Chapter 1

General Introduction

1.1 Introduction

India has a rich biological diversity due to its varied climatic, altitudinal variations and ecological habitats. It is one of the 12 mega-diversity countries of the world with a rich diversity of biotic resources (Niraj, 2012). Out of the 34 hotspots recognised, India has two major hotspots - the Eastern Himalayas and the Western Ghats. The Eastern Himalaya is situated between the Kali Gandaki river in central Nepal in the west and Myanmar in the east, occupying southeast Tibet in China, Sikkim, North Bengal, Bhutan and North-East India. The Western Ghats or Sahyadri is a mountain range that runs parallel to the western coast of Indian peninsula. The range runs north to south along the western edge of the Deccan plateau, and separates the plateau from the narrow coastal plain, called Konkan, along the Arabian sea.
India harbours about 47,000 species of plants of which 17,000 are angiosperms (Bapat et al., 2008), of which 5,800 (aprox) species are present in the Western Ghats and around 8,000 species are found in the Himalayas out of which 5,000 species are found in the Northeastern region (Tandon and Kumaria, 2005; Rao, 1984). Many of these valuable plants have now become rare or endangered and some are approaching extinction.

There is a growing concern about the depletion of the natural resources especially affecting the biodiversity of the ecosystem. The uncontrolled exploitation and the human dominance since the industrial revolution have markedly reduced the diversity of species within many habitats in the biosphere leading to its accelerating extinction. A great number of plant species in the Northeast India including several unique and irreplaceable varieties are becoming extinct and many more are awaiting a similar fate (Tandon and Kumaria, 2005). Fragmentation of habitats adversely affects the fate of rare and threatened species which may be reduced to such low numbers that they may not constitute viable populations.

Because of the increasing rate of extinction, the need for conservation is exceptionally high and of great importance to preserve plants heritage for posterity. This has enacted a collected consciousness on the significance of biodiversity for future human developments; hence the issue of conservation and sustainable utilization of bio-resources has come to the fore.

### 1.2 Medicinal Plants

Plants are one of the most important sources of medicines. The application of plants as medicines dates back to prehistoric period (Mazid et al., 2012). Recent estimates suggest that over 9,000 plant species have known medicinal applications in
various cultures and countries and this is without having conducted comprehensive research amongst several indigenous and other communities (Farnsworth and Soejarto, 1991). It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from or modeled on plant substances (Tripathi and Tripathi, 2003). Extensive exploration of medicinal plants was made with an aim to identify the potential and abundance in distribution for most of the high demand medicinal plants in nature. Human beings have used plants for the treatment of diverse ailments for thousands of years (Sofowara, 1982; Hill, 1989; Yuan et al., 2010; Yadav et al., 2010; Kumari et al., 2014). Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine (Iwu et al., 1999; Idu et al., 2007; Mann et al., 2008; Ammar et al., 2009). According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements (Rabe and Van Stoden, 2000), since they cannot afford the products of western pharmaceutical industries (Salie et al., 1996), together with their side effects and lack of healthcare facilities (Griggs et al., 2001). The World Health Organization (WHO) estimates that a minimum of 20,000 plant taxa has recorded medicinal uses. It is estimated that up to 70,000 plant species are used in folk medicine (Farnsworth and Soejarto, 1991) and a majority of these species are found in the Asia-Pacific region. Therapeutical properties of medicinal plants are very useful in healing various diseases and the advantage of these medicinal plants are natural (Kalemba and Kunicka, 2003). In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years (Ali et al., 1998; Barbour et al., 2004; Yasunaka et al., 2005).
However, the uses of medicinal plants are faced with many constraints. Some of these constraints include: plants with medicinal values not fully identified, invented and characterized, information and knowledge not being adequately documented and disseminated, many issues are not addressed and resolved (i.e. equity and sustainability), and the alarming commercial over-exploitation and consequent genetic erosion of medicinal plants. As an initial step towards resolving this constraint there is a need to develop a sound research strategy and utilization. In view of the growing world population, increasing anthropogenic activities, rapidly eroding natural ecosystem etc the natural habitat for a great number of herbs and trees are dwindling. Many of them are facing extinction. To cope up with alarming situation the recent exciting developments in biotechnology have come as a boon. One of them is the use of plant tissue culture technique. Conventionally, most of the plant raised through seeds are highly heterozygous and show great variations in growth and yield and may have to be discarded because of poor quality of products for their commercial release. Likewise, majority of the plants are not amenable to vegetative propagation through cutting and grafting, thus limiting multiplication of desired cultivars. Moreover many plants propagated by vegetative means contain systemic bacteria, fungi and viruses which may affect the quality and appearance of selected items.

Habitat loss and unchecked commercialization of wild medicinal plants is threatening the future of vital resources, as well as the beauty, diversity, and natural heritage of our planet. As wild-lands are destroyed or degraded, we lose unique and precious species, and with them potential resources to combat hunger, poverty, natural disasters, and social and economic insecurity. This loss of diversity may also take with it important cure for diseases — both those we face now and those that may emerge in the future. Unchecked commercialization may render important
traditionally used medicinal plant resources inaccessible and unaffordable to populations that have relied on them for centuries - as well as to the rest of the world. Medicinal plants are at increasing risk from destruction of their habitats, bio-prospecting for new sources, and overharvesting of known medicinal species. As of 1995, less than 1 percent of all tropical plant species had been screened for potential pharmaceutical applications (world mangrove atlas, 1997). Habitats are being destroyed more quickly than scientists can investigate them. At current extinction rates, experts estimate that the Earth is losing at least one potential major drug every two years (world mangrove atlas, 1997).

1.3 Status of Medicinal plants in India

Asian region which is a bio-geographically unique region, has the maximum degree of endemism in the - it supports about 18,440 species of plants, of which 25.3% are endemic, and has a large repository of medicinal plant species (Mills and Lee, 2011). India which is under the Asian region, is home to a great variety of ethnomedicinally important plant species, and is ranked sixth among 12 mega-diversity countries of the world. India is blessed with two mega centers of biodiversity - the Eastern Himalayas and the Western Ghats, where ecological, phyto-geographical and evolutionary factors favour high species diversity. Medicinal plants as a group comprise approximately 8,000 species and account for around 50% of all higher flowering plant species of India. Floristically rich, India has about 141 endemic genera of 5,150 species belonging to 47 families of higher plants. Among the different endemic species, 2,577 are distributed in Himalayas, 1,788 species in the peninsular region and 785 species in the Andaman and Nicobar islands. About 43,000 plant species are said to exist in India, of which 7,500 plant species are referred to in Indian folklore but only about 1,700 plant species have actually been documented in old
literature (Ramakrishappa, 2002). The increasing demands for herbal medicines by consumers in both developing and developed countries, has renewed interest by the multinational pharmaceutical industry in bio-prospecting. But the lack of national legislation or effective international agreements on conservation and sustainable use of bio-diversity has resulted in ‘slaughter harvesting’ of medicinal plants and massive depletion of biodiversity. Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments (Chopra et al, 1956), which also forms a rich source of knowledge.

The North-east India (located between 87º 32’ E to 97º 52’ E latitude and 21º 34’ N to 29 º 50´ N latitude) is a part of both Himalaya as well as Indo-Burma biodiversity hotspots in the world. It forms a unique biogeographic province encompassing major biomes recognized in the world. It has the richest reservoir of plant diversity in India and is one of the ‘biodiversity hotspots’ of the world supporting about 50% of India’s biodiversity. All types of vegetations right from the grassland, meadows, marshes, swamps, scrub forests, mixed deciduous forest, humid evergreen forests, temperate and alpine vegetation are found here. The varied forests types found in the region are home to numerous plants and animals. Specially, the region exhibits the richest diversity in orchids, zingibers, yams, rhododendrons, bamboos, canes and wild relatives of cultivated plants (Mao et al, 2009). Comparing to other part of the country, population density in the region is less and most of the land was unutilized by the public which needs to be protected to enrich the medicinal plant flora (Majumder, 1981; Mudaiya et al., 1987; Rawat et al., 1997; Shankar et al., 1999; Website IUCN, 2009). Various attempts have been made for exploration of medicinal wealth in different forest land of the Region (Kharkonger and Joseph, 1981; Tiwari et al., 1993; Mahanti, 1994; Sharma and Sharma, 1994; Rai and Sharma, 1994;
1.4 **Genus Panax**

*Panax* is a genus of approximately eighteen species distributed disjunctly in Asia and North America. *Panax*, commonly known as ginseng has also been called as green gold (Persons, 1986), is one of the world's most valued plants. It is an economically important crop in international trade for its highly valued rhizome. Chinese have used ginseng for thousands of years as a wonder drug, cure-all, and aphrodisiac (Proctor and Bailey, 1987). In Li Shih-Chen's Pen-ts'ao Kang-mu, ginseng was described as a superior article among traditional Chinese herbals (Hu, 1976). The pharmacological properties of ginseng is because of its active ingredient saponins, also commonly known as ginsenosides (Homok, 1992).

1.4.1 **Panax species**

The north-east India harbours at least five species *viz.*, *Panax* pseudoginseng Wallich, *P. bipinnatifidus* Seemann, *P. wangianus* S.C. Sun, *P. assamicus* Ban and *P. sikkimensis* Ban, although correct taxonomic identity of these species is yet to be established. As such, the taxonomy of Indian *Panax* is highly controversial (Banerjee, 1968) and the relationships of Indian taxa of Araliaceae are not well understood. *Panax pseudoginseng* Wall, was described by Wallich in 1829 based on the specimen collected from Central Nepal and its circumscription has presented many problems to many taxonomists (Pandey *et al.*, 2002, 2004). *P. wangianus* was classified as endangered (IUCN, 2004). All the five species have high medicinal value under traditional medicine systems. *Panax assamicus* Ban. was described by Banerjee (1968) on material collected from Shillong area (Pandey *et al.*, 2002). The plant is commonly known as ginseng by
the local people and it is available in the wild. The rhizome is being used by the local herbal practitioners, where the rhizomes are collected from the wild and sold by the local people to the practitioners or in the local markets. As a result of which the plant has become rare and endangered (Pandey and Ali, 2001). Some important *Panax* species from other countries are, Asian ginseng or *P. ginseng* C.A. Meyer, American ginseng or *P. quinquefolius* L , which was listed on Appendix II of CITES in 1973, Japanese ginseng or *P. japonicus* C.A.Meyer, and Sanchi or *P. notoginseng* [Burk] F.H.Chen. American ginseng is a perennial herbaceous plant with a solitary stem, whorled leaves, and a thick taproot (1982; Proctor and Bailey, 1987). Ginseng flowers are small and pentamerous (Lewis and Zenger, 1982). American ginseng grows naturally under the canopy of hardwood forests. It is a shade-loving plant. The aboveground portion of the plant dies at the end of each growing season, and the new stem develops in the following spring from the bud on the rhizome (Hu et al., 1980). The rhizome continues to grow each growing season. Among the Asiatic species, several Himalayan taxa are problematic due to sympatry of morphological distinct taxa and the existence of morphological intermediates (Wen and Zimmer, 1996).

### 1.4.2 Scientific classification

According to Bentham and Hooker

Kingdom - Plantae

Division - Magnoliophyta

Class - Magnoliopsida

Order - Apiales

Family - Araliacea

Genus - *Panax*
1.4.3 Distribution

The two species in India, *P. assamicus* Ban. and *P. pseudoginseng* Wall. are found mostly in the North-eastern regions of India. It is found wild in the hills of Arunachal Pradesh, Manipur, Meghalaya, Nagaland, and also in Darjeeling area of West Bengal. Both the species, *P. assamicus* collected from East Khasi Hills and West Khasi Hills district of Meghalaya and *P. pseudoginseng* collected from Phek District of Nagaland (Fig1.1 & table1), are fast vanishing from the habitats due to over exploitation. Initiative needs to be taken immediately to conserve the diversity of this medicinal important taxon.

1.4.4 Morphological description

Ginseng is a dicotyledonous plant, the tuber is single, fascicled or fusiform. Rhizome is elongated and the base of aerial stem is covered with persistent glabrous and membranous scales. Stem is glabrous, 3-4 leaves with persistent stipules. Inflorescence with 1-3 umbels each with 40-60 flowers. Fruits of both the species are crimson red when ripened and with the black topping when fully ripened. 1-2 seeded, seeds are broadly kidney shaped, 7-7.5 mm long, 6-6.5 mm wide, 5-6 mm thick (fig 1.3).

The main difference between the two species i.e, *P. pseudoginseng* and *P. pseudoginseng* are- *P. pseudoginseng*, leaflets are broad and leaf margins are crenate, uppersurface with briskly hairs along veins and veinlets, lower surface glabrous. Whereas *P. assamicus* Ban exhibit tall habit compared to *P. pseudoginseng*, linear leaflets, minutes serrate margins, inflexed apiculate petals, much longer stamens and completely free styles (Wen, 2001; Pandey *et al.*, 2002) (fig 1.2).
Fig 1.1 States from where Panax species were collected
Table 1: Different localities from where *Panax* species were collected

<table>
<thead>
<tr>
<th>State</th>
<th>District</th>
<th>Locality</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>East Khasi Hills</td>
<td>East Khasi Hills</td>
<td>Nongkrem sacred grove</td>
<td>N25°29.580'</td>
<td>EO91°52.506'</td>
<td>1838</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mawphlang sacred grove</td>
<td>N25°26.598'</td>
<td>EO91°44.967'</td>
<td>1622</td>
</tr>
<tr>
<td>Meghalaya</td>
<td>Laitkor</td>
<td>N25°31.996'</td>
<td>EO91°51.064'</td>
<td>1924</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Upper Shillong</td>
<td>25°37.120'</td>
<td>EO91°47.111'</td>
<td>1880</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tyllang</td>
<td>N25°33.280'</td>
<td>EO91°21.987'</td>
<td>1635</td>
<td></td>
</tr>
<tr>
<td>West Khasi Hills</td>
<td>Upper Laitkseh</td>
<td>N25°29.266'</td>
<td>EO91°25.453'</td>
<td>1632</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marngor</td>
<td>N25°27.804'</td>
<td>EO91°25.282'</td>
<td>1490</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mawkyrwat</td>
<td>N25°22.078'</td>
<td>EO91°26.807'</td>
<td>1654</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jakrem</td>
<td>N25°24.426'</td>
<td>EO91°32.450'</td>
<td>1592</td>
<td></td>
</tr>
<tr>
<td>Nagaland</td>
<td>Phek</td>
<td>Khezhakeno (Zirri forest)</td>
<td>N25°30.090'</td>
<td>EO91°13.556'</td>
<td>1810</td>
</tr>
</tbody>
</table>
Fig 1.2 *P. pseudoginseng* a. Plant in habitat; b. Close up view of the leaf *P. assamicus* c. Plant in habitat; d. Close up view of the leaf.
Fig 1.3 *P. assamicus* a. Flower b. Fruits c. Rhizome *P. pseudoginseng* d. Flower e. Fruits f. Rhizome
1.4.5 Chemical constituents

Ginseng saponins called ginsenosides are the major active constituents or the active compounds. The therapeutic importance of ginseng has led to the development of a wide spectrum of analytical methods for the determination of the total saponin content, group-specific analysis, and target compound determination. Ginsenosides belong to a family of steroids with a four trans-ring rigid steroid skeleton. Most ginsenosides share a unique triterpenoid saponin structure of the dammarane type (Fuzzati, 2004). Ginsenosides differ from one another by the type of sugar moieties, sugar number, and site of sugar attachment at positions C-3, C-6, or C-20. The structural isomerism and stereoisomerism, the number and site of attachment of hydroxyl groups, and available modified side chain at C-20 also increase their diversity. Ginsenosides from ginseng are divided into several groups. Protopanaxadiol ‘PPD’ (Rb1 group- Rb1, Ra, Rb2, Rc and Rd) and protopanaxatriol ‘PPT’ (Rg1 group- Rg1, Re, Rf and Rg) groups are the main constituents, while ocutillol and oleanane groups are minor ones (Qu et al., 2009). The PPD group has sugar moieties attached to the β-OH at C-3 and/or C-20, and the PPT group has sugar moieties attached to the α-OH at C-6 and/or β-OH at C-20 (Wang and Yuan, 2008; Jia and Zhao, 2009). The ocutillol group has a five-membered epoxy ring at C-20, and the oleanane group has a modified C-20 side chain (Yoshikawa et al., 1998).

1.4.6 Pharmacology

The individual ginsenosides provide significantly different pharmacological effects, their activities are cardiovascular diseases, cancer, immune deficiency, and hepatotoxicity. Moreover, recent research has suggested that some of ginseng's active ingredients also exert beneficial effects on aging, central nervous system (CNS) disorders, and neurodegenerative diseases. In general, antioxidant, anti-inflammatory, anti-apoptotic, and immune-stimulatory activities are mostly underlying the possible
Ginseng mediated protective mechanisms. Next to animal studies, data from neural cell cultures contribute to the understanding of these mechanisms that involve decreasing nitric oxide (NO), scavenging of free radicals and counteracting excitotoxicity (Liu and Xiao, 1992; Attele et al., 1999; Xiang et al., 2008; Yuan et al., 2010). The active ingredients also express pharmacological actions, such as antitumor, antidiabetic, anti-inflammatory, anti-allergic, and neuroprotective effects (Wang et al., 1999). Ginsenoside is an active triterpenoid obtained from Panax ginseng is used in treatment of chronic diseases like, obesity, diabetes, cardiovascular and arthritis inflammation (Yadav et al., 2010). The active compound is also used for stimulating immune function, improving physical and athletic stamina, improving cognitive function, concentration, memory, and work efficiency. It is also used for depression, anxiety, chronic fatigue syndrome (CFS), Pseudomonas infection in cystic fibrosis, chronic bronchitis, breast cancer, ovarian cancer, liver cancer, lung cancer, and skin cancer. Also used for anemia, diabetes, gastritis, neurasthenia, erectile dysfunction, impotence and male fertility, fever, hangover, and asthma. It is also used for bleeding disorders, loss of appetite, vomiting, colitis, dysentery, insomnia, neuralgia, rheumatism, dizziness, headache, convulsions, disorders of pregnancy and childbirth, hot flashes due to menopause, and to slow the aging process (Chauhan et al., 2012).

1.4.7 Constraints in conventional propagation

The plant can be propagated through both seeds and rhizome. However there are two key factors limiting the cultivation of P. assamicus and P. pseudoginseng. The first one is difficulty in obtaining seeds due to over exploitation of the big plants by the common man from the habitat. And the second one is the difficulty in obtaining enough seedlings due to long period of seed dormancy which is about
eighteen months or even longer. The seeds are of morphophysiological dormancy type and need a long stratification period. The other factor being the plant is very slow in growth, taking about four to five years to flowering and another three to four years to develop rhizome suitable for harvesting. Hence the conventional of the rhizomes takes too long to meet the medicinal demand.

1.4.8 Need for in vitro propagation

The availability of Panax are nearly destroyed due to the aggressive collection of this important herb over the last few decades. At the present the rhizomes are harvested directly from the wild without any cultivation measures. The plant which once used to be abundant in the hills of the North Eastern states have become very rare due to exploitation. Interactions with the local people during the field visit showed that the plants were being collected and exported to the Indo-Myanmar border by the name ‘Ginseng’ without knowing the importance of the plant and its medicinal uses. These important medicinal plants along with others has been reported that tons and tons of rhizomes collected from Manipur, Nagaland, Arunachal Pradesh and Meghalaya are traded to Myanmar (Mao., et al 2009).

1.5 Objective

Keeping in mind, the importance of Panax species and for conservation purposes, the present research work was undertaken with the following objective:

To develop a protocol for mass propagation of Panax species of North-East India through somatic embryogenesis using suitable in vitro and in vivo explants like rhizome, leaves and petioles.