CHAPTER 1

GENERAL INTRODUCTION

"Plant Biotechnology is yet another miracle of man, in his never-ending quest for truth, and realization of his dreams"

1.1. Status of the Problem

Groundnut or peanut (*Arachis hypogaea* L.) is grown primarily for its seeds which are used for human consumption and oil extraction. It is susceptible to diseases, pests, salinity and aridity and hence yield and oil content levels are low (Ozias-Akins et al., 1993; Vajranabhaiah et al., 1993). Early (*Cercospora arachidicola* Hori.) and late (*Cercosporidium personatum* Berk and Curt.) leaf spots commonly referred together as tikka is an important disease of groundnut which, if not managed properly, affects the yield to a considerable extent (Gorbet et al., 1990). Without fungicides, these diseases can cause a yield loss of 50% or more (Knauft et al., 1988; Pixley et al., 1990). The search for the selection of plants, resistant to the pathogen have been going on for a century, but to date resistance has not been found. In Tamil Nadu, losses due to late leaf spot are of primary concern. Oil content in the kernel is a valuable component, which decreases appreciably due to tikka disease (Gupta et al., 1988). Cultivars with resistance to both diseases reduced production costs but yield loss still occurred (Wells et al., 1994).

Plant regeneration from cultured tissues of groundnut is being reported since for nearly 15 years. Thus, the exploitation of cell and tissue culture techniques in callusing and efficient plant regeneration of *Arachis hypogaea* L., is a pre-requisite for the development of disease resistant plants in this oilseed crop. Excised segments of the epicotyl, hypocotyl, cotyledon, cotyledonary node, immature leaflet and petiole explants from *in vitro* grown seedling have been
cultured on different media by various workers (Atreya et al., 1984; Bajaj et al., 1981; Eapen and George, 1993b; McKently et al., 1990; 1991; Mroginski et al., 1981; Narasimhulu and Reddy, 1983; Seitz et al., 1987). Plant regeneration from cultured tissues of groundnut (*Arachis hypogaea* L.) occurs at a low frequency (Cheng et al., 1992; Ozias-Akins, 1989).

*In vitro* regeneration of plants via somatic embryogenesis has much potential for use in plant propagation and gene transfer (Baker and Wetzstein, 1992; Durham and Parrott, 1992). However, an efficient conversion of somatic embryos into plants remains a problem (Baker et al., 1995; Chengalrayan et al., 1994). Recently, there has been a great deal of interest in *in vitro* regeneration of groundnut plants from immature embryos, embryonic axis and immature leaflets (Baker and Wetzstein, 1992; Baker et al., 1995; Chengalrayan et al., 1994; Durham and Parrott, 1992; Eapen et al., 1993; Hazra et al., 1989; McKently, 1991; Ozias-Akins, 1989; Ozias-Akins et al., 1992; Ramdev Reddy and Reddy, 1993) via somatic embryogenesis. In spite of these studies, there are no efficient protocols for regeneration of plantlets from other explants viz. hypocotyl, epicotyl, cotyledons and immature leaflet via somatic embryogenesis (Chengalrayan et al., 1994).

Advances in plant, cell and tissue culture may provide novel ways to recognize, select, modify and transfer the genes involved in disease resistance. However, a pre-requisite for applying these methods to any species is reproducible plant regeneration from explants or protoplasts (Eapen and George, 1993b). Grain legumes, many of which are used as pulse crops, are difficult to regenerate *in vitro*, especially from protoplasts. Although cell division and subsequent callus formation were observed from cultured protoplasts isolated from immature and mature leaves, cotyledons and root tips of *Arachis hypogaea* (Bajaj and Gosal, 1988; Mhatre et al., 1985; Oelck et al., 1982; Rugman and Cocking, 1985), data on plant regeneration from protoplasts of *Arachis hypogaea* are rather limited. To date there is only one report where success has been achieved in regenerating plants from wild groundnut at low frequency (Li et al., 1993).

Selection of tissue cultures resistant to fungal toxins has been investigated for several plant species and pathogens (Daub, 1986). In recent years, pathotoxins
have been identified as useful agents for the induction and selection of disease resistant mutants in depth (Larkin and Scowcroft, 1983). Pathotoxic culture filtrates and purified toxins have been used for \textit{in vitro} selection and regeneration of disease resistant plants in many crops (Behnke, 1979; 1980; Biondi \textit{et al.}, 1991; Hartman \textit{et al.}, 1984; Hohl \textit{et al.}, 1991; Mabellos, 1991; Palit and Reddy, 1990; Pijut \textit{et al.}, 1990; Song \textit{et al.}, 1994; Thanutong \textit{et al.}, 1983; Vidhyasekaran \textit{et al.}, 1990; Yu \textit{et al.}, 1990). As no resistance to \textit{Cercosporidium personatum} has been found in groundnut by conventional methods (Wells \textit{et al.}, 1994), an alternative approach may be to use pathotoxic culture filtrates as screening agent to select the callus lines and regeneration of plants resistant to \textit{Cercosporidium personatum} through tissue culture technology.

However, thereafter tissue culture methods could not be successfully applied to groundnut for evolving disease resistant plants in part because organogenic, embryogenic regeneration and protoplast culture systems lack adequate regeneration rates. Further these tissue culture protocols could not be duplicated readily. Thus, detailed protocols for efficient green plant regeneration and techniques for desirable mutant induction need to be investigated for many of the most important local groundnut cultivars. Perhaps, equally pressing is the need for more research to develop simple media, hormonal combinations for quick and efficient plantlet development.

\textit{Mutation technique has been extensively used in many crops and large number of varieties with improved quantitative and qualitative characters have been developed (Mieke \textit{et al.}, 1990). Though considerable improvement in the productivity of cereals has been achieved, the crop improvement in oilseed crops like groundnut is limited. The major problem in groundnut breeding, which is a autogamous in nature, is the limited natural variability (Jagadeeswaran, 1989). The improvement in groundnut through induced mutations has been achieved in the past (Ashri and Goldin, 1965; Ashri and Herzog, 1972; Emery \textit{et al.}, 1964; 1965; 1970; Gowda and}
Nadaf, 1992; Gregory, 1955; 1956; 1968; Patil, 1966, 1969; 1973a; 1977; Patil and Bora, 1961; 1963; Ramanathan and Rathinam, 1983). Both physical and chemical mutagens have been earlier employed to generate the desired variability in groundnut. Intensive studies on the effect of physical and chemical mutagens in inducing genetic variability for high yield and oil content with disease resistance in groundnut genotypes are required to derive the maximum benefit from these tools in groundnut.

It is well recognized that application of biotechnological tools in crop improvement programmes can be effective in three different, complementary ways: speeding up the processes of conventional breeding, creating somaclonal variations through tissue culture technology and evolving disease resistant plants through in vitro selection technology. The present work is an effort in the above mentioned three ways, in two important local groundnut cultivars viz., Virdhachalam-2 (VRI-2) and Tindivanam-7 (TMV-7) with the following objectives.

### 1.2. Objectives

1. To study the response of different somatic explants viz. hypocotyl, epicotyl, cotyledon, cotyledonary node, immature leaflet, mature leaflet and immature leaflet petiole etc. for callus induction

2. To determine the optimal conditions for efficient and quick plant regeneration from the above

3. To study the effect of various auxins and cytokinins on callus induction, plant regeneration, multiple shoot induction and root initiation through in vitro culture technology

4. To determine the proper hormone combinations for induction of embryogenic callus and plantlet development via somatic embryogenesis from selected groundnut explants
5. To develop a protocol for isolation, purification and culture of protoplasts and regeneration of plants via somatic embryogenesis from protoplast derived callus

6. To develop methods for in vitro selection of resistant callus and regeneration of plants against the pathotoxic culture filtrates of *Cercosporidium personatum* through tissue culture technology

7. To study the effect of gamma rays, EMS and sodium azide on various morphological, physiological and productive characters and selection of desirable mutants for high yield, oil content and disease resistance and

8. To assess the performance of plants regenerated through tissue culture technology and those obtained by mutagenesis in the field.