitro* propagation of *Picrorhiza royle* Ex. Benth. has been reported by Upadhyay *et al.* (1989). An efficient protocol has been developed for the induction of direct shoot multiplication in *Kaempferia rotunda* Linn. by Anand *et al.* (1991). MS medium supplemented with BAP and IAA were proved ideal for both shoot and root induction.

O'Dowd & Richardson (1993) reported *in vitro* organogenesis of *Ephedra*. Adventitious bud formation was obtained from stem internodal explants of *Ephedra fragilis* using a modified MS medium supplemented with BAP, 2,4-D and 3% sucrose. Bhardwaj & Ramawat (1993) reported effect of anti-oxidants and adsorbents on tissue browning associated metabolism in *Cocculus pendulus* callus cultures. Direct organogenesis from internodal
segments of *in vitro* grown shoots of Ipecac (*Cephaelis ipecacuanha*) Rich. was reported by Yamuna *et al.* (1993).

Anther culture of *Allium fistulosum* L. was studied by Saiju *et al.* (1994). Anthers of *Allium fistulosum* were cultured on N₆ medium supplemented with 2,4-D and kinetin. The callus developed from cultured anthers was subcultured on MS medium with coconut milk for the formation of green embryo like structures. These developed shoots after transfer to MS medium supplemented with BAP formed roots after transferring to non-sterile sand.

Enhancement of organogenesis by manipulating the medium and growth regulators in *Artemisia pallens* was studied by Usha & Swamy (1994). Modified MS medium with combinations of NAA and BAP induced proliferation of more callus and MS medium supplemented with kinetin induced more number of multiple shoots. The medium supplemented with IAA and also White's medium supplemented with NAA developed more number of roots.

*In vitro* morphogenesis of *Solanum psuedocapsicum* L. was reported by Liang *et al.* (1994). Micropropagation and callus induction of *Aristolochia bracteolata* Lam. was studied by Remeshree *et al.* (1994). Callusing was observed on MS medium containing combinations of kinetin and NAA supplemented with IAA. Shoots were raised from a single nodal segment when cultured on MS medium containing BAP, Kn and IAA from their basal region of shoots, roots were formed on the same medium without being transferred to any rooting medium.

Micropropagation of *Moringa pterygosperma* Gaertn. from hypocotyls and cotyledonary explants has been achieved by Mohan *et al.* (1995). Initially, multiple shoot regeneration was induced on MS medium containing 3% sucrose with NAA and kinetin. The regenerated shoot tips produced well-developed roots when grown on MS medium containing IBA.
Harikrishnan and Hariharan (1996) reported direct shoot regeneration from nodal explants of *Plumbago rosea* Linn. On MS medium supplemented with BAP and IAA nodal explants produced shoots without the intervention of callus. Shoots were transferred to the medium supplemented with IBA for root induction.

Gokul and Tejavathi (1996) reported *in vitro* culture and production of alkaloids in *Cissampelos pareira* L. (Menispermaceae).

*In vitro* clonal multiplication of *Alpinia calcarata* Rose. was reported by Agretious *et al.* (1996). Maximum number of shoots was obtained from rhizome bud explant on MS medium supplemented with BAP and IAA. The growth and multiplication rate was directly related with the number of subculture. These multiple shoots produced roots from the basal region in the same media. About 80% of the plantlets survived under field conditions.

*In vitro* protocol was developed by Naseem & Jha (1997) for mass propagation of *Cleome gynandra* DC, an important medicinal plant, was standardized using thalamus, gynandrophiore and root segments as explants. Optimum culture response was found in presence of NAA and BAP. Rooting from isolated shoot was obtained on medium (1/2 MS salts) containing IBA.

*In vitro* multiplication of *Alpinia galangal* (Linn.) Willd was studied by Anand & Hariharan (1997). On MS medium along with BAP and NAA, 80% explants produced an average of 6.9 shoots. 70% of the shoots produced roots on MS medium containing NAA.

*In vitro* organogenesis in *Aristolochia indica* (L.) (1997) was reported by Remeshree *et al.* Callus of *Aristolochia indica* was obtained from stem explant on MS medium supplemented with BAP. Bud and leaf initiation from the callus
was also observed within 40 days of culture on MS medium with BAP and NAA.

Sahoo and Chand (1998) described a protocol for rapid and large-scale propagation of the woody aromatic and medicinal shrub *Vitex negundo* through *in vitro* culture of nodal segments from mature plants. MS medium supplemented with BAP was most effective in inducing bud break. Rooting was best induced in shoots excised from proliferated shoot cultures on half strength MS medium augmented with an optimal combination of IAA and IBA.

Micropropagation of *Gmelina arborea* Roxb. through axillary bud culture was reported by Thirunavoukkarasu and Debata (1998). Axillary buds collected from actively growing young shoots of the epicormic and crown regions of a six year old elite *Gmelina arborea* Roxb. tree was used to initiate shoot cultures. Explants from the epicormic region produced shoots when cultured on McCown’s medium for woody plants supplemented with 2% sucrose. About 80% of the *in vitro* regenerated shoots rooted on IBA supplemented McCown’s medium within 7-9 days of culture. Plantlets were established in sterilized soil in a humidity chamber and transferred to field conditions with 100% survival.

Rajendra & D’Souza (1998) reported direct organogenesis from internodal segments of Mature *Murraya koenigii*. The internodal segments of mature *Murraya koenigii*, a medicinal and aromatic plant belonging to Rutaceae produced adventitious shoots with different concentrations of BAP and kinetin. Best response was obtained with BAP. Of the various auxin treatments for rooting of the shoots IBA proved to be the best. *Ex vitro* rooting of microshoots was achieved only with a combination of IBA and phloroglucinol.

Kumar *et al.* (1998) reported micropropagation of *Actinidia deliciosa* from axillary buds. The axillary shoot bud development was higher with BAP
alone and in combination with IBA. Shoot multiplication from axillary buds was achieved on a medium supplemented with BAP along with IAA. *In vitro* raised shoots were rooted in MS medium containing IAA.

Comparative study between normal and tissue cultured plants of *Bacopa monnieri* (L.) Pennell was studied by Tejavathi & Rao (1998).

Sahoo and Chand (1998) described a protocol for rapid clonal propagation of a medicinally important plant species, *Tridax procumbens* L. through *in vitro* culture. High frequency bud break and multiple shoot formation were induced from nodal segments on MS medium supplemented with BAP. Rooting of the excised shoots from secondary or subsequent cultures was best induced on half-strength MS medium containing IBA. Vermicompost was the most suitable planting substrate for hardening; its use ensured high frequency survival (96%) of regenerated plantlets prior to outdoor transfer.

A rapid and efficient protocol for regenerating shoots from aseptic seedlings of *Artemisia annua* L. was developed by Usha and Swamy (1998). MS medium supplemented with BAP was optimum for multiple shoot induction. *In vitro* shoots developed roots in MS medium supplemented with IBA.

*In vitro* propagation of *Pisonia alba* (L.) spanogae (Lettuce tree), a threatened species was reported by Jagadishchandra et al. (1999). *In vitro* culture of axillary buds on MS medium supplemented with cytokinins (BAP and
Kn) resulted in the development of multiple shoots. Roots were induced in multiple shoots on half strength MS medium fortified with auxins (IBA and NAA each). Regenerated plantlets were acclimatized on sand and soil (1:1) before transferring them to field condition, 95% of the plants survived the transfer.

Kathiravan & Iganacimuthu (1999) studied the micropropagation of Canavalia virosa (Roxb.) Wight & Arn. Multiple shoots were induced from nodal explants of field grown plants of Canavalia virosa when cultured on MS medium supplemented with a combination of BAP, GA$_3$ and kinetin. The MS medium containing BAP and kinetin stimulated multiple shoot formation, and BAP and GA$_3$ stimulated shoot elongation. MS medium containing IBA induced root formation. These clonally propagated plants were successfully transplanted to the field after hardening.

Tejavathi & Shailaja (1999) studied regeneration of plants from the cultures of Bacopa monnieri (L.) Pennell. Stem, leaf and flower bud explants were cultured on MS medium supplemented with various growth regulators either singly or in combination. Multiple shoots with roots were obtained from the callus derived from all the explants on MS medium supplemented with auxin/cytokinin individually or in combination. Maximum number of plantlets were obtained on a medium containing IAA or/and Kn. Regenerated plantlets were hardened on sterile distilled water and then tap water sequentially and transferred to soil.

Shahzad & Siddiqui (2000) achieved multiple shoot regeneration from nodal explant callus in Ocimum sanctum L. The explants cultured on MS medium supplemented with phytohormones released phenolic exudates that adversely affected the culture response; the explants turned brown and ultimately necrosed. The addition of ascorbic acid (AA) to MS medium checked
the release of phenolic exudates, and a slight callusing occurred on different hormonal supplements.

Rani et al. (2000) developed an in vitro propagation protocol for *Acorus calamus* from rhizome explants. Maximum shoot multiplication frequency was obtained on MS medium supplemented with BAP and IAA. Regenerated shoots were rooted in vitro or directly transferred to sterile soil.

Emmanuel et al. (2000) studied micropropagation of *Wedelia calendulacea*. It was micropropagated using axillary and shoot tip explants on MS medium containing Gamborg's vitamins. Nodal explants produced prolific multiple shoots on a medium containing BAP and kinetin. For rooting of the excised shoots IBA in half strength MS medium was found suitable.

A method for large scale multiplication of *Curculigo orchioides* through bulbil formation from leaf explant in shake flask culture was developed by Suri et al. (2000). Leaf explants were cultured in B5 liquid medium containing BAP, IBA and PVP.

Naomita & Rai (2000) studied in vitro regeneration of *Crotalaria lutescens* (Dalz.). In vitro shoots were induced from hypocotyls and cotyledonary node explants of *Crotalaria lutescens* on MS medium supplemented with plant growth substances. These shoots were rooted on MS medium containing different auxins with NAA. The plantlets were hardened in a 2:1 potting mixture of sand and soil and were established under greenhouse conditions. In vitro flowering was observed on a medium supplemented with BAP and NAA.

Sivakumar & Krishnamurthy (2000) reported the micropropagation of *Gloriosa superba* L. Saini & Jaiwal (2000) studied the in vitro multiplication of *Peganum harmala*. Of the various seedling explants, cotyledonary node
exhibited maximum shoot regeneration frequency from axillary region on MS medium supplemented with BAP. Addition of NAA enhanced the efficiency of BAP for multiple shoot regeneration as well as improved the growth of shoots. Regenerated shoots were rooted on MS medium containing IBA with 80% efficiency.

Philomina & Rao (2000) reported micropropagation of *Sapindus mukorossi* Gaertn. Bud break and multiple shoots were induced in apical and axillary meristems on MS medium supplemented with BAP. Excised shoots were rooted on MS medium supplemented with IBA. The regenerated plantlets were successfully acclimatized and transferred to soil.

Thoyajaksha & Rai (2001) reported the micropropagation of *Dictyospermum ovalifolium*. Nodal segments and shoot tips of *Dictyospermum ovalifolium* were used as explants for *in vitro* culture establishment. The best rate of shoot proliferation was obtained on MS medium supplemented with BAP. *In vitro* rooting of shoots was induced after 4 weeks on IBA.

Rapid *in vitro* multiplication of *Celastrus paniculatus* Willd. sub sp. *paniculatus* (Celastraceae) was reported by Nair & Seeni (2001).

Thiruvengadam and Jayabalan (2001) studied *in vitro* flowering of *Vitex negundo* L. *In vitro* regeneration and flowering were achieved from two explants on MS medium containing different concentrations of auxins and cytokinins. The highest percentage of flowering was noticed from nodal explants cultured on MS medium supplemented with BAP and NAA combination. Of the two explants tested, maximum number of flower buds was obtained from nodal explants than shoot tip explants. The regenerated shoots were transferred onto MS medium fortified with BAP and NAA for flower maturation and root induction. The regenerated plantlets have been successfully established in vermiculite soil.
Baruh et al. (2001) developed a protocol for high frequency plant regeneration in *Hypericum patulum* by shoot tip culture. Multiple buds were initiated from shoot-tips cultured on MS medium supplemented with BAP and kinetin. Addition of Thiamin HCl, Ca-pantothenate and biotin enhanced multiple shoot formation. Upon transfer to phytohormone free liquid medium following a brief exposure to auxin, root formation occurred from the micro shoots.


Bhattacharya & Bhattacharya (2001) reported high frequency *in vitro* propagation of *Phyllanthus amarus* Schum. & Thom. by shoot tip culture. Shoot tips were cultured in MS medium supplemented with kinetin/BAP singly or in combination with IAA. Kinetin was superior to BAP and kinetin-IAA combination was more suitable than kinetin alone. The cluster of proliferated shoots elongated and rooted simultaneously under the same treatment following another subculture, thus shortening the total time schedule of micropropagation. Micropropagated plants were successfully established in soil with high survivability (80%).

Ahmed *et al.* (2001) devised a protocol for high frequency shoot regeneration from nodal and shoot tip explants in *Holarrhena antidysenterica* L. Shoot tip and nodal explants when cultured on MS medium containing BAP with NAA and BAP with Kn produced multiple shoots. Addition of Urea in the medium increased the number of shoots. For best rooting, the shoots were excised from the culture flask and implanted individually on half strength MS medium with each of IBA, IAA and NAA. About 90% of plantlets survived under open field conditions.
Micropropagation of *Hyptis suaveolens* (L.) Poit. (Labiatae) through nodal culture was studied by Britto *et al.* (2001). High frequency of multiple shoots was achieved with nodal explant culture on MS medium supplemented with BAP and IAA. The elongated shoots were cultured for rooting on MS medium with NAA. The rooted plantlets were successfully established in soil.

Rani *et al.* (2001) reported micropropagation of *Tagetes erecta* L. through nodal segments. The shoots were initiated from nodal stem segments on MS medium supplemented with Kn.

Fast growing root culture of *Decalepis arayalpathra*, a rare endemic medicinal plant was established by Sudha & Seeni (2001) from leaf and internodal explants of *in vitro* raised shoot cultures in MS medium containing BAP, 2-ip and NAA.


Rudra & Juwarkar (2002) reported an *in vitro* multiplication system to fasten the propagation of a hybrid variety of *Punica granatum*, GBG-1 (Ganesh), on MS medium supplemented with BAP and NAA. Efficient rooting was achieved in half strength MS medium fortified with a combination of NAA and IAA.

Effect of copper and zinc on growth, secondary metabolite content and micropropagation of *Tinospora cordifolia* has been studied by Kumar *et al.* (2003). A successful *in vitro* micropropagation protocol from nodal explants of *T. cordifolia* has been formulated. Regeneration was obtained within three
weeks on MS medium supplemented with NAA and BAP. NAA along with BAP induced both shoots and roots.

A high frequency and rapid regeneration protocol via callus and directly from various explants was developed in *Withania somnifera* (L.) Dunal by Govindraju *et al.* (2003). Regeneration was observed from callus of internodal segments, leaf, root and petiole on MS medium fortified with BAP or in combination with IAA. Direct differentiation of shoots from leaf, nodal segments and shoot tips occurred within 2 weeks on MS medium supplemented with BAP in combination with IAA alone or along with IAA.

High frequency induction of multiple shoots from nodal explants of *V. negundo* L. using sodium sulphate was studied by Chandramu *et al.* in 2003. Highest percentage of explants for shoot induction and multiple shoot production were observed in the combination treatment of BAP, NAA and 5% sucrose supplemented with sodium sulphate. IBA was found most effective in inducing roots. The rooted plantlets were shifted to glasshouse for acclimatization and later transferred to the field with cent percent survival.

An efficient method was developed for *in vitro* propagation of *Solanum surattense* Burm.f. by Seetharam *et al.* in 2003. Multiple shoot formation was noticed on MS medium supplemented with BAP and Kn from nodal explants and BAP and Kn from leaf explants. Efficient rooting was achieved on half strength MS medium supplemented with IBA and Phloroglucinol.

Nagaraja *et al.* (2003) reported an efficient protocol for plantlet regeneration through the leaf calli of *Andrographis alata* Nees. Shoot bud induction was recorded in MS medium supplemented with BAP and NAA. The microshoots rooted well on MS medium containing BAP and NAA.
Velayutham and Kumari (2003) reported direct shoot regeneration from leaf explants of Chicory (*Cichorium intybus* L.). *In vitro* plantlets were directly regenerated from the leaf explants in MS medium supplemented with different concentration of IAA/IBA in combination with BAP. Roots were initiated in MS medium supplemented with IAA/IBA/NAA. The rooted plantlets were well established in the field.

An efficient protocol for *in vitro* clonal propagation of *Rauvolfia serpentina* L. was developed by Tiwari et al. (2003).

Sudhakaran and Sivasankari (2003) developed a protocol for *in vitro* plant regeneration in *Ocimum basilicum* L. Callus initiation from the leaf and internodal explants were observed on MS basal media supplemented with 2,4-D and kinetin. Of the two explants used for micropropagation, apical buds showed minimum response whereas the nodal explants exhibited maximum response. Of the two cytokinins used, both BAP and kinetin were 100% responsive in shootlet formation. NAA was found to be more suitable for rootlet formation.

Micropropagation of *Centella asiatica* through axillary buds have been studied by George et al. (2004). Of all the concentrations and combinations tried, BAP either alone or in combination with IBA was found most effective for axillary bud multiplication. Best result for rooting was obtained on medium supplemented with NAA. Well rooted healthy shoots were transferred to small polycups containing 2:1 potting mixture of sand and soil.

Vadawale et al. (2004) established a protocol for axillary bud multiplication and indirect organogenesis for *Withania somnifera*. MS medium with BAP, kinetin and IBA induced an average of five shoots per node. Shoots regenerated *in vitro*, rooted on MS medium with IBA. Ninety percent of the rooted shoots survived when transferred to green house and subsequently to the field.
Hiren et al. (2004) studied in vitro regeneration of Curculigo orchioides. Morphogenetic compact callus derived from leaf and rhizome explants differentiated shoots on MS medium along with BAP and Kn. Good rooting response was observed when regenerated shoots were inoculated on modified White root culture medium. Such plantlets were successfully transferred to soil after hardening, with a high rate of survival.

Vijay & Kumar (2004) reported in vitro regeneration studies on Asparagus racemosus. Nodal segments produced multiple shoots while apical meristems and cladodes proliferated into callus. Among the media tested, MS medium with NAA and Kn favoured the multiple shoot induction in 80% cultures while the increased amount of NAA promoted the callus induction.

Swamy et al. (2004) studied in vitro propagation of Solanum surattense. Maximum number of shoot bud proliferation was observed in MS medium supplemented with kinetin. The in vitro regenerated shoots produced more number of roots on half strength MS medium containing IBA followed by IAA.

Plant regeneration and in vitro flowering from leaf and nodal explants of Solanum nigrum (L.) has been reported by Jabeen et al. (2005). High frequency and maximum number of multiple shoots were obtained from leaf and nodal explants on MS medium supplemented with BAP and IAA. Regenerates when transferred to rooting medium initiated flowering along with rooting. In vitro flowering facilitates in vitro pollination and fertilization.

Ray et al. (2005) for large scale propagation using young capitulum explants in Gerbera jamesonii developed an efficient protocol. MS medium supplemented with BAP in combination with IAA was found to be most effective in initiating multiple shoots at the rate of 10 shoots per explant. Rooting was achieved with IAA in combination with IBA.
Shashikala et al. (2005) reported in vitro regeneration of Centella asiatica L. (urb.) (Apiaceae) and preliminary estimation of secondary cell constituents in methanolic extract of in vitro regenerated plants. Nodal segments were used as explants for in vitro regeneration on MS media supplemented with BAP and NAA. In vitro rooting was induced when MS medium was supplemented with IAA.

Yadav & Padmaja (2005) established a simple protocol for plantlet development through multiple shoot induction, elongation and their rooting from mature seed explants of Cajanus cajan (L.) Millspaugh cv ICPL 93715. MS medium supplemented with BAP appeared to be adequate for inducing an average of 8-18 shoots/explant. MS with BAP promoted shoot elongation, while half strength MS medium with IAA/IBA along with Kn facilitated rooting.

In vitro shoot multiplication from nodal and shoot tip explants was achieved by Kumeri et al. (2005). Maximum number of shootlets was obtained on MS media containing BAP & IBA and IBA was found to be best for root induction.

Jain and Chaturvedi (2005) achieved plant regeneration via multiple shoots in Hyptis suaveolens. The apical buds of the three days old seedlings could be stimulated to undergo multiple shoot formation on MS medium supplemented with BAP with Kn and NAA. On the same medium rhizogenesis was noticed after 15 days of culture initiation.

Generally, medicinally important plant products are “secondary metabolites” which are synthesized at variable periods of growth and developments. By definition these metabolites are either biologically or pharmacologically active compounds. As they are produced in low amounts, substantial quantity of raw plant material is required to extract only a few grams of the useful products. Therefore, novel methods have to be adopted that would
help to enhance the biosynthesis of secondary products so that the prevention of further loss of plants can be augmented with yield. The work on secondary metabolites representing one or the other biological properties has earlier been received by Bell (1980), Bell & Charlwood (1980), Luckner (1980), Balandrin et al. (1983) and Balandrin and Klocke (1988).

The earliest detailed reference of plant cell cultures as an industrial route to natural product synthesis dates back to 1956, when Routien & Nickell applied for a patent between 1950 to mid 1970's. Production of secondary metabolite was enhanced in cell cultures than those expressed by parent plants, e.g. ginseng saponins and harmin (Reinhard et al., 1968) diosgenin (Kaul et al., 1969).

After 1973, a turning point in cell culture technology demonstrated reasonable yields of derived secondary metabolites (Zenk, 1978; Nickell, 1980; Fowler, 1982; Srivastava et al., 1993). Since then many workers have reported the products of secondary metabolites by cell cultures where the yield and synthesis of compounds exceeded or approached to the levels occurring in the natural plant species (Constabel et al., 1974; Butcher, 1977; Khanna, 1977, 1980; Staba, 1977, 1980; Styles, 1977; Tabata, 1977; Bohm, 1978; Misawa & Samejima, 1978; Tabata et al., 1978; Zenk, 1978; Dougall, 1979 a, b; Kurz & Constabel, 1979; Barz & Ellis, 1981; Deus & Zenk, 1982; Shargool, 1982; Beellin, 1984; Fowler, 1984; Maldonado-Mendoza & Loyala-Vargas, 1995; Purohit et al., 1995 a,b; Gulati et al., 1996; Datta & Srivastava, 1997).

An enhancement in the yield of secondary product synthesis was earlier believed to be dependent on prolonged tissue culture or organ formation in the cultures. Subsequently, it was realized that the ability of product biosynthesis may continue throughout during the culture regime and can be detected at specific stages of growth and differentiation (Dougall, 1979a, b).
For obtaining high yield of specific secondary metabolites cell suspension systems have proved very effective (Berlin, 1984; Heinstein, 1985; Collinge, 1986). The cell growth and secondary metabolites both can be manipulated by changing the media and culture conditions. Increase in yield has been observed in systematic studies that were based on biosynthetic pathways and localization of secondary metabolites (Shuler et al., 1984; Staba, 1985). Stress conditions have proved crucial in increasing the yields (Di Cosmo & Towers, 1984; Heinstein, 1985; Srivastava & Purohit, 1994). In addition it has been possible to select and isolate cell lines that are capable of producing several fold higher yields of the active principles than those obtained from the natural plants (Anderson et al., 1983).