Chapter 1

Introduction
INTRODUCTION

Plants have been used in traditional healthcare system from ancient time, particularly among tribal communities. The World Health Organization (WHO) has registered 20,000 medicinal plants globally and India’s contribution is 15-20%. According to the WHO estimate, about 80% of the population in the developing countries depends directly on plants for its medicines. In India about 2,000 drugs used are of plant origin (Laloo et al. 2006). Almost all cultures from ancient times till today have used plants as medicine. In recent years there has been renewed interest in natural medicines that are derived from plant parts or plant extracts. The global demand for herbal medicine is not only large, but growing (Srivastava 2000). The international market for traditional products is amount US $ 62 billion and is expected to reach US $ 5 trillion by 2050. The market for Ayurvedic medicines is estimated to be expanding at 20% annually in India (Subrat 2002), while the quantity of medicinal plants obtained from just one province of China (Yunnan) has grown by 10 times in the last 10 years (Pei Shengji 2002). Today medicinal plants are vital to the global economy, as approximately 85% of traditional medicine preparations include the use of plants or plant extracts (Nalawade and Tsay 2004).

1.1 India: A mega biodiversity centre

India, on the tropic of cancer, is one of the twelve mega biodiversity centre with 8% of the global biodiversity in 2.4% land. The country is 10th amongst plant rich countries of the world and 4th amongst the countries of Asia. Two of the world’s 25 hot spots – are in India. The Western Ghats as a whole is an abode of rich diverse flora; the natural forests of the region are the birthplace of nearly 500 medicinal plants used for traditional and folk medicinal practices (Krishnan et al. 2011).

India is sitting on a gold mine of well recorded and traditionally well practiced knowledge of herbal medicines. The country is perhaps the largest producer of medicinal herbs and is rightly called the “Botanical garden of the world”. There are very few medicinal herbs of commercial importance which are not found
Medicinal plants as a group include approximately 8,000 species and account for about 50% of all the higher flowering plant species of India. This proportion of medicinal plants is the maximum known in any other country against the existing flora of that country (Kala et al. 2006). Very small amounts of the medicinal plants are lichens, ferns, algae etc; the majority of the medicinal plants are higher plants (Sharma et al. 2010). India is having 45,000 plant species; its diversity is supreme due to the 16 different agroclimatic zones, 10 vegetative zones and 15 biotic provinces (Samy and Gopalakrishnakone 2007). The country has rich floral diversity (Table 1).

<table>
<thead>
<tr>
<th>Species</th>
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<tr>
<td>Higher plants</td>
<td>15,000-18,000</td>
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<tr>
<td>Fungi</td>
<td>23,000</td>
</tr>
<tr>
<td>Algae</td>
<td>25,000</td>
</tr>
<tr>
<td>Lichens</td>
<td>1,600</td>
</tr>
<tr>
<td>Bryophytes</td>
<td>1,800</td>
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<tr>
<td>Microorganisms</td>
<td>30 million</td>
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</tbody>
</table>

1.2 Plants in traditional medicine

Plants have been used in traditional medicine for several thousand years. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani, Sidha and Homeopathy. There are considerable economic benefits in the production of indigenous medicines and in the use of medicinal plants for the treatment of various diseases. Due to less communication means, poverty, ignorance and unavailability of modern health facilities, most people especially rural people are still forced to practice traditional medicines for their common day disorders. A vast knowledge of how to use plants against different diseases may be expected
to have accumulated in areas where the use of plants is still of pronounced significance (Muthu et al. 2006).

Traditional medical knowledge of medicinal plants and their use by indigenous cultures are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the present and future. Medicinal plants are moving from fringe to main stream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently considerable attention has been paid to utilize ecofriendly plant based products for the prevention and cure of different human diseases. Considering the adverse effects of synthetic drugs, the western population is looking for natural remedies which are safe and effective. Officially, over 3,000 plants have been recognized in India for medicinal value. Also, it has been estimated that over 6,000 plants are in use in traditional folk medicine and herbal medicine representing about 75% of the medicinal needs of the Third world countries. There are very few medicinal herbs of commercial importance such as Allium sativum, Aloe barbedensis and Panax species which are available in India. Moreover, there are about 7,000 firms manufacturing traditional medicines with or without standardization (Dubey et al. 2004).

The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these plants bioactive chemical constituents (phytochemicals and infochemicals) are alkaloids, flavonoids, tannins and phenolic compounds (Kwada and Tella 2009).

### 1.3 Threat to medicinal plants

The search for natural products to cure diseases represents an area of great interest in which plants have been the most important source. The unscrupulous collection of plants from wild habitats, biopiracy and overexploitation by traders has threatened the very existence of valuable medicinal plant resources (Dubey et al. 2004). As a result 20-25% of existing plant species in India became endangered. Also, the degree of threat to medicinal plants has been increased since more than 90% of medicinal raw materials used for herbal industries in India and also for export are drawn from natural habitat. Medicinal plants are
now under great pressure due to their excessive collection or exploitation. Plant resources are depleting globally at an alarming rate and if the condition remains same a number of economically and medicinally important plant species will soon be extinct. In the last few decades overexploitation of forest resources has led to many species loss.

Continuous utilization of plant species from the natural population and extensive loss of their habitats during the past 15 years have resulted in the population decline of many high value medicinal plants over the years (Kala 2003). There are many other potential causes of scarcity in medicinal plant species, such as habitat specificity, narrow range of distribution, land-use disturbances, introduction of non-natives, habitat alteration, climatic changes, heavy livestock grazing, explosion of human population, fragmentation and degradation of population, population bottleneck and genetic drift (Kala 2000; Weekley and Race 2001; Oostermeijer et al. 2003 and Kala 2005).

1.4 Conservation of plants through *in vitro* techniques

Global concern about the loss of valuable genetic resources has stimulated many new programs for the conservation of plant genetic resources. Within past decade several conservation strategies were developed mainly in the terms of *in situ* (within natural habitats) and *ex situ* (outside natural habitats) conservation (Paunescu 2009). Conservation of medicinal plants is possible through either *in situ* or *ex situ* or preferably through a combination of both. The major constraints in the conservation of these plants include slow regeneration rates, over exploitation and destruction of their natural habitats. Therefore, alternative techniques need to be developed for their propagation; advances in biotechnology, especially *in vitro* techniques and molecular biology provide some important tools for multiplication, conservation and management of medicinal plant resources.

During the last three decades, plant biotechnology has grown into a mature area of study and may be defined as a set of biological tools and techniques used in plants for the development of products of commercial value. One of the most important and widely accepted application of plant biotechnology is cell, tissue and organ culture which is used for various purposes including:
- micropropagation or multiplication of elite plants
- production of virus free plants
- regeneration of transformed cells or protoplasts or tissues required for development of transgenic crops with novel traits.

The history of plant biotechnology can be traced back to the history of cell and tissue culture, which had its birth with the demonstration of totipotency of plant cells by G. Haberlandt (1902). In the middle of 20th century the production of virus free plants of Dahlia and potato (Morel and Martin 1952; 1955) through shoot meristem culture laid down the initial milestone in large scale production of important plant species using the technique of micropropagation. Further, Skoog and Miller (1957) discovered the role of plant hormones, especially of cytokinins in shoot morphogenesis and in the inhibition of apical dominance. The clarification of the role of cytokinins in apical dominance inhibition, which subsequently results in the release of axillary meristem from dormancy, was the major break-through in plant tissue culture (Sachs and Thimann 1964). The successful application of such fundamental discoveries to the multiplication of plants by micropropagation has been a key factor in the development of this technology, not only for mass propagation of the existing stocks of germplasm for biomass production, but also for the conservation of economically and medicinally important plant species. The conventional propagation practices for propagation of such plants are time consuming and labor intensive. During the past couple of decades, there has been an increased interest in problems related to the large scale plant production as well as in its cost reduction for commercial micropropagation (Donnan 1986 and Andrea-Kodym and Zapata-Arias 2001). At present, being the only commercially exploited tool of plant biotechnology, the micropropagation technique has been applied to about 1000 plant species including crop plants, ornamental plants, medicinal and aromatic plants and trees (Mehrotra et al. 2007).

Plant tissue culture (PTC) techniques offer an integrated approach for the production of standardized quality phytopharmaceutical through mass production of consistent plant material for physiological characterization and analysis of active ingredients (Debnath et al. 2006). Since conventional breeding methods
are unable to meet these requirements therefore, the novel PTC technique can be employed for regenerating plants of medicinal value. The recent development in micropropagation of plants through tissue culture techniques has been of great help in the cultivation of medicinal plants by providing planting material of standard quality (George and Sherrington 1984).

Although modern technology in the field of medical service has accomplished miraculous achievements but still plants have their own importance. Nowadays screening of medicinal herbs as potential sources of new bioactive components of therapeutic value has increased. With an ever increasing global inclination towards herbal medicine, there is an obligatory demand for a huge raw material of medicinal plants. In vitro propagated medicinal plants furnish a prepared source of uniform, sterile and compatible plant material for biochemical characterization and identification of active constituents for their vast uses (Banerjee and Shrivastava 2006 and McCoy and O’Connor 2008).

Micropropagation or in vitro regeneration is a potential biotechnological tool that provides a solution to the problem of medicinal plants decimation. It refers to the in vitro cultivation of plants, seeds or plant parts (organs, tissues, cells or isolated protoplasts) on a nutrient medium under aseptic conditions. It relies on the fact that many plant cells have the ability to regenerate whole plant (totipotency). Micropropagation can be broadly described in four stages:

1. Establishment of aseptic cultures
2. Proliferation and multiplication of shoots in culture
3. Rooting of microshoots and
4. Hardening and acclimatization of tissue culture raised plantlets.

Tissue culture has now become a well established technique for culturing and studying the physiological behavior of isolated plant organs, tissues, cells, protoplasts and even cell organelles under precisely controlled physical and chemical conditions. The importance and application of plant cell and tissue culture in plant science are vast and varied. The last few years of research into plant cell, tissue and organ culture had shown the emergence of technology from technique. The establishment of micropropagation for rapid propagation, the use
of shoot tip culture to produce nuclear stock free from parasites, especially viruses, and the application of a variety of procedures including anther and pollen culture to speed up the process of producing better varieties, protoplast culture for hybrid plant production, and genetic manipulation all have contributed to the acceptance of plant tissue culture not only as a valuable tool for plant propagation and multiplication but also for the plant improvement and conservation.

Most of the medicinal plants either do not produce seeds or seeds are too small and do not germinate in soils. Furthermore, the plants raised through seeds are highly heterozygous and show great variations in growth, habit and yield and may have to be discarded because of poor quality of products for their commercial release. Thus, mass multiplication of important medicinal plants is a great problem. In this regard micropropagation is the best alternative way to provide true to type, rapid and mass multiplication under disease free or aseptic conditions. Callus mediated organogenesis exhibit great chances of somaclonal variations that could be exploited for developing superior varieties particularly in vegetatively propagated plant species. Recently, micropropagation protocols have been developed for a wide range of medicinal plants through different developmental pathways using various explants (Pandey et al. 2002; Martin 2002; Sahrawat and Chand 2002; Thomas and Jacob 2004; Thomas and Philip 2005; Karuppusamy et al. 2006; Bouhouche and Ksiksi 2007; Husain et al. 2008; Parveen et al. 2010; Sahai et al. 2010b; Shahzad et al. 2011; Parveen et al. 2012 and Parveen and Shahzad 2012).

1.5 Plant Description

Fabaceae (Leguminosae) is the third largest and extremely diverse family of angiosperms with about 18,000 species classified into about 650 genera. The family is further divided into 3 sub families - Papilionoideae, Caesalpinoideae and Mimosoideae, which are sometimes recognized as three separate families i.e. Papilionaceae, Caesalpinaceae and Mimosaceae. In terms of economic importance Fabaceae is the most important family in the Dicotyledoneae and a large number of crude drugs used in Ayurvedic System employ plants of this legume family. Cassia is one of the most important genus of the family Fabaceae
(sub family Caesalpinoideae) and includes about 600 species which are mostly distributed in Tropics and Subtropics. The members of family Caesalpinaceae include trees, shrubs or rarely herbs which exhibit mostly astringent and mucilaginous properties; some have a pectoral and laxative or cathartic action and some are used as tonic (Parveen et al. 2012).

There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases. Keeping in mind the use of in vitro approaches for the conservation of valuable medicinal plants, the present study was focused for in vitro propagation of two medicinally important Cassia spp. i.e. C. angustifolia and C. sophera through various explants via direct regeneration, indirect organogenesis and somatic embryogenesis.

1.5.1 Cassia angustifolia Vahl.

Synonyms: C. senna L.; C. acutifolia Delite; C. obovata Baker

Common name: Senna; Alexandrian/Bomabay/Tinnevelly senna

Systemic Position:

Kingdom: Planteae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Fabales
Family: Fabaceae
Subfamily: Caesalpinoideae
Genus: Cassia
Species: angustifolia

1.5.1.1 Distribution

Senna is a shrub native to Egypt, Sudan, Nigeria and North Africa, as well as India, Pakistan and China. In India, it is mainly cultivated in the states of Tamil Nadu, Maharashtra, Gujrat, Rajasthan and Delhi.
The plant has been put in the priority list of National as well as State Medicinal Plant Board for development. It is one of the principal herbal drugs having export potential for developed countries. India is the major supplier of the leaves and pods (shells) as well as senna glycosides to the world market. Approximately 75% of the senna produced in India is exported. Presently the most important markets are Germany, Japan, Czechoslovakia, USA and Hong Kong. Annual demand of the plant in 2001-2002 was reported to be 6462.5 tonnes, which has gone up to 11677.5 tonnes by the year 2006 (Shrivastava et al. 2006).

1.5.1.2 Morphological characters
A small shrub about 1.0 to 1.8 m in height with pale subterate or obtusely angled erect or ascending branches, leaves usually 3-9 pairs, leaflets oval, lanceolate, glabrous (Figure 1). Racemes axillary, erect, brilliantly yellow, waxy many flowered. Pods light green when young to dark brown or black when mature, flat, thin, oblong, 3.5-7.0 cm long contains 5-7 obovate dark brown and nearly smooth seeds.

1.5.1.3 Medicinal uses
Its medicinal uses were first described in the writings of Arabian physicians Serapion and Mesue as early as the 9th century AD, the name senna itself is Arabic. The leaves and pods are usually used in the Ayurvedic and Unani systems of medicine as infusion and considered a great tonic. In India, several household preparations such as decoction, powder, syrup, infusion and confection are made with senna. Besides being an excellent laxative the senna is used as a febrifuge in splenic enlargemens, anaemia, typhoid, cholera, biliousness, jaundice, gout, rheumatism, tumours, foul breath and bronchitis and probably in leprosy. It is employed in the treatment of amoebic dysentery, as an anthelmintic and as a mild lever stimulant.

The leaf is one of the constituent of patented drug reported to have protective effects on the lever. The leaf in the form of confection of senna is used for certain skin diseases and the powdered leaves in vinegar are applied to wounds and burns and to remove pimples. The callus cultures and crude alcoholic extract (50%) showed a strong anti-microbial activity against gram positive bacteria (Anonymous 1992a).
Figure 1. *Cassia angustifolia*
1.5.1.4 Active compounds
Two active anthraquinones, sennosides A \((C_{42}H_{38}O_{20}, \text{M.P. } 200-240^\circ\text{C})\) and B \((C_{42}H_{38}O_{20}, \text{M.P. } 180-186^\circ\text{C})\), which are optical isomers, have been isolated from the leaves and pods. Beside these sennosides, presence of sennosides C \((C_{42}H_{40}O_{19}, \text{M.P. } 197-205^\circ\text{C decomp.})\), D \((C_{42}H_{40}O_{19}, \text{M.P. } 210-220^\circ\text{C decomp.})\), G \((C_{42}H_{38}O_{20}, \text{M.P. } 162-176^\circ\text{C decomp.})\), III and A, is also reported. Three sennidins are also present in the leaves. The leaves, pods and roots contain rhein, chrysophanol, emodin and aloe-emodin. Several mono and di-glucosides of anthrone are present in the seedlings, leaves and roots. The leaves also contain mono-\(\beta\)-D glucosides of rhein and aloe-emodin and a water soluble glycoside, which is supposed to be responsible for the synergistic effect.

1.5.1.5 Commercial products
Senna fluid extract, senna fruit, compound senna powder (compound licorice powder), senna syrup, senna tablets, senna tea, yashtyadi churna, shataskar churna.

1.5.1.6 Other uses
Senna is a potential crop for honey bee culture also, as blooming senna provides food supply for honey bee round the year. *C. angustifolia* is native of Saudi Arabia and has been naturalized in India. The plant is ecofriendly and has been recommended for developing wastelands and does not require frequent irrigation. Its drought resistance, quick establishment and perennial nature provide permanent green cover on sand in vegetation deficient arid zones (Agrawal and Sardar 2003).

1.5.1.7 Propagation
Conventionally it is propagated only through seeds. However poor seed viability and low seed germination frequently restricts its propagation on a large scale. The plant grows well in red soil and black soil; even it grows well in saline and alkaline soil. The pH range for better growth is between 7.0 and 8.5 with adequate drainage.
1.5.2 *Cassia sophera* Linn.

**Synonyms:** *Senna sophera*

**Common name:** Pepper leaved senna, African senna, Kasondi, Baner, Kalkasonji

**Systemic Position:**
- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Fabales
- Family: Fabaceae
- Subfamily: Caesalpinoideae
- Genus: *Cassia*
- Species: *sophera*

1.5.2.1 Distribution
Originates from tropical America, but is now pantropical. It occurs throughout tropical Africa, being common in West Africa, but in East Africa and Madagascar it is probably rare. Found throughout India ascending the Himalayas up to an altitude of 750 m. It is common in wastelands, on roadside and in the forest.

1.5.2.2 Morphological characters
A diffuse, sub-glabrous, shrubby herb or under shrub, 0.7-3.0 m in height (Figure 2). Leaves foetid, unipinnate 20-30 cm long, leaflets 8-12 pairs, oblong or lanceolate, flowers yellow in short axillary or terminal corymbose racemes, pods slightly falcate, subterete or terete, 6.5 cm x 0.6 cm, seeds dark brown in colour and about 30-40 seeds per pod.

1.5.2.3 Medicinal uses
The plant exhibited anticancer activity and is reported to be extensively used in Homeopathy. The leaves possess purgative and antidiuretic properties. It is used as an infusion or decoction or as an expectorant for cough, cold, bronchitis, asthma, hiccups and jaundice and in subacute stages of gonorrhoea. Internally, it is reported to act as a febrifuge in rheumatic and inflammatory fevers and in
Figure 2. *Cassia sophera*
skin diseases. A paste of the leaves is used for washing syphilitic sores. The seeds are cathartic and also used as a febrifuge (Anonymous 1992b). The chemical analysis of the seeds revealed the presence of the ascorbic acid, dihydroascorbic acid and β-sistosterol (Bilal et al. 2005).

The powdered seeds mixed with honey are used in diabetes. An ointment made from the seeds is used to cure ringworm, sores, scabies and psoriasis. The bark like the leaves and seeds is cathartic in action. Its infusion is considered useful in diabetes and the juice in asthma. Kasondi is described in unani literature to be repulsive of morbid humors (especially phlegm), resolvent, blood purifier, carminative, purgative, digestive, diaphoretic and reported to be useful in epilepsy, ascites, dyscracia of liver, skin disorders, piles, fever, articular pain and palpitation. In ethnobotanical literature, it is reported to be effective in the treatment of pityriasis and convulsions of children (Bilal et al. 2005). The methanol extract of the leaves of *C. sophera* can be used as a source of natural antioxidants with potential application to reduce oxidative stress with health benefits (Rahman et al. 2008).

### 1.5.2.4 Propagation

Conventionally it is also propagated through seeds. However, hard seed coat and seed dormancy prevent its germination in nature.

### 1.6 Plant tissue culture studies on *C. angustifolia* and *C. sophera* till date

Different regeneration pathways such as direct regeneration, indirect organogenesis via callus formation and somatic embryogenesis has been explored in past by several workers in *Cassia angustifolia* and *C. sophera* to get optimum multiplication rate utilizing different explants. The work done on the *C. angustifolia* and *C. sophera* has been summarized below in table 2:
Table 2. Current status of tissue culture work done on two medicinally valuable Cassia species viz: *C. angustifolia* and *C. sophera*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Explants</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td><em>C. angustifolia</em></td>
<td>CN, NS, ST</td>
<td>Agrawal and Sardar 2003</td>
</tr>
<tr>
<td></td>
<td>L, C</td>
<td>Agrawal and Sardar 2006</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Agrawal and Sardar 2007</td>
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</tr>
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<td></td>
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<td></td>
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</tr>
<tr>
<td><em>C. sophera</em></td>
<td>CN</td>
<td>Parveen and Shahzad 2010</td>
</tr>
</tbody>
</table>

C: Cotyledon, CN: Cotyledonary node, L: Leaf, NS: Nodal segment, P: Petiole, R: Root, ST: Shoot tip

1.7 Objectives

The following objectives were carried out to achieve the goals under the broad prospective described above:

1. To germinate seeds of *C. angustifolia* and *C. sophera* under aseptic conditions.
2. To establish *in vitro* cultures from various explants.
3. To select best suited media and culture conditions for direct and indirect organogenesis and to evaluate the specific role of various plant growth regulators.
4. To standardize reliable protocol for induction of somatic embryos.
5. To encapsulate the vegetative tissues for synthetic seed production.
6. To evaluate optimal medium for root induction in microshoots, germination of somatic embryoids and synthetic seeds followed by successful acclimatization.
7. To study the developmental pathways through histology of differentiated tissues.
8. To study various physiological parameters during \textit{ex vitro} establishment of \textit{in vitro} raised plantlets.