Chapter-1

General Introduction
1.1 INTRODUCTION

Plants are the miniature repositories of nature providing food, spices, fuel, fiber, wood, resins, gums, essential oils, fragrances, medicines and several other important compounds of great medicinal value. Since the ancient times natural products from plants have been employed to cure human suffering. The detailed description of medicinal plants, their properties and their uses for assisting and improving various body functions have been documented in Indian system of medicine ‘Ayurveda’ in ‘Sushruta Samhita’ and ‘Aushtang Ayurveda’. ‘Charaka Samhita’ (900 B.C.) and ‘Sushruta Samhita’ (500 B.C.), dealing with the Ayurvedic pharmacopoeias, were compiled on the basis of knowledge contained in the ‘Rigveda’ and ‘Atharvaveda’, one of the oldest repositories of human knowledge.

History and importance of plants as a vital source of raw materials dates back to early part of this century when medicinal plants were used as much for their ritual magical powers as for their medicinal qualities. During the Rigveda period, the Indo-Aryans were acquainted with a large number of medicinal plants and described ‘Soma’ as the first medicinal plant used by the ancient man. During 1500 B.C. plant like *Aegle marmelos* was considered to be sacred to specific divinities in the Hindu religion, whereas the western culture believed that plants like *Sambucus nigra* possess spiritual powers restricting the trees to be cut down. By the 500 B.C. the myth associated with these herbal plants began to give way to the Indian and Chinese systems of medicine which formed the base of traditional and modern medicine systems. Later on science of medicine started separating from the magical and spiritual world. Hippocrates (460-377 B.C.) known as ‘Father of medicine’ considered the illness to be cured only through proper medication and not through magical or spiritual powers. With this concept, herbal traditions changed all over the world, setting the stage for the re-investigation of lower and higher plants for new biologically active constituents. Plants are extensively used throughout the world in two distinct areas of health management, (i) traditional systems of medicine and (ii) modern system of medicine. Recent upsurge in use of herbal medicines due to its lower toxicity and side effects compared to allopathic medicines has led to sudden increase in the number of herbal drug manufacturers. Capability of Ayurvedic system of medicine to treat all types of illness from minor coughs and colds to life threatening diseases such as tuberculosis, malaria, asthma, arthritis and irritable bowel syndrome for which till date, no conventional treatment exists, makes it a better stream of medication. Popularity of
healthcare plant-derived products in developing as well as developed countries has been traced to their increasing acceptance and use in the cosmetic industry as well as to increasing public costs in the daily maintenance of personal health and well being through modern medicine such as allopathy. Indeed botany as a science in the western world, is rooted in the use of herbs as medicines. The existence of traditional medicine depends on plant diversity and the related knowledge of their use as herbal medicine. In addition both plant species and traditional knowledge are important to the herbal medicine trade and the pharmaceutical industry whereby plants provide raw materials and the traditional knowledge pre-requisite information.

According to the World Health Organization (2002), 80% of the world population is dependent upon plants for primary health care, particularly in the developing countries, where local communities are offered immediate access to safe and effective products so as to treat ill health through self medication. Some of the most potent remedies used today e.g. aspirin, digoxin, codeine, morphine, vinblastine, vincristine, pilocarpine, cocaine, atropine, emetine and ephedrine are derived from the herbal wealth of the world (Natesh and Mohan Ram, 1999).

Detailed chemical screening of just 90 species of flowering plants have yielded 120 widely used drug molecules of known biological activity with practically no synthetic substitute. For nearly 50% of these bioprospection herbs, cultivation technology has been developed on a commercial scale. It is estimated that during next five years, nearly 350 new herbal drugs will enter the global market of modern pharmaceuticals. In view of tremendous demands of the plants throughout the world in medicines, phytochemicals, nutraceuticals, cosmetics and other products, they have become a major sector of trade and commerce. The value of medicinal plants as a source of foreign exchange for developing countries depends on the use of plants as raw materials in the pharmaceutical industry. It provides numerous opportunities for developing nations to advance rural well being.

India recognized as one of the 12 megabiodiversity centers of the world, is floristically a very rich country (Anonymous, 1996). India has 2.4% of world’s area with 8% of global bio-diversity. With over 45,000 species of medicinal herbs spread over three environmental hotspots in the Western Ghats, Eastern Himalayas and the Andaman and Nicobar Islands, India is termed “the world herbal garden”. A significant proportion of these species is employed for medicinal purpose in a variety of ways in allopathic or modern medicine, in traditional system of medicine, in tribal and folk practice, beauty care
and for export of raw material. Traditional systems such as Ayurveda, Unani, Siddha, Homeopathy and Naturopathy are reported to make use of about 1,000 plant genera and 2,500 species. Today, western herbal medicines make use of maximum herbal plants indigenous to Europe, Australia, Africa and America, however, the Indian traditional system of Ayurvedic medicine still remains the dominant herbal tradition. Estimates suggest that as many as 3226 of the 4752 communities in India-representing 70% of the population are dependent on traditional plant based medicines (Gadgil and Rao, 1998). There are around 25,000 effective plant-based formulations, used in folk medicine and known to rural communities in India (Ramakrishnappa, 2002).

Estimates of the volume and value of herbs and herbal products in India vary widely. There are 1650 herbal formulations in Indian market and number of major plants involved in their formulation is around 540 (Polshettiwar and Kudal, 2006). The turnover of herbal medicines in India as over-the-counter products, ethical and classical formulations and home remedies of Ayurveda, Unani, and Siddha systems of medicine is about $ 1 billion with a meagre export of about $ 80 million (Kamboj, 2000) The worldwide market of herbal medicines is of the order of $ 60 to $ 80 billion (WHO, 2002; Mathur, 2003) which is growing by 7 to 12 per cent annually and is likely to become a $ 5-trillion industry by 2050. China and India are two largest users of medicinal plants. China's share in world herbal market is $ 6 billion while India's share is only $ 1 billion which is expected to rise (Rawat, 2002; Dubey et al., 2004), (Fig.-1). Inspite of the rich and diverse plant resources in our country, our export performance is not impressive.

Fig-1: Percentage share of major developing countries in worldwide market of herbal medicines
The major share of market volume is contributed by drugs like ginseng, ginkgo, taxol, vincristine, vinblastine, aloe gel and many more plant based chemical extracts and formulations (Kumar, 2000). All the major herbal-based pharmaceutical companies are showing a constant growth of about 15 per cent or more, next only to information technology industry. There is no doubt that herb exporting countries will get more revenue following the widening of market for herbal products in developed countries. It is estimated that about 25% of modern medicines are obtained from plants. Almost, 70% modern medicines in India are derived from natural products (Choudhary, 2002). Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs of higher efficacy or as models for pharmacologically active compounds (Mukherjee, 2003).

Natural products play a dominant role in the pharmaceutical industry and the systemic investigation of natural resources for the discovery of new drug molecules has been a primary objective today. Prospecting for new potential pharmaceuticals, from sources such as natural products which are traditional in nature or derived from folklore or from little known rain forests have also become an important part of industry. The active principles of some medicinal plants after undergoing pharmacological, toxicological and clinical trials were introduced as drugs in the modern medicine. With the changing scenario and to keep pace with an annual growth rate of 10-12% there is an urgent need to fix priorities and creating capabilities for developing new drug molecules, advanced drug delivery systems, biotechnologies including fermentation technology and emerging approaches such as advanced genomics, high throughput screening, combinatorial chemistry and biology and computer assisted de novo drug design.

Many of these commercially valuable phytomolecules, ‘secondary metabolites’ perform a variety of physiological roles and they are usually not essential to plant growth. The term ‘secondary metabolites’ was coined by Czapek (1921). Secondary metabolites which are used commercially as biologically active compounds in pharmaceutical, flavour, fragrance and pesticide industries are the more valued compounds in the global market. These compounds are produced in small amounts and often accumulate in specialized tissues. Being synthesized in specialized cells at distinct developmental stages make their extraction, isolation and purification difficult, thus making them high value-low volume products. Non-availability of cost-effective synthetic procedures has been the driving force for the extraction of these compounds from cultivated plant sources and has been the
major mode to economically produce these important secondary metabolites (Balandrin et al., 1985; Di Cosmo and Misawa, 1995). Long growth periods and maturity of these crops which in turn are affected by many factors i. e. diseases and climate changes always affect the level of secondary metabolites. These types of environmental and biological problems associated with conventional cultivation provided impetus for various advancements in plant cell and tissue culture technology to overcome these basic problems. Though the bulk of tissue culture studies carried out in medicinal plants were directed towards in vitro secondary metabolite production, the success rate has been disappointing (Taylor, 1998). The reason for this failure has been traced to a rigid spatial, temporal and developmental regulation of many secondary metabolite pathways in plants. With advancement in our understanding of biogenetic routes for the synthesis of these metabolites, it is becoming increasingly clear that in terms of cellular economy these chemicals are metabolically expensive to be produced and transported to specialized cell type at distinct developmental stages of plant growth (Robins et al., 1991; Facchini, 1999). Longer cell doubling time, shear sensitivity, repression of crucial biogenetic pathway steps as a function of inadequate cytodifferentiation, poor sink capacity, genetic instability and finite resources and carrying capacity of the undifferentiated cultures are therefore, the primary constraints of such an approach. These realizations on one hand and increasing demand of the natural products on the other hand have forced us again to look plant as sole source of many major drug molecules.

This resurgence of intense interest in traditional herbal medicines and nutraceutical products has posted unprecedented pressure on:

1. Inventorization and conservation of natural flora and wild reserves of these herbs.
2. Sustained supply of raw plant materials to the industry.
3. Necessity to breed genetically superior genotypes.
4. Derivatization of high throughput methods for propagule generation.
5. Search for alternative methods for producing desired drug molecules.

Nature through evolution has taken millions of years to bring the plants to the present stage of development. However, the earth’s diverse and rich forest cover has shown drastic decline and disappearance due to urbanization process, the consequences of which are seen in the loss of wild plants of high economic value. National studies have shown 120 medicinal plants are rare or endangered in India. Unfortunately, only a small
fraction of medicinal herbs traded today are solely cultivated; over 90% of these are collected from the wild as non-descriptive land races. All these compulsions demand a constant supply of planting material of medicinal herbs for their commercial cultivation. Medicinal plants with established market demands coupled with some inherent problems associated with their field cultivation such as prolonged juvenile phase, poor flowering, low seed set or viability and strong recalcitrant nature are therefore fast attracting the attention of micropropagation industry to meet these demands. An excellent and well established alternative for the conservation of diversity is to resort to “test tube breeding” or what is better known as “in vitro plant cell culture”.

Plant cell and tissue culture technology utilizes a wide range of in vitro techniques including clonal propagation, development of new variants, and production of genetically modified plants through genetic transformation. Modern techniques of biotechnology are being currently used in many areas including agriculture, bioremediation, food processing and energy production. In agriculture, genetic engineering is being used to produce plants that are fast growing, high yielding and resistant to insects, weeds and plant diseases, thus allowing researchers to manipulate desired traits. Plant tissue culture also plays an important role in the conservation of elite and rare species of plants. Plant cell and tissue culture has developed over the years into a science/technology with the hope of producing new plants of novel gene combinations with high agronomic value. This technique is now being used as a regular tool for the study of various basic problems not only in plant physiology, cell biology and genetics but also in agriculture, forestry and horticulture industry. One of the important techniques of plant cell and tissue culture technology, micropropagation has emerged as an invaluable aid for the large scale production of elite clones and is playing a key role in the development of high quality and true to type plantation, foliage, horticultural, medicinal and other economically important plant species. The conventional propagation techniques are time consuming and labour intensive, making availability of large number of plants for plantation or afforestation a difficult and challenging task. These problems can be overcome by using micropropagation techniques and quality propagules can be produced at a comparatively faster rate and in a more economical manner.

Micropropagation is the first major and widely accepted practical application of plant biotechnology and now it has gained the status of a multibillion dollar industry being practiced in hundreds of biotechnology laboratories and nurseries throughout the world. The other method of in vitro plant propagation based on the induction of somatic
embryogenesis has now been proved as potentially most efficient and economic method for the large scale clonal propagation of plants. The plants developed from somatic embryos are normally true to type or express less variability compared to the plants developed via shoot and root morphogenesis. Somatic embryogenesis is a versatile technique for rapid multiplication of plants and offers a superior possibility for developing scale-up technology as compared to organogenesis. During the last few years interest in *in vitro* technology, particularly the *in vitro* mass propagation of medicinal plants has enabled the breeders to be benefited from this technique for various reasons:

1. To increase the propagation rate of plants, it allows rapid multiplication of sterile plants and those showing low germination capacity.

2. The clonal multiplication results in disease-free plants. Shoot meristem culture results in production of virus free plants.

3. Since the culture conditions such as light, temperature and humidity are specifically controlled during the procedure, the plants can be produced independently of season and other environmental conditions.

4. Under controlled culture conditions and proper management, a large number of elite stock plants can be maintained in a small space and for a long time.

5. Leads to resistance of plants to insects, diseases and herbicides.

6. Produces uniform plants of a selected genotype because micropropagated plants are genetically homogeneous.

7. Produces uniform clones from highly heterozygous plants.

8. Conserves genetic resources of threatened plants and species.

9. Plant improvement by regeneration techniques in conjunction with *in vitro* cell multiplication.

Recent advances in plant cell cultures and genetic manipulations have demonstrated the discovery of novel plants and genetic variants which do not exist in nature and can be obtained in a shorter time period. During the last two decades, dramatic progress has been made in developing and refining various tissue culture techniques to make them competent enough to meet the growing demand in the global market.

In spite of these merits, the micropropagation technique has certain demerits also which have limited the use and exploitation of this technique at industrial level. The major
limitation is the higher cost of plant production. Therefore, to overcome this limitation, a number of cost reduction strategies have now been employed. The use of shake cultures utilizing liquid culture medium alone or in combination of semi-solid culture medium etc. have been developed and used by various workers as one of the many approaches. To reduce the intensive labour requirements along with the production cost during plant propagation by tissue culture technique, there is an immense need of developing scale-up systems and automation (Aitken-Christie, 1991). Progress in tissue culture automation will depend upon the use of liquid cultures in bioreactors

Bioreactors require totally different culture conditions than shake flasks. The results of yield improvement studies in shake flasks may not be directly applicable in bioreactor, therefore necessitating many more studies on improvement of biomass production and secondary metabolite production in bioreactors are needed. Except for few examples where potential for industrial application in these novel areas has been recognized, industries largely are still hesitant about adopting plant cell and tissue culture approaches in above mentioned areas due to difficulties in growth rate, product yield, downstream processing and complexity involved in different operational procedures which significantly increase cost of the end products. Commercially viable production levels can be attained by cultivation of cells, tissues and organs at large scale. This large scale production of cells, tissues and organs requires cultivation in novel bioreactors (using liquid medium) that provide optimum conditions possible for growth and expression of a set of genes responsible for yield of desired product. Bioreactor technology involving large scale operations can reduce labour cost and time factor which translates into economic advantages in commercial processes. In bioreactors generally automated control of physical and chemical environment during growth phase of plant cultures is also possible. The need for developing bioreactor configurations suitable to culture plant cells, organs and mass propagation of plants have long been recognized and for this purpose a wide variety of bioreactor configurations have been tried and a lot of literature related to this aspect has appeared (Lee, 1999; Ziv, 2000; Curtis, 2005; Hvoslef-Eide et al., 2005; Ziv, 2005).
1.1.1 General description of the test plant system

1.1.1.1 Occurrence and distribution of the plant

*Chlorophytum borivilianum* commonly known as ‘Safed Musli’, is a monocotyledonous plant which belongs to family Liliaceae. In English it is known as ‘Indian Spider Plant’, in Sanskrit, ‘Shevata Musli’ while in Arabic it is known as ‘Shaqaque’. It has been mentioned in ancient Indian texts (Nighantu, 16th Century A.D; Shaleegrama Nighantu, 20th Century A.D) as ‘Musalee’, ‘Talapatre’, ‘Khalinee’ or ‘Talamoolika’. In Raja Nighantu (17th Century A.D), two types of musli viz. Shveta (white) and Krishna (black) are described.

The family Liliaceae contains about 254 genera comprising about 4075 species, scattered in both the hemisphere of the earth. In India this family is represented by 35 genera and about 189 species occurring mainly in Himalayas. Important genera of this family include *Allium, Aloe, Asparagus, Chlorophytum, Cholchicum, Gloriosa, Yucca*, etc. There are about 215 species in the genus *Chlorophytum*. The name *Chlorophytum* has been derived from Greek word, Chloros-meaning green and phyton-plant (Rochford and Grover, 1961). The probable centre of origin of species is believed to be tropical and subtropical Africa, where about 85% of the species are found. Most species have originated from Africa and are distributed throughout the warmer regions of the world (Li *et al.*, 1990; Kativu and Nordal, 1993). In *Genera Plantarum*, Bentham and Hooker (1880) reported 40 species of the *Chlorophytum* distributed in Asia, Tropical Africa, America and Australia. Cooke (1908) also mentioned about 40 species distributed in the tropical and subtropical parts of the world. Several species of genus *Chlorophytum* are cultivated for their ornamental value. *C. comosum* is native to South Africa. It is commonly known as ‘Spider Plant’. It is widely used as an ornamental plant. It is used for the treatment of bronchitis, fracture, and burns as a part of traditional medicine. *C. malayense* is indigenous to South-East Asia and South-West of Yunnan province of China. Other species in genus *Chlorophytum* are *C. capense, C. elatum, C. undulatum and C. laxum*, while the first three species originated from South Africa, *C. laxum* originated from West Africa. It is also found in India. The species found in India are *C. heyneanum* Wall, *C. breviscapum* Dalz., *C. arundinaceum* Baker, *C. glaucum* Dalz., *C. tuberosum* (Roxb.) Baker, *C. khasianum* Hooker, *C. attenuatum* Baker, *C. malabaricum* Baker, *C. undulatum* Wall. syn., *C. nepalense* (Lindl) Baker, *C. glaucoides, C. orchidastrum, C. laxum* R. Br. and *C. borivilianum* Sant. et. Fernand. (Hooker, 1894; Santapau and Fernandes, 1955; Purohit
et.al, 1994c). Eight species are considered endemic to the subcontinent (Nair, 1974). Distribution of some important species of Chlorophytum found in India is presented below in Table-1:

**Table-1: Distribution of some important species of Chlorophytum in India**

<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. borivilianum</em> Sant. et. Fernand.</td>
<td>Southern Rajasthan, Northern Gujarat, Western Madhya Pradesh and some parts of Maharashtra. In Rajasthan it occurs in moist forests or reserve sanctuaries in the Aravalli Hills of Udaipur, Banswara and Chittorgarh districts.</td>
</tr>
<tr>
<td><em>C. arundinaceum</em> Baker</td>
<td>Foothills of North-Eastern Himalayas (Assam, Bihar and West Bengal), Orrisa, parts of the Vindhya, the Satpura and Himalayas upto 5300 feet.</td>
</tr>
<tr>
<td><em>C. tuberosum</em> (Roxb.) Baker</td>
<td>Deccan Peninsula and Central India, parts of Konkán to Travancore in Kerala, Eastern Himalaya, Bihar and West Bengal.</td>
</tr>
<tr>
<td><em>C. laxum</em> R. Br.</td>
<td>Katki Hills, Belgaum, Dharwar, North and South Kanara, Deccan Peninsula.</td>
</tr>
<tr>
<td><em>C. attenuatum</em> Baker</td>
<td>West Peninsula and Western Ghats from Kanara southwards to the Coimbatore.</td>
</tr>
<tr>
<td><em>C. breviscapum</em> Dalz.</td>
<td>Sikkim Himalaya at the foot of the hills, Belgaum in Western Ghats, Sikkim and coastal traits in West Peninsula.</td>
</tr>
</tbody>
</table>

*C. tuberosum* is most widely distributed throughout India along with other species. It is not commonly used but certainly administrated as an adulterant with the commercial material. Perhaps this is the reason that this species is still present in abundance throughout the country. Other species have restricted distribution as they vanished from the common sites in major parts of the country. Safed musli plants grow in a variety of places in nature, starting from open rocky places to shady and highly humus-rich soil in the forests. All species mentioned in the Table-1 are commonly known as safed musli. But for commercial purpose, three species are important namely *C. borivilianum*, *C. arundinaceum* and *C. tuberosum*. Out of these three species, *C. borivilianum* is economically most important species.

**1.1.2 Botanical description of the plant**

**1.1.2.1 Taxonomy**

Class: Monocotyledons

Series: Coronarieae

Family: Liliaceae

Genus: *Chlorophytum*

Species: *borivilianum*
Santapau and Fernandes (1955) described *Chlorophytum borivilianum* for the first time from India. *C. borivilianum* is a small perennial rhizomatous herb with a condensed stem disc from which a whorl of leaves originate. It perennates by fleshy adventitious roots/root tubers. The fibrous roots of the plant are modified into fascicular roots (fleshy roots) which are used commercially. They are sessile, cylindrical, 1 to 9 in number, brown to black skinned and white after peeling, the tubers are 3-10 cms long at maturity, 1 cm in diameter. Leaves are sessile, radicle, cylindrical, 6-13 in number, 10-40 cms in length and 0.6-4 cms in breadth, spirally imbricate at the base, linear ovate, acute apex and slightly narrowed at the base. The leaves are spreading horizontally and its lower surface is rough; margins are wavy with parallel venation. It has a solitary scape, 15-30 cms long, terminal, unbranched; it bears flowers over its upper ¾ of length. The inflorescence is raceme. Flowers are white, bracteate, pedicillate, usually arranged in alternate clusters, each consisting of 3 flowers, the flower clusters are closer on the upper part of the scape. The bracts are linear papery, purplish. The pedicle look whitish, jointed and kneed at the joint. The perianth consists of six tepals arranged in two heteromorphous series having three tepals in each series. The androecium consists of six stamens arranged opposite to tepals. The pistil is longer than stamen and the stigma is arranged away from the stamens, its filaments are glaborous. Anthers are longer than filaments, yellow, linear and dehisce by longitudinal slits, style slightly larger than stamens, swollen at the apex, ovary is 3-lobed, green, globose and sessile. Fruit a loculicidal capsule, green to yellow, trilobed and bears 3-12 seeds inside, triquetrous to 3-sulcate, almost equal in length and width, seeds are onion like in appearance, flat with angular edges.

An identification key developed by Shah (1978) on the basis of root morphology for three important species of *Chlorophytum* is as follows:

1. Plants with fasciculated roots, scape upto 30 cms long:
   a. Fasciculated roots are attached at the ends of fibrous roots…… *C. tuberosum*.
   b. Fasciculated roots are sessile…… *C. borivilianum*

2. Plants without fasciculated roots, scape upto 10 cm long…… *C. malabaricum*.

Geetha and Maiti (2002) observed variability in the morphology and colour of fleshy roots of *C. borivilianum*. The colour of fleshy roots varied from dark purple/brown to light yellow/off white. The length of the fleshy root varied from 7.0 to 20.5 cms, the diameter was 0.4 to 1.0 cms. Recently the taxonomic position of genus *Chlorophytum* has been revised (Marais and Reilly, 1978). It has been placed close to the genus *Anthericum*.
and is distinguished by the enlarged middle part of stamen filament and capsule with three wings.

1.1.2.2 Cytology

Cytological studies carried out in the genus revealed the occurrence of two basic chromosome numbers, \( x = 7 \) and \( x = 8 \) (Darlington and Wylie, 1955; Sheriff and Chennaveeraiah, 1972; Naik, 1977; John et al., 1989). It is observed that the species with \( x = 8 \) chromosomes are mostly diploid species which are mainly distributed in the semi-arid regions with relatively smaller and short lived shoots. The group of species with \( x = 7 \) chromosome number are either diploid or at different levels of ploidy (triploid, tetraploid and polyploid) and they are distributed at higher altitude with relatively humid and cool atmosphere. These are mostly long lived or perennials. However, exceptions to these criteria were also observed (Naik and Nirgude, 1981). The chromosome number of \( C. tuberosum \) was reported as \( 2n = 2x = 16 \) (Kumar and Rao, 1958). Pahuja and Kumar (1969) studied the cytogeography of three species: two Indian species, \( C. arundinaceum \) and \( C. tuberosum \) and an African species \( C. elatum \) to ascertain the existence of a geographical distribution pattern and karyotype evolution. All the three species showed asymmetrical karyotypes. The chromosome number was \( 2n = 6x = 48 \) in \( C. arundinaceum \) and \( 2n = 4x = 28 \) in \( C. elatum \).

The chromosome number of \( C. borivilianum \) was first reported as diploid (\( 2n = 2x = 16 \)) (Raghavan et al., 1977). However, extensive examination of chromosomes in various accessions by Geetha and Maiti (2004) revealed that the chromosome number in the species is \( 2n = 4x = 28 \), indicating the species as tetraploid with basic number \( x = 7 \), but an octaploid number (\( 2n = 8x = 56 \)) is also observed in few somatic cells of some plant samples.

Lavania et al. (2005) also confirmed the chromosome number \( 2n = 4x = 28 \) of \( C. borivilianum \). They applied the Fluorescence In Situ Hybridization (FISH) technique to elucidate physical localization and measurement of the rDNA sites using two rRNA multigene families homologous to 45S and 5S rDNA in \( C. borivilianum \) and \( C. comosum \). Results revealed the presence of as many as five pairs of 45S rDNA sites in \( C. borivilianum \) and three pairs in \( C. comosum \) suggesting their multi-genomic origin.
1.1.2.3 Propagation

Plant regenerates in nature either by sexual means (seed production) or by vegetative propagation (Jat, 1993). The plants are difficult to locate in forests before or after the growing season as the aerial parts dry and wither away. This is an insect pollinated crop (Dalal et al., 1987) and the capsule matures in succession in a raceme inflorescence, therefore seed collection from the forest plant is difficult. Poor seed set, viability and germination is common in *C. borivilianum* (Bordia, 1993; Ramawat et al., 1996). The percentage of germination in different cultures from different regions varies between 11 and 24% (Jat and Bordia, 1990). The seeds remain under dormant condition for about 10 months. The development of plants as well as roots by the means of seeds in the first year is not vigorous enough as compared to the vegetatively propagated plants. Seed raised plants produce small tuberous roots in their first season and large roots of commercial value are produced at the end of the second season. (Bordia et al., 1995). Therefore, alternative methods of propagation are necessary. More vigorous plants are produced by vegetative propagation than by means of seeds because of available stored food material in the fleshy roots for supporting initial growth. Further, it was observed that the vegetative propagation produces three times more yield of fleshy roots over the seed-raised crop. Besides this the perennating plants are more vigorous as compared to plants grown from individual fleshy roots. Apart from this, the secondary roots (feeding roots developed in sexually propagated plants were smaller in comparison to vegetatively sprouted plants (Bordia and Jat, 1990a). In vegetative propagation fleshy roots bearing shoot buds are used as propagule. During May and June, dormancy breaks and new shoot buds develop from the stem disc portion attached to fleshy roots. Roots without stem disc do not sprout (as they lack the apical bud). These stem have a direct role in vegetative propagation, otherwise sprouting will not take place although they may produce roots (Bordia and Jat, 1990b). These separated fleshy roots could be stored till next season. Degeneration of tuberous roots during storage reduces the effective vegetative multiplication of the plant in the following season (Bordia, 1993). In order to ensure maximum sprouting, the fleshy roots should be separated before initiation of sprouts. These sprouted fleshy propagules should be planted in the field in the first or second week of June, followed by irrigation (if rains do not occur). The plant matures in about 90 days followed by withering of the leaves. Seperation of the tuberous roots along with the stem results in the formation of the new plants. Such plants produce a new crop of tuberous roots towards the end of the growing season.
The roots are collected from the mature plants at the end of monsoon season (September-October) when leaves have dried, the skin is removed by peeling before the roots become completely dry and are finally sun dried and bleached. Roots after drying for 10-20% water content can be stored in dry soil (15 cm deep) in shade for planting in the next season (Bordia, 1993).

Like seed, 7-8 month dormancy is reported in the fleshy roots of *C. borivilianum* (Jat and Bordia, 1990) and *C. tuberosum* (Sheta and Goswami, 1989). In this species, ontogeny plays a definite role in reproduction, i.e. the plants raised from 5, 4, 3 and 2 fleshy root bunches produced comparatively more fleshy and vigorous roots than the plants raised from individual fleshy roots (Anonymous, 1993).

### 1.1.2.4 Soil and Climate

The crop requires well-drained and loamy soils rich in organic matter which favours the luxuriant growth and facilitate the development of fleshy roots. Places having warm and humid climatic conditions with high soil moisture during the growing period i.e. June to October are suitable to the cultivation of the crop. It grows well in the areas where the day temperature varies between 25°C and 38°C and the night temperature varies between 16°C and 25°C with well distributed average rainfall of 400-500 mm during its growing period.

### 1.1.2.5 Irrigation

The crop is usually sown at the onset of the monsoon. Light irrigation is required if there is a lack of soil moisture at the time of planting. In the early growth phases of the plant, normally no irrigation is required. Once the monsoon is over, the crop needs irrigation at the interval of 15-20 days. During crop growth two or three weeding cum hoeings are required. In the flat bed cultivation, soil is loosened after receding of the rain.

### 1.1.2.6 Economic importance

(a) **Pharmacological uses**

Safed musli has been named in the ‘Athrvaveda’ as one of the divine herbs, offering cure for many ailments and health related problems. This plant has been described in other ancient Indian literature named Madhava Dravyaguna (12th Century A.D.), Bhavaprakash Nighantu (16th Century A.D), Rajaballabha Nighantu (19th Century A.D), Siddhabhesaja Manimala (21st Century A.D.) as aphrodisiac (Vrishya), adaptogen, anti-ageing (Rasayana), health restorative and health promoting (Urjaskara), tonic (Balya). Amongst all the different species of *Chlorophytum* present in India, *C. borivilianum* produces the
highest root yield and highest saponin content and it has got maximum demand and commercial value (Bordia et al., 1995).

The economically important part of the herb are roots, which are mainly used to cure general and sexual weakness. Dried roots are exported in bulk quantity. The roots are usually consumed in a powdered form with milk or mixed with sweet preparations. The price of the roots has increased in the recent years because tuberous roots are used as an important ingredient in many Herbal, Unani, Homeopathic and Allopathic formulations in India (Kirtikar and Basu, 1975; Ramawat et al., 1998) for the following properties:

1. As non-hormonal restorative tonic;
2. in fatigue, general debility, weakness and as a general health promotive tonic, anti-ageing used for increasing general body immunity and working capacity;
3. used as a natural aphrodisia to enhance male potency. It treats male sexual inadequacies like oligospermia, lack of libido, impotency, sterility etc.;
4. as cardiac and brain tonic;
5. as a curative agent in various diseases like piles, diabetes, rheumatoid arthritis, various gynecological disorders like post menopausal syndrome, albuminorrhoea-leucorrhoea, menorrhoea etc.;
6. as anti-pyretic, sialogogue, galactogue, diuretic, hemostatic properties and

Generally, *C. borivilianum* has been used along with other plants such as *Asparagus ascendense*, *A. racemosum*, *Curculigo orchioides* and *Withania somnifera* in several formulations in Indian system of medicine (Kirtikar and Basu, 1975; Ramawat et al., 1998).

(b) Traditional Uses

It is used in the diet in the form of ‘laddoos’ for increasing lactation of milking mothers and also used in chips/flakes as a nutritious breakfast. It is a major ingredient in ‘Chyavanaprasha’ which is used in the treatment of general debility and as a vitalizer. It is also used in ‘pan’ and ‘gutkha’. Root powder fried in the ‘ghee’ is chewed in case of aphthae of mouth and throat.

Safed musli is thought to have immunomodulatory and adaptogenic properties like ‘Ginseng’. It has also been named as ‘Alternative Shilajeet’. It is considered as an effective alternative to Viagra and has come to known as ‘Herbal Viagra’. Safed musli has been found to be an ideal aphrodisiac with no negative side-effects associated with chemical-based aphrodisiacs. The active principle, saponins are known as stimulants and metabolic enhancers. The extract prepared from dried root tubers of *C. borivilianum* inhibited ^3^H-dopamine-uptake in striatal synaptosomes and thereby, could lead to enhanced
dopaminergic tone in the central nervous system as also act as psychostimulants. This has a beneficial effect on the brain and human body by increasing alertness, mental ability and intelligence, sexual and maternal characters (Arora et al., 2004).

Three new saponins isolated from *C. comosum* (Mimaki et al., 1996) showed an inhibitory activity against $^{32}$P incorporation into phospholipids of HeLa cells as the primary screening test to identify new anti-tumour promoter compounds. The recent economic interest raised in these plants is from the use of steroidal saponins as a starting material for the industrial production of sex-hormones, corticosteroids and steroid derivatives in general.

(c) Market: Consumption and demand

Due to its high therapeutic activity and diversified uses, demand for dried roots of safed musli is increasing rapidly in Indian and International drug market. Amongst the different species of genus *Chlorophytum* in India, *C. borivilianum* has good market both indigenously and globally due to the reasons stated above. It is estimated that the overall demand for safed musli’s dried tubers in Indian and foreign markets is around 35,000-40,000 tonnes per year (Bordia et al., 1995) while the supply is only 500-600 tonnes per year. Current projection of annual demand in country is estimated between 300 to 500 tones. The dried roots have market price between Rs. 1000 to 1700 / kg. Of the available quantum of safed musli, over 95% of it comes through wild habitat, an act prohibited by the various State Governments. The National Medicinal Plant Board has recognized safed musli as the endangered species and included it in the list of prioritized plant species to be protected and promoted for cultivation and processing. *C. borivilianum* is the only species which is under commercial cultivation. There is limited cultivation of safed musli in the country and so industrial processing has been regionally restricted. Due to a significant demand supply gap there is an urgent need to conserve and multiply the plant in bulk amount for its domestication to meet the present demand but various problems have been encountered in multiplying these plants by conventional methods. Plant cell and tissue culture approach to solve these problems in safed musli (*C. borivilianum*) seems promisable and has recently gained interest.

1.1.2.7 Chemistry of the plant system

The roots of safed musli (*C. borivilianum*) are of commercial importance. They are widely used for various therapeutic applications in the Ayurvedic and Unani systems of medicine (Oudhia and Tripathi, 1999). The major constituents of dried roots of safed musli are
carbohydrates (42%), protein (8-9%), root fibre (3-4%) and saponins (2-17%); (Bordia et al., 1995). Steroidal saponins are one of the important constituents of the roots of *Chlorophytum*. These saponins have steroids as the aglycone moiety, called sapogenins. Neohecogenin and neotigogenin are the usual sapogenins of the genus *Chlorophytum* (Fig-2). Steroidal sapogenins are the active principle which are responsible for most of the medicinal properties of safed musli. Tuberous roots of plants are widely used as tonic and aphrodisiac due to the presence of steroidal saponins, viz. neotigogenin, neohecogenin, stigmasterol and tokorogenin (Tandon and Shukla, 1995b). The sapogenins and glycosides isolated from various *Chlorophytum* species are presented in Table-2:

**Table-2. Steroidal saponins and glycosides isolated from different species of *Chlorophytum***

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Source</th>
<th>Activity</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sapogenins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gitogenin</td>
<td><em>C. comosum</em></td>
<td>Anti-tumor</td>
<td>Mimaki et al., 1996</td>
</tr>
<tr>
<td>Hecogenin</td>
<td><em>C. comosum</em></td>
<td>Anti-tumor</td>
<td>Mimaki et al., 1996</td>
</tr>
<tr>
<td>Tigogenin</td>
<td><em>C. comosum</em></td>
<td>Anti-tumor</td>
<td>Mimaki et al., 1996</td>
</tr>
<tr>
<td></td>
<td><em>C. arundinaceum</em></td>
<td>-</td>
<td>Tandon et al., 1992</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td><em>C. arundinaceum</em></td>
<td>-</td>
<td>Tandon et al., 1992</td>
</tr>
<tr>
<td>Neo-hecogenin</td>
<td><em>C. malayense</em></td>
<td>-</td>
<td>Li et al., 1990</td>
</tr>
<tr>
<td></td>
<td><em>C. malayense</em></td>
<td>Anti-tumor</td>
<td>Qiu et al., 2000</td>
</tr>
<tr>
<td>Neo-gitogenin</td>
<td><em>C. arundinaceum</em></td>
<td>-</td>
<td>Tandon et al., 1992</td>
</tr>
<tr>
<td>Tokorogenin</td>
<td><em>C. arundinaceum</em></td>
<td>-</td>
<td>Tandon et al., 1992</td>
</tr>
<tr>
<td><strong>Glycosides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloromaloside A</td>
<td><em>C. malayense</em></td>
<td>Anti-tumor</td>
<td>Qiu et al., 2000; Li et al., 1990</td>
</tr>
<tr>
<td>Chloromaloside B</td>
<td><em>C. malayense</em></td>
<td>Anti-tumor</td>
<td>Li et al., 1990</td>
</tr>
<tr>
<td>Chloromaloside C</td>
<td><em>C. malayense</em></td>
<td>Anti-tumor</td>
<td>Li et al., 1990</td>
</tr>
<tr>
<td>Chloromaloside D</td>
<td><em>C. malayense</em></td>
<td>Anti-tumor</td>
<td>Li et al., 1990</td>
</tr>
<tr>
<td>Chloromaloside E</td>
<td><em>C. malayense</em></td>
<td>Anti-tumor</td>
<td>Yang and Yang, 2000</td>
</tr>
<tr>
<td>Gitogenin glycosides</td>
<td><em>C. comosum</em></td>
<td>Anti-tumor</td>
<td>Mimaki et al., 1996</td>
</tr>
<tr>
<td>Hecogenin glycosides</td>
<td><em>C. comosum</em></td>
<td>Anti-tumor</td>
<td>Mimaki et al., 1996</td>
</tr>
<tr>
<td>Tigogenin glycosides</td>
<td><em>C. comosum</em></td>
<td>Anti-tumor</td>
<td>Mimaki et al., 1996</td>
</tr>
<tr>
<td>Stigmasterol-β-D-</td>
<td><em>C. arundinaceum</em></td>
<td>Anti-tumor</td>
<td>Tandon et al., 1992</td>
</tr>
<tr>
<td>glucopyranoside</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

- Data not available.

The literature reveals that *Chlorophytum* species hold great promise for medicinal market due to the presence of valuable saponins. Twenty five alkaloids have been reported from its roots (Bordia et al., 1990; Seth et al., 1991). Gutpa et al. (1979) identified galactoglucom in fruits of *C. arundinaceum*. Trivedi (1991) reported 1.9 - 3.5% glycosides.
Fig-2: Structure of metabolites isolated from different *Chlorophytum* species: Chloromaloside-B (A), Spirostanol saponins (B), Arundinoside-B (C), 2,2',4,4'-tetrahydroxybibenzyl xyloside (D) and 4-hydroxy-8,11-oxidoheneicosanol (E)
in safed musli collections. It also contains many vitamins, minerals etc. Safed musli roots also contain albumin but no starch. Saponins readily dissolve in water and forms foam when shaken with water. On hydrolysis, the saponins are broken down to sugar and sapogenins. According to Gaind et al. (1968) mucilaginous extract of safed musli is a superior emulsifying agent. Different genotypes present great variability in their biochemical components (Bordia et al., 1995). Furthermore, it was observed that environmental conditions of natural habitat affect expression of certain characters such as fleshy root yield and carbohydrate and protein content (Jat, 1993; Bhagat and Jadeja, 2003). However, there is low environmental influence over sapogenin content, the active principle of the species.

One of the important techniques of plant biotechnology: micropropagation has been an invaluable aid for the large scale production of elite clones and is playing a key role in the development of high quality and true-to-type economically important plant species. The conventional propagation techniques in this plant are time consuming and labour intensive, making availability of large number of plants for plantation or afforestation a difficult and challenging task. These problems can be overcome by using micropropagation techniques and true to type quality propagules can be produced within shorter time and comparatively smaller space independent of seasonal and other environmental conditions, at a comparatively faster rate and in a more economical manner.

Tissue culture and biotechnological studies in safed musli particularly C. borivilianum have so far been very sporadic and scanty. Therefore keeping in mind to devise methods for possible recovery of this endangered and over-harvested medicinal plant species, as well as a method for providing plant materials for extraction of medicinally important compounds through micropropagation approach, present study was under taken.
1.1.3 Aims and objectives of the study:

The major objectives of the present course of investigation are as follows:

1. Collection, maintenance and in vitro initiation and establishment of aseptic cultures of elite clone of *C. borivilianum*.
2. To work out the nutrient and other cultural requirements for optimal growth and biomass (callus/shoots) production.
4. Studies on regeneration and induction of somatic embryos and plantlet regeneration from somatic embryos.
5. Optimization of different culture conditions for up-scaling cultures of shoots/somatic embryos leading to plantlet development.
6. Assessment of genetic diversity of in vitro grown plants derived from callus and/or somatic embryos using molecular techniques.

Keeping in view the above objectives, the present study has been presented in two separate chapters. Each of the chapter comprises of relevant introduction, review of literature, material and methods and detailed results followed by discussion and a combined bibliography.