INTRODUCTION

The Leguminosae (Fabaceae) is the third largest family of Angiosperms comprising of about 600 genera and 12,000 species. The legumes are next to cereals as a source of human food and are known to contain more protein than any other vegetable product and are rich in minerals and vitamin B. As all the plant parts of the legumes are rich in proteins, they are valuable too as field and forage crops. In addition, legumes are also responsible for nitrogen enrichment of soil.

Although many improvements have been brought about by traditional methods of plant breeding, the technique of tissue culture can also play an important role in genetic manipulations at the cellular level in plant improvement programmes in species including legumes. The plants regenerated from explant callus and cell suspensions can acquire genetic changes expressed as morphological and physiological differences as compared to parental plants and can be screened for desired genetic combinations. The isolated protoplasts can also be used in somatic hybridization between species and genera that are difficult to cross by conventional means.
Tissue Culture – History and Perspectives

Schleiden (1838) and Schwann (1839) independently stated the basis of cellular theory and postulated that the cell is capable of autonomy and is totipotent, as it is obvious in eggs and spores which transform themselves into complete organisms. However, there was no experimental evidence to show that somatic cells are also totipotent. Haberlandt (1902) conceived the idea of culturing plant cells and prophesied that they are "totipotent" i.e. if given the proper environment and nutrition, they have the capacity to regenerate into entire plants. However, his attempts to grow embryos from epidermal hairs of Pulmonaria and palisade cells of Lamium and Eichhornia were unsuccessful.

In 1904, Hannig cultured nearly mature embryos of some crucifers (Raphanus sativus, R. landra, R. candatus, Cochlearia donica) and successfully grew them to maturity on mineral salts and sugar solutions. In 1922, Robbins and Kotte working independently reported some of their success with growing isolated root tips of pea and maize. White (1934) made the first successful report of continuously growing cultures of tomato root tips and the medium which he used became the basic medium for variety of cell and tissue cultures.
Kogl et al. (1934) established that growth substance discovered by Went (1926) was indoleacetic acid, and Snow (1935) demonstrated that this substance was able to stimulate cambial activity. These discoveries gave a big push to the development of plant tissue culture technique. van Overbeek et al. (1941) demonstrated for the first time the stimulatory effect of coconut milk (Embryo sac fluid) on embryo development and callus formation in Datura.

The discovery of kinetin by Miller et al. (1955) led to the classic finding of Skoog and Miller (1957), who demonstrated that shoot and root formation could be regulated by a delicate balance between cytokinin and an auxin. Whereas high ratios of auxin to cytokinin promoted rooting, high ratios of cytokinin to auxin supported shoot formation and at a particular combination of these, the tissue tended to grow in an unorganized fashion. This concept of hormonal regulation of organogenesis is now applicable to many plant tissues. However, exogenous requirement of growth regulators for a particular type of morphogenesis varies depending on the endogenous levels of these substances in the tissue.

Steward et al. (1958) demonstrated that free cells of carrot phloem in culture could develop into somatic embryos by a process similar to normal zygotic
embryogenesis. To date somatic embryogenesis has been reported in several plant species (Thorpe, 1988). Guha and Maheshwari (1964, 1966) were able to induce haploids from pollen for the first time in cultures of excised anthers of Datura which led to the production of haploids in several plants of economical importance.

In 1960, Morel reported that when the shoot apex of an orchid, Cymbidium, was cultured in order to obtain virus-free plants, it produced several rooted plants instead of a single one. He estimated that by this method, if the meristems were transferred to fresh media at regular intervals, a few million plants could be produced from a single explant in a year. As a result of this discovery, many commercial tissue culture laboratories were established in many countries for the propagation of orchids by shoot tip or meristem cultures.

The success of the orchid industry was soon exploited by horticulturists and, currently many ornamentals, fruit trees and vegetables are propagated by this method. Attempts to apply this technique for mass clonal propagation of forest trees has already been made.

In 1972, Carlson et al. produced somatic hybrid between Nicotiana glauca x N. longsdorffii by fusing their protoplasts. To date several somatic hybrids have been
produced between sexually compatible and sexually incompatible parents (Bhojwani and Razdan, 1983).

Encapsulation of asexual plant embryos to produce somatic seeds is a recent progress in the field of tissue culture which is another area of in vitro studies which is rapidly developing (Kitto and Janick, 1985; Redenbaugh, et al., 1984).

Morphogenetic studies:

In vitro morpogenesis is a highly complex phenomenon and to evoke organogenesis in many unorganized tissues, exogenous supply of phytohormones is found necessary. Since endogenous levels of phytohormones vary from tissue to tissue and species to species, different explants show specific requirement of these depending upon the cultural environment (Thorpe, 1980). Many different kinds, concentrations and combinations of growth substances have been used to bring about organogenesis from the cultured tissue and there is no single formula that works well for all the tissues and species.
Almost all parts of the plant body have been brought to culture and extensive studies have been made using these explants. The plant parts used include root (Earle and Torrey, 1965; Yatazawa et al., 1967; Gamborg and Evelegh, 1968; Winton, 1968; Mullin, 1970; Mehra and Mehra, 1972; Lazzeri and Dunwell, 1984), stem segments (Skoog and Tsui, 1948; Abo El-Nil, 1977; Winton and Verhagen, 1977; Helgeson, 1979; Raj Bhansali and Arya, 1979; Gupta et al., 1981), leaves (Gupta et al., 1966; Zenkteler, 1972; Landova and Landa, 1974; Gould, 1978; Sondahl and Sharp, 1979; Wernicke and Brettel, 1980; Takayama and Misawa, 1981; Zamora and Scott, 1983; Cheng and Qiang, 1982), inflorescence sections (Kaul and Sabharwal, 1972; Chen et al., 1977; Nakamura and Keller, 1982), mesocotyl (Rangan, 1974, 1976; Harms et al., 1976), seedling parts such as cotyledons (Doerschug and Miller, 1967; Jelaska, 1974; Mehra and Mehra, 1974; Mehra and Sachdev, 1979; Srejovic and Nesovic, 1981), hypocotyl (Coleman and Thorpe, 1976; Kamat and Rao, 1978; Matsuoka and Hinata, 1979; Price and Smith, 1979; Nessler, 1982), endosperm (Straus and LaRue, 1954; Norstog, 1956; Straus, 1960; Bhojwani, 1966; Johri and Nag, 1970) and seed embryos (Yamaguchi et al., 1970; Sommer et al., 1975; Green and Phillips, 1975; Pence et al., 1979, 1980).
There are several reports on induction of callus and organogenesis from various somatic organs (Nakano and Maeda, 1979). In majority of the cases 2,4-D was found to be necessary for callus induction (Yeoman and Macleod, 1977). However, callus induction has also been achieved using auxins such as IAA, NAA and IBA (Padmanabhan et al., 1974; Ramawat et al., 1977; Bornmann and Jansson, 1980). Hooker and Nabours (1977) have shown in sugar-beet that high concentrations of IAA or NAA (10-25 mg/l) were essential for the callus induction. Similarly, Katterman et al. (1977) while working with Gossypium barbadense demonstrated a similar requirement of high level of auxin in the medium. Wang and Chua (1972) used coconut water along with 2,4-D to induce callus from the stem cultures of Cryptomeria japonica. For optimal growth of callus and organogenesis nutrient media are often supplemented with complex mixture of natural origin viz., coconut water, juices of various fruits, casein hydrolysate, yeast extract (Steward et al., 1958, 1964; Hildebrandt, 1962; Vasil and Hildebrandt, 1962; Butenko, 1968; Vasil, 1977).

The most frequently used auxins to induce roots from the cultured tissue are IAA, IBA and NAA. Different explants of Dalbergia lanceolaria produced roots in
presence of IAA or IBA (Anand and Bir, 1984). Whereas high concentrations of various auxins stimulated rooting in callus cultures of *Diascorea deltoidea* (Grewal and Atal, 1976) and *Pinus gerardiana* (Konar, 1975), low concentrations of IBA favoured rooting in stem cultures of *Dalbergia latifolia* (Sudhadevi and Nataraja, 1987). On the contrary, 2,4-D with or without KN promoted regeneration of roots in chick pea cv. T-3 (Singh et al., 1982).

The differentiation of shoot buds on an auxin enriched medium is an interesting observation and has been reported in only a few instances (Haissig, 1965). Rao and Bapat (1978) have reported differentiation of shoot buds in hypocotyl cultures of sandalwood in response to auxins like IAA, IBA, NAA or NOA. In excised root cultures of *Dalbergia sissoo*, NAA at lower concentrations in the medium favoured shoot bud formation (Mukhopadhyay and Mohan Ram, 1981).

Although shoot bud differentiation has been achieved on hormone-free medium in *Medicago sativa* (Sanders et al., 1975; Walker et al., 1978) and *Vigna aconitifolia* (Eapen et al., 1986) but addition of cytokinins was found necessary to cause shoot bud differentiation in many cases. A marked effect of cytokinin in inducing shoot
buds has been demonstrated clearly by Dankwordt-Lilliestrom (1957). Gupta et al. (1966) demonstrated the involvement of kinetin in shoot bud formation in excised leaves and petioles of Nicotiana tabacum. On the contrary, kinetin failed to induce shoot bud formation in root tip cultures of Limnophila indica (Rao and Mohan Ram, 1981). BAP is found to be more effective than kinetin in inducing shoot buds (Murashige, 1974; Kothari and Chandra, 1984a; 1984b). Stimulatory effect of BAP in shoot bud induction has also been reported in Nicotiana tabacum (Skoog and Miller, 1957), Petula alba (Jacquiot, 1966), Populus tremuloides (Winton, 1970) and Antirrhinum majus (Rao et al., 1976).

As it is already mentioned, interactions between plant growth substances play an important role in organogenesis. A specific ratio of cytokinin to auxin was found to be essential for differentiation of shoot buds in callus of Vigna aconitifolia (Eapen et al., 1986), Indigofera enneaphylla (Bharal and Rashid, 1979), Stylosanthes guianensis (Mroginski and Kartha, 1980) and alfalfa (Stavarek et al., 1980). The usefulness of IAA-BA combination for regeneration of organs has also been reported in cultures of pea and winged bean (Gregory et al., 1980; Rubluo et al., 1984).
A perusal of literature on tissue culture published during the past few years reveals that the legumes, in general, are recalcitrant to regeneration (Jelaska, 1974; Vasil et al., 1979; Thorpe, 1980). The concentration of growth hormones for callus initiation in seed legumes are highly specific. Bean seed extract was used for regeneration in Phaseolus vulgaris (Crocomo et al., 1976) and irradiation of seedlings prior to explanting was necessary for regeneration in Cajanus cajan (Shama Rao and Narayanaswamy, 1975). Though hormone requirement seems to be much less specific in forage legumes, as regeneration occurred on hormone-free medium in cultures of alfalfa (Walker et al., 1979) but a wide range of concentrations of growth hormones were found necessary for regeneration in red clover (Phillips and Collins, 1979).

Although herbaceous angiosperms including legumes, have been studied extensively through tissue culture but in general tree species have received comparatively less attention. Economically important tree species like sandalwood (Rao and Bapat, 1978; Lakshmi Sita et al., 1979), eucalyptus (Lakshmi Sita, 1979; Gupta et al., 1981) and teak (Gupta et al., 1980) have been propagated in vitro successfully. A few recent reports on the

In the present investigations, studies were undertaken on both tree and herbaceous legumes which include *Tamarindus indica* L., *Vigna mungo* (L) Hepper cv. KH-3, *Vigna unguiculata* (L) Walp. Sub sp. *unguiculata* cv. BGM-1 and *Cicer arietinum* L.cv. Kabuli gram.

As the refinements are still needed to improve the efficiency of regeneration in legumes, recognition of the culture media, concentration and combination of phytohormones required for regeneration is necessary, hence the experiments were undertaken with a view to

1. establish seedlings and tissue cultures from seedling parts like shoot tip, segments of nodes, internodes, hypocotyl, stem, leaf/leaflet and cotyledon.
11. induce organogenesis from callus cultures.

111. study the effect of auxins /cytokinins individually or in combinations on callus growth and organogenesis.