Chapter-2

Review of Literature
2. GENERAL REVIEW OF LITERATURE

The foremost position of India as a supplier of medicinal plants is due to enormous wealth of these types that India has. Medicinal plants are those, which contain natural properties or substances that promote health and alleviate diseases of men, animals and crops. A large number of these plants are commonly weeds distributed in different parts of the world. Medicinal plants like several others must have evolved much earlier than the pre-historic man. They are particularly concentrated in the countries of the tropical belt, more so in India and China. It is no wonder, therefore, that in these countries medicinal plants are traditionally associated with cultural, economic and social fabric of the local communities. Nearly 50% of India's 17,000 flowering plants are medicinally used in the traditional system of medicine and in tribal lore, where information is passed on from one generation to other orally.

India is unique due to its very distinct phytogeography and agroecological diversity and is blessed with a wide variety of agroclimate zones repository of biological resources. With only 2.4% of the total land area of the world, it contributes 8% to the global diversity (Khoshoo, 1995). Presently about 18000 species of flowering plants exist in India of which nearly one third are endemic. These include 166 domesticated species of economic importance along with over 320 species of their wild relatives and around 500 species of medicinal value (Arora, 1988). The valuable plant genetic resources however have been lost at a very fast rate because of their replacement by high yielding varieties or due to heavy pressure on their natural habitats.

In India about 2500 plant species belonging to more than 1000 genera are used by traditional healers and about 500 plant species are utilized by 159 different pharmaceutical companies (Chandel et al. 1996). Research in the development and application of in vitro techniques for the conservation of the plant germplasm has significantly increased. In future, conservation and
biotechnology are sure to contribute significantly to the sustainable management of the endemic and / or economically important plant species.

Medicinal plants are of great interest to the researchers in the field of biotechnology as many of the drug industries depend on plants for the production of valuable compounds. The popularity of medicines coupled with reduction of natural resources threaten the very existence of a number of wild medicinal plants. Many of our valuable medicinal plants like Coscinium fenestratum and Rauvolfia serpentina are endangered at present. Kaempfria galanga is another medicinal herb, which will soon disappear from the biosphere unless conservation strategies are adopted (Kareem, 1997 and Shanker et al., 1997). As species and ecosystem loss continue unabated, it is feared that 1500 species of higher plants may disappear once and for all during the next 25 years or so (Nayar, 1996).

Realizing the importance of experiments, in vitro conservation of endemic medicinal plants were initiated in the 1980's at several research centres such as the National Botanical Research Institute, Lucknow (Chaturvedi et al., 1982) and Department of Botany, Delhi University, Delhi (Bhojwani et al. 1989). National Bureau of Plant Genetic Resources (NBPGR) was established in 1976 with the national responsibility for the collection, evaluations, conservation and exchange of germplasm of various agric- horticultural crops.

2.1 In vitro propagation

Plant cell and tissue is fundamental to most areas of biotechnology. Plant tissue culture is a technique of growing plant cell, tissues and organ in an artificially prepared nutrient medium static or liquid under aseptic condition. The basic concept of plant tissue culture is "Totipotency, the capacity of living cell to develop into a whole plant". Plant can be separated into their compound parts (organ, tissue, cells), which can be manipulated in vitro and then grown back to complete plants.
German botanist, Haberlandt (1902) developed the concept of *in vitro* cell culture. He was the first to culture isolated fully differentiated cells in nutrient medium containing glucose, peptone and knop's salt solution. Winkler (1908) cultivated segments of string bear and observed some cell divisions but not proliferation.

### 2.2 *In vitro* Conservation

Two approaches have been used for conservation of the medicinal plant biodiversity, namely *in situ* and *ex situ* approaches. *In vitro* conservation comes under the later method.

Clonal propagation of innumerable plant species was achieved through tissue culture methods from shoot tips or axillary buds by providing appropriate culture medium and other culture conditions. Shoot tips or axillary bud clonal propagation of a large number of medicinal plants has already been used to propagate commercially. In contrast to direct shoot multiplication, which faithfully produces clones regeneration of plant from callus does not result in production of clones (Skirvin, 1978 and Shephard, 1982). Clonal multiplication and *in vitro* studies have been reported on certain medicinal and economically valuable genera.

Somatic embryogenesis is an excellent method of plant regeneration allowing rapid production of a large number of true to type plants within a short period. Though somatic embryos are derived from somatic cells; they closely resemble their sexual counter parts and presumably result from the expression of the genes regulating the same development pathway. Somatic embryos are recognized as a superior method to multiple shoot formation for *in vitro* propagation and are considered as the best expression of totipotency.

Artificial seed production through shoot bud encapsulation has been reported in a range of medicinal plants like *Hyoscyamus muticus, Picrorhiza kurroa, Atropa belladona, Dioscorea floribunda, Rauwolfia serpentina* and...
Mentha arvensis (Ahuja et al., 1989), Holastemma ada-kodien and Tylophora indica (Keshavachandran, 1999). John and Keshavachandran (1996) encapsulated axillary shoot buds of Holostemma adakodien in calcium chloride. The beads could be successfully stored at the room temperature on the cotton wool moistened with liquid basal MS for 154 days, which could prolong to 40 days if stored in 4°C.

Lulsdorf (1993) reported that the addition of 0.5% (w/v) of activated charcoal to alginate capsule could significantly enhance the root development and germination of somatic embryos. Ahuja et al., (1989) has reported that for better response of synthetic seeds under glass house conditions, it was better to incorporate antibiotics like ampicillin, chloramphenicol, tetracycline and fungicides like bavistin.

Mathur and Ahuja (1991) made an assessment of various concentrations of sodium alginate and calcium chloride for the formation of beads in Valeriana wallichhi and indicated that 6% solution of sodium alginate upon compensation with 75mM of CaCl$_2$.2H$_2$O solution gave optimal firm and round beads within ion exchange complexation duration of 30 minutes. They encapsulated apical and axillary shoot buds of Valeriana wallichhi in calcium alginate beads and plantlets development was observed under both in vitro (98%) and mother (64%) conditions in pots with vermiculate under glass house conditions. They also found that the shelf life of encapsulated propagules of Selenium candolii, Nicotiana tabacum and Valeriana wallichhi was enhanced by 25-30 days to 150-240 days by overlying with mineral oil. Seitz and Reinhard (1987) reported that ginsenosides producing cell culture of Panax ginsen were cryopreserved successfully with neither the ginsenoside yield nor the ginsenoide pattern being influenced by cryostorage. The procedure consisted of 18 days preculture when the sucrose concentration was increased to 20 percent and the temperature lowered to 4°C, 1 hour cryoprotectant treatment in 0.2M sorbitol and 5 percent dimethyl sulphoxide, freezing at 1°C / min storage, thawing and recovery growth.
Mannonen et al., (1990) preserved cell line producing secondary metabolites of *Panax ginseng* and *Catharanthus roseus* in liquid nitrogen or under mineral oil for 6 months. The growth behaviour and the ability of the culture to produce ginsenosids or indole alkaloids were measured and compared with cultures maintained by frequent subculturing during the same period. It was found that cryopreservation preserves the cell culture productivity and hence can be used for preserving cell cultures producing cell metabolites while preservation under mineral oil can be recommended for maintenance of non-producing cell cultures.

Zhang et al., (1993) cryopreserved unopened mature anther of *Panax ginseng* and *Panax quinquefoliusi* by placing in test tubes, which were sealed and immersed in liquid nitrogen either directly or after stepwise pre-freezing at 1°C/min and kept at −10°C then cooled to −40°C.

Friesen et al., (1991) found that the cell suspension cultures of *Papaver somniferum* could be cryopreserved successfully by cooling at the rate of 0.5°C per minute. Cryopreserved cells were reported to exhibit a more rapid growth rate in suspension compared to unfrozen cells. Sanguinarine production in the frozen sample was seen to reach a maximum of 7-9mg per gram dry weight compared to 3-7mg per gram dry weight in control samples.

Yoshinatsu et al., (1996) found that though hairy root of *Panax ginseng* could be stored up to 4 months at 4°C, cryopreservation through vetrification was better for long-term survival. Root tips were precultured on half strength MS medium containing 0.3M sucrose for 1-3 days at 25°C in the dark and then treated with cryoprotectant solution (2M glycerol + 0.4M sucrose in MS medium) for 10 minutes, followed by the vetrification solution (30% glycerol, 15% ethylene glycol, 15% dimethyl sulphoxide, 0.4M sucrose in MS medium). After a few minutes, half of the solutions were removed and the tubes were immersed in liquid nitrogen.
Decruse et al., (1998) reported that short / medium term preservation under slow growth conditions was developed for several rare medicinal plants. Shoot cultures of *Piper barberi* survived over 840 days in an airtight screw capped bottles both at 20°C and 27°C. Incubation at 20°C instead of 27°C was reported to increase the shelf life of *Baliospermum montanum, Piper longum, Piper hapnium* and *Gloriosa superba* cultures from 300-360 to 510-720 days. Chaturvedi and Sharma (1986) developed a novel method of germplasm preservation of the medicinal plants through long term excised root cultures. Excised root of *Solanum khasianum, Atropa belladonna* and *Rauvolfia serpentina* were established in prolonged culture and maintained by periodic subculture at 3 months interval.

2.3 Isoenzyme analysis

The basic of electrophoresis analysis of isoenzymes was laid down in 1957 (Stabbins, 1989, McMillan, 1983) when Hunter and Muller discovered the isozymes. In 1959, Markert and Muller introduced the concept of isoenzyme, which they defined as the different molecular forms in which proteins may exist with the same enzymatic specificity (Buth, 1984). This means that different variants of the same enzyme have identical or similar functions and are present in the same individual. As such, their importance for understanding gene action in development and differentiation was exploited during the 1960s in both animal and plants.

Nevertheless, isoenzyme played a minor role in research on plant biochemistry until 1966 when genetic polymorphism for isoenzyme within the same population was discovered (Stabbins, 1989, Wendel 1989). That revealed the possibility for population genetics to make precise quantitative estimates of genetic variability based upon one parameter of the molecular structure of the primary product of the genes themselves (Zeidler, 1999).

In general, isoenzymes having common catalytic activity may be widely synthesized under the control of different genes, active in different tissues and
differing in molecular properties (Nash and Davis, 1975). Isozymic variations may also rise from allelic segregation at a single locus representing more subtle changes in the enzyme molecules. Isozyme analysis has been made in several enzymes in many plant species. These are peroxidase, catalase, amylase, leucine aminopeptidase, esterase, acid phosphatase, dehydrogenase, phosphorylase, transaminase and polyphenol oxidase, cytochrome oxidase etc., (Rocha and Ting, 1971).

Peroxidase and esterase are the two enzymes that have been extensively employed by research workers for biochemical tagging of genotypes and elucidation of several genetic phenomena at the biochemical level owing to their presence in most of the organs and easy detection (Mc Millin 1983; Hondge, 1986).

Molecular forms of an enzyme or isozymes can be separated by several biochemical methods including sedimentation, electrophoresis, chromatography and gel filtration and even serological methods of these gel electrophoresis is basically a process of forced diffusion with in an electric field. In this, the proteins of the given sample are moved through a gel or paper or cellulose by using electrical gradient. Different proteins assume different charges, at different pH and depending upon the molecular weight they move on the gel. This results in separation of different bands that can be stained and differentiated. Acrylamide gels are more useful and can withstand wide range of pH. They are also well known for optical clarity (Sako and Stahmann, 1972).

Mc Lune (1961) has reported multiple peroxidases in maize soon after the discovery of gel electrophoresis. Several workers have observed qualitative and quantitative changes in soluble proteins and isoenzyme pattern during development and differentiation of wheat (Bhatia and Nielson, 1969; Macko et al., 1967), Maize (Kadam et al., 1973), Barley (Upadhya and Yee, 1968), Ragi (Veerabhadrappa and Upadhya, 1979), Pea (Cherry and Ory, 1973) and
Sorghum (Johri et al., 1977). In a detailed review of enzyme heterogeneity in plants by Scandalios (1974) has listed 40 isozyme systems in which the pattern of gene expression varies with development condition. The enzyme activity of individual isoenzyme in a specific tissue, independent on maturity and cellular environment. The specificity of enzyme pattern implies the role of specific enzymes and isoenzymes in plant development and differentiation. Changes in isoenzyme activity during development of a tissue can be detected most conveniently by pattern shifts on zymograms subsequent to electrophoresis (Rao et al., 1992).

Brown (1978) has witnessed that electrophoretic analysis of proteins and isoenzymes after an efficient and cost effective method towards evolution of geographical and taxonomic distribution of genetic variation for sampling strategies in germplasm conservation.

In general, the developmental variation appears to be two fold, involving the number of bands and their relative intensities. These studies suggest that several structural and regulatory genes might be involved in the production of different isoenzyme bands at various stages of development (Scandalios, 1974, Johri et al., 1977).

Freeling (1983) has reported that isoenzyme patterns are better biochemical means to analyse intergenomic interation with higher accuracy. Electrophoresis of proteins and isozymes provide a quick method of ascertaining hybrids and derived species relationship in Brassica.

The electrophoretic analysis of proteins or enzymes has been very widely applied ranging from the breeding of new varieties through seed testing production and certification, to the selling of the harvested grain and its commercial processing (Cooke, 1988).

Cooke (1989) has carried out the use of gel electrophoresis of protein and isozyme to distinguish between genotypes of cultivated autonomous crop
species. Electrophoresis was incorporated into schemes for assessing directness, uniformity and stability.

Kephart (1990) has comparatively gathered information from 25 laboratories utilizing enzyme electrophoresis providing access to protocols for extraction, effective storage of plant samples and buffers, efficient screening of taxa for enzymatic activity and interpretation of diploid and polyploid banding patterns.

Tanimoto and Matsumato (1986) have studied electrophoretic pattern of peroxidase and esterase for identification of genetic diversity in different variation of Colocasia esculata and C. gigantia

Hui Han et al., (1986) demonstrated variation in different organs of tea shoots using peroxidase and polyphenol oxidase isoenzyme pattern. Pearl millet lines susceptible and resistant to ergot were analysed for their peroxidase isoenzyme in pattern by Chahal et al., 1986.

Nakabayashi et al., (1980) has studied the browning in white and yellow peaches due to the polyphenol oxidase (PPO) and reported that the degree of browning correlated well with phenol content in peaches.

Woody tissue of grapes was used to distinguish 29 genotypes by isoenzyme pattern (Subden, 1987). Qualitative and quantitative changes of peroxidase, acid and alkaline phosphatase of the shoot tip, nodal segments with buds, internodal segments, leaves and mature flowers in male and female plants of Coccinia grandis were analyzed. Higher peroxidase and phosphatase activity were noted in the explants from male plants than that from the female plants. Variation in isoenzyme pattern was exhibited by different explants obtained from both male and female plants (Gulati, 1989).

Manju agarwal and Kaul (1993) studied electrophoretic pattern for cultivar identification in Anethum graveolens L.
Peroxidase isoenzyme pattern helped in distinguishing embryonic and non-embryonic calli of tea (Anon, 1996). Electrophoretic studies have demonstrated that isoenzymes pattern and intensities are tissue and plant specific and also different developmental stages have specific isoenzyme patterns. However, several factors like mineral nutrition, cold temperature hardening, disease infection injuries etc. affect the intensity of isoenzyme (Shannon et al., 1966).

Barone et al., (1996) used esterase, peroxidase and acid phosphatase for identification of Pistacia vera germplasm. Fruit ripening was studied in Citrus annum using isoenzyme system of ascorbate peroxidase (Schantz et al., 1995) and β-galactosidase (Biles et al., 1997). 400 melons (Cucumis melo) were compared and characterized by 13 isoenzyme systems (Staub, 1998).

Characterization between Solanum melongena and Solanum indicum was done by the aspartate aminotransferase, alcohol dehydrogenase, phosphogluconate, phosphoglucomutase and shikimate dehydrogenase isoenzyme system (Isshiki, 1994). The population of Tulip from different parts of Greece was characterized and studied using esterase and malate dehydrogenase (Protopapadakis and Yannit Swios, 1994). Esterase isoenzymes showed a specific electrophoretic pattern while characterizing 10 sugarcane cultivars. The peroxidase allowed arranging the cultivars in-group, each one with a specific electrophoretic pattern. The isoenzymes of both esterase and peroxidase were constant in a given cultivars (Almeida, 1994). Detection of difference in callus, roots and stem of 1, 2 and 7-year-old Asparagus employed 11 isoenzyme systems including esterase and shikimate dehydrogenase (Qiu and Zhou, 1995). Identification of Tulip population of leaves was done by using glutamate oxaloacetate transaminase (Protopapadakis, 1995).

Isoenzyme polymorphism in bud tissues from Juglans nigra L., Juglans regia L. and their natural hybrids Juglans intermedia CARR was analyzed. The result revealed that those isoenzyme variants of the enzyme systems
aspartate amino transferase (AAT) and phosphoglucomutase (PGM) are suitable to identify hybrids. The use of this method is suggested for forest nursery practice to distinguish hybrids at an early ontogenetic stage (Hussendoerfer, 1999).

Similarity between 77 Chinese and 3 Japanese cultivars was studied using esterase isoenzyme characterization. Malate dehydrogenase, phosphoglucomutase and shikimate dehydrogenase isoenzyme systems were used to classify hybrids and cultivars of Asparagus (Chen and Zhou, 1999).

Handa et al., (2000) used isoenzyme analysis of hybrid plants of Populus ciliata X Populus maximowiczii. Five enzyme systems (peroxidase, leucine aminopeptidase, esterase, catalase, malate dehydrogenase) were tested. Among the five, three enzymes got resolved well in electrophoresis. Peroxidase and esterase were polymorphic whereas Leucine aminopeptidase was monomorphic.

Variations between 12 natural populations in Eucalyptus microtheca were studied using 9 isoenzyme systems (Li, 1999). Isoenzyme analysis of different populations of Bartramia stricta Brid from Southern France and the Mosel valley area in Germany revealed that the Germany populations studied were identical to one of two populations from Southern France. This could be explained by the fact that only one population from Southern France was able to extend its range to the North and survive there (Quandt, 2000).

Activities of peroxidase, lipoxygenase and gel based isoenzyme pattern of esterase in fresh and stored grains of twelve different varieties of rice were identified as major parameters that could be used to characterize basmati and aromatic and non-aromatic basmati varieties (Srivastava, 2002).

Isoenzyme (peroxidase) analysis was done in calli of different ages of Psidium guajava cv to show a direct correlation between peroxidase activity and tracheary element differentiation (Malabadi and Nataraja, 2002).
Activity of enzyme polyphenol oxidase and peroxidase was determined in developing grains and seedling of two late and early maturing varieties of mung bean (*Vigna radiata* L.). Surprisingly the activity of polyphenol oxidase was found to be completely absent in developing grains. In developing seeds the activity of both enzymes was found to increase with the age of seedling (Gupta *et al.*, 2002).

Polypeptide and isoenzyme patterns in *Aconitum heterophyllum* wall ex Royle seeds were studied in five populations collected from Garhwal Himalaya. The zymograms for different enzymes also varied greatly among different population of *A.* *heterophyllum*. Sikkim population of *A.* *heterophyllum* showed one specific band of esterase. All the populations showed similar trend in respect of peroxidase and acid phosphatase isoenzymes (Bahuguna *et al.*, 2003).

The effect of sodium dodecyl sulphate (SDS) on purified tobacco leaf polyphenol oxidase was investigated at various pH and temperatures. SDS increased the activity of polyphenol oxidase due to the formation of SDS-PPO complex (Huijiang *et al.*, 2003).

Proper identification and understanding of genetic diversity in *Phyllanthus amarus* and *Phyllanthus debilis* were carried out using isozyme technique. To assess levels and patterns of genetic diversity of *Phyllanthus* species, twenty individuals from each species were analyzed for isozyme variation using four enzyme systems (esterase, peroxidase, shikimate dehydrogenase and glucose 6-phosphate dehydrogenase) (Britto *et al.*, 2004).

Adventitious rooting of *Ebenus cretica* cutting was studied in order to examine rooting ability of different genotypes in relation to electrophoretic patterns of peroxidase, the activity and electrophoretic patterns of soluble and ionically bound peroxidases (Syras *et al.*, 2004).
Twenty-two sunflower (*Helianthus annuus* L.) genotypes were analyzed by gel electrophoresis (SDS-PAGE) to characterize soluble seed protein spectrum. All the genotypes were distinguished on the basis of presence or absence of specific bands, intensity of bands and relative mobilities (Rm) of bands (Navhale and Rathod, 2004).

Studies on electrophoretic patterns of five enzymes aspartate amino transferase (AAT), glucose phosphate isomerase (GPI), glutamate dehydrogenase (GDH), shikimate dehydrogenase (SKDH) and triose phosphate isomerase (TPI) resulted in identification of 17 phenotypes in 21 accessions of *Solanum melongena* L, *Solanum insanum* L and *Solanum incanum* L. The results provided evidence of 10 isoenzyme loci presented by 20 alleles involved in the control of the isoenzymes (Kaur *et al.*, 2004).

Polymorphism of peroxidase and changes in its activity and in hydrogen peroxide content were studied in buds of grapevine during dormancy. Three isoforms of peroxidase were detected in bud extracts, 2 basic and one acidic, however, the pattern of peroxidase isoenzyme changed with progress of dormancy (Pere and Burgos, 2004).

The literature reveals pros and cons of isoenzyme analysis in population biology, conservation biology and ecology.

**2.4 Studies on Antimicrobial activity of plants**

The use of plants as antimicrobials from wide range of higher plants from time to time has been experimented by several earlier workers (Cowan, 1999; Samy and Ignacimuthu, 2000). The choice of botanicals is more appropriate due to its efficiency and economy and also they are free from side effects. Some examples, *Harpagophytum procumbens* is against rheumatism (Chrubasik *et al.*, 1996), *Clausena dentata* against cancer, muscular pain, malarial fever, ulcer and liver disorders (Rastogi and Mehrotra, 1984). *Valeriana officinalis* (Valerian) is used as sedative.
The stem extract of *Fibraurea chloroleuca* showed the greatest activity over both gram positive and gram negative bacteria, *Candida spp* and dermatophytic fungi (Mohtar et al., 1998), while the extract from 8 medicinal plants of Trinidad was found to be highly effective against *Staphylococcus aureus* (Chariandy et al., 1995). Substantial inhibitory action of *Thymus vulgaris* against *Candida albicans* and *Escherichia coli* and of *Vetiveria zizanioides* against *Staphylococcus aureus* (Hammer et al., 1999) has been reported. Petroleum ether and alcohol extracts of aerial parts of *Argemone mexicana* showed high antimicrobial activity on *Bacillus subtilis* and *Escherichia coli* (Sangameswaran et al., 2004). Essential oils of *Melia dubia* exhibited bacteriostatic and fungistatic activities against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Fusarium oxysporum* and *Candida albicans* (Nagalakshmi et al., 2001). *Dodonaea viscosa* leaf extract at 12.5mg/ml exhibited antimicrobial activities against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and particularly on *Candida albicans* (Kpemissi et al., 2003).

Extracts of eight medicinal plants *Anredara diffusa*, *Asclepias curassavica*, *Cassia tomentosa*, *Cestrum auriculatum*, *Himatanthus sacaaba*, *Krameria triandra*, *Peperomia galioides* and *Sambucus peruviana* were effective against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium diphtheriae* and *Bacillus megaterium* (Catherine et al., 2002). The crude methanol extract of *Alstonia macrophylla* leaves was found to be effective against *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Proteus mirabilis*, *Trichophyton rubrum*, *T. mentagrophytes* and *Microsporum gypseum*.

**2.5 Histological studies on plants**

Significant changes in leaf morphology and anatomy, especially in epidermal characteristics caused by *in vitro* culture conditions, have been reported (Pospíšilová et al., 1999). Sweetgum (*Liquidambar styraciflua* L.)
plantlets cultured \textit{in vitro} had a less developed cuticle, as compared to the well-developed cuticle in leaves of transplanted and field grown plants (Wetzstein and Sommer, 1982). Gilly \textit{et al.} (1997), in studying the cuticle formation of ivy (\textit{Hedera helix} L.) plants \textit{in vitro} and after transferring to \textit{ex vitro} conditions observed a progressive activation of cuticle biosynthesis as the plants adapted to the \textit{ex vitro} environmental conditions.

Histological, histochemical, and ultrastructural aspects of somatic embryogenesis, whether occurring with or without a callus phase, have been extensively reported (Ho and Vasil, 1983; Williams and Maheswaran, 1986; Stamp, 1987; Pedroso and Pais, 1993; Brisibe \textit{et al.}, 1993; Sagare \textit{et al.}, 1995). A system of efficient regeneration parallel to somatic embryogenesis has been proposed (Aitken-Christie \textit{et al.}, 1988; Mc Cown \textit{et al.}, 1988; Warrag, Lesney, and Rockwood, 1991; Teng, 1997) and is based on the formation of organogenic nodules that arise directly from the explants or from callus and cell suspension cultures. Fortes and Pais, (2000) reported the histological events leading to nodule formation and shoot regeneration from internode-derived nodules of \textit{Humulus lupulus} var. Nugget and on the evidence of a starch accumulation/mobilization cycle during this morphogenic process. Their observations indicated that shoot buds in \textit{Humulus lupulus} var. Nugget arose from organogenic nodules derived from explant internodes.
2.6 AIM AND OBJECTIVE

*Naringi crenulata* (Roxb.) Nicols is a tree species belonging to the family Rutaceae. Different parts of the plant have medicinal properties such as purgative, sudorific and stomachic. The root is employed for the cure of colic and cardialgia. The dried fruit is a tonic and useful in malignant and pestilent fevers. Moreover, it is considered to be a sacred tree that is found growing in the premises of Temples.

*Aegle marmelos* (L.) Corr belonging to the family Rutaceae is also a tree that is well known for its medicinal properties such as astringent, digestive, stomachic and diarrhoea. It is one of the ingredients in the ‘Dasamul’ or ten roots, which is an important preparation in Ayurvedic system of medicine.

Propagation of woody species by conventional breeding methods is cumbersome and beset with a number of limitations. Rapid propagation methods for such plants, especially for those which possess useful medicinal properties, are an urgent need. *In vitro* propagation is an important tool for rapid multiplication of medicinal plant (Jha and Sen, 1984) as well as forest trees (Ahuja, 1991).

The present study deals with the following objectives:

- To optimize plant sterilization
- To optimize media for shoot and root induction
- To optimize plant growth regulators for shoot and root induction
➢ To produce protocol for the large scale multiplication

➢ Genetic conformity test by using isoenzyme analysis

➢ Screening of leaf extracts of both plants against few pathogens, viz, *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhi* and *Staphylococcus aureus*.

➢ Isolation and identification of active principle present in the leaf extract of *Naringi crenulata*

➢ Histological studies of both mother plant and *in vitro* raised plants of *Naringi crenulata* and *Aegle marmelos*.