CHAPTER - II

REVIEW OF LITERATURE

During the later part of 19th century there was a vision on experimentation with isolated plant tissues and organs. Various plant scientists described formation of wound callus by performing experiments on isolated stem fragments and root slices under controlled conditions (Trecul, 1853; Votchting, 1878; Rechinger, 1893).

Cell theory proposed by Schleiden (1838) and Schwaan (1839) is the basis for concept of cellular totipotency proposed by Haberlandt (1902). A totipotent cell is one that is capable of developing into a whole organism and this term was probably coined by Morgan in 1901 (Krikorian and Berquam 1969).

Haberlandt (1902) paved a way into this new science of tissue culture and he was the first to cultivate isolated plant cells **in vitro** on an artificial medium. "Experiements on the Culture of isolated plant cells" a translation of Haberlandt's paper, has been published by Krikorian and Berquam (1969), in which Haberlandt stated clearly the desirability of culturing the isolated vegetative cells of higher plants. Inspite of Haberlandt's failure to obtain cell division due to

the absence of sterile environment and bacterial contamination, he suggested the utilization of embryo sac fluids and the possibility of culturing artificial embryos from vegetative cells.

Very little progress was made during the 30 years following Haberlandt's paper. There were several unsuccessful attempts by various workers to culture the cells (Schmucker, 1929; Scheitterer, 1931; Pfeiffer, 1931, 1933; La Rue, 1933). Kotte (1922) and Robbins (1922) developed a technique for the culture of isolated roots (White, 1934). The discovery of hormonal characteristics (Snow, 1935; Went and Thimann, 1937) were used by Nobecourt (1939) and Gautheret (1939), and succeeded in maintaining the long term callus cultures of cambial tissues isolated from carrot.

The war years (1939-45) showed a soothing effect on Botanical research. After the discovery of nutritional quality of coconut milk by Van Overbeek et al., (1941), with the culture of isolated Datura embryos on a medium enriched with coconut milk, there was again a resurgence in the research. Later the effect of 2,4-D and coconut milk factors on carrot and potato tissues cultures was reported (Caplin and Steward, 1948, Steward and Caplin 1951, 1952). The studies by Camus (1949) on the induction of vascular differentiation,

resulting from grafting buds into tissue cultural masses led on to the important studies on factors controlling vascular tissue differentiation by Wetmore and Rier (1963) and Jeffes and Northcote (1967). The discovery of Cytokinins stems from Skoog's tissue culture investigation at the university of Wisconsin. Skoog's group identified and isolated a potent 'cell division factors 6-furfurylaminopurine and named kinetin (Miller et al., 1955). In tobacco callus a basic regulatory mechanisms underlying shoot and root differentiation involves a balance between auxin and cytokinin (Skoog and Miller, 1957). Analysis of culture growth in quantitative terms was first initiated by Steward (1952) and led to the wide use of coconut milk as a nutrient, and to the discovery of embryogenesis (Steward, 1958; Pilet, 1961).

Rapid progress has been made in plant tissue culture, and techniques have been developed for the commercial propagation. Regeneration of whole plants by meristem culture was reported by several workers (Wetmore and Wardlaw, 1951; Morel, 1964). It became evident that plant tissue culture technology was beginning to make significant contributions to the agriculture and industry (Murashige, 1978; Zenk, 1978). According to the estimations of Murashige there were

more than 600 species of ornamental plants exploited for clonal propagation.

More than 100 tissue culture laboratories were engaged in commercial propagation of plants. The progress is being made, especially in China (Loo, 1982).

TISSUE CULTURE OF TREES

The history of regeneration of complete plants in tree species through tissue culture is only about a decade old.

Tissue culture of tree species in India began only during 1960s. Callus cultures of Pinus were established and studied by Konar (see Mascarenhas and Muralidharan, 1989). But only after 1974 there has been a siginificant rise in tissue culture of woody species. Complete plantlets were first reported from hypocotyl segments of Pinus sylvestris on a medium fortified with IAA, BAP or Ammonium Chloride (Sommer and Brown, 1974).

Later complete plantlets were reported in Psegudotsuga menziesi and Picea glauca. Since then many plants have been tried to get regeneration through callus cultures and only a limited number of species have given positive results (Kapoor, 1989).

Gautheret (1934, 1937) was the first to obtain calli but not sub-cultures from cambial explants of angiosperms and conifer trees. The first angiosperm tree callus cultures which were sub-cultured regularly were Salix caprea (Gautheret, 1948), Syringia vulgaris, Crataegus monogyna (Morel, 1948) and Castanea vesca (Jacquiot, 1966). During the first studies of tree tissue culture, cambial explants were used for culturing.

In order to obtain, morphogenesis the most important factor is the selection of explant. Even though the explants were obtained from different parts of the tree, they produced equally fast growing calli, (Barker, 1969; Mehra and Mehra, 1974), but the morphogenetic potential of these calli often varies with the origin of the explant. Although callus cultures have a great deal of potential in the biotechnological aspects of tissue culture, i.e., secondary product formation, callus cultures are not very suitable for plant propagation. The key reason for their unsuitability is that genetic aberrations occur during mitotic divisions in callus growth (D'amato, 1965). Roots, shoots, embryos and sometimes plantlets were obtained either directly or from callus from sections of embryos or very young seedlings of Pinus sps (Greenwood

and Berlyn, 1965; Sommer et al., 1975), Picea glauca (Campbell and Durzan, 1975), Biota orientalis (Konar and Oberoi, 1965), Cryptomeria japonica (Isikawa, 1974) and Santalum album (Rao and Rangaswamy, 1971).

During 1960 Morrel for the first time used shoot apex cultures for rapid clonal propagation of tree species. Culturing of apical meristems and axillary buds is termed as micropropagation of trees. The advantage of a micro-propagation system over conventional seed propagation is that it is possible clonally to multiply plants with a desired genotype (Jones et al., 1982).

There is a clear distinction between bud culture and apical meristem culture. Apical meristem cultures involve only actual apical dome of the shoot, sometimes with a few leaf primordia. These apical meristem cultures are often used to obtain disease free clones (Smith and Murashige, 1970). Rudimentary vegetative shoots are used in bud cultures.

The advantage of this method over propagation by callus cultures is that a shoot apex is a priori present and does not have to be induced, only root induction being required. But there is disadvantage that one explant will form only one propagule, not the hundreds

or thousands that are sometimes produced in callus and suspension cultures. The literature dealing with propagation of trees by bud culture has been reviewed by Bonga (1974).

Beauchesne (1966) obtained little growth in axillary bud cultures of several hard wood species. In Acer platanoides only shoot elongation was observed but rooting was absent (Demaggio and Freeberg, 1969). Similar observation was made by Elliot (1972) in Malus sylvestris. Dutcher and Powell (1972) reported succesful rooting of Malus buds. Buds of Prunus were rooted by Quoirin et al., (1974). Staritsky (1970) reported rooting of buds in Elaeis guinensis. Dormancy is a limitation in bud cultures. Dormant buds of some tree species elongated readily in culture (Al-Talib and Torrey, 1959, 1961; Chalupa and Durzan, 1973). Presence of stimulatory and inhibiting substances and their interactions may affect the rooting. Inhibitors can often be removed from tissues simply by washing in water. Good (1974) described the leaching of an inhibitor resembling abscisic acid from the foliage of Picea sitchensis and Betula pendula. Soaking in water was a prerequisite for successful rooting of nodes of Eucalyptus grandis in culture (Cresswell and Nitsch, (1975). David (1982) suggested that axillary bud

initiation can be promoted by growth substances added to the medium, generally BAP (10^{-5} M and 5 x 10^{-5} M) with auxin either NAA or IBA (10 M), applied over a period have been suggested as the most successful concentrations. Induction of axillary buds on hypocotyl explants of Pinus pinaster with BAP and NAA was reported by David (1978).

The presence of growth substances particularly auxin, in the medium can give rise to callus development on the explant and it can be difficult to distinguish between axillary and adventitious bud initiation when it occurs in the region of callus development (John, 1983).

Mascarenhas and Muralidharan (1989) reviewed the tissue cultural studies carried out in India on forest trees. Many of these studies are directed at large scale micropropagation of economically important trees. These are given below.

Morus alba, Tectona grandis (Narasimham et al. 1970), Terminalia bellerica (David and Vasikar, 1980), Dalbergia latifolia, Eucalyptus globulus, Eucalyptus tereticornis (Mascarenhas et al. 1982), Morus indica (Patel et al., 1983); Mhatre et al., 1985), Sapium sebiferum (Kotwal et al., 1983), Morus alba (Anand and Bir, 1983), Eucalyptus torelliana, Eucalyptus camaldulenisis (Gupta et al., 1983), Dalbergia sissoo (Datta et al., 1983), Ceratonia seliqua (Thomas and Mehta, 1983), Azadiracta indica (Jaiswal and Narayan, 1984, 1985), Dendrocalamus strictus (Nadgir et al., 1984), Holarrhena antidysenterica (Datta and Datta, 1984), Leucaena leucocephala (Dhawan and Bhojwani, 1984, 1985; Datta and Datta, 1985), Santalum album (Bapat et al., 1985), Populus alba (Mehra and Cheema, 1985), Ficus religiosa (Jaiswal and Narayan, 1984), Eucalyptus grandis (Lakshmi Sita and Shobharani, 1985), Lagestroemia flos-reginae (Paily and D'Souza, 1986), Salvadora persica (Rao, 1987), Morus indica (Bapat et al., 1987), Mitragyna parviflora (Roy et al., 1988).

TISSUE CULTURE OF MULBERRY:

The credit for establishing Mulberry in culture for the first time goes to Narasimhan et al., (1970). They embodied different methods of culturing tissues of Tectona grandis (teak), Artocarpus heterophyllus, Morus alba (mulberry) and Populus nigra (poplar). Teak, mulberry and poplar tissues grew well on Murashige and Skoog's medium containing 1.0 ppm Glycine, while jack tissues grew well on Blaydes medium. Ghugale et al., (1971) amply stressed on the effect of auxins and Gibberellic acid on growth and differentiation of Morus alba and Populus nigra tissues in vitro. They reported root differentiation in the presence of IAA, IBA, IPA, α NAA and β NAA and not in 2,4-D, 2,4-TCP and GA_3. Synergistic action of auxin and cytokinin was reported and there was root formation only when the medium contained auxin. Ohyama (1970) described a method for culturing excised tissues, organs and cells. They proposed that the Tissue culture method will become an effective means in future not only in the tree physiology but also in the practical problems of breeding, propagation etc. Since, it requires a long time for general woody plants to attain to their efflerence compared with herbaceous plants, a

problematic point consists in the low breeding efficiency.

Sekih et al., (1971), studied the effect of concentrations of auxin and kinetin on the callus culture of the mulberry stem. Sekhi and Meguro (1972) observed better development of female flower organs in Nitsch medium than White medium. Even better development was observed when Nitsch medium was fortified with IAA (2 PPM).

Oka and Ohyama (1973) studied the effects of constiuents of medium on callus formation in mulberry tree. They reported that nutritional conditions such as five kinds of sugars, concentrations of sucrose, two kinds of nitrogen sources, auxins, cytokinins and some extracts exerted effects on both growth rate and friability of callus.

Effect of different concentrations (0,10,20,30,40, and 50 g/l) of sucrose, galactose, glucose, or fructose in MS agar media with 2 PPM NAA in callus formation and root initiation were examined using excised stem segments of mulberry trees (Minamizawa and Hirano, 1973).

Sekih et al., (1974), determined the effect of several chemicals on the growth rate of callus which

were induced from embryo and endosperm of mulberry seeds and cultured on Murashige and Skoog's basal medium containing 0.2PPM kinetin.

Oka and Ohyama (1974) studied in vitro culture of excised buds in mulberry tree. They reported different effects of growth substance (Benzyladenine) on the development of shoots and organ formation from winter buds. In the year 1975 Oka and Ohyama performed three kinds of experiments in order to find a suitable medium for the culture of axillary buds in mulberry tree. In the first experiment they determined suitable material for successful culture. In the second experiment various aged axillary buds were used as materials. In the third experiment media were changed. Ohyama and Oka (1976) regenerated whole plants from isolated shoot tips of mulberry tree. They reported the fresh weight yeild increase with decrease in agar concentration. They concluded that an exogenous supply of BA and NAA is necessary for morphogenesis leading to the development of complete plant in mulberry trees. Later Oka and Ohyama (1976), concentrated on the hormonal requirement of mulberry callus during subsequent sub-culturing. They used BA, NAA and 2,4-D for such studies. Their interest continued on Mulberry and in 1978 they reported effects of agar concentration, pH and sugars of medium

on the development of shoot from Winter buds. In the same year they have undertaken dormancy analysis by culturing the excised mulberry buds. Normal and abnormal leaves were observed with varying concentrations of benzyl adenine and adventitious buds were initiated by Oka and Ohyama in 1981.

Patel et al., (1983), cultured axillary buds, stem stegments, leaf discs and petiole explants from mature trees of mulberry. Plant regeneration and fruit formation was observed in axillary bud cultures by Ho-Rak Kim et al., (1986). Leaf, hypocotyl, and shoot-tip explants of mulberry derived from embryos cultured with benzyl adenine (BA) were induced to form adventitious shoots. In order to induce adventitious shoots media containing high BA with or without a low concentration of Naphthalene acetic acid (NAA) was used.

Mhatre et al., (1985), regenerated plants from the cultures of leaves and axillary buds in mulberry (Morus indica L.).

Narayan et al., (1989), raised callus cultures from internodal segments of mature plants of Morus alba on Murashige and Skoog's medium supplemented with 1.0 mg/l 2,4-D and 0.5 mg/ BAP. Shoots were regenerated. Chattopadhyay et al., (1989) proposed methods for quick

in vitro production of mulberry (*Morus alba*) plantlets for commercial purpose. High multiplication rate complete plants were obtained within 8 weeks of culture period from a single axillary bud of a mature *Morus alba* tree var. Mandalaya, Rao *et al.*, (1989), discussed a detailed account of application of plant cell Tissue and organ culture in Mulberry improvement. Callus was initiated from leaf explants of *Morus alba* S1 Strain, on MS medium fortified with BAP, Kn and 2,4-D. After certain age browning of callus was observed due to acumulation of phenolics by Tewary *et al.*, 1989. Rapid clonal propagation of the mulberry plant through shoot apex culture has been dealt by Tewary and Subba Rao (1990). They reported multiple shoots along with proliferation of callus when shoot apex explants were cultured on M.S. medium fortified with different concentrations of BAP.