REVIEW OF LITTERATURE
Review of Literature

2.1. General

The term Tissue Culture covers a wide range of techniques involving induced growth of explants under controlled aseptic conditions of light, temperature and humidity and where plant organs such as shoots, roots or embryos or alternatively the culture of tissues or single cells or even cells devoid of cell wall known as protoplasts. It serves as an experimental tool in basic studies in physiology and biochemistry, genetics and plant breeding. This technique has become extremely significant for a number of practical applications in agriculture, horticulture and forestry. Tissue culture methods can therefore, serve as aids to plant breeders in two ways, viz., as a tool to supplement in the conventional methods of breeding and as a method to enhance genetic variability through the development of ploidy levels and somaclonal variation. While culturing of embryos, ovules and ovary can be used for the former, protoplast culture and fusion techniques will be useful for the latter. Adoption of cells, anther or microspore during tissue culture are relevant to both the methods. One of the areas in which tissue culture has assumed tremendous significance is the vegetative propagation of difficult to propagate plants or in plants wherein asexual methods of propagation do not exists or are not successful. The methods that are available for the asexual propagation of plants via tissue culture are (a) activation of the develop-
ment of pre-existing apical and axillary buds (meristems) and induction of neoformation of organs or embryoids from differentiated or dedifferentiated tissue(s). Since the thesis deals mainly on axillary and apical bud (meristems) culture and also on organogenesis from callus of derived from various segmental origin of *A. chinensis* this review is therefore, relatively restricted to these topics only.

2.2 Shoot Tip And Axillary Bud Culture:

All plant populations which are propagated through seeds resulting from cross pollination exhibit fairly a large amount of variation. Besides this heterogeneity, in many temperate and semi temperate perennial taxa the phenomenon of juvenility further lengthens the aestivation period before these plants attain flower and fruit bearing. Efforts to minimize this prolonged juvenility and practice began ever since agriculture became organized. However, rooting of cuttings is difficult and/or almost impossible in many orchard trees. It is a common practice to resort to budding and/or grafting of cuttings on good root stocks wherever possible. This technique has also met with limited success in view of its inherent disadvantages. All these have necessitated to look for an easy and new alternative method which is more rapid and efficient. In recent years *in-vitro* technique of plant propagation has come to fill in a major gap in this realm. Thus, quite a few temperate orchard and nut species are now being routinely multiplied rapidly by tissue culture methods (Skirvin et
The potential of tissue culture technique and its refinement could be exploited in a number of recalcitrant species for their rapid multiplication (George and Sherrington, 1984; Rao and Lee, 1986).

Developmentally shoot tips are nothing but condensed stems of several internodes with a growing apical meristem and few associated foliar initials and nascent axillary bud sites. In angiosperms, each leaf axil (at the junction of leaf to the stem possesses one or two presumptive sites of axillary buds (Esau, 1977)). However, in nature, due to the phenomenon of apical dominance these axillary bud meristems remain suppressed (dormant) and the ones farther away from apex only grow and develop. Under appropriate cultural conditions these axillary buds situated very close to the growing apical meristem can be induced to resume their growth and development. This is the basic premise of shoot tip and/or axillary bud culture.

The concept of employing shoot tip culture for rapid multiplication of plants could be credited to Georges Morel (1960; 1964). Basically his initial intention was to recover virus free Cyathidium by isolating and culturing the shoot meristems from infected plants. Generally viruses are absent in the apical meristem region. In this exercise, he observed later that the initial protocorm-like bodies (PLBs) developing from the cultured shoot tips could be sub-divided and each of which may be induced to form PLBs
under appropriate *in-vitro* milieu. Thus, from a single shoot tip he recovered many PLBs which further grew as plantlets. It could be continued *ad infinitum*. These procedures were further refined by various workers and have been used routinely by orchidologists for rapid multiplication of several orchids. The refinement of this technique further paved the way for *in-vitro* multiplication of other higher plants.

Despite the success of recovering *virus free* *Cymbidium* through shoot tip culture and the exploitation of "mericloning", the technique almost remained confined only to the recovery of specific pathogen-free plants in certain agriculturally and horticulturally important species and in other orchids. In spite of sporadic reports appearing in various journals from time to time on the attempts to recover plantlets via neoformation of organs and embryoids, it took almost a decade to realize the usefulness and extension of this technique to other important crop plants (Murashige, 1974).

Essentially there are some sequential steps involved in successful micropropagation schedule employing shoot tip and axillary bud meristems. Murashige (1974) classified and elaborated these into three stages viz., (a) *in-vitro* establishment of the initial explant, (b) multiplication of propagules and (c) rooting of isolated shoot buds and preparation for growing in natural environment (harden­ing and acclimatization). However, the stages may vary
depending upon the species and its nutrient requirements, plasticity of genotype and progress of cultures (Maene and Debergh, 1986).

It has been generally observed that explants from seedlings and juvenile phases respond to cultural manipulations much more readily than those from mature trees. For example, Lagerstroemia flos-reginae explants from mature flowering tree needed highest level of 6H.1N6—benzylaminopurine (15 mg/l) to induce maximum multiple shoot production. Shoot production from mature tree was lower and the time required for shoot formation was longer than those in young non-flowering explants (Pally and V'souza, 1986). In the case of Liquidambar styraciflua the rooting percentage of mature material was lower than that from juvenile material (Sutter and Barker, 1983). De Fossard et al. (1977) used axillary buds from young actively growing stem sprouts to raise Eucalyptus plants. His attempts to culture shoot tips and axillary buds from mature trees were not successful. However, Chalupa (1977) obtained the proliferation of shoots from axillary buds of some 10–20 years old trees like Ulmus species, Quercus rubra, Fagus sylvatica and Acer pseudoplatanus ssp. He succeeded in rooting these under in-vitro proliferated shoots ex-vitro in a peat perlite mixture with about 80% survival. From these observations it may be surmised that though seedling materials may be ideal for employment in tissue culture based multiplication programme, juvenile tissues and/or actively growing shoot tips and
axillary buds from mature trees responded well at least in certain cases, provided appropriate nutrient conditions are incorporated in the medium.

In the first stage of *in-vitro* establishment of the initial explant, there are two main constraints, viz. contamination (bacterial and fungal) and browning of explants. The major problem confronting tissue culture scientists is contamination of explants from woody perennials, especially if the explants are isolated from trees growing outdoors. Elimination of this requires utmost care in handling of specimens at two stages, (i) prior to the collection of explants and (ii) after excision of the materials. Pretreatment of the mother plant by spraying a mixture of antibiotics and systemic fungicide in the field, before collecting the explants was found to be effective in partially preventing subsequent fungal or bacterial infestations from spores those remain unsterilized in many cases during the culture. However, spraying of chemicals causes logistical problems especially in case of large trees (Reussard et al., 1977) after excision. Generally the explants are treated with some chemical sterilants like calcium hypochlorite, sodium hypochlorite or mercuric chloride which invariably helps in surface sterilization.

Lethal browning or blackening of the nutrient media and/or explants by leachates from inocula is yet another problem. This is believed to be the result of oxidation of polyphenols which exude from the cut surface of explants (Rhodes and Wootorton, 1978). Further, the
polyphenols and their oxidised products react with constituents of the medium and these compounds often can be toxic to the explant (Hu and Wang, 1983; Compton and Preece, 1986). To overcome this, substances like activated charcoal, polyvinylpyrrolidone, ascorbic acid, citric acid and/or L-cysteine are added in the nutrient media (George and Sherington, 1984).

The addition of activated charcoal must be undertaken cautiously as it absorbs some of the growth regulators like auxins and cytokinins (Preil and Engelhardt, 1977; Anderson, 1987). Modification of redox potential by immersing explants prior to inoculation in solutions of dithiothreitol and glutathione are also found to be effective in preventing blackening of tissues due to the removal of quinones through polymerization (Loomis and Battaile, 1966).

Other methods for the alleviation of this blackening include incubation of cultures in the dark, e.g., roses (Pittet and Monocousin, 1982) and/or reducing the mineral concentration of the nutrient formulation, e.g., apple, and tea (Werner and Boe, 1980; Sharwar, 1985). While the former could be attributed to the inhibition of the light induced polyphenoloxidase activity, the latter could be due to the interaction among nutrient ions. From this it is clear that browning of media and/or explant could be overcome in many cases by certain modification of nutrient components or by adding some specific enzyme inhibitors or adsorbents in the medium.
The orientation of inocula on the culture media is important especially for polarity and directed (vertical) supply of nutrients (Sinnott, 1960). For example, the response of the internodal explants of tea varied depending on the orientation of inoculum in the culture medium (Ogutu-ga and Northcote, 1970). On the other hand in apple, nodal explants cultured vertically with proximal end in the medium produced more shoots than nodes placed horizontally when the majority of culture had only single shoots (Hutchinson, 1982; Zimmerman and Fordham, 1989).

Role of phytohormones in the overall growth and development phenomena both in-vitro and ex-vitro is well documented. In majority of cases, for the successful micropropagation, incorporation of plant growth regulators at different concentration has been found to be indispensable at various stages of development. For shoot proliferation, cytokinins are an important component of the medium. This is quite understandable as they have been implicated in sprouting of buds and shoot growth in several in-vitro and in-vivo systems (Nii and Furoiwa, 1980). Ever since the classical work of Skoog and Miller (1957) on the interaction between auxin and cytokinin in root and shoot bud differentiation in tobacco callus cultures, it was surmised that organogenesis is regulated by appropriate ratios of auxins and cytokinins which are essential for triggering specific morphogenetic phenomena. However, this ratio varies with each cultivar explant and/or species. For example, for the in-vitro proliferation of pecan shoots the combination
of BA and NAA (indole-3-butyric acid) at 4:1 was found to be suitable (Wood, 1982), whereas BA and NAA (1-Naphthaleneacetic acid) at the ratio of 74:1 was found to be best suited for multiple shoot differentiation in sour cherry (Borkowska, 1983). Welander (1985) in his investigation observed that a ratio of 44:1 of BA and IBA was optimal for the proliferation of Ribes grossularia shoot tip culture. While in certain cases, the presence of an external supply of auxin was observed to be essential for rapid proliferation of shoot buds, there are other instances where incorporation of auxin proved to be inhibitory. For example, the presence of NAA with BA in the medium increased shoot tip necrosis in the cultures of Pistacia vera (Barghchi and Alderson, 1985), as opposed to BA alone. Similarly, Passey and Jones (1983) reported that auxin had no effect on shoot proliferation of cocoa. In fact auxin was inhibitory when IBA or NAA was added with BA in proliferation medium. Thus, it is very clear that at least for shoot tip establishment and proliferation, the presence of an external supply of auxin is not absolutely essential in all systems. On the contrary, in certain cases it has even proved to be inhibitory instead of promotory.

In many cases the optimal responses of the explants in terms of proliferation rate, vigour of the bud sprouts, and subsequent rooting percentage varied depending upon the kinds of cytokinins present in the nutrient medium.
In the case of *Salix matsudana* the maximum number of shoot buds proliferated when BA was incorporated in the medium (Bhojwani, 1980). Of the two other cytokinins tested, while 2iP (2-isopentenyl adenine) was completely ineffective, but kn induced much lesser number of shoots than BA when added to the medium. In the case of olive, out of seven cytokinins investigated, zeatin induced the most satisfactory shoot proliferation from shoot buds cultured *in vitro* (Rugini, 1981). *Azalea* shoot tips proliferated abundantly in zeatin containing nutrient formulation compared to that of BA or kn incorporated nutrient milieu (Fordham et al., 1982). *Camellia japonica* shoot buds responded much better in terms of proliferation in the presence of BA while kn had no effect (Samartin, et al., 1984; Rajasekaran and Raman, 1986). On the contrary, the cytokinin, viz., kn, 2iP and zeatin were inferior to BA on shoot multiplication of *Atractylodes lancea* (Hiraoka et al., 1984). Incorporation of BA in the culture media produced the greatest number of shoots whereas substitution 2iP by BA completely failed to evoke axillary bud proliferation in *Quercus shumardii* (Bennett and Davies, 1986). Zeatin containing media was capable to produce longer and more vigorous shoots than those having BA but did not promote the multiplication of axillary shoots in the case of Chinese chestnut (Qi Guang et al., 1986). Even though almost all the cytokinins like kinetin, zeatin and 2iP have been found to be effective in inducing multiplication of shoot buds. Thus, BA seems to be the most potent cytokinin in a large number
or species for shoot bud multiplication.

Needless to mention, as with almost all chemicals, besides the kinds, the concentration plays a major role in eliciting the morphogenetic phenomena in vitro culture. For example, in Vitis, even though shoot production occurred from 1 to 40μM BA, it was maximum at 5 μM. Higher concentrations are detrimental to shoot multiplication and lower ones less proliferative (Chee and Pool, 1985). In Chinese chestnut nodal explant cultured with BA at 0.44μM promoted the proliferation of axillary shoot bud but in creasing of concentrations from ten hundred folds inhibited budding and elongation rather they promoted callus formation (Qi Guang et al., 1986). Shoot production of Gardenia jasminoides was achieved from cultured shoot tips in low salt medium supplemented with 20 to 90μM 2,4D (Economou and Spanoudaki, 1985). However, they observed that optimum concentration of 2,4D was 45 μM for shoot proliferation. Of the different concentrations of BA tested (1.3 and 6 μM) for the shoot multiplication of Leucaena leucocephala, 3 μM concentration proved to be the best (Dhawan and Bhujwani, 1986).

According to Murashige (1974), the third stage in the micropropagation scheme is rooting of in vitro produced shoots. Rooting can be induced quite readily in most herbaceous species, but has not been easier in many woody species especially if initial explants are from mature trees. Though there are three phases involved in micro-
genesis viz., induction, initiation and elongation (Hu and Wang, 1985). It is rather difficult to isolate one phase from the other. Similarly, the kinds and concentrations of auxins as well may affect the intensity and rapidity of rooting. However, in some cases the root initiation and development take place in the basal medium itself, devoid of growth regulators. This is believed to be due to the young developing shoots which themselves possess the sites of auxin synthesis, thereby supplying the necessary amount of such auxins for root initiation and development. Under such circumstances, incorporation of auxin to the medium may be unnecessary and/or even may prove to be supraoptimal and thus become inhibitory (Hasegawa, 1980; Papachatzis et al., 1983; Evaldson and Weiander, 1985).

Based upon the ease with which rooting takes place, the shoots may be characterised as (a) those which initiate roots on transfer to ex-vitro media such as soil, perlite and/or vermiculite (Hosier et al., 1985; Zimmerman, 1988), (b) those which require a transfer to a hormone-free basal medium and (c) those which have to be subjected to a brief spell of culture in a nutrient medium with auxin and later transplanted to an auxin-free medium (Sutter and Barker, 1986; Mascarenhas et al., 1982). In the first category of shoots, rooting can again be improved qualitatively and quantitatively by quick old method prior to planting ex-vitro. Lane and McVaghaid (1982) termed this as chronic auxin treatment. and it was helpful in root induction in several species (Vieitez et al., 1987; Bennett
and Davies, 1985; Samartin et al., 1980; Hildebrandt and Hosier, 1985; Sobczykiewicz, 1984). However, certain cultures will not root if transferred directly *ex-vitro*. These have to be cultured in a hormone free basal medium during which rooting is initiated. In such cases a factor critical for root initiation and development is the concentration of minerals in culture (Kartha et al., 1981; Kato, 1985; Cockrell, 1986; Sebastian and McClomo, 1986). In some cases, this two step-rooting procedure is usually necessary in order to allow root extension since the inhibitory effect of auxin on root elongation is well known (Thimann, 1977) and also the continued presence of auxin leads to excessive callusing at the base of the shoot.

Residual cytokinins from shoot proliferation medium is known to inhibit root induction in many cases (Takayama and Misawa, 1980; San Jose et al., 1984; Van Nieuwkerk et al., 1986). In order to circumvent this, many workers incorporated charcoal in the rooting media. This helps in absorbing the residual cytokinin as well as toxic substances in the medium, thereby improving the initiation and development of roots (Shir and Erez, 1980; Takayama and Misawa, 1980; Sobczykiewicz, 1984).

In many cases, it has been observed that liquid media with filter paper wicks (Vaccov and Van, 1981; Kato 1985; Pink and Waineko, 1984) or with sterile foam (Gebhardt, 1985) fared better than the semisolid agar media with regard to the initiation and development of roots. It is, there-
fore, surmised that one of the limiting factors could be the poor availability of oxygen in agar based media. However, in most of the cases agar based media were successfully used for rooting.

The ultimate success of any commercial tissue culture programme entirely depends upon the ability to successfully transplant the intact plants into the soil. This is a crucial phase and if not handled properly, it may result in heavy causality. Essentially, it is an acclimatization process of plants that so far were growing in highly protective, sterile and humid environment. Since plants derived in-vitro are very soft, lacking a thick cuticle, the rooted plants can be hardened off briefly in a high humidity level of the environment which can then be lowered gradually over one or two weeks period. Light intensities during hardening period also need to be increased slowly so that the plantlets become self-sufficient in terms of their own evapotranspirational and photosynthetic characteristics (Hutchinson and Zimmerman, 1987). During transplanting, the plants should not be exposed to the full intensity of sunlight immediately. It is wise to provide protective shades with cover material until the plants have adjusted to the new environment. The survival rate of saplings depend upon shoot/root ratios of plants (Sommer and Caildas, 1981). Sanitation should be practiced to minimize transplantation loss associated with infections.

The plants derived from shoot tip (McLown and Amos, 1979; Hammerschlag et al., 1987), axillary bud
Barvey et al. (1984) and meristem cultures (Hurasnige, 1974b) are uniform and do not exhibit any variability. Such plants are more valuable because they are genetically uniform and stable. In fact they are clonal offspring, having genetic stability unlike those produced by other in-vitro methods like organogenesis and embryogenesis especially from dedifferentiated tissues via callus media. Even though in axillary bud proliferation the initial multiplication rate may be slow, it nevertheless increases during subsequent subculture cycles. By using shoot tip and axillary bud culture, millions of plants can be produced from a single explant within a very short time-span depending upon the nature of the species (Boxus et al., 1977; Kuzic, 1983; Hiraoka et al., 1984). It has also been reported that tissue cultured plants are more robust, attractive and uniform compared to those raised conventionally (Murasnige, 1974; Zimmerman, 1988).

In any clonal selection programme, micropropagation method can be used advantageously not only for rapid multiplication of new selected genotypes available in limited numbers but also to establish propagation of stocks in a much shorter time span (Chee and Pool, 1985; Economou and Sparondaki, 1986). In commercial nurseries, micropropagation can also be used to minimize the growing space usually provided for the maintenance of stock plants (George and Sherrington, 1984). Further, in-vitro vegetative propaga-
tion techniques could also be used for germplasm preservation and easier international exchange (Kartha, 1981).

Despite several attempts on tissue culture studies of plantation crops like coconut, ginger, turmeric, coffee, rubber, cocoa and cardamom, it is doubtful whether any of these except cardamom, turmeric and ginger to a limited extent have attained a level where it can be adapted for commercial exploitation, (Kuruvinashetti and Iyer, 1981; George and Sherrington, 1984; Rao and Lee, 1986). This is especially true in the case of *A. chinensis* where there is an urgent need to adopt the in-vitro methodologies mentioned earlier.

Unlike the other tree species legume tree tissue culture is comparatively recent. Earlier work on leguminous plant tissue cultures was devised to study the infection of legumes roots with *Rhizobium* (Raggio and Raggio, 1956; Raggio, Raggio and Torrv, 1957). The excised roots of *Glycine max* and *Phaseolus vulgaris* were provided with organic nutrients via basal cut ends of the root inserted in a small vial filled with medium, while apical (root cap) portion was allowed to grow into an inorganic medium in a petri dish. The arrangement stimulated *in-vivo* conditions, i.e. the root base normally absorbed the organic metabolites, whereas the inorganic metabolites are absorbed through the root tip region. The *Rhizobium* infects the roots via root hairs, so the inorganic medium was inoculated with the microorganisms. Cartwright (1967) and Torrv (1978) reviewed these technique for symbiotic studies of root culture. Later on Bonner (1942)
cultured the isolated roots of Accacia leonocystis and
seelinger(1958) tried on Robinia pseudoaccacia.

Since 1980's there were sporadic reports on suc-
cessful in-vitro culture of many legumes mainlly Albizia
lebbeck(Gnyrayai and Maheswari, 1981; 1982), A. odoratissima
(Phukon and Mitra,1983), A. richardiana (Tommer and Gupta
,1988) Robinia pseudoaccacia(bargnchi, 1987), Leucana
(Dhawan and Bhojwani,1985).

In 1983,Phukon and Mitra were first to obtained
direct multiple shoot - buds from apical shoot tips of A.
odoratissima seedlings using BA in MSBM. Borthakur (1994) obtained multiple shoots when germinated seeds were incubat-
ed on semisolid MSBM with 1mg/l BA. Multiple shoots de-
veloped from the cotyledonary nodes of Accacia tortilis when
cultured in MSBM supplemented with different concentration
multiple shoots from epicotyl and hypocotyl segments excised
from in-vitro grown seedlings of Accacia auriculiformis in
half strength of MSBM supplemented with BA, NAA and BA5.
Rajasekaran (1990, 1992, 1993) was the first who did mass
scale propagation from field grown plant of Maesopsis
eminii, Acrocarpus fraxinifolius using Woody Plant Basal
Medium (McCown and Lloyed, 1981) with BA and NAA. Multiple
shoots were obtained from the nodal segments of 20 years old
plant of Accacia auriculiformis using BA and NAA in MSBM
with 2% sucrose (Keddy, 1975). Raghavan Swamy et al1 (1972)
obtained multiple shoots from the nodal explants of root
suckers of 10 years old rosewood (*Delbergia latifolia* KoO&b.) using BA, NAA, L-glutamine in MSBM. Induction of multiple shoots from excised hypocotyl and shoot tips of *in-vitro* grown seedlings of rosewood was reported by Nataraja and Sudhadevi (1984), Ravishankar Rai and Jagdish Chandra (1989).

2.3 Organogenesis

**Callus** is a loosely arranged unorganised mass of thin walled parenchymatous cells, arising from the proliferating cells of parent tissue. Frequently, as a result of wounding, callus is formed at the cut end of stem or root. The most important characteristics of callus is that this abnormal growth has the potential to develop into normal roots, shoot and embryos which can be converted into plantlets.

Establishment of callus from the explant was divided into three steps (Dodds & Roberts 1985) induction, cell division and differentiation. During the initial stage of induction, metabolism is stimulated on the cells preparing for division. The length of the phase is depending upon the culturing condition and the physiological status of the explant. 2nd phase is of active cell division and the 3rd phase is the cellular differentiation stage (Aitchison *et al.*, 1977). Some callus growth are heavily lignified and hard in texture, whereas others break easily into small fragments and are called friable callus.
The main problem associated with callus culture is genetic instability created in the cell populations due to chromosomal and genetic variations within the cultures. There may be chromosomal aberrations, nuclear fragmentation and endoreduplication resulting in polyploidy (Hick, 1980). The frequency of abnormality is dependent upon the age of the culture and concentration and type of plant growth regulators used in the culture medium (Evan et al., 1986). The nutritional requirement for the initiation of callus varies considerably for primary explants of different origin. The majority plant organ require one or more plant growth regulators in the medium in order to stimulate the callus development (Yeoman & Macleod, 1977). Explants can be subdivided into their growth factor requirement i.e. (a) auxin (b) cytokinins (c) auxin and cytokinin (d) complex natural extracts. Cytokinin as a growth promoter and has some role in stimulating bud or shoot formation and growth, whereas auxin stimulate growth in culture capable of differentiation of organs.

The induction of callus formation in different explants such as stems, tubers, leaf and root etc. require an environment which induces some cells to become meristematic and resume division. With humidity, temperature and aeration, the nutrient media are the major factors. For induction of callus in tobacco pith culture (Murashige and Skoog 1962) and soybean hypocotyl culture (Gamborg et al., 1968)(E6) were well accepted by all. The two media
differed with respect to nitrogen and phosphorus content. Hormone level however, are flexible.

The use of callus culture for propagation of woody plants has been reviewed by Barbee et al. (1972 & 1976), Diaz - Rondero and Alcaraz - Melendez 1987), and Haanet et al. (1973), Beloualy (1991) Islam et al. (1995). The general approach was to derive callus from different type of explants for regeneration plantlets.

Many investigator induced callus from different parts of the leguminous plants. Phukon & Mitra (1983) obtained callus from the different seedlings parts eg. hypocotyl, leaf cotyledon and root using different auxin in MSBM and BSBM. Tomer & Gupta (1988a & 1988b) induced callus and obtained plantlets by culturing hypocotyl explants of A. lucida, A. amara and A. richardiana.

2.3.1. Leaf explant:

Phukon and Mitra (1983) obtained callus from leaf explants of A. odoratissima but failed to regenerate plantlets from that callus. Rao and Forkmann (1985) produced anthocyanin rich callus from Callistephus chinensis. Raman (1985) reported morphogenetic development from Salpiglosis leaf discs callus. Sinha and Mallik (1991) obtained callus from leaflets of the woody legume Sesbania bispeciosa (Jacq) using 0.5 mg/l of BA and 2 mg/l of 2,4-D in MSBM and successfully regenerated shoot buds in 2 and 4 mg/l of BA with
15% (v/v) coconut milk supplemented with MSBM. Diaz-Rozdero and Alaraz-Melendez (1987) obtained callus from leaf explants of *Turnera diffusa* by using MSBM and B5BM supplemented with 2,4-D and regenerated plantlets from the leaf callus.

Economou and Read (1982) observed shoot production by treating with NAA and BA in a modified MSBM in petunia leaf. Alderson and Barghchi (1982) regenerated shoot bud from the leaf explant of *Matricaria chamomilla* in MSBM with NAA and in some culture without changing the culture media regenerated shoot buds. Celizarova et al. (1982) observed morphogenesis of leaf explants of *Matricaria chamomilla* and able to obtain roots. Danq et al., (1981) produced callus from *Datura innoxia* leaf explant by using each of 1 mg/l of Kn and 2,4-D in MSBM. Using IAA, Kn and Casein hydrolysate supplemented with MSBM. Vesseur and Sene obtained callus from leaf explants *Cichorium intybus*. Regeneration of plantlets from callus of leaf explants of *Mimordica grosvenorii* was obtained by Lim et al., (1981) using 0.5 mg/l of BA added with MSBM. In *Stylosanthes humitae*, Maijer (1982) standardized a protocol to raise plantlets from callus of petiole explant in MSBM supplemented with 1 mg/l of BA and 1 mg/l of NAA. The MSBM containing 2,4-D, Kn, IAA and Casein hydrolysate favoured somatic embryo formation in *Gossipium klotschianum*. 
2.3.2 Root culture:

The first work on tissue culture of leguminous plants was to study the infection of *Rhizobium* species on legume roots (Raggio and Raggio, 1956; Raggio, Raggio and Torry, 1957; Cartwright, 1967 and Torry, 1978). Later on other workers tried on callus production, organogenesis and embryogenesis from the root callus. In 1983, Phukon and Mitra obtained callus from the root explant of *A. odoratissima* using 2,4-D in addition with BA and Kn in MSBM but they have failed to differentiate shoot from the root callus. Barna and Wakhalu (1989) successfully differentiated shoot from the root callus of *Plantago ovata* using IAA and Kn. Cellarova et al. (1982) observed morphogenesis from the root callus of *Matricaria chamomilla*, and obtained roots only. In 1984 Vasser and Sene observed proliferation of small root explants using Kn, IAA and casein hydrolysate in MSBM.

Phukon and Mitra (1983) obtained callus from the root portion of *A. odoratissima* in MSBM fortified with NAA or 2,4-D incorporated with BA or Kn but failed to regenerate plantlets. Adventitious buds from root callus of *Plantago ovata* was obtained by Barna and Wakhalu (1989), using 11.4 uM IAA and 0.9 uM Kn. Cellarova et al., (1982) observed morphogenesis of callus produced from roots of *Matricaria chamomilla* and obtained roots only. A mass of root proliferated from small root explants was observed by Vasser and Sene (1984), in MSRM supplemented with Kn, IAA and Casein hydrolysate(CH). The first successful root culture was
reported by White (1934) who obtained less success using tomato roots.

2.3.3. Hypocotyl explant

Number of workers obtained callus and differentiated shoot buds from hypocotyl callus from the seedlings of different species. Phukon and Mitra in 1983 obtained callus from the hypocotyl explants of *A. odoratissima* using NAA, IBA and 2,4-D along with BA and Kn in MSBM and B5BM and regenerated shoots by using NAA and BA in MSBM. Goyal and Arya (1981) differentiated shoot buds from the hypocotyl callus of *Prosopis ciceraria*. In MSBM supplemented with 2,4-D and Kn produced callus from hypocotyl explants of *Plantago ovata* Forssk. Eight days old calli of the same plant when placed in MSBM supplemented with IAA and Kn developed roots (Barma and Warhlu, 1989). Tommer and Gupta (1988) obtained callus from hypocotyl explant of *A. Richardiana* using BA in B5BM and found that 2% sucrose was optimum for the production of somatic embryo, embryogenesis was inhibited by 4% or higher sucrose concentration, While organogenesis was suppressed at 6% or higher sucrose concentration. Hypocotyl explant of three leguminous species of *Albizia lucida*, *A. amara* and *A. richardiana* have differentiated shoot buds in B5BM. Maximum number of shoot per explant developed on basal medium augmented with 2,4-D in *A. amara*, BA for both *A. lucida* and *A. richardiana* (Tommer and Gupta, 1988b). Tommer
and Gupta also observed that higher concentration of auxins in the medium, enhanced rooting and callusing but cytokinin promoted the growth of green calli, BA enhanced the differentiation of shoots in three species. Arrillaga et al. reported that hypocotyl explants of *A. julibrissin* when cultured in the B5BM supplemented with various level of paclobutrazole, uniconazole, prohexadione, calcium or GA3, callus formation was observed within one week. Green nodule like structure have arisen from distal end of the explant within 10 days and developed into shoots (Sankhala et al., 1993). Excised hypocotyl explants from *in-vitro* grown seedlings of *Accacia auriculiformis*, proliferated shoots from cotyledon axillae and rooting was induced in the 1/2 strength of MSBM with combination of BA (1 - 2 mg/l), NAA (0.02 mg/l) and GA3(0.5 mg/l) (Ide et al., 1994). Brew et al. (1984) induced callus from hypocotyl tissue of *Medicago sativa* cultivars in B5BM supplemented with 2, 4-D and Kn. Somatic embryos were obtained from these callus subcultured in different basal media and observed wide variations among 5th cultures in respect of callus and somatic embryogenesis. Hypocotyl callus of *Stylosanthes humilis* was obtained by culturing *in-vitro* grown seedling hypocotyl in MSBM supplemented with 1 mg/l each of BA and NAA when subcultured in the same medium with 0.5 mg/l each of the same plant growth regulator. Induction of shoot bud observed in 1 mg/l of BA alone in MSBM (Meijer, 1982). Embryogenesis from hypocotyl callus was obtained when cultured the cultivar of
golden apple the hypocotyl callus in each of White or Nitsch’s medium supplemented with 2 mg/l of NAA and BA (Menra and Satchev, 1980). In 1984, Jose and Vieteq got callus from hypocotyl explant when cultured in the medium containing BA, NAA or IBA and adventitious buds arose from the callus culture. The adventitious shoot buds were induced from hypocotyl callus of *Morus alba* in the medium containing high concentration of BA (Kim et al., 1985). Hypocotyl explants of 3 weeks old golden delicious apple seedlings when placed in modified MSBM containing B5 vitamins and supplemented with various concentrations of BA and NAA and produced adventitious buds when placed the culture under light (Liu et al., 1983). Uka et al. (1982) cultured *Eucalyptus globulus* hypocotyl explants on a Schnerr and Hildebrandt medium with NAA and BA produced roots and shoots with or without callus formation. From the hypocotyl of *in vitro* grown seedlings of *Melilotus perviflora*, Bajaj and Gospal (1981) obtained callus, and differentiated shoot buds in the medium containing BA or Kn. They reported that BA was superior for induction of shoot bud than Kn and addition of NAA induced roots from the callus derived shoots.

2.3.4 Cotyledon explant:

Sinha and Mallick (1971) obtained callus from cotyledon explants of *Sesbina bispinosa* using BA (0.5mg/l) and 2,4-D (2 mg/l) in MSBM medium. Cotyledon explant of *A. odoratissima* produced callus with NAA and 2,4-D in combination with BA or Kn in MSBM and got rhizogenesis by using IBA.
in the medium (Phukon and Nitra, 1983). Ozcan et al. (1992) using 0.3 mg/l of BA and 4 mg/l of NAA in MSBM regenerated adventitious shoot from the cotyledon of *Pisum sativum*. From the callus of *Phaseolus coccineus* immature embryo, Ganga and Allavena (1991) obtained morphogenesis using 2iP and NAA in MSBM. *Sesbania grandiflora* cotyledon produced shoot-bud directly in the medium containing 1 mg/l each of NAA and BA (Detrez et al., 1994). The regeneration of shoot from *Acacia auriculiformis* cotyledon callus has been obtained by Das et al. (1993) using BA in MSBM. Hallik and Sinha (1991) obtained organogenesis and plantlets from cotyledonary callus using MSBM with IBA, 1BA, NAA, 2,4-D, Kinetin, BA and Coconut milk. Franklin et al. (1991), Kyselý and Jacobsen (1970) produced plants from cotyledon and embryogenic axis of two varieties of pea.