Chapter 1

Introduction
Plants are an important source of medicines and play a key role in world health (Constabel 1990). Almost all civilizations from ancient times to today have used plants as medicine. The medicinal plants are important to the global economy (Srivastava et al. 1995), as approximately 85% of traditional medicine preparations involve the use of plants or plant extracts (Vieira and Skorupa 1993). The past decade has witnessed a tremendous resurgence in the interest and use of medicinal plant products (Hoareau and deSilva 1999; Davicino et al. 2006).

During the last few decades, the public awareness in plant based medicine has increased significantly. At the same time, the activity of pharmaceutical industries has also increased several folds in recent time. As a result of overexploitation many of the medicinal plants are reported to be under severe threat (Faisal et al. 2005).

Owing to their importance scientists all over the world have attempted to develop syntheses of such components, but due to structural complexity, the resulting multistep organic synthesis of compounds rarely find application in largescale production as required by pharmaceutical industries. Thus, the need of plant derived compounds are usually met by extraction from the living plants. The plant secondary metabolites are not found in all plants, have a specific distribution, and are also found in a very low quantity. Depending on the plant species, traditional agriculture methods often requires months to year to obtain a crop (Kieran et al. 1997). Further more, the levels of secondary metabolites are often influenced by many other factors, including pathogens and climate changes.

Advanced biotechnological methods of culturing plant cells and tissues provide new means of conserving and rapidly propagating valuable, rare, and endangered medicinal plants. Combination of in vitro propagation techniques (Fay 1992) and cryopreservation (Wang et al. 2002) may help in conservation of biodiversity of locally used medicinal plants. Plant tissue culture has emerged as a potential tool and forms the backbone of plant biotechnology. In the recent years, tissue culture combined with genetic engineering has emerged as a promising technique for the
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improved production of medicinal plants. *In vitro* propagation (micropropagation) is a miniature version of conventional propagation, which is carried out under aseptic conditions (Durzan 1988). Plants raised through micropropagation are of uniform quality, pathogen free, show improved vigor and can be produced much more rapidly as new cultivars could become commercially available with in 2 to 3 years from development rather than 5 to 10 years needed using conventional propagation. The advent of *in vitro* tissue culture technique has offered a new approach to the morphogenetic investigations. It allows a living system to be studied under controlled environmental conditions. This also enables a study of the complex biological phenomenon in parts.

*In vitro* clonal propagation involves a few stages, as (i) Initiation and establishment of aseptic culture, this involves explant isolation, surface sterilization and establishment of culture on an appropriate medium; (ii) Multiplication/Regeneration of propagules, this stage is concerned with rapid multiplication of the regenerative system for obtaining large number of shoots/regenerants; (iii) Rooting and pretreatment prior to transfer to soil, this stage is characterized by preparation shoot clusters for successful transfer to the soil; (iv) Transfer to natural environment, this stage deals with transfer of plantlets in pots, their hardening and establishment in soil. A successful tissue culture method of propagation must result in re-establishment in soil of a high frequency of the tissue culture derived plants.

Plant regeneration via somatic embryogenesis

Somatic embryogenesis (SE) is the process by which somatic cells, under inductive conditions, generate embryogenic cells, which undergo a series of morphological and biochemical changes resulting in the formation of somatic embryos (Zimmerman 1993; Schmidt et al. 1997; Komamine et al. 2005). SE forms the basis of cellular totipotency that is unique phenomenon in higher plants. Differing from its zygotic counterpart, somatic embryos are easily tractable, culture conditions can be controlled and lack of material is not a limiting factor for experimentation (Kawahara and Komamine 1995). These characteristics have made SE a model for the study of
morphological, physiological, molecular and biochemical events that occur during the onset and development of embryogenesis in higher plants (Jimenez and Bangerth 2001). It also has potentially rich biotechnological applications such as artificial seeds, micropropagation, transgenic plants etc.

Since the first report of somatic embryogenesis (Steward et al. 1958; Reinert 1959) this *in vitro* technique has been studied extensively in diverse plant groups (Jimenez and Bangerth 2001). Studies indicate that several cultural conditions/inducers influence cultivated tissues and make the cell competent in demonstrating embryogenic efficiency (Feher et al. 2002).

**Induced Mutations**

Induction of mutations based on the use of chemicals or ionizing radiations is one of the major breeding approaches for plant improvement (Ahloowalia and Maluszynski 2001). Combination of such techniques with a variety of *in vitro* culture methods can speed up breeding programmes, from the generation of variability, through selection, to multiplication of the new genotypes (Maluszynski et al. 1995). Tissue culture also allows for the handling of large populations for mutagenic treatment, selection, and cloning of selected variants. It also offers the possibility to rapidly execute the propagation cycles of subculture aimed to separate mutated from non-mutated sectors. (Ahloowalia 1998).

The impact of mutation techniques has been evaluated, during the last 3 decades approximately 1900 mutant varieties have officially been released, nearly 1300 are in agricultural crops and over 500 in ornamental plants (Sigurbjörnsson and Maluszynski 1995). In contrast, very limited work (Kaul and Choudhary 1975; Kak et al. 1982) has been conducted on the application of mutation techniques to raise new varieties of medicinal plants that may, overproduce the desired secondary metabolites.

A variety of plant derived alkaloids are used as pharmaceuticals which has been isolated from whole plant extraction process. This causes rapid depletion of natural
valuable flora and has now become an important socio-economic issue. Alternatively, plant cell culture technique offers a continuous renewable source of phytocompounds that may or may not be produced by microbial cells or by chemical synthesis. As there are several other advantages, these in vitro technologies have often been exploited for the production of alkaloids and biochemicals (Dicosmo and Misawa 1995; Zhao et al. 2001; Mulabagal and Tsay 2004).

The indolic alkaloids, vinblastine and vincristine are used in cancer chemotherapy for treating various leukemias, Hodgkin's disease and solid tumors (Dicosmo and Misawa 1995). The drugs are solely produced commercially by extraction of large quantities of Catharanthus plant material. However, the intact plant, contains low concentrations of drugs (0.0005% dry weight basis). As an alternative to whole plant extraction, plant cell cultures have been employed in efforts to produce vinblastine and vincristine (Zhao et al. 2001).

General description and Importance of the selected plant

Catharanthus roseus is commonly known as Madagascar periwinkle is an important medicinal as well as ornamental plant of family apocynaceae. It has been widely cultivated for hundreds of years and can now be found growing wild in most warm regions of the world, including India. The plants grow one or two feet high, have glossy, dark green leaves (1-2 inches long) and flowers all summer long. The blooms of the natural wild plants are pale pink with a purple "eye" in their centers, but horticulturists have developed varieties with colours ranging from white to hot pink to purple. The plant has historically been used to treat a wide assortment of diseases. It was used as a folk remedy for diabetes in for centuries (Nammi et al. 2003). The juice from the leaves was used to treat wasp stings. Also, it was used as an astringent, diuretic and cough remedy (Nammi et al. 2003).

Cell and tissue culture techniques have been used for a long time to improve alkaloid yield in a variety of medicinal plants including Catharanthus. For such purpose, various plant parts (shoot, root, callus, organ, suspension etc) have been used to