Chapter 1

INTRODUCTION
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Medicinal plants are the important and valuable source of various medicinal preparations as well as pure form of medicines. Since prehistoric time natural products from plants have been employed to heal several human ailments throughout the world. Eighty percent of the world population is dependent upon plants for primary health care, particularly in the developing countries (Akerele, et al 1992). Even today plants are treated as the most exclusive source of drugs for the majority of world’s population and plant products constitute about 25% of prescribed medicines (Farnsworth and Bingel, 1977; Principie, 1989).

Recognised as one of the 12 mega biodiversity centres, India is floristically a very rich country (Shah, 1982). India has a vast and inexhaustible resource of drugs of plant origin. The systematic investigation of these drugs used in indigenous medicine on modern scientific lines, gained momentum since independence. A significant portion of these species is employed for medicinal purpose in a variety of ways, in allopathic or modern medicine, and traditional systems of medicines.

Over 90% of the plant species are collected from the wild, very often in a destructive and unsustainable manner and less than 10% of the medicinal plants traded in the country are cultivated. Overharvesting, loss of habitat, increasing urbanization has resulted in significant decline in the volume of raw materials produced. More significantly it has resulted in irreversible loss of population of medicinal plant whose size will be too diminished to survive through natural means of asexual reproduction. A renewed interest in plant based medicine including nutraceuticals in the West has fuelled greater demand for raw materials all over the world. As a result many plants, which are especially in demand, are endangered and some are on the verge of extinction (Sterenson and Narr's, 1980). The plants growing on wild are dependent on soil, seasons and weather conditions and hence they may not be available throughout the year. Moreover, accidental collection of unwanted plants often leads to unintentional
adulteration of the collected material. Therefore search for an alternative method of propagation and for preserving germplasm and for sufficient supply of raw material irrespective of season and weather condition, has become utmost important. Conventional method of propagation takes years to get sufficient amount of plant material for commercial distribution. It is therefore imperative to conserve our medicinal plant wealth on a scientific basis. Biotechnology has opened up new vistas in the conservation of medicinal plants by way of in vitro propagation. Developments in the technology of plant tissue culture since its pioneering experiments by White (1934, 1937) and Murashige and Skoog (1962) have contributed in establishing a strong foundation for the application of this versatile technology. The important morphological application of plant tissue culture is micropropagation. The use of micropropagation has also become of much importance in medicinal plants due to its different advantages. Looking at the alarming rate of extinction of medicinal plant, the trend has naturally been diverted to utilize plant tissue culture technology to rapidly propagate the elite genotypes of different medicinally important plants. Use of tissue culture technique for conservation of rare and endangered medicinal plant species also help them to be multiplied with minimum loss of time, which can be reinforced for establishment in their natural habitats.

Gloriosa superba L. (liliaceae) is an overexploited and important medicinal plant likely to be originated in Sikkim and Khasi Hills and distributed throughout India. All of its plant parts find diverse uses in indigenous systems of medicine (Sivkumar and Krishnamurthy, 2000). Corms and seeds are rich source of colchicine and many other related alkaloids and possess thermogenic, abortifacient, alexeric, and antipyretic properties. Leaves are used to treat ulcers, piles scrofula and to expel placenta and seeds are used to cure cancer related diseases (Evans et al., 1981). The colchicine content being higher in the seed than in the corm, there is a great demand for seeds in the export market (Samarjeewa et al., 1993). The conventional method of propagation is through corms since poor germination of seeds restricts their use in multiplication. On the other hand, the practice of using corms for multiplication does not provide enough planting material to meet the demand of raw materials, as one corm gives at the most only two
plants a year and 4-5 vegetative cycles are needed before the flowering stage is reached (Somani et al., 1989). Owing to its widespread use in traditional system of medicine indiscriminate overexploitation from its wild habitat is occurring over time, which might result in extinction of this valuable plant species from the nature in near future.

The most common active principle, colchicine present in *Gloriosa superba* has been used for a long time in the treatment of gout (Wildman, 1970). During the period, 1940-50, the genetic potentialities and possible therapeutic values of this compound in combating cancer was apparent and became the subject of extensive chemical (Dewan, 1945) and medical investigation (Capraro, 1984). There have been several reports of tissue cultures giving yields to secondary products, which have exceeded those present in the explant. It has been established that slow growing callus accumulates more secondary products (Ramawat, 1990) and the composition of the culture media for establishing callus and plantlets have influence on the production of biomass and on the synthetic machinery for both primary and secondary metabolites. Thus plant tissue culture technology has considerable scope for the production of important secondary metabolites. This technique has provided an alternative to whole plant cultivation for obtaining such metabolites (Purohit et al., 1994).

Medicinal properties of *Gloriosa superba* however have not been exploited extensively for its secondary metabolites both *in vivo* and *in vitro* and hence many of the compounds remain unidentified. Therefore bioprospecting of this plant species has become important for the isolation of several new compounds. One of the most common uses of plant cell and tissue culture technology is, *in vitro* cloning or asexual propagation of plants. Tissue culture approaches are needed for the rapid propagation and conservation of the plant (Finnie and Van Staden, 1989). Clonal propagation for *Gloriosa superba* is a potentially valuable method to accelerate the production of the plant throughout the season as natural propagation occurs through corms, which grows only during the rainy season. The poor germination of seed, uncontrolled collection by pharmaceutical industries necessitates rapid micropropagation technique for this species.
Plant tissues and cells in culture undergo variations. These variations sometimes lead to unwanted results. Analysis and characterization of genetic variation is fundamental to any conservation strategy, whether in situ or ex situ (Westman and Kresovioch, 1997). Also strategies to assess the genetic stability of in vitro derived plants such as karyological analysis or isozyme markers (Isbel et al., 1993) are in use. Studying isozyme profile is one of the methods to detect genetic diversity. Keeping these few aspects in mind the present experiment was designed with the following objectives.

1. Micropropagation of *Gloriosa superba* using different plant parts as explants.

2. High frequency rhizome production and subsequent regeneration.

3. Comparative isozyme studies to assess any variation in wild and in vitro grown plants.

4. Extraction of colchicine and their qualitative and quantitative comparison in wild and tissue cultured plants.