INTRODUCTION

The technique of tissue culture is being used in many areas of plant science and has become an attractive tool for the study of growth and regeneration. The plant parts cultured in vitro are known to produce roots, shoot buds directly and through callus formation. In addition, the regeneration and multiplication of plants through micropropagation and large scale production of somatic embryos of agronomic crops and their packaging into artificial seeds have also been proved to be useful commercially.

A brief history of plant tissue culture:

As early as 1902, Haberlandt conceived the idea of tissue culture and put his aim as "aus vegetativen Zellen Kunstliche Embryonen Zuzuchten", i.e. it is possible to grow embryos from somatic cells. He himself tried to grow the mature leaf cells of Lamium purpureum and the hair cells of Tradescantia and Pulmonaria in Knop's solution with 1-5% sucrose without any success. Later, Robbins (1922) and Kotte (1922) independently succeeded in growing excised root tips of maize and pea. In 1934, White established continuously growing cultures from root tips of tomato. Gautheret (1934) obtained the callus from cambium of Salix capraea and Populus nigra. Kogl et al. (1934) established that the growth substance discovered by Went (1926)
was indoleacetic acid and Snow (1935) demonstrated that this growth substance was able to stimulate cambial activity. However in 1939, Nobecourt induced root formation from callus cultures of carrot. In the same year, White succeeded in producing leafy buds from tobacco stem tissue cultures. Thereafter the culture of embryos (van Overbeek, 1941), single cell culture (Muir et al., 1954) and initiation of suspension cultures (Steward and Shantz, 1955) were reported.

The discovery of Kinetin (Miller, et al., 1955) led to the classic finding of Skoog and Miller (1957) in which the concept of hormonal control of organ formation was demonstrated, which holds good to quite many plant tissues. In 1958, Steward et al. proposed the theory of totipotency of plant cells and both Steward et al. (1958) and Reinert (1958) reported somatic embryogenesis in carrot cultures.

Bergmann (1959) described the biological technique of cloning of single cells of higher plants. Guha and Maheshwari (1964) induced for the first time haploid embryos from pollen in anther cultures of *Datura innoxia* which led to the production of haploids in several plants.

The report of the technique of enzymatic isolation of protoplasts (Cocking, 1960) gave the idea of their fusion (Power et al., 1970). Takebe et al. (1971) reported successful
regeneration of plantlets from isolated protoplasts derived from mesophyll cells of tobacco. Since then interspecific and intergeneric hybridization were reported for many species (Carlson et al., 1972, Kao et al., 1974). In 1976, Jones et al. reported the fusion between human cells and tobacco protoplasts.

The production of artificial seeds by encapsulating asexual embryos (Redenbaugh et al., 1984, Kitto and Janick, 1985) and transgenic plant by transformation of fire-fly luciferase-gene to tobacco plant (Ow et al., 1986) have been successfully demonstrated.

The technique has also provided an opportunity to study the various morphogenetic events occurring in a plant body and is being applied to basic and applied studies in genetics, breeding, pathology, physiology and biochemistry of plants (Street, 1976).

**Morphogenetic studies:**

The regeneration of plants from explants of various origin has been successfully accomplished in many plants (Bhojwani and Razdan, 1983). Almost all parts of the plant body have been used as explants to initiate the cultures; and many of them have produced callus, roots, shoot buds and/or embryoids.
Lakshmi Sita et al., 1980) and seed embryos (Yamaguchi et al., 1970; Sommer et al., 1975; Green and Phillips, 1975; Pence, 1979, 1980). In addition, the calli derived from these explants were also made use for the studies on organogenesis, and plantlets have been regenerated (Simonsen and Hilderbrandt, 1971; Jordan et al., 1982, Narasimhulu and Reddy, 1983; Kothari and Chandra, 1986).

The amount of growth regulator/s in the culture medium is/are critical for the control of growth and morphogenesis. Skoog and Miller (1957) reported that generally a high concentration of auxin and a low concentration of cytokinin in the medium promotes abundant cell proliferation with the formation of callus and often 2,4-D alone is also used to initiate callus. On the other hand, low auxin and high cytokinin concentrations in the medium result in the induction of shoot morphogenesis. Flick et al. (1983) demonstrated that auxin alone or with a very low concentration of cytokinin is important in the induction of root primordia. But such a phenomenon differs from species to species as a few species of compositae exhibited callus initiation on a medium with auxin/KN ratio < 1 (Bowes, 1970; Landova 1979; Subramaniyan and Subba Rao, 1980). Steward et al. (1967) pointed out that various growth regulating stimuli may need to be applied to cells, not only in the right amounts but also in right sequence and under the right culture
conditions. Chen and Galston (1967) used such a sequential treatment to induce shoot and root formation in *Pelargonium* pith callus. Earlier kinetin was the commonly used cytokinin but now it is often replaced by benzylaminopurine (Jacquiot, 1966; Lam, 1975; Gamborg *et al.*, 1979; Yang and Chang, 1979); isopentyladenine (Helgeson, 1979; Fassuliotis *et al.*, 1981; Welsch and Sink, 1981) and zeatin (Meredith, 1979; Power and Berry, 1979).

In some plant species either exogenous auxin or cytokinin alone may be sufficient to bring about morphogenesis. In *Coriander* petiole segments, whereas low concentration of 2,4-D favoured meristemoid formation but NAA at higher concentrations favoured embryogenesis (Steward *et al.*, 1970a,b; Zee, 1981). On the otherhand, in excised root cultures of *Dalbergia sissoo*, NAA at lower concentrations in the medium favoured shoot bud formation (Mukhopadhyay and Mohan Ram, 1981). But in embryos of norway spruce (van Arnold and Erikson, 1978) and in embryonic tissue of radiata pine (Reilly and Washer, 1977) buds were induced with the addition of cytokinin. But *Armoracia rusticana* (Sastri, 1963) auxin in the medium induced shoot bud differentiation but kinetin suppressed the bud formation. Thomas and Street (1970) found that omission of auxin from the medium stimulated rooting in cell aggregates of *Atropa belladonna* and the process was speeded up and amplified by the application of anti-auxins like
tropic acid and 1-naphthoxyacetic acid. The anti-auxins, 5-hydroxy nitrobenzyl bromide, 7-azaiondole and 8-azaguanine promoted somatic embryo development in Citrus cultures (Kochba and Spiegel-Roy, 1977).

In addition to the phytohormones some of the complex additives of natural origin have also been used in the media to induce growth of callus and subsequent regeneration of plantlets. These additives are used singly or in combination with phytohormones. Most commonly used natural additives include coconut water (Steward et al., 1958; Nataraja, 1968, 1971; Sen and Gupta, 1979; Vasil and Vasil, 1981); casein hydrolysate (Shama Rao and Narayanaswamy, 1975; Bajaj and Mader, 1974); yeast extract (Yamane, 1974; Bingham et al., 1975); malt extract (Kochba and Spiegel-Roy, 1973) and juices of various fruits (Jelaska, 1972, 1974).

Mode of plant regeneration:

There are three principle methods of plant regeneration in tissue cultures and one of which is organogenesis. The latter may be direct from explants or it may be indirect through callus formation in explants. As early as 1939, White observed shoot differentiation in tobacco hybrid and Nobecourt noted root differentiation in callus cultures of carrot. Since then direct
morphogenesis (organ differentiation directly from cultured explants) or morphogenesis mediated through callus phase has been observed in many species (Vasil and Vasil, 1980; Evans et al., 1981; Flick et al., 1983). The second method of plant regeneration is through enhanced axillary branching. In a number of plant species adventitious buds are produced in vivo from different organs such as root, leaf and bulbs and the rate of their development can be enhanced considerably under culture conditions. The apical meristem of Begonia under appropriate nutrient conditions regenerated plants through axillary branching which are genetically identical to parent plants and are pathogen-free (Reuther and Bhandari, 1981). Axillary buds also regenerate shoots and eventually plantlets (Patel et al., 1983).

The third method is the regeneration of plantlets through embryogenesis, a process by which haploid or diploid cells develop through characteristic natural embryological stages giving rise to embryoids and these are bipolar structures with a root/shoot axis. This process occurs naturally in a species from both reproductive and somatic tissues but in tissue cultures it has been induced from almost all parts of the plant body (Steward et al., 1966; Konar and Nataraja, 1969; Raghavan, 1976; Bhojwani and Razdan, 1983). Somatic embryogenesis can occur directly (Konar and Nataraja, 1965; Backs-Husemann and Reinert, 1970) or through callus (Tisserat et al., 1979; Kohlenbach, 1978; Sharp et al., 1980; Vasil and Vasil, 1986). To date embryoid formation has been reported in more than 130 species including angiosperms and gymnosperms (Thorpe, 1988).
Extensive investigations on somatic embryogenesis have indicated that two important factors namely auxin and source of nitrogen influence the process. Among the auxins, 2,4-D in the range of 0.5 - 2.0 mg/l has been found to be the most useful and is being used in 57.1% of successful embryogenic cultures (Evans et al., 1981). Other auxins such as NAA (Gleddie et al., 1983) IAA (Bapat and Rao, 1979) or NAA and IBA (Jelaska, 1974) have also been used. Along with these auxins some more uncommon auxins like 2-benzothiazole acetic acid (BTOA) (Rao and Narayanaswamy, 1972), parachlorophenoxy acetic acid (PCPA) (Abo El-Nil, 1977) and picloram (Collins and Phillips, 1980) have been successfully employed. Cytokinin like zeatin is also found to play a promotive role for embryogenesis in carrot (Fujimura and Komamine, 1980). Gibberellins, generally inhibit somatic embryogenesis (Tisserat and Murashige, 1977, Fujimura and Komamine, 1975).

The role of nitrogen compounds in embryogenesis has been studied. Tazawa and Reinert (1969) observed that embryogenesis in vitro could be induced by both inorganic (KNO₃, NH₄⁺, NO₃⁻) and organic (aminoacids, amides) compounds. But the nitrogen content of the culture medium seems to influence the morphogenetic effect of growth substances (Rucker, 1982). Christianson et al. (1983) postulated that a co-ordinate change in nitrogen source and auxin level could evoke somatic embryogenesis in embryogenically competent cultures.
Although the tissue culture studies have been extensively exploited in agriculture for the improvement of crop plants, but the oil yielding crops like sunflower and niger have received comparatively little attention. The conventional method of vegetative propagation permit production of limited number of plants in coffee. Therefore the present investigations were undertaken on these plants with a view:

1. to establish tissue cultures from different seedling and mature explants.

11. to study the morphogenetic responses of callus derived from different explants on various nutrient media.

111. to study the effects of auxins and cytokinins individually and in a few combinations on callus growth, organogenesis and embryogenesis.