# Population Studies and Habitat Characteristics of *Swertia chirayita* (Roxb. ex Fleming) H. Karst., a Threatened Medicinal Herb in Sikkim Himalaya, and Its Conservation

A thesis submitted to
THE UNIVERSITY OF NORTH BENGAL

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in
SCIENCE (BOTANY)

LIBRARY
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# DECLARATION

I, Bharat K Pradhan, hereby declare that the thesis entitled, "**Population Studies and Habitat Characteristics of *Swertia Chirayita* (Roxb ex. Fleming) H. Karst., A Threatened Medicinal Herb in Sikkim Himalaya, and its Conservation**" submitted for the degree of '**DOCTOR OF PHILOSOPHY**' in **Science (Botany)** to the **University of North Bengal,** is a *bona fide* and original piece of research work carried out by me under the supervision of Dr Hemant K. Badola, Scientist 'E' (Conservation of Biodiversity), at G.B. Pant Institute of Himalayan Environment & Development, Sikkim unit, Pangthang-Gangtok, Sikkim as a regular and full time research fellow. I am registered with the University of North Bengal, Raja-Rammohunpur, Dist. Darjeeling, for the Ph.D. with Registration No.168/Ph.D./Bot/1657(R)06(4), dated 11.9.06.

No part of this thesis work has been submitted for the award of any other degree or equivalent elsewhere and is entirely original.

**Date:** 13/06/2011

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CHAPTER 1

# Introduction

The plants have been the source of medicine since time immemorial. Millions of people largely from the developing countries are known to have dependency on traditional medicine for their primary health care needs (WHO 2002, Badola and Aitken, 2003), for which the medicinal plants are the important source. The increased popularity of the traditional medicine has led to massive rise in its economic importance. Plants and their derivatives contribute to 25% of the medical drugs in developed countries (Principe, 1991); there is rapid increase in the international consumer demand for the traditional plant based medicines (Lambart *et al.*, 1997; Hamilton, 2004). Majority of the population in the European North have been paying attention to application of medicinal plants, vitamins, natural and active biological derivatives in the treatment and prevention of many diseases (Galambosi *et al.*, 2010). The growing use of plants as a source of medicine has resulted in the over exploitation of several medicinal plant species from the wild (Badola and Pal, 2002; Schippmann *et al.* 2002; Badola and Aitken, 2003, 2010; Dhyani and Kala, 2005, Butola and Badola, 2008a) thereby increasing the risk of extinction of important plant species and/or loss of genetic diversity. Furthermore, the multifold increase in the use, marketing and trade as a consequence of growing industrial demand for raw materials (Badola and Pal, 2003, Badola, 2009), have been experienced during the past couple of decades exerting unprecedented pressure on the natural habitat of important medicinal plants, This has led to increased threat of depletion of their populations, as majority of the medicinal plants are largely collected from the wild.

The medicinal and aromatic plants (MAPs) form an important source of income for the rural communities in several south Asian countries (Karki *et al.*, 2003) mainly in the Himalayan region. In many parts of Himalaya, the communities inhabiting the mountain areas sustain their livelihood by collecting and marketing the important medicinal plants (Farooquee and Saxena, 1996; Dobriyal *et al.*, 1997; Tandon, 1997; Badola, 1998, 2009; Olsen and Larsen 2003; Olsen, 2005, Larsen and Olsen, 2007).

Nevertheless, over collection has been identified as one of the serious threat to medicinal plant populations; other threats endangering the existing populations of medicinal plants include habitat alteration and specificity, narrow range of distribution, overstocking and over-grazing of areas by domestic animals, etc. Besides, some of the identified natural threats are pathogens, herbivores, seed predators, etc., which are also threatening the natural population of many of the rare and endangered medicinal plants (Dhyani and Kala, 2005; Badola and Pal, 2003). This has led to worldwide concern over the conservation of commercially utilized medicinal plant resources (Hamilton, 2004; Larsen and Olsen, 2007; Badola and Aitken, 2010) as well as the on the diminishing populations, loss of genetic diversity, local extinctions and habitat degradation of the important medicinal plants (Canter *et al.*, 2005).

It is estimated that the number of higher plant species used worldwide for medicinal purposes is more than 50,000 i.e., 20% of the world's vascular plants (Schippmann *et al.*, 2002; Hamilton *et al.*, 2006), the two-third of which is collected from the wild (Turcker, 2003). In India, approximately 8000 plant species are used for medicinal purposes (Planning Commission, 2000), of which, 90% of the medicinal plants having commercial value are harvested from the wild, and over 70% of the plants collected through destructive harvesting. Threat assessment of medicinal plants using IUCN (International Union for Conservation of Nature and Natural Resources) designed CAMP (Conservation Assessment of Medicinal Plants) methodology suggests that about 112 species in southern India, 74 species in northern, northeastern and central India and 42 species in the high altitude of Himalayas are threatened in the wild (Sharma *et al.*, 2010). To develop conservation strategies for the threatened medicinal plant species, it is imperative to have better understanding on the nature of the threats to their survival (Badola and Aitken, 2003) at population levels, addressing habitat niches and viability.

## 1.2 Medicinal plants – growing importance in the global market

The growing popularity of medicinal plants is well acknowledged in the international market, having a worth around US $60 billion (World Bank, 2004), which is increasing at the rate of 7% per year (Koul and Wahab, 2004). The World Health Organization reports international market for herbal drugs to be around $ 43 billion a year, which is expected to touch $5 trillion by the end of year 2050 (Christie, 2001). Approximately, 2500 species of medicinal plants are known in trade in the international market (Schippmann *et al.* 2002); the major markets include Europe, North America and Asia. In 1991, the herbal medicine market in the European countries alone was about $6 billion, with Germany accounting for the highest trade of $3 billion (50% of the European market), followed by France for $1.6 billion and Italy for $0.6 billion, while in other countries the trade touched to $0.8 billion mark. In European countries, in the span of 4 years (1991-1994) the herbal market recorded a rise of $4 billion reaching to $10 billion; the herbal market was about $4 billion, $1.0 billion and $5 billion, respectively, in USA, India and other countries (Prajapti *et al.*, 2003). In 1997, the European market alone reached to about $7.0 billion followed by France ($1.8 billion), Italy ($700 million), the United Kingdom ($400 million), Spain ($300 million) and the Netherlands (about $100 million) [Calixto, 2000]. The present global market of herbal drugs has reached to US $250 billion.

The nutraceuticals (nutritionally or medicinally enhanced foods with health benefits) are in the huge demand in developed countries like USA and Japan. Its market in the USA and Japan alone is about US$ 80–250 billion (with a similar market in Europe) and US$ 1.5 billion, respectively. As per the annual Herbal Supplement Usage Study for the year 1997, it was revealed that one in five adults in USA take herbal supplements (Wood, 1997).

## 1.2 Changing market trend of medicinal plants in India

For having 2.5% of the world's total geographical area and bestowed with 8% of the global biodiversity, India ranks sixth among 12 mega biodiversity countries of the

world. Of the 34 hotspots in the world, India forms the part of two biodiversity hotspots, (i) Indo-Burma and (ii) Western Ghat. Of about 45,000 plant species (nearly 20 per cent of the global species) in the Indian subcontinent, approximately 8000 species (both higher and lower plant groups) are of medicinal value (Planning Commission, 2000). Different types of forest systems prevailing in the country harbour about 90% of the medicinal plant biodiversity. Tremendous increase in the domestic demand for the medicinal plants has been observed with the resurgence of Indian traditional medicinal systems in the current past (Badola and Aitken, 2003). Approximately, 289 species of medicinal plants were reported to be used as traditional medicine during Vedic period, the number increased to 650 species from 1500 BC to 500 AD, and by 1900 AD, it reached to 1814 species (Rath, 2005).

Today India stands as second largest exporter of herbal products/drugs in the world; despite having rich biodiversity, the country has market share limited to only 1 to 2%. The volume of medicinal plants exported from India were 32882.8 tons, 37976.3 tons and 36617 tons with monetory value of Rs.2159.518 million, Rs.2377.952 million and Rs.1764.799 million, respectively, during the year 1996-97, 1998-99 and 1999-2000. The export of medicinal plants and its product from India was estimated to be Rs. 4.46 billion in the year 2000 (NMPB, 2002) and Rs. 3.16 billion in 2000-01 (Rawal, 2003). In spite of assuming the conventional growth rate at 15%, India still lags behind China. It was reported that when transactions of China and Thailand were Rs.2500 crore and Rs.1000 crore, respectively, India could achieve a figure of Rs.436 crore only. India uses approximately 8000 plants species compared to approximately 5000 plant species used in traditional Chinese medicine (Rath, 2005); but still, traditional Chinese medicine is well established compared to Indian Ayurvedic medicine in the international market (Aneesh *et al.*, 2009). The demand and supply ratio of the raw materials to the pharmaceutical sectors are not balanced in the country. For example, the gap between demand and supply of MAPs was estimated to be about 40,000 to 200,000 tons in year 2000 and was estimated to increase from 52,000 to 400,000 tons in 2005 (Planning Commission, 2000). In year 2002, the demand of medicinal raw drugs in the country was 2.72 lakh tons as against an estimated supply of 1.20 tons (Rath, 2005). The volume of the annual demand of raw

materials by the pharmaceuticals in India in the year 2005-2006 was 3,19,500 metric tons having worth value of Rs. 1069 crores (Ved and Goraya, 2007). Currently, the sale of total herbal products in India is estimated at $1 billion (Joshi *et al.*, 2004) and the export of herbal crude extract reached about $80 million (Samy and Gopalkrishnakone, 2007).

Currently, the Indian herbal industry has an annual turnover of Rs. 7,500 crore as against Rs. 14,500 crore of the pharmaceutical industry which is estimated to double by the year 2015 at the current growth rate of 15 percent. The global herbal industry is estimated to grow to Rs 70,000 crore by the year 2015 from the current level of Rs 30,000 crore (www.niir.org). India has potential to compete in the global herbal market, which largely depends on the quality of the products it produces.

## 1.3 Medicinal Plants - in context to Indian Himalayan Region

The Himalaya region, considered as an exceptional landscape in the world for its biological and cultural richness, is particularly gifted with one of the richest medicinal plant species diversity (Badola and Aitken, 2003, 2010), largely for having diverse agro-climatic conditions. Nonetheless, the region is under considerable anthropogenic threats due to extraction of a wide variety of natural resources for subsistence as well as for the commercial purposes. Indian Himalayan Region (IHR) harbours over 1748 species of medicinal plants. Of the total medicinal plants species of IHR included in the Red Data Book, 31% are native, 15.5% are endemic and 14% have already assessed as threatened (Samant *et al.*, 1998). On the regional scale, Uttaranchal harbours the maximum species of medicinal plants (Kala, 2004); cumulatively, however, Sikkim and Darjeeling hills represent maximum number of medicinal plant species (Badola and Aitken, 2003). Nevertheless, the continuous exploitation of several medicinal plant species from the wild (Kala, 2003, Badola, 2010) and substantial loss of their habitats during past 15 years (FAO, 2003) have resulted in population decline of many high value medicinal plant species (Badola and Pal, 2002, 2003). Consequently, many of the valuable medicinal plants can now only be found growing in small scattered populations in remote areas (Hussain and Sher, 1998).

## 1.4 Medicinal plants and current problems

The studies on medicinal plants of Indian Himalayan region have mostly been focused on their inventory, distribution and uses; a meager proportion of researches have been dedicated on population and habitat relationships. Dhar *et al.*, (2000) reviewed the status in this regard and revealed that 50% of the study were focused on the distribution and availability, 16.3% on status (21% on population studies, 79% general), 24.4% on cultivation and propagation, 4.7% on extraction and trade and 4.7% on conservation of medicinal plants of IHR. Such insufficient knowledge base hampers scientists and natural resource managers to make proper assessment of the quantitative harvesting of medicinal plants from the wild (Badola and Aitken, 2003). Data covering different climate and geographical regions of the Himalaya, especially location specific, are imperative to identify relative threats to natural populations of plant species; particularly those treated threatened (Badola and Pal, 2003). In recent years, some studies concerning population structure of threatened Himalayan medicinal plant taxa have appeared for different localized parts; these either focused a single taxa (Airi *et al.*, 1997, 2000; Bhadula *et al.*, 1996; Nautiyal *et. al.*, 2003a) or a group of taxa (Kala, 2000; Negi and Upreti, 2000; Uniyal *et al.*, 2002). Few have attempted identifying habitat preferences of the targeted taxa using quantitative tools (Airi *et al.*, 1997, 2000, Uniyal *et al.*, 2002). Different populations of a plant species may exhibit noticeable inter and intra variations (Bhadula *et al*, 1996). For example, Nautiyal *et al.* (2001) assessed the differences in active ingredients for two types of leaf variants of *Picrorhiza kurrooa*. Understanding and identifying morphological and genetic traits, specific to each targeted taxa, may have high importance in identifying superior germplasm for the conservation programme (Badola, 2002, Badola and Aitken, 2003). In general, insufficient studies are in hand which cover different regions of Himalaya and limited to some taxa; that means, many of the highly sensitive and valuable taxa of conservation value need immediate attention focusing detailed studies.

Approximately, 80% of the medicinal plants used by contemporary Ayurvedic industry in India are collected from the natural populations (Gupta, 1993). There are

factors such as slow growth rate, low population density and narrow geographical ranges of several medicinal plants make them more prone to extinction (Nautiyal *et al.*, 2002). There are several other causes such as land use changes, introduction of non-native species, habitat alteration, climate changes, fragmentation and degradation of the plant populations and genetic drift (Kala, 2000), which hastens the threat of extinction to those medicinal plants already considered threatened. Therefore, an urgent need is felt to formulate and implement regeneration / conservation strategies using both *in-situ* and *ex-situ* mechanisms targeting the over exploited medicinal plant species with an aim to strengthen the maintenance of biodiversity as well as preservation of the indigenous knowledge.

In addition, the growing demand for raw material of several medicinal plants at both national and international market has exerted unprecedented harvesting pressure on their natural habitats, and Indian Himalayan plant diversity victimized the most (Badola and Pal, 2002; Badola and Aitken, 2003). Resultantly, several medicinal plant species found their inclusion in the Indian Red Data Book, and in IUCN threatened list (Ved *et al.*, 2003a,b), and over a dozen have been prioritized by an international forum of experts for their immediate conservation through *ex-situ* cultivation in Himalaya (Badola and Pal, 2003). To strengthen either, *in-situ* or *ex-situ*, mechanism assessment of potential populations and appropriate technologies for propagation and mass multiplication *vis-à-vis* developing accurate information on the cost-benefit ratio of the cultivation are a must steps. The awareness amongst masses in *in-situ* conservation and the involvement of stakeholders, especially farmers in the cultivation demonstrations and in furtherance of the efforts would be important at the same time.

## 1.5 Medicinal plants – a concern in Sikkim

Sikkim harbors over 500 species of medicinal plants that occur right from the humid river valleys to the high cold trans-Himalayan desert. Prior to the governmental ban imposed on the collection of medicinal plants in the year 2000, Sikkim was one of the important sources of *in-situ* raw material harvesting and had traditionally been one of the major suppliers of the medicinal herbs to the markets in Siliguri, Kolkata and New

Delhi. Amongst high value medicinal plant species harvested from these areas, some of prominent ones were *Aconitum heterophyllum, A. ferox, Bergenia ciliata, Cordycep sinensis, Dactylorhiza hatagirea, Nardostachys grandiflora, Panax pseudoginseng, Picrorhiza kurrooa, Podophyllum hexandrum and Swertia chirayita*, etc. The State government is seriously concerned about the rapidly depleting medicinal plants resources in the state caused by the large scale collection from the wild in the recent past. As an initiative to conservation, (i) collections of medicinal plants have been banned in the State; (ii) numbers of medicinal plant species have been promoted in nursery cultivation in the state; and (iii) farmers who are interested in medicinal plant cultivation are being provided with subsidy loans. Further, the government is also planning to have large collection centres for the medicinal plants in which farmers can deposit their products which will go directly to the Pharmaceutical companies. With this, the farmers get the direct and maximum benefits. However, it must be pointed out that, the current conservation efforts are not enough to ensure the survival of threatened medicinal plant species in wild, where their potential and viable populations are expected to be maintained as vital gene banks. There is an urgent need in the state to carry out research firstly on population assessment for their quantum availability, community structure, potentiality of the regeneration and habitat characteristics of the threatened medicinal plant taxa, which is essentially required in designing and implementing the suitable and effective conservation management plans. Secondly, the research is imperative on identifying and prioritizing viable populations for long term storage and developing the standard propagation and mass multiplication protocols, strengthening *ex-situ* cultivation programmes. Also, it would be important to develop and highlight the nondestructive and sustainable harvesting methodologies of threatened medicinal plant species. So far in Sikkim Himalaya, there is highly insufficient research work available on above aspects; however, some fragmentary publications are available in the form of scattered or isolated efforts, aiming mostly towards medicinal use of the items or taxonomic findings. In fact, preparation of an inventory alone would not be able to meet the growing need of planned utilization of medicinal plant resources within the state. It is essential to assess the level and extent of exploitation of medicinal plant from the wild stock and to put in place necessary checks. The knowledge in this field would enable

introduction of timely cultivation in desired cases and maintain the required balance between the sustainable use and exploitation of limited natural resources. At the same time, *in-situ* level understanding on the habitats supporting potential populations of the threatened medicinal plant taxa and their distribution is a vital step in planning conservation, particularly in Sikkim Himalaya.

## 1.6 Importance of current research and the focal species

The current research lucidly addresses the above mentioned priority in relation to rich plant biodiversity of Himalaya, focusing mainly on one of the highly marketed and threatened medicinal plants, the *Swertia chirayita*. Despite the great demand of the species in Indian and International market (Badola and Pal, 2002), the plant is still collected from the wild, as such the existing population of *S. chirayita* is depleting at an alarming rate in different parts of Himalaya. Concerning over the declining population of the species, the IUCN (International Union for Conservation of Nature and Natural resources) has categorized the species as critically endangered (Ved *et al.*, 2003b). Species is also listed as endangered in Indian Red Data Book and has been assessed as critically rare and virtually endemic to Indian Himalaya (Samant *et al.*, 1998), and vulnerable to Sikkim (Ved *et al.*, 2003a). It has been prioritized at the top amongst 14 species for immediate conservation through *ex-situ* cultivation by an international forum of experts (Badola and Pal, 2002), and also prioritized by the National Medicinal Plant Board (Government of India) as well as State Medicinal Plant Board (Government of Sikkim) in Sikkim.

At present, the available publications and reports available so far, focusing on the conservation of *Swertia chirayita*, suggests several existing constraints, which need to be addressed:

1. So far, meager effort has been made to assess the availability of the species and its microsite preferences in natural habitats. Only very limited work is restricted to covering very few populations from the central and north-western Himalaya

2. Assessment of the threat status of the species has been done on the basis of IUCN's CAMP (Conservation assessment of medicinal plants) method, no field assessment data are available indicating its exact status
3. No information is available on the regeneration potential of the species from the eastern Himalaya; the scattered available information indicates very low seed viability in *S. chirayita* and majority of studies have given emphasis on its micro-propagation.
4. Assessment of seed viability over a long period of storage, as essential tool for successful conservation plan, is completely ignored aspect.
5. Available information reveals problems in domestication of the species
6. So far, no scientifically validated agro-techniques have been published on the species
7. Phenological studies, an important tool in determining the seed sowing, seedling transplantation as well as harvesting time, is completely absent for the species.
8. Current information, except the present studies, is not sure about the exact life span of *S. chirayita*

There is a wide scope for exploring different aspects of *S. chirayita*. Such gaps in the related research field raise questions regarding the vulnerability of the species, emphasizing the need for focused research.

### *1.6.1 The genus 'Swertia'*

The genus '*Swertia* L.' belongs to family Gentianaceae. It is named by Linnaeus (1753) in honor of Emanuel Sweert (rarely spelled Swert, 1552–1612), a Dutch gardener and illustrator who published an important "Florilegium" (a catalogue with over 560 different flowers in 110 plates - all in contemporary hand colouring) in the early 1600s. The genus *Swertia* is large, and taxonomically a distinct and difficult genus (Suksathan, 2001; Rijal, 2009) and has always been debated due to the morphological similarities of the species of *Swertia* to its related genera i.e., *Halenia*, *Loatogonium* and *Veratrilla* (Chassot *et al.*, 2001; Joshi and Joshi, 2008). Clarke (1885) divided the genus *Swertia* into three subgenera: *Ophelia*, *Euswertia* and

*Poephila*. It is cosmopolitan and mainly distributed in temperate regions of the northern hemisphere (Ho and Pringle, 1995). However, the genus is also represented in tropical regions and in the southern hemisphere (Meusel *et al.*, 1978). It is known from all the regions except Australia, New Zealand, South and Central America, and its highest species diversity are in the Himalaya and south-western China (Meusel *et al.*, 1978; Suksathan, 2001) due to which, Ho *et al.*, (1994) assumed south-western China to be the major centre of the origin of the genus. According to them, *Swertia* has diversified and dispersed to south-east Asia as well as to Africa and North America from China and have formed two secondary centre of diversification. In addition, Sudan-Zambezian region in Africa is a second distribution centre (Ho *et al.*, 1994). The genus *Swertia* is highly polymorphic (Chassot *et al.*, 2001) and their superficial resemblances often pose difficulties in species delimitation (Nemomissa, 1998). The genus *Swertia* is not a monophyletic taxon, instead it is highly a paraphyletic group and its closest genera include *Obolaria*, *Gentianella* and *Halenia* (Joshi, 2011).

Globally, the genus *Swertia* comprises of about 170 species, of which approximately 79, 27 and 40 species are distributed in China, Nepal and India, respectively. Sikkim alone, a northeastern Indian State harbours 13-14 species of *Swertia*. The *Swertia* species are rich sources of xanthones, flavonoids, iridoid and secoiridoid glycosides and triterpenoids. The species of genus *Swertia* are very well-known for their pharmacological properties and its bitterness. Several species of *Swertia* finds extensive use in folk medicine as an alternative, febrifuge and anthelmintic as well as a bitter tonic; they are known for their therapeutic potential against fever, cold, cough, stomachache, hepatitis, pneumonia, dysentery, scabies, spasm, pain, and malaria, and used as antidepressant, mutagenic, antipsychotic, tuberculostatic, choleretic, antioxidant, antiinflammatory and antidiabetic drug. In China, 30 species, of about 79 species of genus *Swertia*, are well acknowledged in the folk traditional medicines (Tian *et al.*, 2008) and about 13 species have been registered in the provincial book as folk medicine (Chen *et al.*, 1999).

### 1.6.2 The species distribution

*Swertia chirayita* [(Roxb. ex Fleming) H. Karst] is indigenous to temperate Himalaya and distributed from Afghanistan to Bhutan through Nepal, normally grows along 1200-3000m asl. In India, *S. chirayita* is found in Kashmir, Himachal Pradesh, Uttaranchal, Darjeeling (1500-2500), Sikkim (1500m - 2900m), Arunachal Pradesh, and Meghalaya (1200m -1500m). In Bhutan, species grows between 1900m to 3000m asl. Its synonyms are *Swertia chirata*, Buch.-Ham., *Ophelia chirata* Griseb., *Agathotes chirayita* D. Don., *Gentiana chirayita* Roxburgh., *Gentiana chirata* Wall., and *Gentiana floribunda* Don. In Sikkim, *S. chirayita* is known as chirowto, pothi chirowto, kalo chirowto, rato chirowto (Nepali); Rung kyen (Lepcha); and Titto (Bhutia). It is also called Chirayita, Charaita, chirata (Hindi); Charayatah, Chirayita (Marathi); Chirayatin (Gujarati); Chireta (Bengali), Nelabevu (Kannada); Chiaravata, Chiraita shireen (Urdu), Qasabuzzarirah (Arab, Farsi); East Indian Balmony or Indian Gentian (English) and Viktaka (Sanskrit) etc.; in Nepal, it is known as Chirrato and Chiraita; and in Bhutan, it is known by the name Khalu due to its bitter taste.

### 1.6.3 Plant morphology

*S. chirayita* is about 3-5 ft tall triennial erect herb, with single stem bearing numerous branches but sometimes un-branched. However, in some cases in nature, *S. chirayita* grows up to 7ft under good habitat conditions (*personal observation*). The stems are robust, smooth, purplish red in colour; the lower portion is cylindrical while the upper portion is quadrangular containing easily separable yellowish pith. The leaves are sessile, opposite, lanceolate, acute, entire, 3-5 nerved; the lower leaves are much larger. The roots are yellowish, simple, short, stout, tapering, *ca.* 0.5 - 1m long and 1 – 1.5 cm in thickness near the crown. Flowering in *S. chirayita* is in the form of large panicle, leafy, many flowered and the whole inflorescence is approximately 2 ft. long. Flowers are hermaphrodite, small, stalked, greenish-yellow, tinged with purple colour, rotate and tetramerous. The corolla is twice as long as the calyx and divided near the base into four ovate – lanceolate segments. The upper surface of the petal bears a pair of nectaries covered with oblong scales and ending as fringes. Capsules are small, ovoid-acute, unilocular, two-valved containing numerous minute seeds. The seeds are

many-sided and angular. *S. chirayita* has three years life cycle; in the first and second year, the plants remain in rosette form; however in the third year, plant grows abruptly, bears flowers, fruits and the plant totally senescence; in rare cases, *S. chirayita* completes its life cycle in three years (*personnal observation*). The whole process of flowering organ development to seed shedding completes within 6-7 months from late June to mid December. The mode of pollination is the cross pollination (Koshoo and Tandon, 1963) as bumble bee, honey bee and other insect, particularly bee family, were observed collecting nectars from the flowers of *S. chirayita*; however, Chakroborty *et al.*, (2009) emphasized it to be the self pollinator.

### *1.6.4 Chemical constituents in Swertia chirayita*

*Swertia chirayita* is known to contain amarogentin, amaroswerin (Korte, 1955; Medda *et al.*, 1999; Keil *et al.*, 2000); xanthones derivatives like1,5,8-trihydroxy-3-methoxyxanthone, 1-hydroxy-3,5,8-trimethoxyxanthone, 1-hydroxy-3,7,8-trimethoxy xanthone, 1,8-dihydroxy-3,5-dimethoxy-xanthone, 1,8-dihydroxy-3,7- dimethoxy xanthone, 1,3,6,7-tetrahydroxy xanthone C-2-β-D-glucoside (mengiferin), 1,3,8-trihydroxy-5- methoxy xanthone, 1,3,5,8-tetrahydroxy xanthone and 1,3,7,8-tetrahydroxy xanthone (Ghosal *et al.*, 1973); chiratol (Asthana *et al.*, 1991), swerchirin (Bajpei *et al.*, 1991; Saxena and Mukherjee, 1992; Saxena *et al.*, 1993), enicoflavine (Dalal and Shah, 1956), gentiamine, swertiamarin (Bajpei *et al.*, 1971); syringareinol, sweroside, (Chakravarty *et al.*, 1994) sweroside-2′-O-3″, 5″-trihydroxy biphenyl-2″carboxylix acid ester (Chaudhury and Daniewshi, 1996), chiratol, gentianine, gentiocrucine (Purushothaman *et al.*, 1973a,b), swertanone, swertenol, gammacer-16-en-β-ol, 21-α-H-hop-22(29)-en-3-β-ol, taraxerol, ursolic acid (Chakravarty *et al.*, 1991), swerta-7,9(11)-dien-3β-ol, lupeol, kairatenol, pichierenol, monohydroxy terephthalic acid, 2,5-dihydroxy terephthalic acid (Chakravarty *et al.*, 1992a), Kairatenol (Chakravarty *et al.*, 192b), chiratenol (Chakravarty *et al.*, 1990), oleanolic acid (Sharma, 1983; Yeung and Che, 2009), β-amyrin, erythrodiol, Ø-taraxasterol (Sharma, 1982, 1983), episwertinol, chiratanin (Mandal and Chatterjee, 1987), mangostin (Mandal, 1992); pentacosanoic acid (Srivastava *et al.*, 2010). The herb is also known to contain ophelic acid (an amorphous bitter principle), chiratin (yellow bitter glucoside) discovered by Hohn in 1869. It also possesses resin, gum,

carbonates and phosphate of potassium, calcium and magnesium but does not contain tannin. (www.henriettesherbal.com/eclectic/kings/swertia-chir).

### 1.6.5 Use of Swertia chirayita in Ayurveda

The species bears high pharmacological importance; whole plant is used in traditional medicine; however the root is mentioned to be the most effective part. According to Lloyd (1911), *"this drug has long been held in esteem by the Hindoos being frequently mentioned by Susruta. It first attracted attention in England in 1829 and was introduced into the Edinburgh Pharmacopoeia in 1839. The plant was first described by Roxburgh in 1814. However, there is no record of its introduction into Indian medicine or to its original usage is possible, other than that it is a gift of native medication and is described in Sanskrit writings"*. Its medicinal efficacy is officially registered and recognized in Indian pharmaceutical codex, the British and the American pharmacopoeias and in different traditional systems of medicines such as the Ayurveda, Unani and Siddha http://crdd.imtech:res.in/indipedia/index.php/ Chirayita). In Ayurveda, *S. chirayita* is described as bitter in taste (*rasa: tikta*), it is light and dry (*guna: laghu, ruksha*) because of which, it is easily digestible; it has cooling potency (*virya: shita*) and produces pungent post digestive effect [*vipaka* (post digestive effect): *katu* (pungent)]. It act as *raktasodhaka* (blood purifier) and *vranasodhana* (wound cleaner). It alleviates kapha and pitta doshas *(kaphapittahara)*, stimulates bile from the liver *(saraka)*, relieves from sensation of excessive thirst *(trishnasodham)*, alleviates burning sensation from the body *(dahanut)*, it is useful in respiratory discomfort and cough *(shwas kasanut)*, alleviates skin diseases *(kushtha pranut)*, can be employed effectively in edema and related conditions *(shotha pranuta)*, and is useful in hemorrhoids and other problems of the anal region *(arsha hara)* [http://www.ayurvedicdietsolutions.com/Chirayata.php; http://logayurveda.com/plant-profiles/171-swertia-chirata.html]. The use of *S. chirayita* have been mentioned in *Caraka Samhitha Sutra* (an early ayurvedic text on internal medicine), the origin of which dates back to Maurya Period (3rd to 2nd Century BCE). In *Charaka Samhita*, Kiratatikta is referred to as *jvaraghna* (antipyretic) and *stanyasodhana* (lactodepurant). The name of the plant itself indicates the origin of plants from Kirata desa (country of the Kirantis). Over centuries it has

been used in Ayurveda as a bitter tonic, febrifuge and anthelmintic. (www.herbalcureindia.com/herbs/swertia-chirata). In ancient Indian literature, *Mādhavachikitsā* text, chapter *Jvarachikitsā*, one of the most important post *Caraka Sushruta Samhita* mentions effectiveness of *S. chirayita* in *Jvara* or fever (Mishra, 2009). Plant is used separately or in combination with other herbs in Ayurvedic, Siddha, Unani, Tibetan and Chinese traditional medicine for treating different health problems.

### 1.6.6 Medicinal importance of Swertia chirayita

The whole plant of *S. chirayita* has long been used in India, and later has been introduced into Europe. Plant is used to treat fever, malaria, gastritis, scanty urine, hypertension, melancholia, hepatitis, cold, cough, intestinal worms, diarrhoea, stomachache, bronchial asthma, dyspepsia, debility, liver diseases, and is effective in curing gastric ulcers (Biswas and Chopra, 1982; Kirtikar and Basu, 1984; Rafatullah *et al.*, 1993; Rastogi and Srivastava, 1996), mental disorder (Chopra *et al.*, 1958) and arthritis (Kumar, P. *et al.*, 2004). *S. chirayita* is used in India to offer alcoholic a temporary distaste from alcohol (Lockhart, 1989). It improves and tones up the digestive system as a whole. The decoction or powder is beneficial in increasing the appetite and relieving the constipation. The decoction of *S. chirayita* (chirata) and *Eclipta alba* (bhrngaraja) with honey controls hyperacidity. When taken along with camphor and shilajit, plant is effective in rheumatic diseases, syphilis and chronic fever. On taking the powder or decoction of *S. chirayita* with honey, it effectively controls vomiting sensation in pregnant women. However, if taken in large quantity, plant nauseates and upset the stomach. In India, this plant has been employed in intermittents and remittents, combined with the seeds of *Guilandina Bonducella*. It can be used in number of forms as powder, infusion, tincture, or fluid-extract (www.herbalcure-india.com/herbs/swertia-chirata; www.henriettesherbal.com/eclectic/kings/swertia-chir).

The whole plant is soaked in water overnight and the bitter juice is taken tonic the next morning during malarial fever; indigestion, worms in stomach especially in children (Pradhan and Badola, 2008). The decoction of the root is taken to cure liver

diseases; paste of the plant is also used in common ailments like cough, cold, asthma, headache and fever; the roots crushed and paste rubbed over joints for quick relief from pain; leaves warmed and paste prepared with mustard oil applied over boils and scabies. The whole plant is used to treat fever in swine and ruminants and to in stimulating the appetite in swine (www.divineremedies.com/swertia_chirata). Concoction of chirata *(S. chirayita)* with cardamom *(Ammomum subulatum)*, turmeric *(Curcuma longa)* and kutki *(Picrorhiza kurroaa)* is given for gastrointestinal infections, and along with ginger it is considered good for fever (Kirtikar and Basu, 1984). When given along with neem *(Azadirachta indica)*, manjishta *(Rubia manjith)* and gotu kola *(Centella asiatica)*, it cures different skin problems. It is used in combination with other drugs in cases of scorpion bite (Nadkarni, 1976). The root of *S. chirayita* impart yellow colour to cotton and is used to dye linen (Jain and Tarafdar, 1970). The anti-feedancy properties of *S. chirayita* against jute semilooper *Anomis sabulifera* (Malic *et al.*, 1985) and hairy caterpillar *Spilosoma oblique* (Sharma *et al.*, 1992) have been reported. The beverage industry has discovered an alternative bitter product from *S. chirayita* (i.e. used in the liquor industries to impart bitter flavour to mouth).

### *1.6.7 Pharmacological properties of Swertia chirayita*

*S. chirayita* has blood sugar lowering (Mukherjee and Mukherjee, 1978; Chandrasekhar *et al.*, 1987; Saxena *et al.*, 1991, 1993, 1996; Saxena and Mukherjee, 1992) or hypoglycemic (Chandrasekhar *et al.*, 1990; Asthana *et al.*, 1991; Bajpai *et al.*, 1991; Banerjee *et al.*, 1994; Kar *et al.*, 2003) or anti-diabetic (Arya *et al.*, 2010); anti-inflammatory (Mandal *et al.*, 1992; Chowdhary *et al.*, 1985, 1995; Banerjee *et al.*, 2000; Kumar *et al.*, 2002, 2003; Kumar, P. *et al.*, 2004); anti-hepatotoxic (Karan *et al.*, 1999a,b) or hepato-protective (Chakravarty *et al.*, 1994; Mukherjee *et al.*, 1997); anti-tuburculous (Komatsu *et al.*, 1971); anti-pyretic (Bhargava *et al.*, 2009); anti-tumor (Balasundari *et al.*, 2005); anti-fungal, anti-bacterial (Bhargava and Garg, 2007); anti-malarial (Bhat and Surolia, 2001; Brahmachari *et al.*, 2004); anti-oxidant (Balasundari *et al.*, 2005; Tripathi *et al.*, 2005; Kumar *et al.*, 2011); anti-carcinogenic (Lin *et al.*, 1996; Das *et al.*, 2004; Saha *et al.*, 2004, Saha and Das, 2010; Nawab *et al.*, 2011); anti-platelet, anti-viral (Verma *et al.*, 2008), anti-helmintic (Iqbal *et al.*,

2006; Kekuda *et al.*, 2009); anti-cholinergic (Rafatullah *et al.*, 1993); anti-microbial (Alam *et al.*, 2009; Kekuda *et al.*, 2009), central nervous system depressant (Leslie and Chungath, 1987; Srivastava *et al.*, 2010), analgesic (Alam *et al.*, 2010), chemopreventive (Saha *et al.*, 2004), anti-arthritic (Kumar *et al.*, 2002, 2003; Kumar P. *et al.*, 2004) properties. It can also reduce hyper-proliferation (Kar *et al.*, 2003; Reen *et al.*, 2001; Tripathi *et al.*, 2005; Saha *et al.*, 2006). Medda *et al.*, (1999) have reported the anti-leishmanial activity of the plant extract against Leishmania donovani in golden hamsters. The herb can also be used as natural larvicide (Balaraju *et al.*, 2009) and possesses wound healing property (Manjunath *et al.*, 2006). Ray *et al.*, (1996) reported that the herb inhibits the catalytic activity of topoisomerase I of Leishmania donovani. The efficacy of an oral formulation consisting of *S. chirayita, Fumaria indica, Tephrosia purpurea, Sphaeranthus indicus* and *Zizyphus jujuba* in treating scabies has been reported by Ali *et a*l., (2006).

### 1.6.8 Herbal and Ayurvedic preparations from Swertia chirayita

The plant is practiced in the preparation of herbal drug: Diabegon, D-400, Chirayita tablet, GlucoBuster (anti-diabetic tablet), DBCare (which lowers high blood sugar), Himoliv, and Melicon V (an herbal antiseptic and antifungal veterinary ointment). It is one of the ingredients in Kabdeen (a poly herbal non pharmacopoeial Unani formulation for treating viral hepatitis), Sudarshan churna (remedy for pyrexia), Chandra Prabati (the ayurvedic drug for curing cancer), Mahasudarsana churna, Palas abijadi chhurna, Kiratatiktadi kvatha, Bhunimbadi kvtha, Tikatapancaka kvatha, Kiratadi taila, etc. Also, plant is one of the ingredients of Dermafex oil, Suvarna hair vitaliser and Sisa hair vitaliser for men (www. banlab.com/Healingherbs/chirayta), etc.

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### 1.6.9 Economic importance of Swertia chirayita

*S. chirayita* is extracted both for domestic use as well as for the commercial benefits from different parts of the Himalaya (Dutta, 2004; Olsen, 2005; Anonymous, 2008; Dhital, 2009). During 1992-93, 18 tonnes of *S. chirayita* was collected by the farmers in Bhutan which increased to 20 tonnes in 1993-94 worth Nu.1,000,000 or US$35,714

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(Dhital, 2009). During 1997-98, amongst the top five harvested medicinal plants in Nepal, *S. chirayita* contributed 14.5% of the total trade, which was estimated to be 9870 quintal as whole plant dry biomass (Olsen, 2005). The current market rate of raw dried *S. chirayita* is Rs. 300/kg (Nepal currency) in Nepal (Phoboo and Jha, 2010). The local collector used to get Rs. 800/kg (Nepal currency) in the past in Nepal (Phoboo *et al.*, 2008); however, owing to adulteration, the rate has come down (Phoboo and Jha, 2010). Sikkim (India) had a figure of 3440 kg of *S. chirayita* trade from the wild during the year 1990-92 (Rai *et al.*, 2000), which provides a gloomy state of population loss of the species. Several instances of wild harvesting of *S. chirayita* in the state are in the record indicating the availability of illegal markets motivating harvesters to go for regular extraction of the species. For example, in 2002, the forest official seized over 120 kg of *S. chirayita* being smuggled from south Sikkim (India). In India, in spite of fluctuating price of the dry material, i.e. Indian Rs. 286/kg in 2001, Rs. 148/kg in 2002, Rs. 118/kg in 2003 and Rs. 105/kg in 2004 (Nagpal and Karki, 2004), the annual demand of *S. chirayita* increased by 10 percent, i.e. from 965.2 tonnes/year in 2001-02 to 1284.7 tonnes/year in 2004-05 (Chauhan, 2006). The total revenue generated from *S. chirayita* trade between 1997-98 to 2001-02 was Rs. 2,96,025 in Arunachal Pradesh (Dutta, 2004) for which the West Kameng district forms the major centre of harvesting of *S. chirayita* marketed from the state. In order to meet out ever growing raw material demand of *S. chirayita* by the pharmaceuticals, the material is largely imported from adjacent countries like Nepal and Bhutan. A task force report on conservation and sustainable use of medicinal plants indicates that, in 1997-98, over 27.2 tonnes of *S. chirayita* (valued at over Rs 2.3 million) was imported from Nepal. Gurung (2007) reports average annual import of 25 metric tons of *S. chirayita* from Nepal to India. Further, India had a demand of 1,285.0 metric tons of *S. chirayita* in the year 2004-05 (Gurung, 2007). Another report reveals that over 60,000 kg to 1,00,000 kg of *S. chirayita* was being imported annually into the country (Chauhan, 1999). The disparity in the market prices has also been observed within the country. In general, the prices may fluctuate between Rs 250/kg and Rs. 270/kg in different markets. A survey conducted by CERPA has shown an increase in the market price from 1997 to 2000 for *S. chirayita*. For example, in 1997-98, it was Rs 274/kg; in 1998-99, the price was Rs 282/kg, in 1999-

2000, the price was Rs 300/kg, but a survey by FRLHT (Bangalore) in 2005-06 revealed its price to be 200-225/kg in the country. Fluctuation in the market price of *S. chirayita* as, Rs. 60-80/kg (Solan), Rs. 160-180/kg (Saharanpur), Rs. 200-230/kg (Dehradun); Rs. 230/kg (Amritsar), Rs. 165/kg (Delhi) is also reported by Butola and Badola (2008a)

This clearly reveals that *S. chirayita* is a very important high value-high traded species. It can play significant role in upliftment of the status of the poor rural villagers. At the same time, it is not always possible to fulfill the growing pharmaceutical demands for the raw material supply of *S. chirayita* by collecting merely from the wild, but the only option is the *ex-situ* cultivation, which will not only check the *in-situ* extraction of the species but also help in conservation of the species (Badola and Pal, 2002) and its genetic diversity and minimizes the risk of adulteration. The local entrepreneurs can generate substantial income and may take it as a lucrative option for empowerment through *ex-situ* cultivation of *S. chirayita.* Reintroduction of the species produced through *ex-situ* means to its natural habitat will strengthen the efforts on recovery of the species in the areas which have faced extinction risk. The cultivation will help maintain its balance in nature as well as provide sustainable income to the villagers who uphold their life through its collection. Although, at present the livelihood dependency of people in Sikkim on medicinal plants does not seem to be substantial; nonetheless, there is clearly a scope for enhancing this dependence and the resource's conservation by large-scale cultivation and even domestication of the more valuable species like *S. chirayita.*

## *1.7 Rational of the study*

It is clear that *Swertia chirayita* is a very important high value and traded (domestic and global) and threatened species in Himalaya and under constant *in-situ* threats. It is used in multiple drug formulations in Ayurveda, Unani, Chinese, Tibetan traditional medicine as well as in pharmaceutical industries. The species plays significant role in upliftment and sustenance of life of the rural folks through *in-situ* harvesting and marketing in many parts of Himalaya. The disappearance of species from the nature may have huge impact on the traditional medicines, pharmaceutical industries and life

of the poor people who sustain lives through the collection of *Swertia chirayita*, as well as the wholesalers who makes huge profit from the species. Above all, the species make an important element of Himalayan biodiversity. However, still, the species is neglected; no proper evaluation on the exact status of its populations has been done. Also, no effort or meager effort have been undertaken to conserve the species both *in-situ* and *ex-situ*. The assessment of wild populations for their habitat niches and viability and identifying potential genetic bank and subsequently the development of conventional propagation protocol and field trials for both *ex-situ* cultivation as well as an aid to species recovering programme in its natural micro-habitats would be vital for the safe future of *Swertia chirayita* in Himalaya.

## 1.8 Objectives of the study

The present research is undertaken with the aim of evaluating the threat status and identifying suitable conservation measures for *Swertia chirayita*. The present research has been undertaken with the following objectives:

1. Systematic assessment of *Swertia chirayita* for its status of prevalence and quantum of availability in nature, and identifying its habitat preferences.
2. Identifying and assessing morphological traits of plants in understanding their adaptive mechanism.
3. Assessing natural populations for their propagation potential under laboratory and nursery conditions.
4. Assessing effects of storage on seed germination potential.
5. Determining appropriate pre-sowing chemical treatments to improve seed germination of the selected populations
6. Determining appropriate substrate combinations for improved germination and vigorous seedling growth
7. Assessing periodical phenology of selected populations under nursery conditions and in natural habitats, to understand devices for their adaptation and potentiality for species conservation.
8. Developing sustainable harvesting techniques and conservation plan of *Swertia chirayita*.

CHAPTER 2

# The Study Area

## 2.1 Physiography

Sikkim is the 22nd state and 2nd smallest state of the Indian Union. The state has the total area of 7096 sq. km and is situated between $27^0 46'$ to $28^0 7'48''$ N Latitude and $88^0 0'5''$ to $88^0 55'25''$E longitude in the eastern Himalaya (Figure 1). As an important trans-boundary landscape, it shares border with countries like Bhutan (*ca.* 30 km long) represented by Pangolakha range in the east; Tibetan Autonomous Region of China (*ca.* 220 km long) represented by Chola range in the east and Trans-Himalaya region in the north; and Nepal in the west (*ca.* 100km long) represented by Singhalila range; however, towards the south, Sikkim shares border of *ca.* 80 km with Darjeeling district (West Bengal) of the Indian Union which is segregated by three river system viz., Teesta chu, Rangit chu and Rongpo chu. It stretches approximately 64 km from east to west and 114 km from north to south. The altitude in Sikkim ranges between 220m asl to 8598m asl.

Sikkim is bounded by some of the world's most important mountain ranges which are interspersed with numerous passes (La) connecting it to the neighboring countries. These passes are Jelep La (ca. 4300m asl), Nathu La (ca. 4300m asl), Cho La, Bhutan La (ca. 4000m asl) in the east; Dongkia La (ca. 5500m asl), Kongra La (ca. 5200m asl), Chorten Nyima La (ca. 5800m asl) in the north; and Chuwa Bhangyang (ca. 3200m asl) in the west. Sikkim is a state of mountainous terrain with cliff, deep gorges and the valleys. World's third highest mountain, Mt. Khangchendzonga (8,598m) is situated in Sikkim. Other important high mountains with above 6000m altitude in Sikkim are Mt. Siniolchu, Mt. Pandim, Mt. Kabru, Mt. Tinchenkhang, Mt. Rathong, Mt. Kokthang, Mt. Kanglakhang, Mt Paunhari, Mt. Pandim, Mt. Talung, Mt. Simvo and Mt. Jonseng. The peaks with little lesser altitude than 6000m are Mt. Masunyange, Mt. Yabukjakchen, Mt. Narsing and Mt. Lamaongden.

There are 84 glaciers in Sikkim; the most important of which are Zemu glacier (largest glacier in Sikkim; ca. 26 km long), Rathong glacier, Zum-thul Phuk glacier, Onglathang glacier, Tasha Khang glacier, Lhonak glacier, Hidden glacier, Talung glacier, Tista Khangse glacier, North Lhonak glacier, South Lhonak glacier, etc. The water from these glaciers is discharged into two major river systems in Sikkim, Tista and Rangit which is considered as the life line of Sikkim. Tista originates from Tista Khangse glacier in the north-eastern part of north Sikkim and Rangit has its origin from Rathong glacier in the western part of Sikkim; however, both the river systems takes its course towards southern direction.. The major tributaries of river Tista include Lachung chu, Zemu chu, Bakcha chu, Rangpo chu, Dikchu chu, Rangyong chu; at the same time, Tista receives water from many other tributaries also. Similarly, river Rangit receives water mainly from Rathong chu, Kalej khola, Relli chu and Rangbhang khola. There are about 104 small and big rivers and streams in Sikkim.

Sikkim has more than 227 lakes and wetlands and numerous hot springs located along different altitudes. Majority of these lakes and hot water springs are confined to high altitudes areas. Some of the high altitude lakes include, Tso Lhamu, Gurudongmar, Dudh Pokhari, Tsomgo, Samiti, Lam pokhari, Menmecho, etc. Similalry, the important hotsprings in Sikkim are Phur-tsa-chu, Yumay samdomg or Momey samdong, Yumthang, Ralong-tsa-chu, Borong, etc.

## 2.2 Geology

Major portion of the Sikkim Himalaya is in the Lesser and Greater Himalayan zone; however, Sikkim is located in eastern part of the Indian Himalayan Region, and is covered by Precambrian rock assumed to be much younger in age. Sikkim is considered as a young mountain system, still in the process of formation, with highly folded and faulted rock strata at several places. The mountain system in Sikkim generally runs in east-west direction, while the chief ridges run in more or less north-south direction. The northern portion of the state is deeply cut into escarpment, while the southern portion is comparatively open which is basically due to the direction of the main drainage (southern). The northern, eastern and western portion of the state constitutes of hard massive gneissose rocks which are capable of resisting denudation;

however, the central and southern portion constitutes of soft, thin, slate and half-schistose rocks which are highly susceptible to weathering. Owing to the presence of gneissic group of rock in Sikkim, the soil represents brown clay type and is generally shallow and poor. Soil is coarse-loamy to fine-loamy in texture, contains large amount of iron oxide, neutral to acidic in nature and very poor in mineral content, while it is rich in organic content. In fact, voer 50 percent of the land is barren and inhospitable in Sikkim. In many places, the soil is good for the crops; it is sandy alluvial in the lower regions and black, white or red in the higher hills.

## 2.3 Climate

Sikkim has a peculiar climatic condition which varies from tropical heat in the lower valleys to the freezing cold in the higher altitudes. State is basically divided into: sub-tropical humid type (upto 1500 m asl), semi-temperate type (1500 m asl to 2000 m asl), temperate type (2000 m asl to 3000 m asl), alpine snow forest type (3000 m asl to 4000 m asl), alpine meadow type (4000m asl to 6000m asl), and arctic type (above 6000 m asl) [Chaudhury, 1998]. The zone above 6000 m asl remains permanently snow covered throughout the year due to its closeness to the tropic of Cancer; however snow comes down upto 2500 m asl during winter.

Sikkim Himalaya is presumed to be the most humid place in the entire Indian Himalaya because it is situated very close to Bay of Bengal and is directly exposed to moisture laden southwest monsoon. Sikkim is one of the rainiest regions in India as it comes direct on the path of the monsoon clouds; nonetheless, the rainfall varies with varying altitude. Sikkim receives rain from mid-February to early-November, but, the intensity is higher during late-May to early-September. The monsoon normally occurs from June to August, of which July is the wettest month of the year receiving total rainfall upto *ca.* 5000 mm (Pangthang, east Sikkim), which is the highest in the eastern Himalaya. The downpour is very less in the northern part of Sikkim because the monsoon cloud dries out by the time it reaches the northern barrier.

The temperature is not uniform in the state due to variability in the altitude. At lower altitudes (220 m asl to 1500 m asl), the temperature varies between $6^0$C and $35^0$C;

moderate altitude (1500 m asl to 2000 m asl) experiences temperature between $2^0$C and $26^0$C; and the altitudes between 2000m asl and 3000m asl experiences temperature between $0^0$C and $15^0$C. At altitudes between 3000m asl and 4000 m asl, the temperature drops below freezing point during winters and at altitudes above 4000 m asl, the temperature drops down below freezing point almost every night and the temperature goes below $-40^0$C during winter. The relative humidity remains above 70% throughout the year in most part of the state.

## 2.4 Flora

Sikkim is a part of the Indo-Burmese biodiversity hotspot and has unique geographical features having wide range in altitude, high precipitation, etc., which makes it a home to a rich diversity of flora and fauna from tropical region to arctic region which differs from valley to valley. The state has 46 percent of its total geographical area under the forest cover harboring approximately 4500 species of flowering plants (26 percent of the country). There are a record of over 450 tree species, 36 species of rhododendrons, 500 species of medicinal plants, 523 orchid species, 362 species of fern and its allies, 8 tree fern species, 11 oak species, 16 conifer species, 23 bamboo species, 60 primula species and 150 species of wild edible plants. Sikkim is famous for its orchids and rhododendrons and harbors 72 percent of the rhododendron species of the country. For having various potential biological resources, Sikkim offers greater possibilities for nature based entrepreneurships to people (Badola and Aitken, 2010).

On the basis of variability in altitudes, Sikkim is divided into three different vegetation zones, tropical, temperate and alpine with their distinguishable plant species composition. For example, the tropical regions (220 m asl to 1500 m asl) are covered with the forests of *Shorea, Adnia, Dalbergia, Dillena, Artocarpus, Ficus, Bahunia, Litsea, Terminalia, Schima, Syzgium, Engelgardtia, Castanopsis, Pandanus, Cythia, Michelia; Saurauia* sps, etc. Apart from these, several varieties of orchids, laurels, bananas are available in this region. In the temperate regions (1500 m asl to 4000 m asl), forests comprises of *Alnus nepalensis, Acer, Betula, Magnolia campbellii, Rhododendron, Larix, Berberis, Michelia, Engelhardtia spicata, Quercus,*

*Nyssa sessiliflora, Abies densa, Tsuga dumosa, Larix griffithiana, Taxus baccata,* etc. Besides, numerous species of herbs of medicinal importance such as, *Aconitum, Podophyllum, Picrorhiza, Rheum, Swertia, Nardostachys, Polygonatum,* etc., are found in this zone. The Alpine region (4000 m asl to 6000 m asl) consists of shrubs and scrubs of rhododendrons, junipers, etc. In addition, there exists various species of *Primula* and *Mecanopsis*. The altitude above 6000 m asl remains permanently under snow cover.

Sikkim harbours several endemic floral species, viz. *Acronema pseudotenera, Anaphalis cavei, Anemone demissa, Coelogyne treutleri, Mahania sikkimensis, Podophyllum sikkimense, Rhododendron sikkimense,* etc. The species of the eastern Himalaya endemic to Sikkim includes *Abies densa, Betula utilis, Larix griffithiana, Rhododendron baileyi, Rhododendron camelliiflorum, Rhododendron ciliatum, Rhododendron glaucophyllum, Rhododendron grande, Rhododendron lanatum, Rhododendron lindleyi, Rhododendron wallichii, Rhododendron wightii,* etc. The endemic orchid of Sikkim includes *Bulbophyllum trichocephalum* var.*capitatum*, *Calanthe anjanii*, *Calanthe yuksomnensis*, *Cremastra appediculata* var. *sonamii*, *Cymbidium whiteae*, *Dendrobium eriiflorum* var. *sikkimensis*, *Pantlingia paradoxa*, etc. For its floral richness, several botanical explorations have been conducted in Sikkim by famous botanist and naturalist all over the world including Sir J.D. Hooker, Sir G. King, C.B. Clarke, G.H. Cave, W.W. Smith, and J.M. Cowan who accounted their collection in different books.

## 2.5 Fauna

The beauty of variety of plant diversity in Sikkim is further enhanced multifold by the availability of wide variety of faunal components, which include 150 mammal species, 552 bird species, 690 butterflies and 1500 moth species, 29 reptile species, 10 amphibian species, and over 48 fish species.

Amongst most important and rare animals found in Sikkim, the Snow leopard, Red Panda, Musk deer, Barking deer, Himalayan Thar, Goral, Blue sheep, Serow, Gazella, Tibetan wolf, Himalayan black bear, Clouded leopard, Leopard cat, Jungle cat,

Himalayan marmot, etc. are prominent. Many of these species are now listed in the IUCN red data book. Some of these animals are now included in the list of schedule I species of the Wildlife (Protection) Act, 1972 and are given special protection which includes Blue sheep, Clouded leopard, Himalayan Thar, Musk deer, Red Panda, etc.

With regard to the bird species diversity, Sikkim represents highest bird richness in terms of total geographical area. About 50 percent of the bird species of the Indian sub-continent (1400 species) are present in Sikkim. Nevertheless, bird species like Blood Pheasant, Crimson horned Pheasant, Forest eagle owl, Himalayan golden eagle, Monal Pheasant, Lammergeyer, Tibetan snowcock, etc., have been listed in schedule I of the Wildlife (Protection) Act, 1972; bird species like Black naked Crane, Himalayan Griffon, Tibetan Snowcock, Snow Partridge, Snow Pigeon, Snow Finch, Mountain Finch, etc., have become endangered in Sikkim.

## 2.6 Protected Area Network

With a view to conserve the rich biodiversity, wide areas of Sikkim have been included in the protected area network. There are 8 protected areas in Sikkim viz., Khangchendzonga Biosphere Reserve, Fambong Lho Wildlife sanctuary, Kyongnosla Alpine Sanctuary, Barsey Rhododendron Sanctuary, Maenam Wildlife Sanctuary, Singba Rhododendron Sanctuary, Kitam Bird Sanctuary and Pangolakha Wildlife Sanctuary covering 46.93% of the total geographical area).

## 2.7 Social profile

As per the official Provisional Population Census 2011, the population of Sikkim is approximately 0.61 million (0.05% of the total population of the country) with 0.32 million male population and 0.29 million female population. The gender ratio of females per 1000 males is 889; the state has overall literacy rate of 82.20% (male: 87.30%; female 76.53%). The overall density of the population in the state is 86 per sq. km. East district, especially the capital, Gangtok being cosmopolitan, is the most populated while north district is the least populated. There are mainly three ethnic communities in Sikkim, viz., Nepali, Bhutia and Lepcha; however, many other communities from different parts of India have made their way to Sikkim and are

mostly involved in trade and other related works. Nepali constitutes more than 50 percent of the total population of the state as it includes many sub-communities, followed by Bhutia and Lepcha. There are three official languages spoken in the state: Nepali, Bhutia (Sikkimese) and Lepcha; however, Nepali has become the lingua franca of the state and English as the medium of education. Other languages spoken in Sikkim include Hindi, English, Gurung, Limboo, Magar, Rai, Sherpa, Tibetan, Tamang, etc. Hinduism and Buddhism are the main religion followed by the people of Sikkim; nevertheless, Sikkim also has a small population of Christians and Muslims.

## 2.8 Economic profile

Sikkim is an agrarian state. Agriculture forms the backbone of the economy of the state and animal husbandry forms an integral part of majority of the household in Sikkim (Badola, 2009). The people practice mixed farming. The major cash crops grown in the state are cardamom and zinger; the state is the largest producer of large cardamom and has the largest area under cardamom cultivation in India. Besides, people grow potatoes, mandarin, guava, kiwi fruit, passion fruit, maize, paddy, finger millet, mustard, barley, wheat, and other vegetables; however, the production is still at low level and yet to reach the commercial level. Beside, small area in South Sikkim has tea plantation which is rated very high in the international tea market.

In Sikkim, livestock farming comprise of cattle, sheep, pigs, goats, poultry, yak, etc. depending upon the altitude which plays a significant role in the economy of the rural household. Tourism is the major upcoming industries in Sikkim which has increased the revenue of the state multifold times. The state is one of the most important destinations for nature, cultural and religious tourism. The other main industries in Sikkim are distilleries, breweries, pharmaceuticals, juice factory, etc. Also, there are multiple small scale industries in Sikkim which are important in boosting the economy of the state. Apart from these, upcoming several small and big Hydel power projects will be another main source of revenue to the state. There exists several minerals such as, copper, zinc, lead, coal, quartzite, dolomite graphite, etc., which are mined from Sikkim.

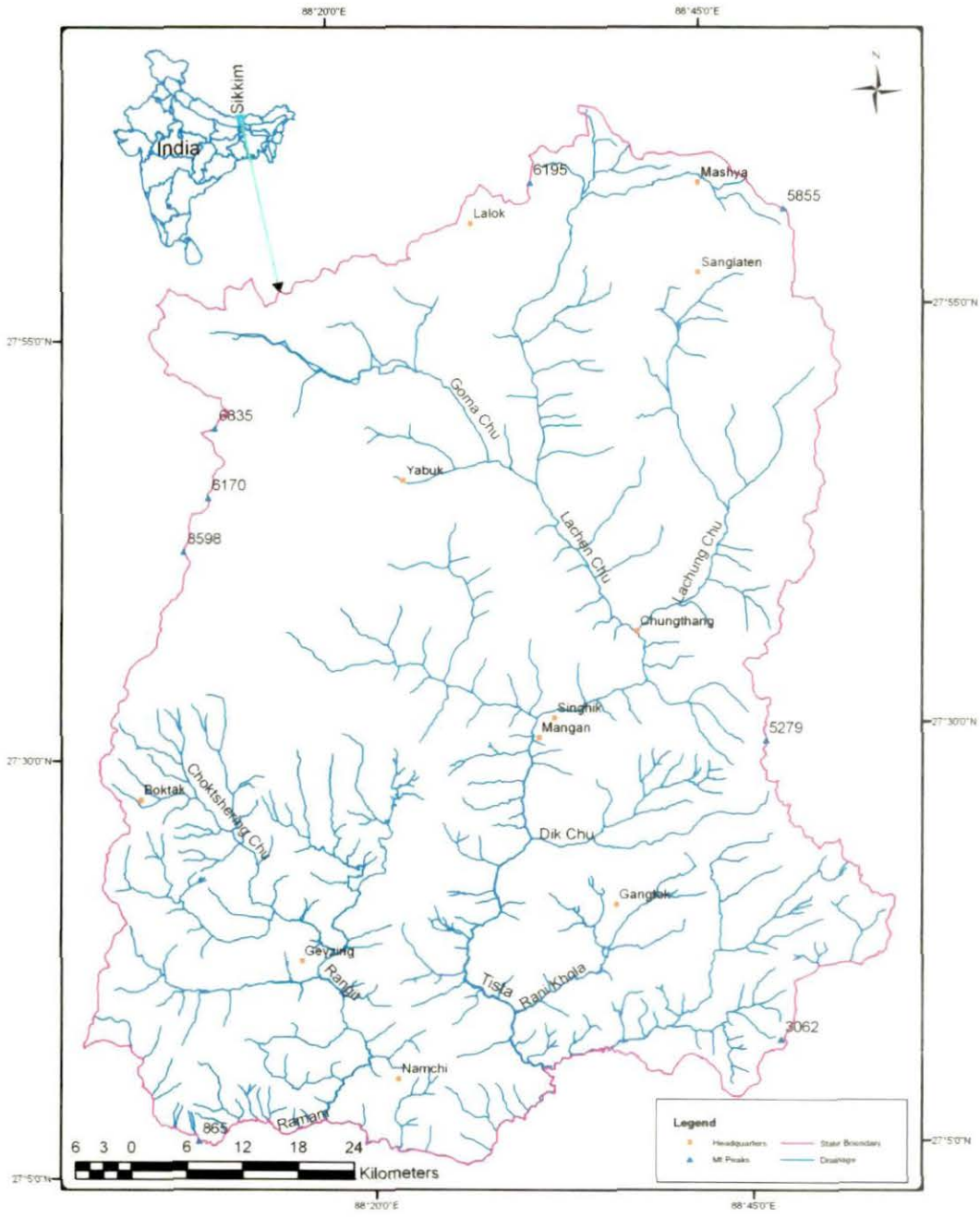



**Figure 1.** Location map of Sikkim

CHAPTER 3

# Review of Literature

## 3.1 Introduction

Human dependence on the traditional medicine to cure various diseases is an age old practice. Even now, majority of the people especially from developing countries (80%) largely depends on the traditional medicine for their primary health care needs (IUCN 1993; WHO 2002; Badola and Aitken, 2003) for several reasons such as low cost, easy availability, high efficacy, non-occurrence of side effects, environmental friendliness, lasting curative properties, etc. Being highly effective, the pharmaceutical industries have diverted their attention towards deriving basic compositions from traditional medicines to prepare modern drugs, the source for which is the medicinal plants. Dhar *et al.*, (2000) reviewed that *ca.* 175 species (most of them are threatened) of medicinal plants from the Indian Himalayan Region (IHR) are used for the preparation of over 316 drug formulations. With increasing human population, several new diseases have emerged leading to the discovery of new medicinal drugs thereby increasing the demand for the raw materials. This resulted in unprecedented harveting pressure on many of the high value medicinal plants in their natural habitats, as 90% of the raw material demands of the pharmaceuticals are met from the wild, leading to over exploitation of the existing resources. Un-scientific and unsustainable over-harvesting in the wild has caused severe damages to the quality and quantity of natural populations of medicinal plants, thereby accelerating the threat of their extinction and/or loss or genetic diversity (Badola and Aitken, 2010). The over extraction have resulted in wiping out of the entire populations of many of the important species from the nature in many parts, due to which, it has become very difficult for the pharmaceutical companies to obtain the required amount of raw materials as the demands for medicinal plant species have increased by 50%, whereas their availability in nature has declined by 26% (Anonymous, 2001). To overcome these problems, the pharmaceutical sector has substantially increased the prices for the raw materials, thus aggravating the extraction pressure, leading to non-recoverable

damage to the biodiversity, as many species have already shown sign of extinction and many are assessed as threatened. This has forced the biodiversity scientists all over the world to come together and raise concern over the declining populations of the rare medicinal plants and finding ways to conserve endangered medicinal plant species (Badola and Pal, 2002). Therefore, the conservation of these high value threatened medicinal plants has become the priority of the environmental policies in many countries (Zhang *et al.*, 2010; Badola, 2009). Thus the need of the hour is to assess the availability and distribution pattern and identification of the suitable habitats of the threatened medicinal plants in the wild, and equally important to develop suitable propagation and mass multiplication protocols, strengthening both *in-situ* and *ex-situ* conservation of prioritized Himalaya taxa, such as *Swertia chirayita* (Badola and Pal, 2002).

This review of literature tries to look into the findings of the studies made on the medicinal plants from Indian Himalayan region and other parts of the world on the aspects falling within the objectives of the present study.

## 3.2 Population assessment and habitat characteristics of medicinal plants

The population assessment has been identified as an important tool for the prediction of extinction risk and the future population size (Guedje *et al.*, 2003) of the important plant species. In addition to assessing resource availability, the micro-habitat assessment is very important which helps in determining performance of the species under different growing conditions and provides vital information on the specialized ecological requirements of the species (Badola and Pradhan, 2010a,b; Uniyal *et al.*, 2002b), which is crucial for developing conservation management strategies and suitable agro-techniques for cultivation of the important plant species (Shrestha and Jha, 2008). Assessment of the site specific populations with those covering micro-habitats will strengthen conservation approaches for threatened plant species, especially at *in-situ* level, which further may provides clues for taking not only elite populations but the type of potential micro-habitats within the targeted sites for their management. Successful conservation and management planning of the threatened

plant species also depends on the insight of the effect of the natural or man-made change on habitat (Hegland *et al.*, 2001), their ecological requirements, status of the existing populations (Kalliovirta *et al.*, 2006) and identification of the superior germplasm, etc. Moreover, the identification of potentially viable populations provides an opportunity to conserve the important medicinal plants through various propagation methods (Bisht *et al.*, 2006; Butola and Badola, 2006a).

In recent years, several studies covering population structure and habitat assessment of threatened medicinal plants have appeared from different localized parts focusing either on single taxa or a group of taxa from IHR, Western Ghat (India) and other regions. Bhadula *et al.*, (1996) through their study in 6 populations revealed restricted distribution and small population size of *Podophyllum hexandrum* in Garhwal Himalaya. Furthermore, their long term observation revealed decrease in population size as well as disappearance of the species from some of the populations. Airi *et al.*, (1997, 2000) assessed the status of *P. hexandrum* and *Nardostachys jatamansi* in western Himalaya applying vertical belt transect method using stratified random quadrats. They recorded very low availability as well as patchy or scattered distribution of *P. hexandrum* in all the populations and high availability of *N. Jatamansi* on the west facing on dripping moss-laden rocks and moist boulders. They concluded *N. jatamansi* to be restricted to some specialized habitat and are subject to destructive harvesting, in addition. Nautiyal *et al.*, (2003a) evaluated the threat status of *Nardostachys jatamansi* in the Garhwal Himalaya and found that the species had restricted distribution in small pockets and are vulnerable. They also pointed out that the species performing well on moist, rocky or boulder habitat with rich organic content.

The population structure of *Angelica galuca* in western Himalaya and part of Trans-Himalaya applying different quadrat approach (Bisht *et al.*, 2006), disclosed the low density of the species in all the 8 populations studied, with random distribution in 63% of the total sites. Vashistha *et al.*, (2006) reported very low availability of *A. glauca* and *A. archangelica* in Garhwal Himalaya and categorized them as critically endangered to endangered and stressed on immediate conservation initiative. Nautiyal

*et al.*, (2004), Bhatt *et al.*, (2005a) and Giri *et al.*, (2008) divulged increasing habitat disturbance with time leading to decline in population of *Dactylorhiza hatagirea* in Garhwal Himalaya and western Himalaya regions. For Himachal Pradesh, a study assessed and reviewed the conservation status of 17 high value threatened medicinal plant species by taking habitat, quantitative availability in nature and known conservation status (Butola and Badola, 2008a).

Bhatt *et al.*, (2007) revealed the low availability of *Swertia angustifolia* in Kumaon Himalaya; they recorded random distribution of the species; however had high frequency of occurrence and good performance of the species on the southeast facing dry slope under the canopy. Small population size and patchy or scattered distribution was attributed to the habitat degradation in *Gaultheria fragrantissima* in Darjeeling region (Bantawa and Mondal, 2008). Very low density and frequency percent was assessed for *Malaxis muscifera,* a medicinal orchid in Garhwal Himalaya, attributing to several reasons, viz., over collection, poor regeneration, low seed germination and seedling establishment, habitat loss, grazing, forest fire, competition, etc, and suggesting the species as rare and adapted to specific microhabitat condition, i.e., moss-laden moist slope (Chauhan *et al.*, 2008).

Duchok *et al.*, (2005) studied the impact of disturbance in the form of fruit/seed harvesting, firewood collection, etc., on population structure and regeneration of *Illicium griffithii*, a medicinal tree from the eastern Himalaya. Their study disclosed low regeneration of the species as well as decreasing number of adult individuals. Similarly, Laloo *et al.*, (2006) studied the status of four important medicinal tree species viz., *Camellia caduca, Cinnamomum pauciflorum, Erithroxylum kunthianum* and *Picrasama javanica* in eastern Himalaya. They recorded high regeneration of all the four species in disturbed sites; however, its conversion into sapling was highly affected.

The availability and habitat preferences of different medicinal plants in the Kumaon Himalaya and the western Himalaya indicated their species specific habitat requirement (Uniyal *et al.*, 1998, 2002). Kala (2000) studied the distribution pattern,

population structure and conservation status of rare and endangered medicinal plant species in Spiti sub-division of Himachal Pradesh in the Indian trans-Himalaya. He recorded the localized and patchy distribution of 23 rare and endangered medicinal plants distributed over 10 major habitat types. He also observed the large differences in the number of rare and endangered medicinal plant species within six different zones and the species number was high in areas close to the Great Himalayan range. In determining the population status of *Aconitum balfourii, A. heterophyllum* and *A. violaceum* in Garhwal Himalaya, Nautiyal *et al.* (2002) recorded fragmented distribution in *A. balfourii* and *A. heterophyllum* due to specific habitat requirement and uninterrupted harvesting, while good availability in majority of populations in *A. violaceum*. Very low density of the targeted medicinal plant species in Kedarnath Wildlife Sanctuary was assessed and attributed to the species specific habitat requirements, restricted distribution and high pressure, legal and illegal exploitation etc. (Semwal *et al.*, 2007); the study further highlighted the positive responses of the species to the conservation effort.

Remarkable impacts of habitat disturbance as well as unsustainable harvesting technique on the distribution, availability and regeneration of the important medicinal plants in Gyasumdo valley, Nepal were reported (Ghimire *et al.*, 1999). In *Aconitum naviculare* and *Neopicrorhiza scrophulariiflora* habitat assessment study revealed that the populations of either species were fragmented and small with complete absence of *A. naviculare* from the open habitat, confining to alpine scrub due to collection and livestocks trampling; whereas, for *N. scrophulariiflora*, receding of the glacier was the emerging threat to its habitat (Shrestha and Jha, 2008). Assessing the high density of *A. naviculare* in Manang district (Central Nepal), a study reported the disturbance intensity dependent variability in plant availability amongst the populations, as the populations were either fragmented or confined to single patch and more common in open areas than within the patches of shrubs (Shrestha and Jha, 2010). Duraisamy and Paulsamy (2010) observed the variations in population density of six medicinal plant species from Nilgiri hills; very low density was related to the severity in climatic condition and illegal exploitation.

The review of literature discloses that there is hardly any publication on the systematic evaluation of *Swertia chirayita* in its natural habitats, except Bhatt *et al.*, (2006) who assessed availability of *S. chirayita* in 5 populations from central Himalaya. They accounted very low availability of the species in the area which were randomly distributed in 3 populations and showed regular distribution in 2 populations. Semwal *et al.* (2007) found the localized distribution of *S. chirayita* restricted to single habitat in entire study area in Kedarnath Wildlife Sanctuary, central Himalaya. A complete lack of substantial population data poses a question on a perceived estimated assessment in categorization of *S. chirayita* under endangered or vulnerable category. Nevertheless, maximum studies on the medicinal plants are conducted either in the central or the western Himalaya region of the IHR; however, meager efforts have been made on the assessment on the availability and habitat characteristics of the important medicinal plant from the eastern Himalaya region of the IHR, including *S. chirayita.* In this research, an attempt has been made to assess the availability of *S. chirayita* covering 22 populations covering all the four districts in Sikkim, India. Further, its suitable microhabitat have been identified which will be useful in species recovery programme for the conservation of *S. chirayita.*

## 3.3 Morphological variations amongst populations

Habitat degradation results in fragmentary and isolated distribution of the plant species which may develop different morphological and physiological characters in a plant (Ouinsavi and Sokpan, 2010). In such fragmented and isolated natural plant communities, a reduced gene flow level consequently leads to the loss of genetic diversity thereby increase the extinction risk of important plant species (Ouinsavi and Sokpan, 2010). In addition to altitude and seasonal variation (Stech and Tesitel, 2005), adaptation to varying climatic and habitat conditions may also be reflected upon the variability in morphological characters viz., plant height, numbers of branches, scent and colour of flower, number, shape and size, pigmentation of leaves, etc., the study of such variation has become significant in conservation biology. Assessing variability in plant morphology is the traditional way of determining diversity within and amongst plant populations (Bayorbor *et al.*, 2010). Different populations of a plant species may exhibit noticeable inter and intra variations

(Bhadula *et al*, 1996) and identifying such morphological variation, specific to each targeted taxa, may have high importance in identifying superior germplasm in conservation programme (Badola, 2002) and can easily be identified in the field.

Considerable amount of work is available on the assessment of morphological variatiosn amongst plant population (Davis and Gilmartin, 1985; Van, 1992; Cordell *et al.*, 1998; Ralph *et al.*, 1998; Allen *et al.*, 1996; Casas *et al.*, 1999; Krishnan *et al.*, 2000; Lynn and Waldren, 2001; Ojeda *et al.*, 2001; Sapir *et al.*, 2002; Bruschi, 2003; Ellison *et al.*, 2004; Puijalon and Bornette, 2004; Karam *et al.*, 2006; Del *et al.*, 2007; Tesitel and Stech, 2007; Pavlova, 2009; Serebryanaya and Shipunov 2009; Thiebaut and Nino, 2009; Bayorbor *et al.*, 2010; Ouinsavi and Sokpan, 2010). Yet, few scattered literatures are available with regard to assessment of morphological variation (plant architecture) in medicinal plants.

Inter and intra variation in plant morphology, viz., plant height, leaf characteristics, fruit weight, seed weight, seed colour, etc., in different populations of *P. hexandrum* from Garhwal Himalaya is reported and related to growing conditions (Bhadula *et al.*, 1996). Similar variations in morphological characters such as, number of leaves/plant, leaf shape, etc., in *P. hexandrum* was observed by Sharma *et al.* (2000). Vriations in plant morphology viz., plant height, stem diameter, rhizome length, rhizome diameter, leaf area, etc., in the same species from Kumaon Himalaya has been reported (Airi *et al.* 1997). The inter-population variation in plant morphology has also been reported in *Aconitum atrox* (Kuniyal *et al.*, 2002). To identify the suitable germplasm for domestication, Nautiyal *et al.* (2003a) evaluated the morphological characters of *Nardostachys jatamansi* from 6 populations from Garhwal Himalaya. They found the availability of superior germplasm on moist rocky or bouldery habitats with rich carbon and nitrogen contents.

Morphological variation in *Astilbe rivularis* and *Plantago major* amongst population in Darjeeling Himalaya indicated the decrease in leaf length and width, stem length and diameter, and root length and diameter with increase in altitude in *P. major* and noticed variation in recorded morphological parts in *A. rivularis* (Chettri *et al.*, 2005).

Vashistha *et al.* (2006) observed significant difference in morphological characters in both *Angelica glauca* and *A. archanglica* from Garhwal Himalaya and recorded superior germplasm of both the species on moist habitats with rich humus content. By examining the morphological variations in populations of *Picrorhiza kurrooa* from Garhwal Himalaya, Purohit H. *et al.* (2008) presumed that in addition to microhabitat conditions, soil nutrient plays a significant role in determining variability in plant morphology.

On the basis of morphological assessment of *Gaultheria fragrantissima*, Bantawa and Mondal (2008) concluded the population of Maneybhanjyang, Darjeeling to be superior germplasm compared to other assessed populations and can be commercially exploited. Significant variations among the genotype in *Andrographis paniculata* were observed, indicating maximum variation in leaf colour Sharma S.N. *et al.*, (2009). Morphological characters of *Acacia caesia* var. *caesia* from different populations from Western Ghat revealed that the arid condition suppresses the vegetative growth but enhances reproductive ability in plant species (Satishkumar *et al.*, 2010). However, for the morphological variability amongst 28 populations in *Prunella vulgaris* in China, geographical isolation was not the only contributing factor (Liao *et al.*, 2010).

The above information reveals that not much work has been done in this regard in case of medicinal plants from north-east Indian states including Sikkim, and for *S. chirayita*, no literature on the assessment of morphological variation amongst natural populations is available, except Raskoti and Shakya (2004) from Nepal. At international level also, such studies on medicinal plants are missing except few from China. The current effort targets to assess the existing variation in morphological characteristics amongst different populations of *S. chirayita* in Sikkim (India).

## 3.4 Propagation of medicinal plants under laboratory condition

### *3.4.1 Effect of microhabitat, light and temperature on seed germination*

Seed germination in the field conditions is strongly influenced by internal as well as external environmental factors (Baskin and Baskin, 2001) including seed traits and microhabitat conditions (Kitajima, 2007) Seed responses greatly varies depending on the type of microhabitat it is exposed too (Navarro and Guitian, 2003), because some microhabitat may offer better condition than others, while some provide appropriate condition at different times (Bisigato and Bertiller, 1999; Guariguata, 2000; Nilsson *et al.*, 2000; Oleskog and Sahlen, 2000; Isselstein *et al.*, 2002; Castro *et al.*, 2005). In addition, the temperature, light and soil humidity greatly impacts the seed germination and seedling establishment (Baskin and Baskin, 1988). The optimal growth response and development in plants depends on the amount of light they receive (Maloof *et al.*, 2000).

In natural habitats, several monitoring of seedling emergence as affected by the microhabitats (Eckrt *et al.*, 1986; Smith and Capelle, 1992; Caccia and Ballare, 1998; Ibanez and Schupp, 2002; Castro, 2006; Gul *et al.*, 2007; Sharma *et al.*, 2009; Yu *et al.*, 2009) and the effect of light and temperature on the seed germination/seedling emergence (Jensen, 1973; Thompson *et al.*, 1977; Ellis, 1980; Probert *et al.*, 1985; Washitani and Saeki, 1986; Tooren and Pon, 1988; Meyer *et al.*, 1989; Dickie *et al.*, 1990; Bai and Romo, 1995; Baskin *et al.*, 1995; Navarro and Guitian, 2003; Pinheiro and Borghetti, 2003; Zaddy *et al.*, 2003; Godoi and Takaki, 2004; Sugahara and Takaki, 2004; Ren *et al.*, 2005; Tilki and Cicek, 2005; Albrecht 2006; Cicek and Tilki, 2006; Serrano-Bernardo *et al.*, 2007; Yilmaz and Aksoy, 2007; Kandari *et al.*, 2008; Simao and Takaki, 2008; Socolowski *et al.*, 2008; Silveira *et al.*, 2010; Vieria *et al.*, 2010) are reported from other regions. In India, especially on Himalayan medicinal plants, insufficient attention has been given on those aspects. Amongst few, Sultan *et al.*, (2006) studied the effect of light on the seed germination of *Podophyllum hexandrum*; Kandari *et al.*, (2008) observed the effect of temperature

and light on the seed germination of *Arnebia benthamii*; and Sharma *et al.*, (2009) studied the effect of microhabitats on seedling emergence of *Cinnamomum tamala* in natural habitat.

Extensive review of literature revealed that not much work on this aspect of microhabitat, light, temperature effect on the seed germination in medicinal plants, especially on *S. chirayita* have been carried out so far.

### 3.4.2 Effect of storage period, storage condition and storage temperature on seed germination

For the sustenance and conservation of threatened medicinal plant resources in nature, it is imperative to have knowledge on seed biology (Vazquez Yanes and Orozco-Segovia, 1993). Studies have suggested that the seed germination varies within and amongst the populations, and understanding on such variability offers useful clues on the genetic make-up of the species and its existence in the natural settings (Baskin and Baskin, 2001). Seeds do not emerge immediately after ripening and seed sowing immediately after harvesting is very rare (Oneykwelu and Fayose, 2007), the process follows drying and storage at least for some period of time. Many plant species do not develop seeds every year, hence, the seed storage serve as important back-up for the threatened and high value plant species and is a necessary initiative to support the long term conservation of genetic resources. Seed deterioration during storage is the natural phenomenon (Nasreen *et al.*, 2000); however, inappropriate storage aggravates the process (Romanas, 1991) whereas, suitable storage minimizes the rate of seed deterioration. Therefore, to attain long term viability in seeds, storage conditions play a significant role (Bonner, 1990; Takos, 1999; McCormack, 2004).

From India, a significant numbers of publications are available on the effect of long term storage, storage condition and storage temperature on the seed germination (Mukherjie, 1966; Sastry *et al.*, 2006, 2007; Rao *et al.*, 2009; Suthur *et al.*, 2009) and other regions (Muenscher, 1936; Jones, 1962; Harrison, 1966; Janssen, 1973; Roberts, 1973; Ellis and Roberts, 1980; Scoweroft, 1988; Barnett and Momonov, 1989; Dickie *et al.*, 1990; Ellis *et al.*, 1991ac; Wang *et al.*, 1993; Zarnstorff *et al.*, 1994; Ramiro *et*

*al.*, 1995; Garcia-Fayose and Verdu, 1998; Sivritepe and Dourado, 1998; Walters and Engels, 1998; Acharya *et al.*, 1999; Gonzalez and Mendoza, 1999; Dahiya *et al.*, 2000; Kitchen and Monsen, 2001; Takos and Merou, 2001; Chen and Chian, 2002; Cupic *et al.*, 2005; Espinar *et al.*, 2005; Liu *et al.*, 2005; Albrecht, 2006; Tilki and Cicek, 2005; Cicek and Tilki, 2006; Maara *et al.*, 2006; Martinkova *et al.*, 2006; Ma and Liang, 2007; Beardmore *et al.*, 2008; Huang *et al.*, 2008; Ekpong, 2009; Getys and Dumroese, 2009; Sakcali and Serin, 2009; Salimi, 2010; Silveira *et al.*, 2010; Liu *et al.*, 2011). Nevertheless, in respect to medicinal plants, very few works have been done to assess the effect of storage period, temperature and condition on the germination potential of the medicinal plants from India as well as other regions.

Significant difference in the germination between different storage periods in seeds of *Strychnos nux-vomica,* and further 92% germination in the seeds, with 10% initial moisture content, after storage for 30 weeks at ambient room temperature was observed (Sivakumar *et al.*, 2006). Chauhan and Nautiyal (2007) stored the seeds of *Nardostachys jatamansi* collected from 6 different populations in two different conditions, refrigerator (0 to $4^0$C) and room temperature (10 to $35^0$C) and tested its viability after every two months for 2 years. They observed significant fall in the moisture content, seed viability and germination percentage with increase in storage period and noticed the loss of seed viability at faster rate in room temperature than the refrigerator. Further, it was revealed that, in either condition, the seeds stored in polythene bag retained their viability for longer period, relatively. They discovered that the seeds stored at room temperature lost viability in majority of the populations after one year; however seeds stored in refrigerator showed over 60% germination by the end of $2^{nd}$ year.

Budvytyte (2001) tested the seed viability of 6 medicinal plants species and 4 vegetable species prior and after storage for 1 year subjecting to ultra-desiccation and freezing. For medicinal plants, he observed fall in germination percent in ultra-desiccated seeds stored in freezer compared to initial test in 4 species, while for two species, increase in germination occurred. Similarly, for vegetable species, seed germination improved in majority of the species, and related such improvement snd

breaking of seed dormancy to ultra-desiccation and freeze storage. Steady decline in seed viability within the period of one year storage from 95% to15% was recorded in *Newbouldia laevis* (Ehiagbonare and Onyebi, 2009).

The review shows that the studies on the long term effect of storage and storage conditions are missing for *S. chirayita*. In addition, studies examining the differences in germination potential amongst populations for single species are very rare (Thompson, 1981).

### 3.4.3 Chemical stimulation of seeds germination

Poor seed germination have been one of the several causes endangering the existence of the plant species including many of the important medicinal plants. Hence, the control and timing of the germination process is crucial for the survival of the plant populations whose principle mode of reproduction is from the seeds (Harper, 1977). The seed germination requirements are species-specific (Harper *et al.*, 1970; Stebbins, 1971; Fenner, 1985; Meyer and Monsen, 1991; Schutz and Milberg, 1997); seed remains inactive until it receives appropriate environment triggering the embryo growth. Seeds often respond to specific blend of light, temperature, and soil moisture that are most favorable for their establishment (Baskin and Baskin, 2001). Some seed germinates instantly once exposed to appropriate environment, while many attain dormancy which may prolonged to several years (Hartman *et al.* 1997; Pons, 2000). Over the years several methods have employed to improve germination and in breaking dormancy in seeds which includes scarification to chemical or hormonal treatments etc., depending on the size and texture of the seeds.

For Indian Himalaya, several publications have appeared reporting stimulatory effect of chemicals and growth regulators on the seed germination of medicinal plants (Bhadwar and Sharma, 1963; Choudhary *et al.*, 1996; Kant and Vashist, 1998; Pandey *et al.*, 2000; Beigh *et al.*, 2002; Joshi and Dhar, 2003; Manjkhola *et al.*, 2003; Butola and Badola, 2004ab; Bhatt *et al.*, 2005b; Bhuwan *et al.*, 2006; Sharma *et al.*, 2006; Sivkumar *et al.*, 2006; Sultan *et al.*, 2006; Chauhan and Nautiyal, 2007; Kandari *et al.*, 2007, 2008; Sreenivasulu *et al.*, 2008; Vashistha *et al.*, 2009; Joshi and Pant,

2010) and other regions (Genova *et al.*, 1997; Prasad BN., 1999; Coelho *et al.*, 2000; Abdel-Hady *et al.*, 2008; Ali *et al.*, 2010; Amri, 2010).

Chaudhary *et al.* (1996) observed no effect of $GA_3$ (5 and 10 mM) on breaking seed dormancy in *Podophyllum hexandrum*. In *Nardostachys jatamansi* from 4 populations in Garhwal Himalaya, Kant and Vashist (1998) used 4 different concentrations of $GA_3$ (100, 500, 1000 and 1500 ppm) to test its efficacy on seed germination and found $GA_3$ (100 ppm) most stimulatory, which varied amongst populations. Pandey *et al.*, (2000) disclosed significant improvement in seed germination in *Aconitum heterophyllum* with $GA_3$ (250 µM) but was inhibitory to *A. balfourii*. They further reported that seed germination significantly improved in *A. balforii* on pretreatment with $KNO_3$ (50 and 150 µM), whereas, the treatment showed marginal effect in *A. heterophyllum*.

The efficacy of $KNO_3$ and $GA_3$ (100, 500 and 1000 ppm) on seed germination in *Heracleum candicans* from 4 populations from Himachal Pradesh was reported; all the concentrations of KNO3 was stimulatory over control, however, highest seed germination obtained for $GA_3$ 100 ppm (Joshi and Dhar, 2003).

In *H. candicans*, Butola and Badola (2004a) also studied the seed germination enhancing properties of $GA_3$ (25, 100 and 250 µM), $KNO_3$ (50 and 100 mM) and $NaHClO_3$ (15, 30 and 45 minutes), and recorded effectivness of all treatment over control except for $GA_3$ (100 µM), with best germination and lowest mean germination time for $GA_3$ (250 µM), $KNO_3$ (100 mM) and $NaHClO_3$ (30 minutes). Another study showed that $GA_3$ (25 µM), $KNO_3$ (150 mM) and $NaHClO_3$ (30 minutes) treatments were highly stimulatory on the seeds of *Angelica glauca* for germination and reducing mean germination time using over control (Butola and Badola, 2004b). In *Arnebia benthamii* seeds collected from alpine and sub-alpine population from Western Himalaya, Manjkhola *et al.*, (2003)assessed the effect of $GA_3$ (100, 200 and 400 µM) and $KNO_3$ (50 and 100 mM) on seed germination and revealed the inhibitory effect of $GA_3$ in alpine population, while stimulatory effect in sub-alpine population.

In *Swertia angustifolia* effectiveness of $GA_3$ (100, 200 and 400 µM) and $KNO_3$ (100, 200 and 400 mM) with variability in seed germination with particular concentration of $GA_3$ as well as $KNO_3$ amongst the population were observed (Bhatt *et al.*, 2005b); the study further disclosed $GA_3$ (100 µM) and $KNO_3$ (200 mM) to be the best treatment.

In *Picrorhiza kurrooa,* 11-fold increase in seed germination using $GA_3$ (250 µM) was reported (Bhuwan *et al.*, 2006). Bisht *et al.*, (2006) observed the variability in stimulatory effect of $KNO_3$ (100, 500 and 1000 ppm) and $GA_3$ (100, 500 and 1000 ppm) on seed germination on *Angelica glauca* amongst populations. However, they obtained highest seed germination with $KNO_3$ (500 ppm) and $GA_3$ (100 ppm) for the majority of the populations. However, the efficacy of $KNO_3$ (0.2%) and $GA_3$ ($10^{-3}$ and $10^{-4}$ M) on seed germination in seven medicinal plants from Lahaul and Spiti (Himachal Pradesh) was tested (Sharma *et al.*, 2006), which observed $GA_3$ ($10^{-3}$ M) to be more effectivefor all the medicinal plants; however, $KNO_3$ (0.2%) was effective only for *Inula racemosa* and *Saussurea costus*, and proved detrimental to the remaining species.

Maximum seed germination (90%) and lowest mean germination time using $GA_3$ (100 ppm) was observed in *Nardostachys jatamansi* (Chauhan and Nautiyal, 2007). In *Angelica glauca* and *Pleorospermum angelicoides*, Kandari *et al.*, (2007) examined the effect of $GA_3$ (100, 200 and 300 ppm) under light and dark condition and different temperature regimes ($15^0$, $20^0$, and $25^0$C). They detected higher concentration of $GA_3$ to be relatively effective at low temperature in dark condition in both the species; the $GA_3$ (100 ppm) incubated at $25^0$C under light condition was highly effective in improving seed germination in both the species. In *Arnebia benthamii* higher concentration of $GA_3$ was found to be highly effective at low temperature, and lower concentration to be effective at high temperature both in dark and light condition; however, increase in mean germination time with increasing concentration of $GA_3$ in both dark and light conditions was observed (Kandari *et al.*, 2008). Improvement in seed germination and reduction in mean germination time with $KNO_3$ and $NaHClO_3$ over control is reported in 4 populations of *Angelica archangelica* from Garhwal Himalaya (Vashistha *et al.*, 2009).

Of the 4 concentrations of $GA_3$ (0.35, 0.50, 1.00 and 1.50 mg/l), Genova *et al.*, (1997) recorded 1.00 mg/l concentration at an exposition of 24h producing the optimal effect on germination process in *Atropa belladonna.* Improvement in seed germination with all concentrations of $KNO_3$ (0.01, 0.02 and 0.03%) in *Descurainia sophia over* control, with highest value with $KNO_3$ (0.02%) was observed (Ali *et al.*, 2010).

Similar scattered reports are available on stimulatory effect of chemicals, growth regulators and chilling effect on seed germination in *Swertia chirayita* (Raina *et al.*, 1994; Prasad, 1999; Bhatt *et al.*, 2007; Mukherjee *et al.*, 2008; Maji *et al.*, 2009). Prasad (1999) revealed that in *S. chirayita*, seeds treated with $GA_3$ (50–400 ppm) can achieve high seed germination but higher concentration can have inhibitory effect. Bhatt *et al.*, (2007) observed significant improvement in seed germination and reduction in mean germination time with $GA_3$ (100 ppm) in *S. chirayita* and found variability amongst the populations.

The review of literature does not show any detailed work focused on seed germination in plants growing in *ex-situ* plots within natural habitats and compared to those obtained from nursery conditions, using stimulatory chemical treatments for seed germination improvement.

## 3.5 Propagation of medicinal plants under nursery condition

### *3.5.1 Influence of chemical treatments on seedling emergence and vigour*

The risk of extinction of threatened species in nature can be reduced by reinforcing the *ex-situ* raised individuals (Bowes, 1999). To raise them, expensive techniques are not necessary, but identifying suitable technique, based on species requirements such as pre-sowing chemical treatments, would minimize the wasting of the resources (Butola and Badola, 2004b, 2007; Fay, 1994; Benson *et al.*, 2000) as well as there would be an option for the production of uniform and vigorous seedlings. There have been insufficent studies covering this aspect in medicinal plants. The productions of

uniform and vigorous seedlings have been achieved in some of the Himalayan medicinal herbs (Kattimani *et al.*, 1999; Nadeem *et al.*, 2000; Butola and Badola, 2004b; 2006a, 2007).

Nadeem *et al.*, (2000) in their study treated the seeds of *Podophyllum hexandrum* with $GA_3$ (10 and 100 µM) and $NaHClO_3$ (30 minutes) before sowing. They recorded the improvement in seed germination with $NaHClO_3$ over control; whereas, $GA_3$ was ineffective. In another set of experiment, they observed seed treatment with $GA_3$ (250 µM) resulting in high seed germination compared to $GA_3$ (25 µM) and control. Butola and Badola (2004b) treated the seeds of *Angelica glauca* with $GA_3$ (25 µM) and $NaHClO_3$ (30 min) prior to sowing in nursery condition (net house, farm yard manure and sandy loam soil, equal proportion), and found both the treatments to be beneficial in enhancing seedling emergence and seedling vigour under nursery condition over control. However, they noticed $NaHClO_3$ (30 minutes) treatment to be more effective than $GA_3$ (25 µM) in improving seedling emergence and seedling vigour in *A. glauca*.

In *Heracleum candicans*, using seeds from two populations, Butola and Badola (2006a) observed the effects of pre-sowing treatment of $GA_3$ (250 µM) and $NaHClO_3$ (30 minutes) and recorded both the treatments to be effective over control in augmenting seedling emergence and vigour in net house; nevertheless, likewise their previous experiment (Butola and Badola, 2004a), they obtained higher seedling emergence and vigour with $NaHClO_3$ (30 minutes) than $GA_3$ (25 µM).

Effect of $NaHClO_3$ (15, 30 and 45 minutes) on seedling emergence, vigour and survival in *Angelica glauca* and *Aconitum heterophyllum* in nursery condition under poly house was assessed, discovering $NaHClO_3$ to be effective in breaking seed dormancy, increasing seedling vigour and maintaining higher seedling survival in both the species in general (Butola and Badola, 2007). However, experiment acquired high seedling emergence, seedling vigour and seedling survival with $NaHClO_3$ (30 minutes) in *A. glauca* and $NaHClO_3$ (15 minutes) in *A. heterophyllum* over control.

## 3.6 Growth and plant phenology

Phenology involves the recording of recurring seasonal events. Such recording in plants provides information about different growth phases and their periodicity, length of growing seasons and of the dormancy (Badola, 1994; Jeffrey *et al.*, 2009). Such studies impart knowledge on the morphological and functional traits offering clues about adaptability mechanisms of the plants under varied and periodically altering climatic conditions in which they grow, and thus help in developing management and conservation plans for valuable plant species (Badola, 1994; Martinez-Calvo *et al.*, 1999; Baumgartner and Hartmann, 2000; Bhat and Murali, 2001; Sanz-Cortes *et al.*, 2002; Hamann, 2004; Tebar *et al.*, 2004; Badola, 2010). In addition, the phenology is also pertinent to other sectors like agriculture, socio-economy and public health within the context of global change (Penuelas and Filella, 2001). Studies suggest that variation in altitude (Butola and Badola, 2006c) and hike in temperature is responsible for such changes in plant phenology (Badola, 1994; Menzel *et al.*, 2006; Parmesan and Yohe, 2003; Hamann, 2004; Walther, 2004).

Over the years, several studies on plant phenological observations have appeared from differnt regions (Holway and Ward, 1965; Wang, 1993; Kubinova and Krahulec, 2009; Martinez-Calvo *et al.*, 1999; Sanz-Cortes *et al.*, 2002; Ramirez, 2002; Tebar *et al.*, 2004; Schaber and Badeck, 2005; Tredici, 2007; Fatimah and Ahmed, 2009; Hudson *et al.*, 2009; Pirzad *et al.*, 2010) including India (Sundriyal *et al.*, 1987; Lodhiyal *et al.*, 1998; Bhat and Murali, 2000; Badola, 2010).

For one of critically endangered Himalayan herb, *Dactylorhiza hatagirea*, Butola and Badola (2006c) performed the comparative analysis of phenophases between domesticated and wild plants. They noticed earlier initiation of phenophases in domesticated plants than wild plants of *D. hatagirea*. Duraisamy and Paulsamy (2010) made phenological observation in six medicinal plant species from Nilgiri Hill, and recorded no variation in initiation and completion of phenophases in all the studied medicinal plants except seed dispersal which was very late in *Anaphalis elliptica*. Vashistha *et al.*, (2010) examined the phenological attributes of *Angelica*

*glauca* and *A. archangelica* under cultivation at two different altitudes (2200 m asl and 3600 m asl). They recorded variation in phenophases between the two altitudes and stressed on the need of developing location specific strategies for cultivation and management of the species.

The review of literature indicates that such phenological studies are limited in medicinal plants. There is no literature available on the phenological and periodical growth recordings in *S. chirayita.*

## 3.7 Development of agro-techniques, harvesting techniques and economic viability of cultivation of medicinal plants

To restrain the over exploitation of medicinal plants for meeting the demand of the pharmaceuticals industries, *ex-situ* cultivation is the only answer which is considered as appropriate tool to *in-situ* conservation of the threatened medicinal plants (Badola and Pal, 2002; Badola, 2009). *Ex-situ* cultivation of medicinal plants further provides better economic returns compared to wild collection, it reduces the chances of adulteration, incorrect identification, etc. It has several advantages over the cultivation of traditional crops, (1) the cost of cultivation of medicinal plants are very low; (2) can be cultivated in degraded and marginal lands, and also as inter crops; (3) can be sold both in national and international market in higher prices; (4) has less chances of pest attacks, diseases and being grazed; and (5) can be stored with minute precautionary measures for long time. However, for cultivation and high productivity, development of quality planting material and appropriate growing media or substrate combinations is imperative (Badola and Pokhriyal, 2001; Badola and Butola, 2003; Butola and Badola, 2005, 2006c). In recent years, suitable agro-techniques for the successful domestication using different growing media has been reported from Indian Himalaya and other regions for many of the important medicinal plants. However, different researcher used varied parts of the medicinal plants such as seeds, rhizome cuttings etc., for their trials.

A comparative study on growth and estimated the productivity of *Aconitum balfourii* and *A. heterophyllum* propagated through tubers under poly house and open field at 3600 m was conducted, recording high growth in plants under poly house than the open fields in either species with estimated multifold profit from poly house compared to open fields. (Nautiyal and Purohit, 2000)

Effect of inorganic fertilizers on growth and yield of *Valeriana wallichii* crop indicated an increase in the total plant biomass and weight of roots (on dry weight basis) with application of NPK fertilizers at 150:75:75 kg/ha compared to lower levels or untreated control (Singh *et al.*, 2000). Study recorded increase in yield of dry roots, root/shoot ratio, and proportion of roots in the whole plant biomass with advancement in age after transplanting. In addition, better crop growth under nylon net-shade compared to natural shade and open condition was noticed.

The germinability and productivity in *Picrorhiza kurrooa* by using both seeds and stolon segments as propagule at 1800m and 2200m asl was assessed, recording high germination under poly house as well as in open beds at both the altitudes (Nautiyal *et al.*, 2001). Study further recorded that year-wise productivity significantly increased during third year of cultivation with high dose (60 kg) of litter treatment in crop raised from both seeds and stolon at either altitude; however, the production was higher in crop raised through vegetative segment than the seeds at both the altitudes.

Nautiyal *et. al.* (2003b) applied the similar approach for carrying out cultivation of *Rheum emodi* at 1800 m asl and 2200 m asl under poly house using nursery soil treated with farm yard manure and forest humus in various concentration. They also sowed the seeds in open beds; however they used only single concentration of the treatments and observed maximum seedling emergence during October in both poly house (nursery soil treated with forest litter, 1:2) and open beds (treated with forest litter), and during June in both poly house (nursery soil treated with farm yard manure, 1:2) and open beds (treated with farm yard manure). They also recorded survival and production under different treatment after transplantation and found highest seedling survival under forest litter treatment in 40 and 60kg concentration at

both the altitude. Of the three different treatments used, yearly productivity was recorded higher with forest litter treatment (60kg per 2m x 2m) by them comparatively at both the altitudes.

The seedling emergence, growth and productivity in *Heracleum candicans* using three different treatments (farm yard manure, compost and forest humus) recorded highest seedling emergence in forest humus treated soil; while seedling growth, survival rate and productivity was recorded higher in compost treated soil (Badola and Butola, 2003); the study concluded that the compost treatment to be most beneficial in enhancing crop productivity and increasing economic benefits in *H. candicans*.

The effect of ploughing depths (30, 45 and 60 cm) on growth and productivity was examined in *Heracleum candicans*, using nursery beds with black sandy loam soil amended with farm yard manure and forest humus; the study obtained highest survival percent in bed with ploughing depth of 60 cm and 30 cm in the first year and second year, respectively (Badola and Butola, 2005). The positive impact of increasing ploughing depth on seedling growth and overall productivity in *H. candicans* was revealed; study recommended deep ploughing for better growth, high productivity and high economic return from cultivation of *H. candicans* (Badola and Butola, 2005).

Chauhan and Nautiyal (2005) carried out the cultivation of *Nardostachys jatamansi* at three different altitudes viz., 1800 m, 2200 m and 3600 m using vegetative propagules and seedling transplantation methods under different treatments. They observed increasing economic yield with the addition of farm yard manure in all the conditions (horizontal bed, vertical bed and poly house bed) and altitudes compared to control; nevertheless, they found significantly high yield in horizontal ridge condition at 3600 m; As per them, cultivation was commercially viable at 2200 m and 3600m in most of the treatments. They disclosed highest profit at 3600m under poly house condition and comparable profit at 2200 m from both vegetatively grown as well as seedling transplanted crop.

In their study, Butola and Badola (2006b) assessed the seedling emergence, growth and vigour in *Angelica glauca* and *Heracleum candicans* using 5 different growing media under poly house and net house. The study revealed that all the substrate combinations to be effective in improving seedling emergence, survival, growth and biomass over control. They identified sandy loam soil + forest humus combination and sandy loam soil + vermin-compost combinations to be highly suitable media for *H. candicans* and *A. glauca*, respectively. They further reported the poly house to be suitable condition for getting higher seedling emergence, better seedling growth and survival in *A. glauca*; they stressed that, though for *H. candicans*, polyhouse appeared suitable condition for higher seedling emergence, the seedlings should be transferred to the net-house for better growth and survival.

The domestication trial for *Dactylorhiza hatagirea* using both seeds and young tubers in soil amended beds mixed with farm yard manure and forest humus disclosed the failure of seeds to germinate; however, study recorded 90% sprouting from tubers with 70% survival frequency, and on comparing, domesticated plants were found better performing with respect to growth and productivity than the plants collected from the wild (Butola and Badola, 2006c).

Collected from 3000 m and 3600 m, the seeds of *Aconitum heterophyllum*, *Angelica glauca* and *Heracleum candicans*, sown in a mixture containing sandy loam soil and farm yard manure under poly house, net house and open condition recorded the maximum seedling emergence as well as lowest mean emergence time under polyhouse condition in all three species; however, growth parameters were high under net house and open condition for *A. heterophyllum* and *H. candicans*, but in the case of *A. glauca*, better growth was obtained under poly house (Butola and Badola, 2008b). The study concluded that each species differ in their growth requirements.

Experiment conducted on *Andographis paniculata* to study the impact of spacing (30 x 30 cm, 30 x 45 cm, 45 x 45 cm and 45 x 60 cm) and harvesting methods (destructive: uproot method and non-destructive: cut method) on quality and productivity of *A. paniculata* revealed that spacing, harvesting time and technique

plays a major role on productivity and quality of herb, and found a significant difference in the yield of the herb in relation to harvesting techniques (Pandey and Mandal, 2008).

Sher *et al.*, (2005) made cultivation trials of six important medicinal plants at different altitudes (1200 m, 1400 m, 1600 m and 1900 m) through vegetative propagation method using rhizome. They recorded highest survival in *Viola serpense* and *Valeriana jatamansi*, as economically feasible species. Sher *et al.*, (2010a) conducted the domestication and cultivation study of six important high altitude medicinal plants using their earlier approach (Sher *et al.*, 2005) at different altitude (1400 m, 1600 m, 1800 m and 2200 m), and recorded highest survival in *Linnum ustatissimum* followed by *Carum carvi*, and concluded their cultivation economically viable for having high market price. Sher *at al.*, (2010d) using the same approach evaluated the growth performance six medicinal plant species at 1200 m, 1400 m, 1600 m and 1900 m altitudes and observed highest survival leading to high productivity in *Hypericum perforatum* and *Bunium persicum* and revealed their economic feasibility of cultivation..

*Swertia chirayita* has been largely neglected with regard to such studies. The present study attempts (i) to develop suitable agro-technique for developing uniform and vigorous seedlings, (2) to evaluate the cultivation prospect and viability of *S. chirayita* which would be very helpful in species conservation.

CHAPTER 4

# Population Studies and Habitat Assessment

## 4.1 Introduction

In recent decades, the use and collection of medicinal plants have gone up from the subsistence level to large scale commercial extraction endangering their natural populations (Ghimire *et al.*, 2008; Badola and Aitken, 2003, 2010). In addition, overgrazing, pathogens, herbivores, seed predation (Albert *et al.*, 2005), etc., are also responsible for the reduction in population size of important medicinal plant species. Natural disturbances such as landslides and avalanches cause habitat alteration, degradation and fragmentation, leading to population isolation and reduction in population size or loss in case of rare plants in mountains (Badola and Pradhan, 2010) particularly for the species having small populations (Coates *et al.*, 2006). Habitat degradation may cause inbreeding and genetic drift in plant species thereby affecting their population viability as well as the survival probabilities of rare species (Hooftman and Diemer, 2002; Hooftman *et al.*, 2004). At the same time, plant fecundity may be reduced due to habitat degradation, fragmentation and destruction of plant populations (Brys *et al.*, 2003). In majority of cases in high value medicinal plants, the unsustainable *in-situ* harvesting to meet raw material demand of pharmaceuticals (Badola and Pal, 2002; Badola and Aitken, 2003, 2010; Butola and Badola, 2008a) has resulted into habitat loss and/or habitat degradation, population fragmentation/isolation, and loss of genetic diversity. Despite of these facts, the studies on the population ecology of important medicinal plants are least considered especially on Himalayan taxa (Ghimire *et al.*, 2008).

The successful conservation and effective management planning of the threatened plant species largely depends on an understanding of the effects of the natural and/or man-made changes on habitats (Hegland *et al.*, 2001), their ecological requirements and demography of the existing populations (Kalliovirta *et al.*, 2006), and their responses to disturbances (Ticktin, 2004). The microhabitat of the site plays a

significant role in the successful regeneration of plant species (Tilman, 1993; Hegland *et al.*, 2001; Jensen and Meyer, 2001; Overbeck *et al.*, 2003) and identification of which is very important for the species conservation (Sypros *et al.*, 2008). The nature of microhabitats is significantly related to the seed viability potential, which in turn decides the regeneration future of a population (refer chapter 6). Insufficient knowledge regarding the effects of disturbance on plant communities and plant populations are the main constraints in designing appropriate management plans, which complicate the conservation initiatives (Fidelis *et al.*, 2008). Envisaging the extinction risk and the future population size based on current population structure, growth and fecundity is the most suitable practical approach in assessing the impact of exploitation (Guedje *et al.*, 2003); which can be used as an appropriate tool in designing future conservation and management planning for threatened species.

*Swertia chirayita* (Roxb. ex Fleming) H. Karst (family Gentianaceae), a critically endangered medicinal plant species of the temperate Himalaya (Badola and Pal, 2002), bears high pharmacological importance (Joshi and Dhawan, 2005); the whole plant is used in traditional medicine (Kirtikar and Basu, 1984; Pradhan and Badola, 2008). Further, the plant is practiced in the preparation of herbal drug: Diabecon (Kohli *et al.*, 2004), D-400 (Sundaram *et al.*, 1996), Himoliv (Bhattacharya *et al.*, 2003); Melicon V (an herbal antiseptic and antifungal Veterinary ointment).

*S. chirayita* have been extracted traditionally both for domestic use, and rigorously for the commercial benefits especially in recent decades from different parts of Himalaya (Badola and Pal, 2002; Dutta, 2004; Olsen, 2005) leading to tremendous pressure on its natural populations. Despite very high conservation value of the species, there have been no efforts to replenish its source. The categorization of this important species as critically endangered (IUCN), endangered (Indian Red Data Book), critically rare and virtually endemic to Himalaya (Samant *et al.*, 1998), and vulnerable to Sikkim (Ved *et al.*, 2003a) has led the biodiversity conservationists internationally to come together to develop concern over the species. As an initiative, a forum of international experts had prioritized conservation of *S. chirayita* through cultivation at the top (Badola and Pal, 2002). However, so far, no initiative for systematic evaluation of the species in its

natural habitats has been taken up, except targeting five populations for central and north-western Himalaya (Bhatt *et al.*, 2006). Further, the documentation on quantitative investigation relating to the impact of such exploitation on population dynamics of targeted taxa is very poor. There is hardly any literature available, including those from north-eastern Himalaya on the populations, community associations and micro-habitat diversity of the species hindering the suitable conservation framework. A complete lack of substantial population data pose a question on, hitherto, a perceived estimated assessment for categorization of *S. chirayita* under threatened status. Therefore, it is essential to have appropriate and sufficient data on populations representing species availability in Himalayan regions before exact criteria is fixed towards conservation status of this important species. At the same time, it would be vital to have micro level assessment of habitats of the species. It would equally be important to know whether different sites have *S. chirayita* as dominant species in represented communities or not. Whether some associates in a community are invasive and their quantitative presence may be detrimental to future regeneration of *S chirayita*, as vital clues to conservation planning? Also, it would be important to assess the site specific population data, with those covering micro-habitats and identifying communities and potential conservation threats. Such studies are imperative in understanding the relationship between the species to its community and habitats (Zotz and Schmidt 2006), and will be helpful in sustainable use as well as designing effective conservation management approaches for *in-situ* conservation of *S. chirayita* as a model species.

The objectives of the present study on *S. chirayita* in Sikkim Himalaya were to, (1) evaluate the status of populations of targeted taxa and associated potential threats; (2) assess the population structures and associates of *S. chirayita* in nature; and (3) identify the microhabitat characteristics and community structures of *S. chirayita.*

## 4.2 Materials and Methods

### *4.2.1 Study area*

The present study was conducted in Sikkim Himalaya (Figure 2), India, which lies between $27^0$04'46" and $28^0$07'48" North latitudes and $88^0$58" and $88^0$55'25" East

longitude in the altitudinal range of 220m asl to 8,598m asl, covering an area of 7,096 sq. km. (0.2% of the Indian sub-continent). It shares border with countries like Bhutan (*ca.* 30 km long represented by Pangolakha range in the east); Tibetan Autonomous Region of China (*ca.* 220 km long represented by Chola range in the east and Trans-Himalaya region in the north) and Nepal (*ca.* 100km long represented by Singhalila range in the west); however, towards the south, it shares border of *ca.* 80 km with Darjeeling district (West Bengal) of the Indian Union. The Sikkim is further represents two major river system viz., Tista and Rangit. It has peculiar climatic condition, as such; the temperature conditions vary from sub-tropical in the southern lower parts to cold deserts towards northern part. It lies very close to Bay of Bengal and is directly affected by moisture laden southwest monsoon making Sikkim one of the most humid places in the whole Indian Himalaya range.

### *4.2.2 Data collection*

Initially, available literatures were reviewed thoroughly to generate idea on the distribution of the species in Sikkim Himalaya. Interaction with field personnel of state forest department, villagers and local folks helped focusing possible areas of species availability and accessibility for targeting the field studies. On the basis of extensive field surveys and accessibility to different sites for data collection, 22 populations along the Tista and Rangit river basins were identified within altitudinal range of 1500m asl to 3000m asl, lying between $27^0$09'58.2"and $27^0$44'34.7" N latitude and $88^0$06'35.2" and $88^0$38'28.08" E longitude in different parts of Sikkim Himalaya. Populations of *Swertia chirayita* were considered different primarily based on their non-continuity with each other by isolation, or by physical barriers such as rivers, mountain and aspects, and longer distances. Secondly, a site of the species located at least 500 m distance apart from other population was considered as different population (Osunkoya, 1999).

For each population, sampling was conducted through vertical belt transects methods following Michael (1990), in which a 20m x 150m long transect was marked as sufficiently large size to sustain a population (based on first hand experiences in Sikkim for the species). Each transect was sub-divided into three stands (20m x 50m),

as lower, middle and upper stand. In each population, density of *Swertia chirayita* was determined by using random 30 quadrats of 1m x 1m size targeting 10 quadrates for each of 03 sub-sites or a stand in which presence and abundance of *S. chirayita* was recorded and counted. The associated species were also enumerated and the number of individuals was counted within each quadrat; the type of microhabitat in each quadrat was also identified and noted. The microhabitat characteristics were used for estimating microhabitat preference of *S. chirayita*. In addition, broad vegetation type at each major site was identified. At each sample site, altitude and coordinates were recorded using hand held GPS (Garmin *etrex*, USA); aspect and slope was recorded using inclinometer (Suunto, Finland). The humus depth was measured using standard ruler, taking 10 samples per stand.

### *4.2.3 Statistical analysis*

Analytical features such as density, frequency percentage (Mishra, 1968), relative density, relative frequency (Jain and Rao, 1977), Importance Value Index (IVI) as a sum of relative density and relative frequency (Michael, 1990) was calculated focusing the ground vegetation only. Diversity index (Shannon and Weiner, 1963) was calculated to observe variability of associated species along different altitudes, which, were later used to relate with the density and availability of the targeted species. Pearson's correlation was used to assess the relation of different parameters amongst themselves and with the altitude. IVI value was used to identify the plant community structure in different population sites of *S. chirayita*. One way ANOVA ($\alpha = 0.05$) was used to compare the differences in means of density between the different population and microhabitats. If the differences was significant, Bonferroni test ($p<0.05$) was applied, and if the difference was non-significant, further testing was avoided.

The abundance to frequency ratio was used to interpret the distribution pattern of the species (Whiteford, 1949). The value <0.025 indicated regular distribution, 0.025 to 0.05 indicated random distribution and >0.05 indicated contagious distribution (Curtis and Cottam, 1956). The patchiness of the population was analyzed indirectly based on density and frequency. If the population has high density but low distribution

frequency, then the population is considered to be patchy in nature, but if the population has high frequency then it is considered to be non-patchy in nature (Rokaya and Ghimire, 2004). On the basis of the availability, the density was classified as: nil (0 individual), low (<5 ind/$m^2$), medium (5 to 10 ind/$m^2$), high (10 to 20 ind/$m^2$), and very high (>20 ind/$m^2$).

## 4.3 Results

A total of 22 populations, along 1500 to 3000m asl, were identified for *Swertia chirayita* under present study in Sikkim Himalaya (Table 1). These sites represented the ridges, forest gaps, forest shrubberies/pastures, rocky slopes, open slopes, shrubberies, landslide debris, etc., having varied terrains / diverse landscapes (Photo Plate I). Broad habitats of these study sites were further characterized by the different types of temperate broad leaved mixed forests, with the presence of single conifer elements in some population sites except for Sc11, which represented the steep open rocky slope in temperate coniferous forest (Table 1). The *Castonopsis tribulides, Alnus nepalensis, Michelia lanuiginosa, Quercus lamellose,* etc were the dominant broadleaf tree species; the dominant conifer element represented the *Cryptomeria japonica* only. The ground associates of *S. chirayita* varied along the change in altitude of the population sites, but in general, the prominent ground associates were *Gleichinia gigantean, Osbeckia sikkimensis, Anaphalis* sp., *Eupatorium cannabinum, Dichroa febrifuga, Artemesia vulgaris, Hedychium spicatum, Eragrostis atrovirens, Swertia bimaculata*, etc. In Sc20 population (altitude: 2332 m), *Anaphalis* sp., was the only ground associates. Of these associates, *Gleichinia gigantean, Osbeckia sikkimensis, Eupatorium cannabinum, Artemesia vulgaris, Anaphalis* sp., were prevalent in majority of the population sites (Table 2). Under all sampling quadrats for 22 population sites for *S. chirayita*, 37 different associate species were recorded. The general slopes of the sites ranged between $25^0$ and $65^0$. The humus depth increased with increasing altitude of the population sites; whereas, the soil pH exhibited significant ($r$ = -0.454; $p<0.05$) negative correlation with the altitude of the same.




**Photo Plate I.** Broad habitat of *Swertia chirayita* in Sikkim
A: Forest shrubberies; B: Forest-agriculture margin; C: Along/near the road;
D: Forest slope; E: Landslide area; F: Open grassy slope

The lowest species richness was recorded for the population Sc20 (at 2332m altitude), while the Sc2 population (1948 m altitude) exhibited highest species richness (Table 3). However, a negative correlation was established between the species richness and altitude of the population sites ($r$ = -0.027). In general, all the populations showed low community plant density, which was maximum for Sc8 and minimum for Sc14 followed by Sc18 (Table 3). Further, the community plant density was significantly declined with increasing altitude ($r$ = -0.406; $p<0.10$). The species diversity was recorded highest for the Sc6 and the lowest for Sc15 followed by Sc1 and Sc16 (Table 3), which showed insignificant negative correlation with the altitude of the population sites ($r$ = -0.342). Observations revealed that the *S. chirayita* occurred at the margin of the forest or in the forest gaps and was barely noticed inside deep forest except few populations.

Variability in *S. chirayita* density in different population sites (Figure 3) and sub-sites within a population (Figure 4) was recorded. The existing differences in plant density within a sub-site in a population were high for some of the populations (Figure 4) indicating the patchy or clustered or fragmentary nature of distribution of *S. chirayita*. The plant density decreased insignificantly with the increasing altitude of the population sites; while, the degree slope and soil pH had insignificant positive effect on the average density of *S. chirayita*; however, the effect of humus depth was poorly significant ($r$ = 0.372; $p<0.10$). Populations, viz., Sc5, Sc6, Sc10, Sc14 and Sc18 recorded very low average *S. chirayita* density (Figure 3); amongst different populations, the lowest and the highest average density was recorded for Sc10 (1.63 $\pm$ 0.69 ind/m$^2$) and Sc8 (21.67 $\pm$ 8.62 ind/m$^2$); Sc7 (18.40 $\pm$ 14.58 ind/m$^2$) followed Sc8 in plant density. Population sites such as Sc5, Sc6 and Sc18 were located near to the human habitation where different type of land use practices such as agriculture, fodder/fuel hood collection, grazing, NTFP collection, etc., were prevalent; however, Sc7, Sc8 and Sc10 were located near or along the road side where frequent disturbance in the form of road clearing/cleaning were common; and Sc14 was located near to the sacred wetland which experiences frequent human visits. Populations, viz., Sc2, Sc4 and Sc12 also recorded good availability of *S. chirayita* (Figure 3). Sc2 and Sc12 were located near to the human habitation while Sc4 was

located in the forest area; however, all the three sites had a history of land use for grazing, fodder collections, etc., as well as collection of *S. chirayita* for domestic use and nursery plantation in the past. Populations namely, Sc3 and Sc22 recorded 9.63 ± 6.39 ind/$m^2$ and 9.63 ± 7.15 ind/$m^2$ respectively (Figure 3). Both these sites were located away from human habitation; however, they were under the immense anthropogenic pressure in the form of grazing, illegal tree felling, etc., in the recent past. Sc4 still faces human pressure because this site lies along the heavily used trail. Other populations such as Sc1, Sc9, Sc17, Sc19 and Sc20 recorded moderate average density of *S. chirayita* (Figure 3). Sc1 and Sc9 were located in the forest area close to the villages and are still frequently exploited for the collection of fodder and NTFP. Population Sc17 was also located in the open forest area where the site was recovering from the old land slide. Population Sc19 and Sc20 existed on the same vertical slope, but both the populations had peculiar characteristics and the nature of disturbances were different. Of all the populations, Sc11 with plant density of 6.50 ± ind/$m^2$ was distinct because it was located in the highest altitude (2841 m asl) of the study sites near or along the road where the developmental activities such as road extension / maintenance is continuous phenomena. The types and level of existing disturbance in different sites are shown in Table 4. In general, the average density of *S. chirayita* were negatively correlated with the species richness ($r$ = -0.04) but positively correlated with species diversity ($r$ = 0.17).

Phytosociological analysis indicated that the relative density of *S. chirayita* to overall stand density was high in majority of the populations, which ranged between 8.27% (Sc6) and 78.38% (Sc12) [Table 2]. Relative density of *S. chirayita* exceeded the value recorded for the associate species in majority of the populations; while in some case, the relative density was lower than the value recorded for some of the associates at the site (Table 2). The dominant associates varied amongst the populations; however, *Eupatorium cannabinum, Gleichenia gigantean, Anaphalis* sp., *Artemesia vulgaris* were the main dominant associates of *S. chirayita*. Interestingly, the *Eupatorium cannabinum* showed greater ecological amplitude and grows in majority of the sites (15 sites) where *S. chirayita* was recorded and in 7 populations, it recorded higher relative density over *S. chirayita* (Table 2). The relative density of *S. chirayita*

showed positive correlation with the altitude ($r$ = 0.511, $p<0.02$; Figure 5); whereas, the degree slope showed insignificant effect on *S. chirayita* availability ($r$ = 0.021) and its frequency of occurrence ($r$ = 0.181).

Of the total 22 populations sites, 13 showed 100% frequency of occurrence of *S. chirayita*, whereas, for the remaining 9 sites, the frequency ranged between 33.3% and 70% (Table 2), which increased with ascending altitude ($r$ = 0.423; $p<0.05$). Highest relative frequency for *S. chirayita* was recorded in Sc19 followed by Sc7 and Sc4; while, the lowest was recorded for Sc1 followed by Sc10 and Sc21 (Table 2). Relative frequency showed insignificant increase with increasing altitude, humus depth and soil pH of the population sites. Comparatively, IVI value was higher for the *S. chirayita* than other associates in majority of the populations, thus forming communities with the domination of *S. chirayita*, the maximum of which was recorded for Sc4 followed by Sc7 and Sc8 (Table 3). Based on the IVI value for different species, 11 different types of ground community structures were identified in 22 population sites of *S. chirayita*, **(1)** *Gleichenia gigantean – S. chirayita* (Sc1); **(2)** *S. chirayita – G. gigantean* (Sc2, Sc14); **(3)** *S. chirayita - Osbeckia sikkimensis - Anaphalis* sp. (Sc3); **(4)** *S. chirayita* (Sc4. Sc9, Sc11, Sc12, Sc19); **(5)** *Eupatorium cannabinum – S. chirayita* (Sc5, Sc6, Sc10, Sc18, Sc21); **(6)** *S. chirayita – E. cannabinum* (Sc7, Sc8, Sc17); **(7)** *S. chirayita – Osbeckia sikkimensis – E. cannabinum* (Sc13); **(8)** *S. chirayita – S. bimaculata* (Sc16); **(9)** *S. chirayita – Artemesia vulgaris – E. cannabinum* (Sc7); **(10)** *S. chirayita – Anaphalis* sp. (Sc20); **(11)** *S. chirayita – Eragrostis atrovirens* (Sc22). Of the 11 plant community structure identified, *S. chirayita* was dominant over other species in 8 community structure in 11 population sites; however, in Sc4, Sc9, Sc11, Sc12 and Sc19 *S. chirayita* formed the pure community with smaller share of other associates (Table 2). In total, *S. chirayita* was dominant in 16 populations indicating its high availability in Sikkim. As regard to distribution pattern, broadly, the *S. chirayita* was found to be patchy in nature since none of the population sites revealed the even distribution for the same (Figure 4). On the basis of abundance to frequency ratio, *S. chirayita* showed contagious/clustered distribution in majority of the populations except for Sc5 and

Sc10, which showed random distribution (Table 2). However, none of the site showed regular distribution for *S. chirayita*.

*S. chirayita* was found growing in a diverse microhabitat conditions viz. boulders/stony areas, rock crevices, moss covered/grassy slopes, tree/root base, tree/bamboo canopy, along streams, rotten logs, shrubberies etc (Photo Plate II). Present study identified a total of 14 microhabitats for *S. chirayita* in 22 population sites along different altitude (Figure 6). Of the 22 populations studied, Sc3 recorded the highest and Sc10, Sc11 and Sc18 had the lowest number of microhabitat niches for *S. chirayita* (Table 3). The number of microhabitats decreased with the increasing altitude ($r$ = -0.158) of the population sites. Amongst the 14 microhabitats identified, open grassy slope recorded significantly ($p<0.05$) highest density (37.57 $\pm$ 16.24 ind/m$^2$; 21.94%) of *S. chirayita* compared to shrubberies, rotten logs/burn/tree stumps, between tree saplings, marshy grassy slope, old landslide/debris and bamboo canopy. The other microhabitats which recorded good density of *S. chirayita* were rock crevices / boulders / stony areas (29.23 $\pm$ 16.45 ind/m$^2$; 17.08%) and below tree canopy (26.73 $\pm$ 9.17 ind/m$^2$; 15.62%); however, marshy grassy slope had the lowest plant density (1.63 $\pm$ 0.69 ind/m2; 0.95%) followed by shrubberies (2.43 + 2.50 ind/m2; 1.42%) [Figure 7]. On individual quadrat basis, the 'open grassy slope' (22.51%) followed by 'below tree canopy' (16.79%) were the most frequently occurring microhabitats, while, 'between tree saplings' was amongst the least frequent (1.66%) occurring microhabitat (Figure 8). On per site basis, 'along tree root base' was the most prominent microhabitat (17.14%) followed by 'open grassy slope' (16.19%); whereas 'bamboo canopy', 'marshy grassy slope' and 'rocky slope' appeared in single population site each (Figure 8). Aspect, cumulatively for the entire study sites, had influenced the availability of *S. chirayita*; the maximum average plant density was recorded along west facing slope (20.03 ind/m$^2$); whereas, cumulatively, the highest (100%) frequency of occurrence for *S. chirayita* was recorded along the north facing slope (Figure 9).



















**Photo Plate II.** Microhabitat of *Swertia chirayita* in Sikkim
A: Rocky slope; B: Tree base; C: Rotten logs; D: Along stream;
E: Shrubberies; F: Stoney areas

## 4.4 Discussion

The present study reveals an overall availability of *S. chiraiyta* to be relatively high in Sikkim Himalaya. Of the total 22 populations, 16 populations recorded medium (5 to 10 ind/$m^2$) to high (10 to 20 ind/$m^2$) to very high average (>20 ind/$m^2$) density of *S. chirayita* plants; only 6 populations had low density of >5 ind/$m^2$ compared to very few reports in western and central Himalaya (Bhatt *et al.*, 2005), where the species density ranged between 1.65 ind/$m^2$ and 2.35 ind/$m^2$. However, the variation in *S. chiraiyta* plants' availability was observed amongst the populations and within the stands in a population in the present study. Here, the *S. chirayita* showed either contagious or random distribution indicating its patchy or scattered or fragmented distribution, as reported in *Podophyllum hexandrum*, an another endangered Himalayan herb (Airi *et al.*, 1997). In such case, the analysis of patchiness nature on the basis of frequency and density could not be applied as suggested by Rokaya and Ghimire (2004), because, even though the species revealed high average density and frequency in majority of the sites, *S. chirayita* was available in patches, the size of which varied amongst the population sites.

The lowest species richness recorded in Sc20 site was might be due to its location near to the road side where frequent road cleaning and clearing process and other disturbances restricted the regeneration of the other ground flora; while the highest species richness in Sc2 could be related to increased intensity of fodder collection as a consequence of ban imposed on animal grazing from forest lands vide Notification No.426/F, dated 17.07.1995, the former activity is known to increase the species richness (Dzwonko and Loster, 2007). The highest species diversity recorded at population site Sc6, which could be due to its low altitude as the species diversity reduces with increasing altitude (Pavon *et al.*, 2000; Wang *et al.*, 2007). In addition, the site is located in the private agricultural field where grazing, fodder/fuel hood collection, land clearing, etc., were very common creating open spaces suitable for regeneration of the ground species (Aikens *et al.*, 2007). While, comparatively the low species diversity in Sc15 could be related to its higher altitude location, compared to Sc6, and further, to its abandoned status created by the cattle herders which gave an

opportunity to growth of unpalatable species especially shrubs suppressing the regeneration of the other herb species. Population Sc1 and Sc16 was located in the forest area where the minimum light penetration to the ground prohibited the survival of monocarpic (light loving) ground species (Gaudio *et al.*, 2011) resulting in low species diversity. Decreasing plant density with increasing altitude is a natural phenomenon as observed in the present study with *S. chirayita*. *S. chirayita* performs well on the slopes as the gradients often do not allows accumulation of water; higher the degree of slope, more is the seedling emergence or plant density as observed in the present study.

The low availability of *S. chirayita* in populations, viz., Sc5, Sc6, Sc10, Sc14 and Sc18 could be possibly due to its proximity to the human habitation. As the Sc5 experiences frequent visitors for fodder/fuel hood collection thus creating open condition/gaps where the individuals of *S. chirayita* can easily be sighted. During such activities, collection of *S. chirayita* plants without considering their age has been observed, for the domestic use, prior to flowering and fruiting. Such practice allowed very few individuals to complete the reproductive phase resulting in low regeneration of *S. chirayita*. Further, major portion of the site had rocky habitat which could restrict the species to crevices and holes creating competitive environment for the species. The Sc6 population site was located along the periphery of the privately owned agricultural field where the species faced tremendous pressure in terms of different land use practices. The plants gets trampled or uprooted along with the other weed species during the clearing / preparation of land to sow crops, as change in land use, in particular the intensification of agriculture is the one of the major cause for the decline in the number of plant populations (Korneck and Sukopp, 1988) as the alteration in demographic rates may rapidly affects population size (Schemske *et al.*, 1994; Fischer and Matthies, 1998). Sometimes, the cattle are left in the field for grazing; being bitter in taste, the *S. chirayita* is not preferably grazed but may result in damage of the whole plant or part of it by trampling which affects the species abundance (Yu *et al.*, 2008) as well as its regeneration. Nonetheless, such activities may further augment the threat of existence to *S. chirayita* in those sites in future because constant disturbance would prohibit the seedlings / saplings to mature leading

to disappearance of the species from the sites as the habitat degradation is regarded as the major cause of species extinction in human modified landscapes (Andren, 1994).

The lowest availability of *S. chirayita* in Sc10 could be possibly due to its closeness to the roadside as well as availability of single microhabitat condition which experiences frequent soil erosion due to marshy habitat. Such condition offers chances of uprooting of the seedlings / saplings, as well as the washing away of the seeds, resulting in low density as well as accumulation of available *S. chirayita* plants in lower and middle stand. Also, the frequent soil erosion prohibited the humus accumulation required for the seedling emergence and growth affecting the availability of *S. chirayita* in Sc10, as the health of a forest floor plays a significant role in plant growth and survival. High moisture availability increases the seedling mortality by increasing the chances of fungal attack (Fidelis *et al.*, 2008). This might be the probable reason for the low availability of *S. chirayita* in Sc14, as the site is located in the vicinity of the wetland. In addition, the wetland being sacred, experiences frequent human visits which cause disturbances to its habitat thereby resulting in lower availability of *S. chirayita* in Sc14. Being closer to the settlement, harvesting of *S. chirayita* for domestic use is also a common practice at this site; further the site is exploited for other forest resources aggravating the problem of habitat deterioration, which lessened the species availability. Similarly, being closer to the villages, population site Sc18 is exploited for fodder/fuel hood as well the collection of *S. chirayita* for personal use. There have been instances where *S. chirayita* juveniles were collected for plantation in the nursery and the harvesting of the adults, before maturing, for trade at the site. This has led to low availability of the species at the site. Further, the site recorded single microhabitat and appeared much vulnerable to deterioration as a result of human interferences; and microhabitat loss results in decrease in species abundance (Goode *et al.*, 1995) ultimately leading to species extinction.

Populations, viz. Sc7 and Sc8 being located nearer to the road are easily accessible and even though they were found to be constantly degraded during the road extension / repairing / cleaning process, both the sites had good availability of *S. chirayita* and

recorded highest density of the same indicating that the species being adapted to such disturbances, as the effect of habitat deterioration on the persistence of plants may strongly differ among species (Brys *et al.*, 2005). At the same time, the sites provided suitable niche (open condition) for the regeneration of the species (Yu *et al.*, 2008). *S. chirayita* showed high availability in lower stand and its availability decreased in middle stand and was completely absent from upper stand in Sc7, as the population was situated in the forest margin, indicating its patchy or contagious distribution at the site; even though, the species showed high availability in all the three stands at site Sc8; the distribution was random in nature. The greater availability of *S. chirayita* in both the sites could also be attributed to the larger number of microhabitats as microhabitat diversity significantly affects species richness and abundance (Yu *et al.*, 2008; De Souza and Eterovick, 2010). However, both the sites had abundance of juveniles and the adult plants were very scarce which signals towards the requirement of high conservation measures because if juveniles are destroyed before they reaches the adult stage and complete its life cycle, the population may become vulnerable to extinction. Populations, viz. Sc3, Sc4 and Sc22 were located in the forest areas in the gaps in similar range of altitude (ca. 2100 – 2200 m asl). All the three population sites were under immense anthropogenic pressure in the form of grazing, NTFP collection, building of cattle sheds, land clearings, etc. Nevertheless, ban in grazing by the state government and the removal of cattle from the forest areas in Sikkim promoted *S. chirayita* to flourish in those areas where open conditions were available. Besides, higher number of microhabitats in all the three population sites proved advantageous (Yu *et al.*, 2008; De Souza and Eterovick, 2010) for the growth of *S. chirayita* leading to high availability of the plants in those sites. Nonetheless, growth of unpalatable species have been observed in abandoned areas in other sites which may become a problem for regeneration of *S. chirayita* in the future leading to species disappearance from those sites

Over 50% of the population site Sc2 was observed as open grassy slope and under continuous pressure for the fodder and NTFP collection due to its closeness to the human habitation. Also, the site had a history of grazing which could be attributed to openness and high species richness at the site. The site also recorded greater number

of microhabitats in addition to the presence of unpalatable species such as *Edgeworthia gardenerii*, *Gleichenia gigantean* and *Osbeckia sikkimensis,* which act as a buffer protecting *S. chirayita* from many disturbances. This looks promising for *S. chirayita* at present; however in the long run, species such as *Osbeckia sikkimensis*, which has a tendency to invade, might cover entire area restricting *S. chirayita* to small pockets. On the other hand, entire slope in Sc12 is completely open; this might be due to mass felling of trees in the past for township development in the nearby area or due to huge landslide that might have occurred in the geological past. In addition, the site recorded varied microhabitats and very low density for the associated species. Further, the presence of unpalatable species like *Osbeckia sikkimensis* and *Rubus ellipticus* protects the species from disturbances like trampling, etc. The abrupt ban on grazing has promoted the species like *Aconogonum molle, Osbeckia sikkimesis, Dichroa fenrifuga*, etc., to spread covering wide area under which *S. chirayita* cannot be sighted easily and escapes ruthless harvesting thereby resulting in good availability in the area. At present, though the *S. chirayita* seems dominant over other species at the site; but, uncontrolled growth of other species makes this species vulnerable to population fragmentation and extinction because *S. chirayita* is a slow growing species, compared to other associated species, which have tendencies to become invasive, and *S. chirayita* cannot withstand them in longer run.

The Sc11 population was explored at the highest altitude (2841m asl) located entirely on the rocky slope and with complete isolation from other populations. Isolation of populations results in the reduced fitness of plants and further decline in population size (Lienert, 2004; Hensen *et al.*, 2005). *S. chirayita* was highly concentrated in the upper stand while it showed medium availability in lower and middle stands thereby resulting in contagious distribution. Though, it had good availability of *S. chirayita*; nevertheless, the population might face risk of extinction in future because the area is prone to landslides / rockslide etc., caused by the developmental activities such as road extension / maintenance. This particular population is very important as the entire area remains under snow cover for almost 3-4 months in a year; to overcome the severity of the climatic condition, the species might have developed special adaptive features such as shorter plant height (refer chapter 5), production of highly

viable seeds (refer chapter 7), healthy seedlings, nectars very rich in sugars attracting insects etc., which needs detailed eco-physiological studies to determine its adaptive mechanism better. However, specialization of plants to specific environment makes them susceptible to various environmental pressures (Korner, 1999), suggesting that the population should be provided special conservation measures.

The association of *S. chirayita* and *Eupatorium cannabinum* was common amongst Sc1, Sc9 and Sc17 populations. These three sites recorded moderate availability of *S. chirayita* and are potential population sites. In the first two sites, *S. chirayita* was dominant over *E. cannabinum* and vice versa in Sc17. All the threes sites were located in the forest area either in the gaps or in the fringe of the forest. The governmental ban imposed on collection of medicinal plants (Exim Bank, 2009) allowed good growth and regeneration of *S. chirayita* in population site Sc1, supported by 100% frequency of occurrence. The site has a history of *S. chirayita* collection for trade in the past; however, ban on grazing has limited the human intervention in population site Sc1 but has permitted the growth of several unpalatable plant species thus threatening the availability of *S. chirayita* at the site. Higher intensity of forest resource use such as fodder/fuel hood, NTFPs, etc., at population site Sc9 resulted in trampling as well as damaging of *S. chirayita* plants or its parts during fodder collection process along with other fodder elements. This site could be a promising population if conservation measures are taken. Population Sc17 is situated in distant location in open slope and the frequency of disturbance was very low; however, the population was available on the land slip area with over 70% adult plant. There is a chance of increase in abundance of the species from moderate at present to high in the future if the landslide does not become active and burry the juveniles and the dispersed seeds under the deep soil. The major threat at the existing population site is the dominance and uncontrolled growth of a weed species, *Eupatorium cannabinum,* which may cover entire area in the future suffocating the growth and regeneration of *S. chirayita.* Sc19 was lying in the margin of agricultural field and the forest area and faced higher degree of disturbances such as grazing fodder/fuel hood collection, land clearing for agriculture purpose, NTFP collection, etc. The increasing density from lower to upper stand reveals higher intensity of pressure at the side nearer to agricultural field. This

will restrict the distribution of the species in a pocket in future at the population site. Population Sc20 was located in the forest area along or near to the road where it encounters disturbances like road cleaning/clearing, chirayita collection for the domestic purpose, etc. Likewise in the previous population, Sc20 had high availability of *S. chirayita* in the upper stand and low availability in the lower and the middle stand indicating increasing threat on the species leading its restriction wtihin small patch in the future. Both the site can be considered a potential population and needs meager conservation initiatives for long term sustainability of the population.

*Swertia chirayita*, for its higher density and higher relative density, emerged dominant over other associated species in 16 of the total 22 populations. *S. chirayita* is not selective in forming associations; rather, the species grows evenly with other species existing in the population sites. However, *S. chirayita* being slow growing species, its domination over other species might get affected in future because some of the associated species like *Eupatorium cannabinum, Osbeckia sikkimensis, Aconogonum molle*, etc., have a tendency to become an invasive element thereby endangering the existing populations of *S. chirayita* .

During the study, it was also observed that the availability of *S. chirayita* being affected by increasing number of associate species ($r$ = -0.040), as reported by Fidelis *et al.*, (2008) in *Eryngium horridum*. The possible reasons could be the low light penetration, increased competition, high moisture climate leading to fungal attack, etc., affecting both germination and establishment of seedlings (Tilman 1993; Foster and Gross 1998; Xiong and Nilsson, 1999; Moles and Westoby, 2004a,b) thereby causing increased seedling mortality (Fidelis *et al.*, 2008). Litter may favour seed germination and establishment. Higher density of plants with increasing humus depth ($r$ = 0.372; $p<0.10$) in the current study indicates that the good humus availability helps to maintain greater plant availability of *S. chirayita*. However, negative effect of accumulation of litter on the seedling recruitment, survival of plant populations (Kalliovirta *et al.*, 2006), species richness and vegetation (Sydes and Grime 1981a,b; Colling *et al.* 2002; Dzwonko and Gawronski 2002) has been reported in several studies. Decline in species richness with the increasing altitudes due to various

phenomenon viz., dry weather condition, decrease in precipitation, short photo period, less soil nutrient, etc., has been indicated in several studies (Hamilton 1975; Gentry 1988; Kitayama 1992; Stevens 1992; Vazquez and Givnish 1998; Odland and Birks 1999). Similar decrease in the species richness along increasing altitudes in the present study has been observed as promising advantageous for the important taxa like *S. chirayita* by providing sufficient area for it to boon.

Quality of habitat/micro-site plays a significant role in the seedling recruitment, establishment and better performance of the species (Turnbull *et al.*, 2000; Kalliovirta *et al.*, 2006). The present study discovered that, *S. chirayita* is not habitat specific, suggesting the species offers high microhabitat niche pliability, in contrary to general assumption that the endangered species are often habitat specific. However, species performs well in the microhabitats characterized by less prevalence of competitor species such as open grassy slope, old landslide debris, rock crevices / boulders / stony areas, tree canopy, open moss covered slope, moist moss laden rocks, etc., as recorded in the present study. Under such habitat conditions, competition pressure for sunlight, precipitation and nutrients is reduced thereby helping *S. chirayita* to flourish. Trapping of soil and organic matter in the bedding/fault planes at the base of the large rock outcrops/on rock outcrops/rock crevices/gaps enabling good germination of seeds can be the possible reason for high density of *S. chirayita* in such microhabitat conditions where the plants were observed in numerous clusters. Tree canopy intercepts the abiotic factors necessary for the growth and survival of many under storey vegetation (Dobrowolska, 2008; Valladares and Niinemets, 2008), which results in high emergence of plant species like *S. chirayita* under such habitat condition in the absence of competitors. The water logging in the soil leading to decomposition of the underground part followed by the complete drying of the above ground part was observed in some of the population viz., Sc4, Sc9, indicating and emphasizing the less water requirement by the species for the growth and survival (refer chapter 8). Bamboo groove acts as a barrier to the human as well as the grazing animals thereby the species like *S. chirayita* acquires protection and an opportunity for the seeds to emerge in high number as seen in the case of Sc18.

## 4.5 Conclusion

The present study concludes that, Sikkim Himalaya offers a high potential and greater availability of *Swertia chirayita* compared to central and northwestern Himalaya (Bhatt *et al.*, 2006), where species is regarded as critically rare / critically endangered. Also, the identification of 14 microhabitats in the present study suggests the species maintains high micro-habitat pliability and that is important for its conservation and management, as a newer finding. However, the distribution is patchy in nature. Study further revealed that the commercial extraction of *S. chirayita* is not common in Sikkim and cannot be consider as a threat because of the governmental ban imposed on its collection from the wild. However, in cases, the impact of past harvesting could be witnessed. The use of the species as a home remedy cannot be considered as a threat to the species. Such practices are necessary to conserve the traditional knowledge (Pradhan and Badola, 2008; Badola and Aitken, 2010), which is also a very important issue. The present study suggests that the only threat to the species is the man made habitat modification or degradation in Sikkim Himalaya. In majority of the population sites, *S. chirayita* was available near to human settlement or in the areas where human interference exists, which indicate that its existence is not affected by minor habitat disturbances caused by the humans but is affected by complete degradation or modification of the habitat. Apart from these threats, natural disturbances viz., landslide, forest fire, etc., are also responsible for the species endangerment which makes it vulnerable as categorized (Ved *et al.*, 2003a), and has no remedy except preservation of the gene pool through *ex-situ* mechanisms (Badola and Pal, 2002).

The necessary steps in the context of conservation of *S. chirayita* would be the (i) identification and conservation of suitable habitat condition and elite populations, (ii) supplementary researches to discover and study more populations in other possible localities, (iii) the development of the cost effective *ex-situ* cultivation packages and improved agro-techniques for mass scale cultivation of the species (refer chapter 8), (iv) development of the *ex-situ* conservation strategies especially in a scenario when

harvesting potential from the wild falls short of the demand for the commercial exploitation (Badola and Pal, 2002; Badola and Aitken, 2010).

At the same time, the relationship between the habitat and population characteristics needs to be considered in wider dimensions, especially when planning management activities need to be placed in order to enhance the regeneration of this threatened species (Kalliovirta *et al.*, 2006). The better performance of the species in nature and difference in secondary metabolism content in comparison to *in-vitro* cultivated plants (Wawrosch *et al.*, 2005), suggest primarily focuses of *in-situ* over *ex-situ* conservation, though later is equally supportive. The insignificant difference in major phytochemicals (amarogentin, mangiferin, swerchirin) between extracts of wild and cultivated plants strengthens the validity of cultivated *S. chirayita* for medicinal purpose and trade (Phoboo *et al.*, 2010) which would be an important direction towards *in-situ* conservation of *S. chirayita* (Badola and Pal, 2002). Future studies should focus on monitoring the populations and estimating the population growth rate to address the best management system of the species (Kusuma and Astuti, 2009).

**Table 1.** Site characteristics of selected populations of *Swertia chirayita* in Sikkim

| Site code | Site Name | Altitude (m asl) | Aspect | Slope ($^0$) | GPS | | Broad habitat |
|---|---|---|---|---|---|---|---|
| | | | | | Latitude (N) | Longitude (E) | |
| Sc1 | Luing (ES) | 2126 | NE | 60 | 27$^0$21'47.60" | 88$^0$34'12.17" | Ridge, mixed forest of *Cryptomeria japonica* and *Castanopsis tribuloides* |
| Sc2 | Railgaon (ES) | 1948 | NE | 25 | 27$^0$21'52.71" | 88$^0$33'47.07" | Shrubberies with scattered tree species of *Cryptomeria japonica* and *Castanopsis tribuloides* |
| Sc3 | Upper Pangthang (ES) | 2176 | SW | 50 | 27$^0$21'13.54" | 88$^0$33'53.37" | Forest-shrubberies of *Quercus lamellose* and *Castanopsis tribuloides* |
| Sc4 | Guransey dara (ES) | 2107 | SE | 55 | 27$^0$20'51.02" | 88$^0$34'07.92" | Moist mixed Forest with the domination of *Cryptomeria japonica* and *Rhododendron* sp. |
| Sc5 | Jaunbari (SS) | 1651 | SW | 45 | 27$^0$11'47.20" | 88$^0$23'33.60" | Moist forest-shrubberies with *Alnus nepalensis* and *Michelia lanuigilosa* |
| Sc6 | Upper Chamgaon (SS) | 1583 | NW | 50 | 27$^0$11'03.80" | 88$^0$22'26.20" | Forest-shrubberies with *Alnus nepalensis* and *Schima wallichi* |
| Sc7 | Tiffin dara1 (SS) | 1667 | W | 55 | 27$^0$11'14.8" | 88$^0$22'46.01" | Moist grassy slope with *Cryptomeria japonica* and *Alnus nepalensis* |
| Sc8 | Tiffin dara2 (SS) | 1744 | W | 55 | 27$^0$11'12.1" | 88$^0$22'45.8" | Forest-shrubberies with *Cryptomeria japonica* and *Alnus nepalensis* |
| Sc9 | Upper Changrang (ES) | 2055 | S | 60 | 27$^0$21'51.97" | 88$^0$35'20.31" | Forest-shrubberies with *Machilus* sp. and *Castanopsis tribuloides* |
| Sc10 | Phinsyonala (NS) | 1761 | NE | 50 | 27$^0$37'01.6" | 88$^0$37'26.2" | Forest-shrubberies of *Alnus nepalensis* |
| Sc11 | Zema1 (NS) | 2841 | NE | 65 | 27$^0$44'34.7" | 88$^0$32'48.0" | Rocky steep slope with *Selenium* sp. and *Artemesia* sp. |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | Moist mixed forest with the dominance of *Cryptomeria japonica* |
| Sc13 | B2 (ES) | 1694 | N | 55 | 27°22'54.12" | 88°38'28.08" | Forest-Shrubberies with persisting landslide with the domination of *Alnus nepalensis* |
| Sc14 | Khecheopalri (WS) | 1867 | SW | 50 | 27°21'06.7" | 88°11'21.6" | Moist mixed forest of *Quercus* sp. and *Castanopsis tribuloides* |
| Sc15 | Fring dara (WS) | 2030 | SW | 40 | 27°20'53.2" | 88°10'51.5" | Shrubberies of *Osbeckia* sp. |
| Sc16 | Yuksam (WS) | 1948 | SW | 65 | 27°23'26.2" | 88°13'09.8" | Mixed forest of *Quercus lamellosa* as dominant |
| Sc17 | Deewani taar (WS) | 2055 | SE | 55 | 27°11'41.3" | 88°13'23.9" | Forest-Shrubberies with the dominance of *Alnus nepalensis* |
| Sc18 | Gumpa dara (WS) | 1978 | SE | 55 | 27°10'13.6" | 88°11'03.9" | Forest –shrubberies of *Cryptomeria japonica-Alnus nepalensis* |
| Sc19 | Hilley (WS) | 2697 | S | 60 | 27°10'44.5" | 88°07'05.4" | Forest-shrubberies with *Lithocarpus pachyphylla* and *Arundinaria maling* |
| Sc20 | Ribdi (WS) | 2332 | S | 60 | 27°09'58.2" | 88°06'35.2" | Moist forest of *Cryptomeria japonica* |
| Sc21 | Tendong (SS) | 2099 | S | 50 | 27°13'45.0" | 88°23'37.0" | Forest-shrubberies with *Castanopsis tribuloides* and *Quercus* sp as dominant |
| Sc22 | Ravangla (SS) | 2160 | S | 45 | 27°18'58.6" | 88°22'20.6" | Mixed forest of *Castanopsis tribuloides, Quercus lamellose, Machilus edulis* and *Cinnamomum* sp. |

ES: East Sikkim; SS: South Sikkim; NS: North Sikkim; WS: West Sikkim

**Table 2.** Phytosociological attributes of *Swertia chirayita* and its associates in Sikkim

| Site code | Species | RD (%) | Freq (%) | RF (%) | IVI | Ab | A/F ratio |
|---|---|---|---|---|---|---|---|
| Sc1 | *Swertia chirayita* | 23.30 | 100.00 | 24.79 | 48.09 | 7.30 | 0.073 |
| | *Anaphalis* sp. | 7.34 | 36.67 | 9.09 | 16.43 | 6.27 | 0.056 |
| | *Clematis buchananiana* | 0.64 | 13.33 | 3.31 | 3.94 | 1.50 | 0.150 |
| | *Eupatorium cannabinum* | 10.43 | 30.00 | 7.44 | 17.86 | 10.89 | 0.225 |
| | *Gleichenia gigantean* | 37.23 | 50.00 | 12.40 | 49.63 | 23.33 | 0.171 |
| | *Osbeckia sikkimensi* | 14.15 | 100.00 | 24.79 | 38.94 | 4.43 | 0.044 |
| | *Swertia bimaculata* | 6.38 | 60.00 | 14.88 | 21.26 | 3.33 | 0.467 |
| | *Symplocos glomerata* | 0.21 | 6.67 | 1.65 | 1.87 | 1.00 | 0.113 |
| | *Symplocos theifolia* | 0.32 | 6.67 | 1.65 | 1.97 | 1.50 | 0.363 |
| Sc2 | *Swertia chirayita* | 42.21 | 100.00 | 32.26 | 74.47 | 11.83 | 0.118 |
| | *Anaphalis* sp. | 9.16 | 43.33 | 13.98 | 23.13 | 5.92 | 0.087 |
| | *Edgeworthia gardenerii* | 0.12 | 3.33 | 1.08 | 1.19 | 1.00 | 0.384 |
| | *Elaeocarpus lanceaefolius* | 0.12 | 3.33 | 1.08 | 1.19 | 1.00 | 0.133 |
| | *Eurya acuminata* | 0.36 | 3.33 | 1.08 | 1.43 | 3.00 | 0.137 |
| | *Gleichenia gigantean* | 34.01 | 36.67 | 11.83 | 45.84 | 26.00 | 0.083 |
| | *Osbeckia sikkimensis* | 5.59 | 43.33 | 13.98 | 19.57 | 3.62 | 0.709 |
| | *Pieris ovalifolia* | 0.12 | 3.33 | 1.08 | 1.19 | 1.00 | 0.900 |
| | *Swertia bimaculata* | 3.45 | 33.33 | 10.75 | 14.20 | 2.90 | 0.300 |
| | *Symplocos glomerata* | 3.80 | 16.67 | 5.38 | 9.18 | 6.40 | 0.094 |
| | *Symplocos theifolia* | 0.48 | 10.00 | 3.23 | 3.70 | 1.33 | 0.300 |
| | *Zanthoxylum alatum* | 0.59 | 13.33 | 4.30 | 4.90 | 1.25 | 0.300 |
| Sc3 | *Swertia chirayita* | 56.67 | 70.00 | 31.82 | 88.48 | 13.76 | 0.197 |
| | *Anaphalis* sp. | 16.86 | 30.00 | 13.64 | 30.50 | 9.56 | 0.042 |
| | *Gleichenia gigantean* | 13.53 | 16.67 | 7.58 | 21.11 | 13.80 | 0.046 |
| | *Osbeckia sikkimensis* | 7.65 | 53.33 | 24.24 | 31.89 | 2.44 | 0.828 |
| | *Rhododendron dalhousie* | 2.55 | 16.67 | 7.58 | 10.12 | 2.60 | 0.319 |
| | *Swertia bimaculata* | 2.75 | 33.33 | 15.15 | 17.90 | 1.40 | 0.156 |
| Sc4 | *Swertia chirayita* | 66.93 | 100.00 | 60.00 | 126.93 | 11.33 | 0.113 |
| | *Eupatorium cannabinum* | 16.93 | 13.33 | 8.00 | 24.93 | 21.50 | 0.200 |
| | *Eurya japonica* | 3.35 | 6.67 | 4.00 | 7.35 | 8.50 | 1.613 |
| | *Gleichenia gigantean* | 5.71 | 6.67 | 4.00 | 9.71 | 14.50 | 0.111 |
| | *Osbeckia sikkimensis* | 5.91 | 30.00 | 18.00 | 23.91 | 3.33 | 1.275 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Swertia bimaculata* | 1.18 | 10.00 | 6.00 | 7.18 | 2.00 | 2.175 |
| Sc5 | *Swertia chirayita* | 12.85 | 70.00 | 58.33 | 71.18 | 3.29 | 0.047 |
| | *Eupatorium cannabinum* | 86.59 | 36.67 | 30.56 | 117.15 | 49.00 | 1.336 |
| | *Golchidion acuminatum* | 0.56 | 13.33 | 11.11 | 11.67 | 2.25 | 0.169 |
| Sc6 | *Swertia chirayita* | 8.27 | 46.67 | 35.90 | 44.17 | 3.93 | 0.084 |
| | *Engelhardtia spicata* | 0.30 | 16.67 | 12.82 | 13.12 | 1.20 | 1.067 |
| | *Eupatorium cannabinum* | 90.23 | 46.67 | 35.90 | 126.12 | 49.79 | 2.100 |
| | *Hedychium spicatim* | 1.05 | 3.33 | 2.56 | 3.62 | 7.00 | 0.072 |
| | *Machiliu edulis* | 0.15 | 16.67 | 12.82 | 12.97 | 1.60 | 0.096 |
| Sc7 | *Swertia chirayita* | 61.81 | 53.33 | 61.54 | 123.35 | 34.50 | 0.647 |
| | *Betula alnoides* | 0.56 | 10.00 | 11.54 | 12.10 | 2.33 | 2.957 |
| | *Eupatorium cannabinum* | 37.63 | 23.33 | 26.92 | 64.55 | 69.00 | 0.233 |
| Sc8 | *Swertia chirayita* | 51.88 | 100.00 | 71.43 | 123.30 | 21.67 | 0.217 |
| | *Eupatorium cannabinum* | 47.73 | 33.33 | 23.81 | 71.53 | 59.80 | 1.794 |
| | *Hedychium spicatum* | 0.24 | 3.33 | 2.38 | 2.62 | 3.00 | 0.900 |
| | *Maesea chisia* | 0.16 | 3.33 | 2.38 | 2.54 | 2.00 | 0.600 |
| Sc9 | *Swertia chirayita* | 50.44 | 100.00 | 51.72 | 102.16 | 7.67 | 0.077 |
| | *Artemesia vulgaris* | 3.73 | 10.00 | 5.17 | 8.90 | 5.67 | 3.300 |
| | *Eupatorium cannabinum* | 21.71 | 10.00 | 5.17 | 26.88 | 33.00 | 0.567 |
| | *Gleichenia gigantean* | 0.88 | 3.33 | 1.72 | 2.60 | 4.00 | 0.133 |
| | *Osbeckia sikkimensis* | 19.74 | 50.00 | 25.86 | 45.60 | 6.00 | 0.120 |
| | *Polygonum molle* | 3.51 | 20.00 | 10.34 | 13.85 | 2.67 | 1.200 |
| Sc10 | *Swertia chirayita* | 13.92 | 60.00 | 25.71 | 39.63 | 2.72 | 0.045 |
| | *Artemesia vulgaris* | 5.68 | 50.00 | 21.43 | 27.11 | 2.13 | 0.250 |
| | *Astilbe rivularis* | 3.41 | 46.67 | 20.00 | 23.41 | 1.57 | 0.043 |
| | *Eupatorium cannabinum* | 76.99 | 76.67 | 32.86 | 109.85 | 19.17 | 0.034 |
| Sc11 | *Swertia chirayita* | 61.71 | 100.00 | 46.88 | 108.58 | 6.50 | 0.065 |
| | *Artemesia vulgaris* | 27.85 | 40.00 | 18.75 | 46.60 | 7.33 | 0.183 |
| | *Astilbe rivularis* | 1.27 | 10.00 | 4.69 | 5.95 | 1.33 | 0.133 |
| | *Selenium* sp. | 9.18 | 63.33 | 29.69 | 38.86 | 1.53 | 0.024 |
| Sc12 | *Swertia chirayita* | 78.38 | 100.00 | 43.48 | 121.86 | 10.63 | 0.106 |
| | *Aconogonum molle* | 8.60 | 33.33 | 14.49 | 23.09 | 3.50 | 0.150 |
| | *Astilbe rivularis* | 2.21 | 20.00 | 8.70 | 10.91 | 1.50 | 0.075 |
| | *Dichroa febrifuga* | 0.49 | 6.67 | 2.90 | 3.39 | 1.00 | 0.044 |
| | *Osbeckia sikkimensis* | 8.11 | 50.00 | 21.74 | 29.85 | 2.20 | 0.105 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Rubus ellipticus* | 2.21 | 20.00 | 8.70 | 10.91 | 1.50 | 0.075 |
| Sc13 | *Swertia chirayita* | 44.64 | 100.00 | 47.62 | 92.26 | 6.67 | 0.067 |
| | *Artemesia vulgaris* | 4.24 | 10.00 | 4.76 | 9.00 | 6.33 | 0.731 |
| | *Eupatorium cannabinum* | 24.55 | 23.33 | 11.11 | 35.66 | 15.71 | 0.112 |
| | *Osbeckia sikkimensis* | 16.29 | 46.67 | 22.22 | 38.52 | 5.21 | 0.084 |
| | *Pteris biaurita* | 8.71 | 13.33 | 6.35 | 15.05 | 9.75 | 0.633 |
| | *Rubus ellipticus* | 1.56 | 16.67 | 7.94 | 9.50 | 1.40 | 0.673 |
| Sc14 | *Swertia chirayita* | 43.38 | 33.33 | 38.46 | 81.84 | 5.90 | 0.177 |
| | *Gleichenia gigantean* | 25.00 | 13.33 | 15.38 | 40.38 | 8.50 | 0.100 |
| | *Hedychium spicatum* | 5.88 | 3.33 | 3.85 | 9.73 | 8.00 | 0.975 |
| | *Osbeckia sikkimensis* | 2.94 | 3.33 | 3.85 | 6.79 | 4.00 | 2.400 |
| | *Pteris biaurita* | 9.56 | 6.67 | 7.69 | 17.25 | 6.50 | 0.450 |
| | *Swertia bimaculata* | 8.82 | 20.00 | 23.08 | 31.90 | 2.00 | 0.638 |
| | *Utrica diocia* | 4.41 | 6.67 | 7.69 | 12.10 | 3.00 | 1.200 |
| Sc15 | *Swertia chirayita* | 36.36 | 100.00 | 33.33 | 69.70 | 5.07 | 0.051 |
| | *Artemesia vulgaris* | 21.53 | 26.67 | 8.89 | 30.42 | 11.25 | 0.041 |
| | *Dichroa febrifuga* | 1.44 | 10.00 | 3.33 | 4.77 | 2.00 | 0.067 |
| | *Eupatprium cannabinum* | 5.26 | 6.67 | 2.22 | 7.49 | 11.00 | 0.104 |
| | *Heracleum wallichii* | 0.24 | 3.33 | 1.11 | 1.35 | 1.00 | 1.650 |
| | *Osbeckia sikkimensis* | 10.53 | 46.67 | 15.56 | 26.08 | 3.14 | 0.200 |
| | *Rumex nepaulensis* | 10.05 | 36.67 | 12.22 | 22.27 | 3.82 | 0.422 |
| | *Swertia bimaculata* | 14.59 | 70.00 | 23.33 | 37.93 | 2.90 | 0.300 |
| Sc16 | *Swertia chirayita* | 33.58 | 100.00 | 35.29 | 68.87 | 6.17 | 0.062 |
| | *Aconogonum molle* | 9.62 | 26.67 | 9.41 | 19.03 | 6.63 | 0.332 |
| | *Arisaema sp.* | 2.18 | 23.33 | 8.24 | 10.41 | 1.71 | 0.073 |
| | *Artemesia vulgaris* | 25.77 | 46.67 | 16.47 | 42.24 | 10.14 | 0.056 |
| | *Eupatorium cannabinum* | 24.32 | 36.67 | 12.94 | 37.26 | 12.18 | 0.167 |
| | *Rubus ellipticus* | 0.91 | 10.00 | 3.53 | 4.44 | 1.67 | 0.248 |
| | *Rumex nepaulensis* | 2.72 | 30.00 | 10.59 | 13.31 | 1.67 | 0.167 |
| | *Utrica diocia* | 0.91 | 10.00 | 3.53 | 4.44 | 1.67 | 0.217 |
| Sc17 | *Swertia chirayita* | 37.19 | 100.00 | 43.48 | 80.66 | 8.90 | 0.089 |
| | *Anaphalis* sp. | 12.12 | 30.00 | 13.04 | 25.16 | 9.67 | 0.357 |
| | *Artemesia vulgaris* | 0.28 | 3.33 | 1.45 | 1.73 | 2.00 | 0.333 |
| | *Astilbe rivularis* | 0.28 | 6.67 | 2.90 | 3.18 | 1.00 | 0.322 |
| | *Eupatorium cannabinum* | 42.48 | 53.33 | 23.19 | 65.67 | 19.06 | 0.150 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Osbeckia sikkimensis* | 1.39 | 10.00 | 4.35 | 5.74 | 3.33 | 0.600 |
| | *Pteris biaurita* | 5.57 | 20.00 | 8.70 | 14.27 | 6.67 | 0.333 |
| | *Rhododendron. cilliatum* | 0.70 | 6.67 | 2.90 | 3.59 | 2.50 | 0.375 |
| Sc18 | *Swertia chirayita* | 29.91 | 33.33 | 33.33 | 63.24 | 6.70 | 0.201 |
| | *Anaphalis sp.* | 16.52 | 13.33 | 13.33 | 29.85 | 9.25 | 0.297 |
| | *Eupatorium cannabinum* | 44.20 | 33.33 | 33.33 | 77.53 | 9.90 | 0.467 |
| | *Strobilanthus* sp. | 3.13 | 10.00 | 10.00 | 13.13 | 2.33 | 0.694 |
| | *Utrica diocia* | 6.25 | 10.00 | 10.00 | 16.25 | 4.67 | 0.233 |
| Sc19 | *Swertia chirayita* | 69.87 | 100.00 | 65.22 | 135.08 | 7.03 | 0.070 |
| | *Anaphalis sp.* | 25.83 | 33.33 | 21.74 | 47.57 | 7.80 | 0.131 |
| | *Rhododendron* sp. | 1.99 | 6.67 | 4.35 | 6.33 | 3.00 | 0.234 |
| | *Rubus ellipticus* | 2.32 | 13.33 | 8.70 | 11.01 | 1.75 | 0.450 |
| Sc20 | *Swertia chirayita* | 55.97 | 100.00 | 50.00 | 105.97 | 8.43 | 0.084 |
| | *Anaphalis* sp. | 44.03 | 100.00 | 50.00 | 94.03 | 6.63 | 0.066 |
| Sc21 | *Swertia chirayita* | 13.23 | 70.00 | 27.63 | 40.86 | 6.38 | 0.091 |
| | *Anaphalis* sp. | 4.44 | 16.67 | 6.58 | 11.02 | 9.00 | 0.519 |
| | *Eragrostis atrovirens* | 18.26 | 50.00 | 19.74 | 38.00 | 12.33 | 0.133 |
| | *Eupatorium cannabinum* | 61.60 | 63.33 | 25.00 | 86.60 | 32.84 | 0.120 |
| | *Osbeckia sikkimensis* | 0.39 | 10.00 | 3.95 | 4.34 | 1.33 | 0.247 |
| | *Pteris biaurita* | 0.99 | 16.67 | 6.58 | 7.57 | 2.00 | 0.113 |
| | *Rubus ellipticus* | 0.49 | 13.33 | 5.26 | 5.76 | 1.25 | 0.094 |
| | *Strobilanthus* sp. | 0.59 | 13.33 | 5.26 | 5.86 | 1.50 | 0.540 |
| Sc22 | *Swertia chirayita* | 77.07 | 70.00 | 53.85 | 130.91 | 13.14 | 0.188 |
| | *Aconogonum molle* | 0.27 | 3.33 | 2.56 | 2.83 | 1.00 | 0.215 |
| | *Anaphalis* sp. | 1.87 | 6.67 | 5.13 | 6.99 | 3.50 | 0.300 |
| | *Eragrostis atrovirens* | 15.47 | 30.00 | 23.08 | 38.54 | 6.44 | 0.150 |
| | *Eupatorium cannabinum* | 2.13 | 6.67 | 5.13 | 7.26 | 4.00 | 2.700 |
| | *Osbeckis sikkimensis* | 0.27 | 3.33 | 2.56 | 2.83 | 1.00 | 0.300 |
| | *Pteris biaurita* | 2.40 | 3.33 | 2.56 | 4.96 | 9.00 | 0.525 |
| | *Rubus ellipticus* | 0.53 | 6.67 | 5.13 | 5.66 | 1.00 | 0.600 |

RD: relative density; RF: relative frequency; IVI: important value index; Ab: abundance; A/F: abundance to frequency ratio

**Table 3.** Diversity and dominance pattern of *Swertia chirayita* pockets in Sikkim

| Site code | Species richness | Community density (ind/m$^2$) | Shannon - Wiener diversity Index | No. microhabitat / site |
|---|---|---|---|---|
| Sc1 | 9.0 | 31.33 | 0.71 | 6.0 |
| Sc2 | 12.0 | 28.03 | 0.89 | 5.0 |
| Sc3 | 6.0 | 17.00 | 1.20 | 8.0 |
| Sc4 | 6.0 | 16.93 | 1.82 | 7.0 |
| Sc5 | 3.0 | 20.57 | 6.52 | 6.0 |
| Sc6 | 5.0 | 25.76 | 8.75 | 4.0 |
| Sc7 | 3.0 | 34.73 | 1.44 | 5.0 |
| Sc8 | 4.0 | 41.77 | 1.44 | 5.0 |
| Sc9 | 6.0 | 15.20 | 1.02 | 6.0 |
| Sc10 | 4.0 | 18.13 | 3.93 | 1.0 |
| Sc11 | 4.0 | 10.53 | 1.54 | 1.0 |
| Sc12 | 6.0 | 13.56 | 3.30 | 7.0 |
| Sc13 | 6.0 | 14.94 | 0.87 | 5.0 |
| Sc14 | 7.0 | 4.54 | 0.82 | 4.0 |
| Sc15 | 8.0 | 13.94 | 0.69 | 7.0 |
| Sc16 | 8.0 | 18.37 | 0.73 | 4.0 |
| Sc17 | 8.0 | 23.93 | 0.95 | 7.0 |
| Sc18 | 5.0 | 7.46 | 0.91 | 1.0 |
| Sc19 | 4.0 | 10.06 | 2.15 | 4.0 |
| Sc20 | 2.0 | 15.06 | 1.50 | 2.0 |
| Sc21 | 8.0 | 33.77 | 1.46 | 5.0 |
| Sc22 | 8.0 | 12.50 | 3.06 | 6.0 |

**Table 4.** Types and intensity of existing disturbances in different populations of *Swertia chirayita* in Sikkim Himalaya

| Sites | Types of disturbance | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grazing | | | Fodder collection | | | Fuel hood collection | | | NTFP collection | | | Agriculture | | | Road cleaning / clearing | | | Human movement | | | Chirayita collection | | | Others (landslides, etc) | | |
| | Intensity of disturbance | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | L | M | H | L | M | H | L | M | H | L | M | H | L | M | H | L | M | H | L | M | H | L | M | H | L | M | H |
| 1 | | | | ● | | | | | | ● | | | | | | | | | ● | | | | | | | | |
| 2 | | | | | | ● | | | | ● | | | | | | | | | | | ● | ● | | | | | |
| 3 | | | | | | | | | | ● | | | | | | | | | | ● | | | | | | | |
| 4 | | | | | | | | | | | ● | | | | | | | | | ● | | | | | | | |
| 5 | | | | | | ● | | | ● | | | ● | | | | | | | | | ● | | ● | | | | |
| 6 | | ● | | | | ● | | | ● | | | | | | ● | | | | | | ● | | | | | | |
| 7 | | | | | | | | | | | | | | | | | | ● | | | ● | | | | | | |
| 8 | | | | | | | | | | | | | | | | | | ● | | | ● | | | | | | |
| 9 | | | | | | ● | | | ● | | ● | | | | | | | | | | ● | | | | | | |
| 10 | | | | | | | | | | | | | | | | | | ● | | | ● | | ● | | | | |
| 11 | | | | | | | | | | | | | | | | | | ● | | | | | | | | | ● |
| 12 | | | | ● | | | | ● | | | | ● | | | | | | | | | ● | | ● | | | | |
| 13 | | | | | | | | | | ● | | | | | | | | ● | | | | | | | | | ● |
| 14 | | | | | | ● | | | ● | | | ● | | ● | | | | | | | ● | | | ● | | | |
| 15 | ● | | | ● | | | ● | | | | ● | | | | | | | | ● | | | ● | | | | | |
| 16 | | | | | ● | | | ● | | | | ● | | | | | | | | | ● | | | ● | | | |
| 17 | | | | | | | ● | | | | | | | | | | | | | | ● | | | | ● | | |
| 18 | | | | | | ● | | | ● | ● | | | | | | | | | ● | | | | | ● | | | |
| 19 | | ● | | | | ● | | | ● | | ● | | | | ● | | | | | | ● | | ● | | | | |
| 20 | | | | | | ● | | | ● | | | ● | | | | | | ● | | | ● | | ● | | | | |
| 21 | | | | | | | | | | | | | | | | | | | ● | | | | | | | | |
| 22 | | | | | | | | | | | | ● | | | | | | | ● | | | ● | | | | | |

L = low, M = moderate, H = high

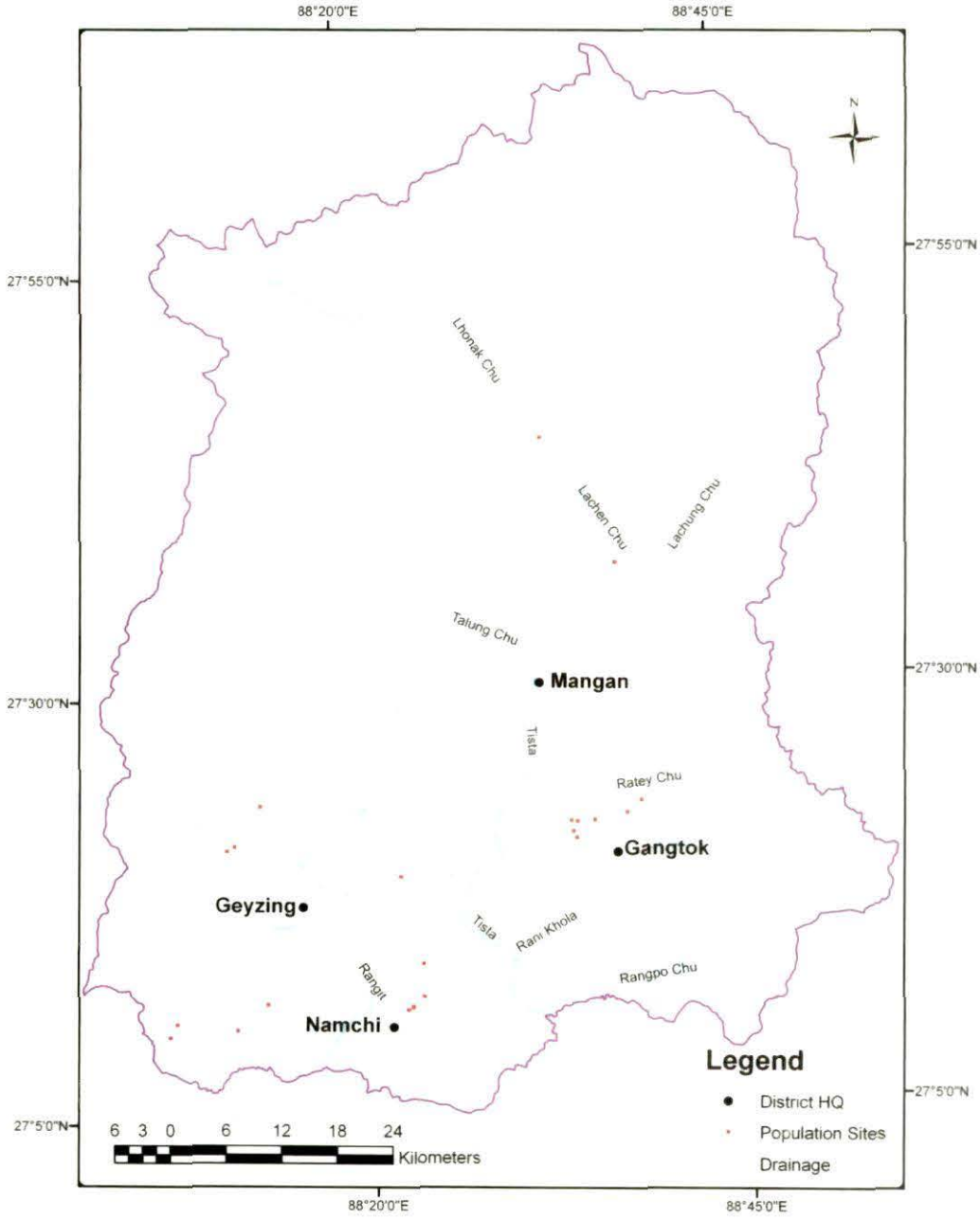



**Figure 2.** Map showing the studied sites for *Swertia chirayita* in Sikkim, India

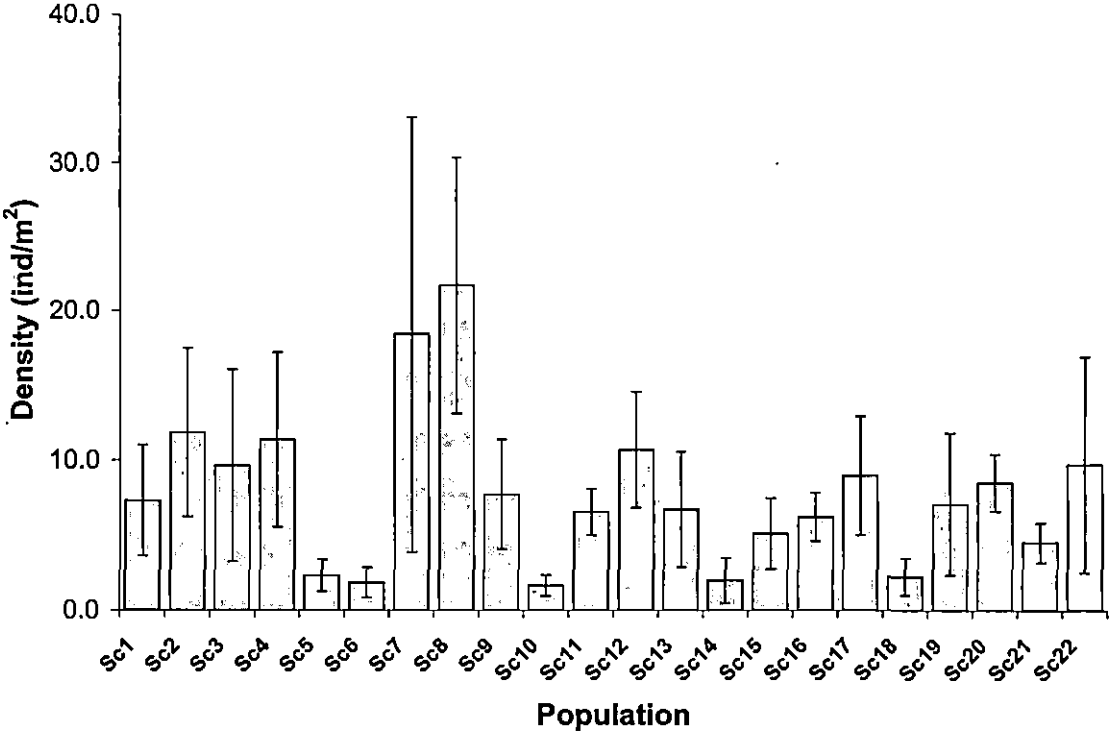



**Figure 3.** Distribution pattern of *Swertia chirayita* in selected population in Sikkim

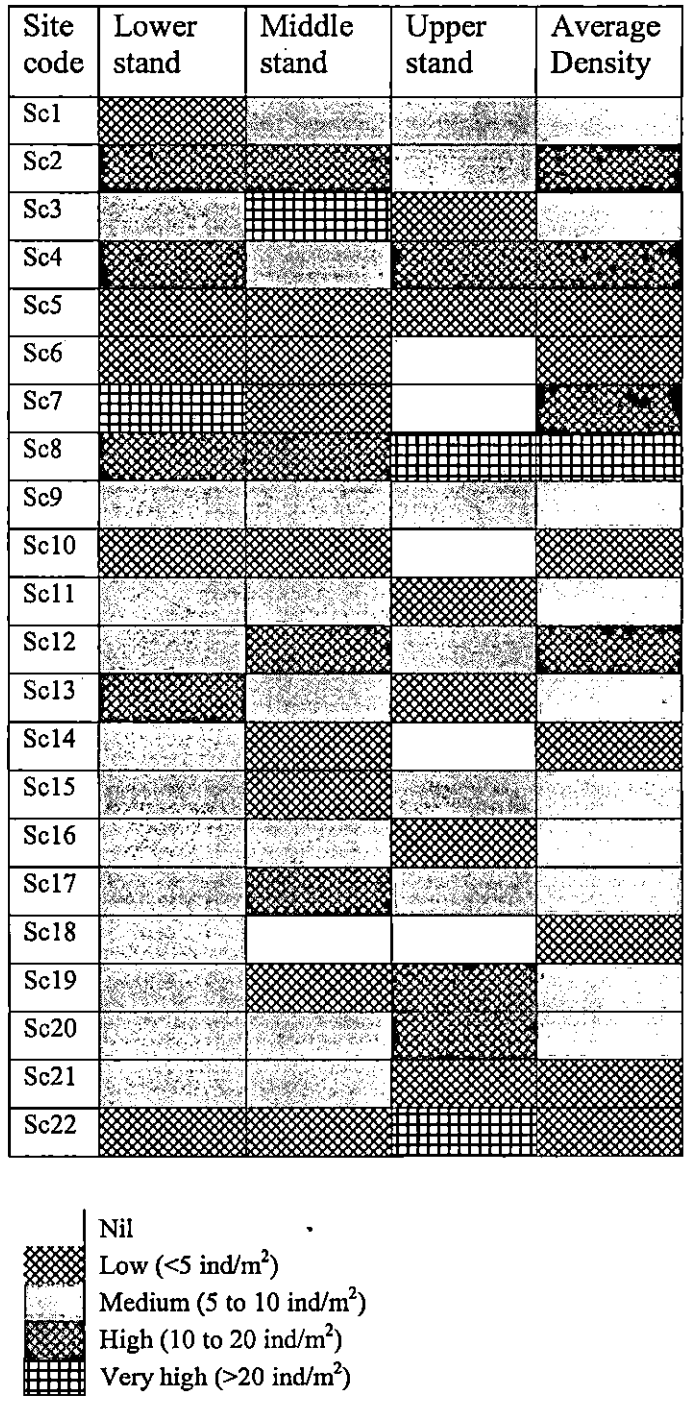



**Figure 4.** Distribution pattern of *Swertia chirayita* in selected population in Sikkim

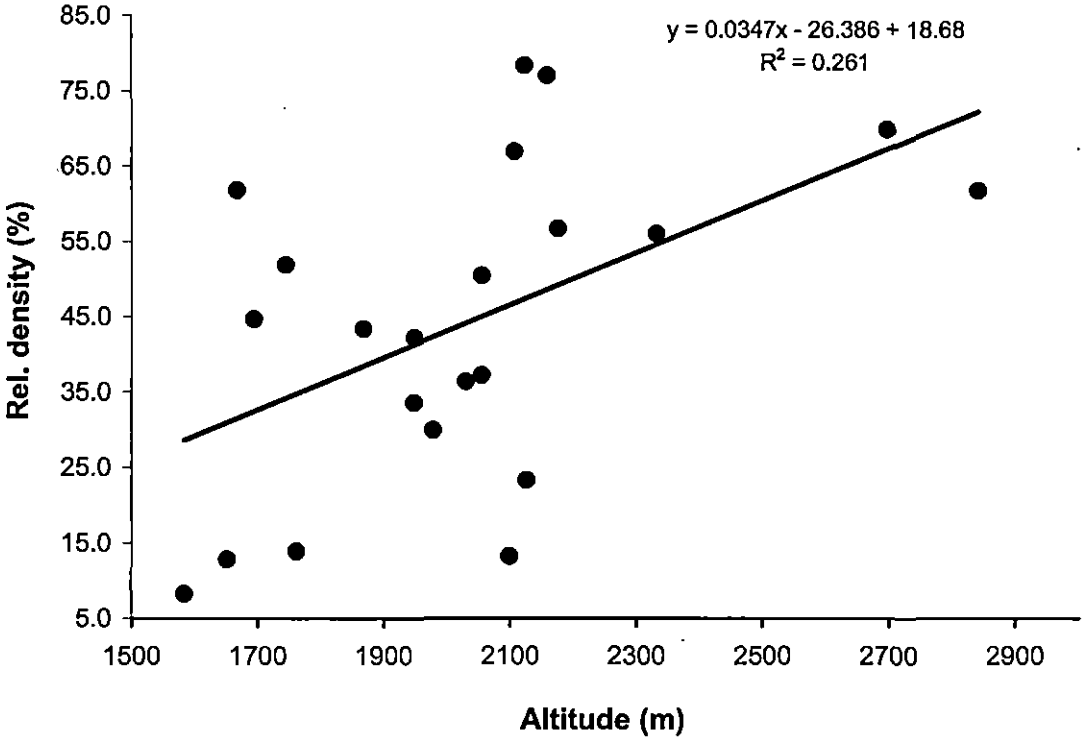



**Figure 5.** Availability of *Swertia chirayita* along altitudinal gradient in Sikkim

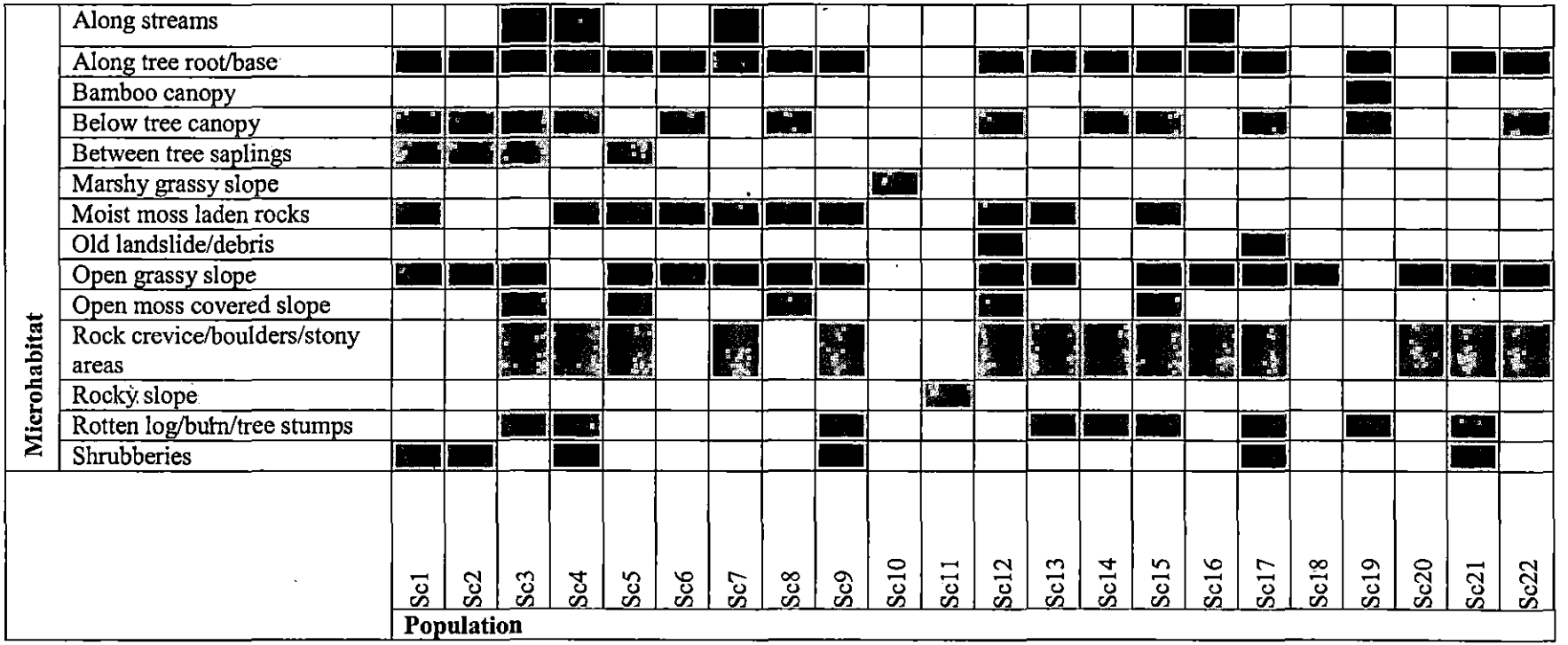



**Figure 6.** Occurrence of microhabitat of *Swertia chirayita* in different populations of Sikkim

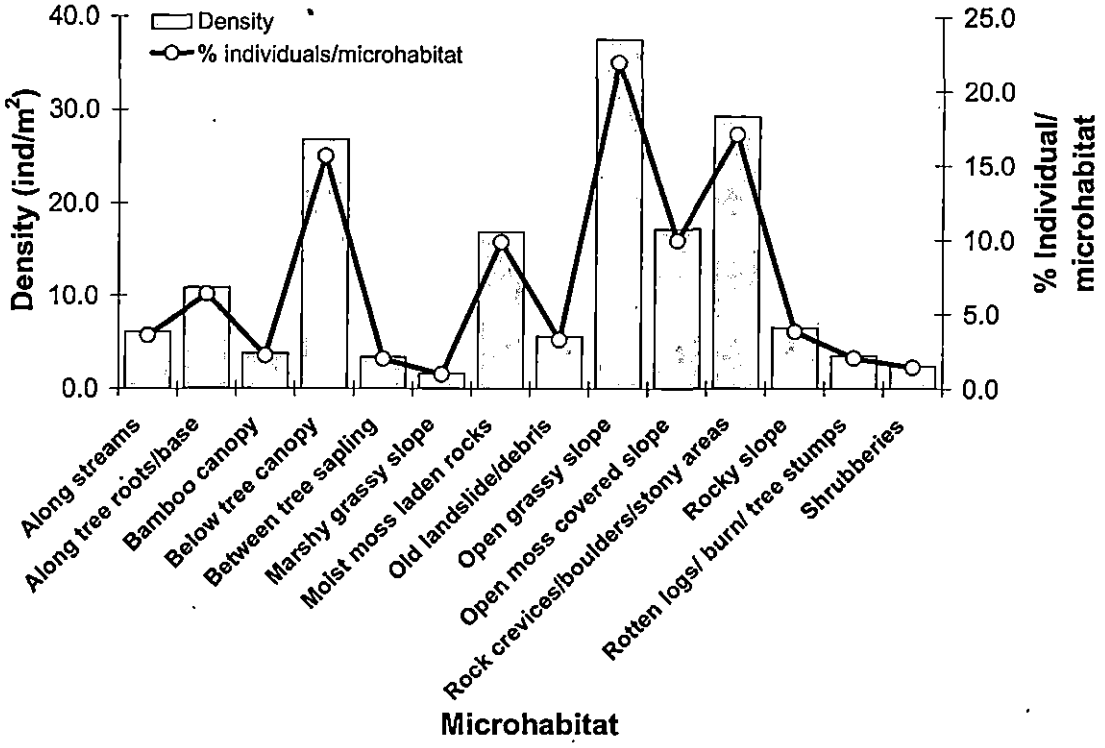



**Figure 7.** Distribution pattern of *Swertia chirayita* in identified microhabitats in Sikkim

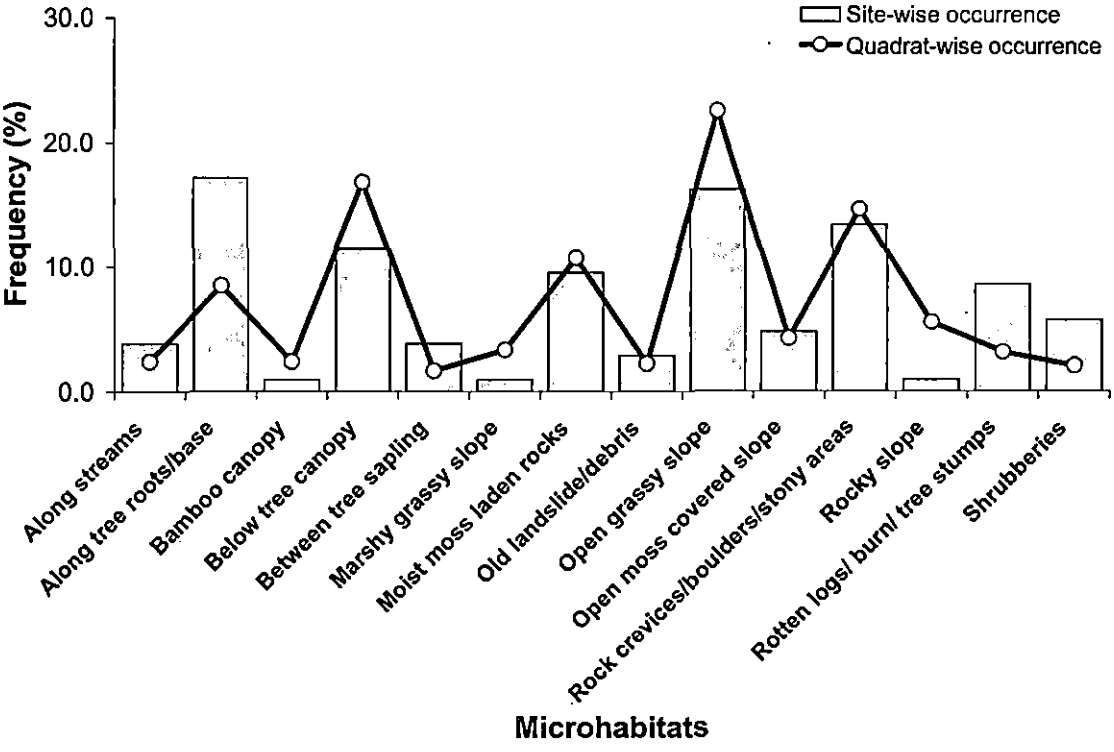



**Figure 8.** Frequency of occurrence of microhabitat of *Swertia chirayita* in Sikkim

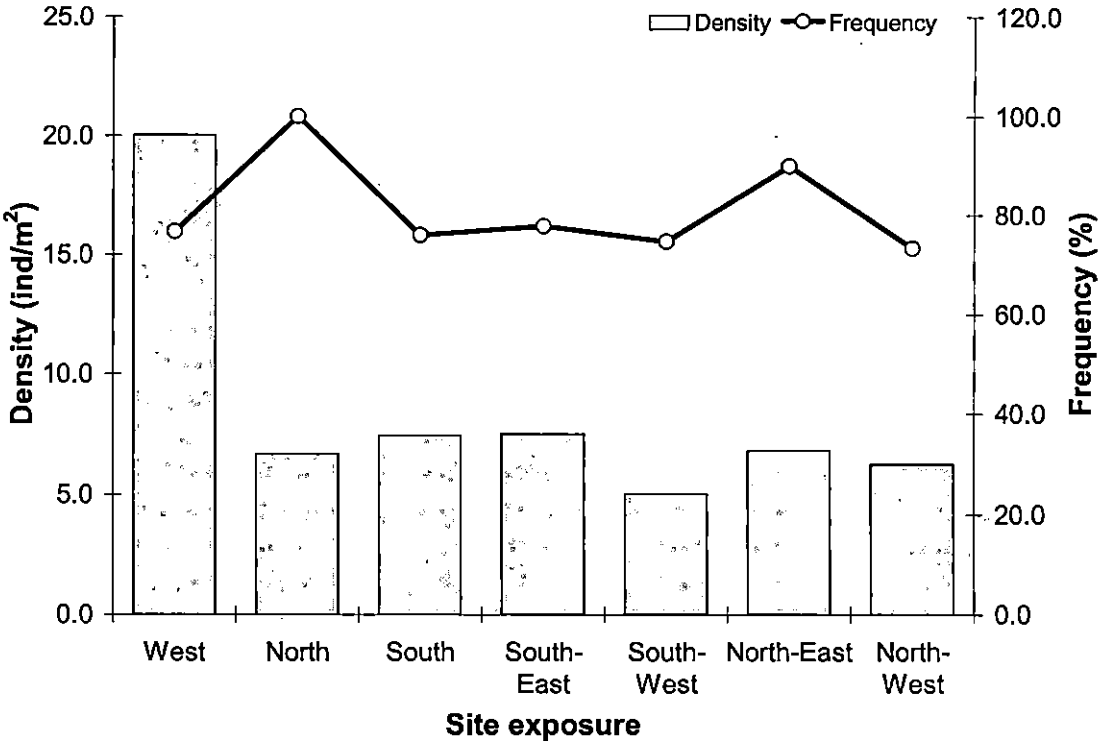



**Figure 9.** Effect of site exposure on the availability of *Swertia chirayita* in Sikkim

CHAPTER 5

# Morphological Variability amongst Populations

## 5.1 Introduction

Variation in plant morphology, influenced by geography, is a function of phenotypic changes in response to local environmental conditions, genetic variation and evolution among populations, and the biogeographic history of an individual species (Ellison *et al.*, 2004). Morphological variation and geographical separation among populations are equally imperative to the formation of subspecies and species (Losos and Glor, 2003). The environment, viz., light condition, climate, and competition with other species (Tesitel and Stech, 2007) is a governing factor in determining population differentiation affecting morphological features in a species and evolvement of specialized populations adapted to particular environment (Lynn and Waldren, 2001); however, the altitude and seasonal variation are amongst other major responsible factors. Assessment of differences in morphology is the traditional way of determining diversity within and between plant populations (Bayorbor *et al.*, 2010). The existence of localized populations each adapted to the particular environmental conditions of their habitat have been revealed in many plant species (Turesson, 1922; Bradshaw, 1960, 1991; McNeilly, 1968; Kik *et al.*, 1990; Geber et al., 1992; Badola and Pradhan, 2010a, 2010). Such adaptation to specific environmental conditions can occur over a relatively short span of time (Lynn and Waldren, 2001), however, such species preferring specific habitat are much prone to habitat loss and extinction than the species with broad habitat range (Badola and Aitken, 2003; Samant *et al.*, 1996). In addition, the habitat destruction and fragmentation are the major causes restricting the natural populations of important plant species to smaller pockets (Parab and Krishnan, 2008) *vis-à-vis* species extinction (Ehrlich and Ehrlich, 1981). Deleterious effects on the genetic diversity within a species can be resulted due to fragmentation of natural plant communities, because there will be a decrease in levels of gene flow, particularly over longer distances (Ouinsavi and Sokpon, 2010). The subsequent effects of genetic drift in small, isolated populations will be the loss of diversity,

inability of plants to adapt to changes in their environment and ultimately increasing the risk of extinction (Wilson and Provan, 2003). At this juncture, it would be vital to know if such fragmentation leads to inter population variation. In addition increasing human populations and land use intensification (e.g., cultivation, grazing, and urban development) have (i) resulted in the loss and subdivision of native habitats, (ii) increased species extinction rates, and (iii) lowered species diversity (Ouinsavi and Sokpon, 2010). Such circumstances call for the establishment of a strategy to conserve the important species *in-situ* (Karam *et al.*, 2006) which will provide an opportunity for the researchers to understand the evolutionary processes of the plants (Arafeh *et al.*, 2002). In addition, an evaluation of the germplasm in nature generally provides an idea about suitable climatic conditions for domestication and cultivation of an important plant species; and, on the basis of phenotypic traits, the elite germplasm can be identified for crop improvement (Vashistha *et al.*, 2006).

*Swertia chirayita*, a high value medicinal herb, has huge demand in the national and international market (Badola and Pal, 2002). Its increasing demand has led to unsustainable harvesting from nature resulting in restricting its distribution to small pockets (ecodemos) and specific habitats in the Himalayan belts. These habitats are being destroyed at an alarming rate due to natural as wells as man made calamities resulting in changes in the immediate environment. Also, the species has wide range of altitudinal distribution in Sikkim from 1500m to 3000m and is not unusual to undergo changes within the species to adapt itself to varying climatic conditions. This necessitates evaluating the forgone changes within and amongst the populations of *S. chirayiata* to adapt itself to changing environmental conditions. The present study was undertaken with broad aims to, (1) assess the most discriminating morphological character for evaluating variations amongst the natural populations in *S. chirayita*; (2) based on morphological traits, the identification of superior germplasm for domestication, crop improvement and conservation of the species; (3) determination of appropriate morphological trait to evaluate the below ground biomass; (4) assess the adaptive mechanism to withstand the varying climatic conditions. The outcome will provide helpful clues and suitable approaches for the sustainable use of the herb as medicine in future, as well as for the *in-situ* conservation of the species.

## 5.2 Materials and Methods

### 5.2.1 Material

Morphometrical data were collected from 22 natural populations covering all the four districts (east, west, north and south) in Sikkim. Two forms (rosette and reproductive) of *S. chirayita* plants were collected during the end of the growing season (November-December) [Table 5 and Table 6]. From each population, 9 plants each (both form) were randomly selected maintaining at least 2-3 meter distance between each individual plant to avoid sampling clones. For each population, the broad habitat and other topographical features were observed. The altitude, aspect and slope of the site, including latitudinal and longitudinal coordinates and humus depth were recorded (refer chapter 4).

### 5.2.2 Morphological characters

In the laboratory, the plant materials were thoroughly washed to remove the soil particles and wiped with tissue roll to remove the additional moisture. In the case of rosette form, the morphological characters measured included collar diameter, root diameter, number of leaves, largest leaf length, largest leaf width, root length while in the case of reproductive form, plant height, stem basal diameter and number of branches in addition to the above characters, were taken into account. After recording the fresh weight, the collected materials were oven dried at $70^0$C to constant weight in order to obtain the plant biomass.

### 5.2.3 Statistical analysis

The SPSS 10.0 for windows (SPSS Inc. 1989) was used for statistical interpretations of the data. Multivariate analysis of variance (MANOVA) was conducted to determine the variation and if significant variation existed, Bonferroni ($p<0.05$) test was performed to test the significant difference in means of the recorded growth parameters amongst populations. The coefficient of variation for morphological characters was calculated for each population. Canonical discriminant analysis (CDA) was used to determine the most variable morphological character marking differences amongst populations. Based on the average linkages (between groups) and Pearson's

correlation, a dendrogram was constructed to evaluate the levels of variation amongst populations. Pearson's correlation coefficient analysis was performed to evaluate the correlation amongst the different morphological characters in both rosette and reproductive form; however, here, the populations were not taken into account.

## 5.3 Results

In the case of the rosette form of plants, the recorded morphological characters in *S. chirayita* except the number of leaves were positively correlated with altitude but showed negative correlation with the humus depth; the growth parameters were positively correlated with soil pH except leaf length (Table 7). Similarly, in reproductive form, all the recorded morphological characters showed positive correlation with the altitude except for the plant height and number of branches; however, the parameters were negatively correlated with the humus depth and positively correlated with soil pH (Table 7). In terms of total plant biomass, altitude and soil pH showed positive correlation while humus depth showed negative correlation in rosette form of *S. chirayita*. In reproductive form, altitude, humus depth and soil pH had positive effect on the total plant biomass (Table 7).

Variability in microhabitat condition has been observed in each of the sites in the present study which resulted in differences in morphological characters amongst different populations in *S. chirayita*. All the recorded morphological characters are presented in Table 5 (rosette form) and Table 6 (reproductive form). One way multivariate analysis of variance (MANOVA) revealed a significant ($p<0.0001$) variation in the recorded morphological characters including total plant dry biomass amongst populations in both rosette and reproductive forms in *S. chirayita* (Table 8). The collar diameter ranged from $2.73 \pm 0.4$ (Sc5) to $7.99 \pm 1.1$mm (Sc21) in rosette form; similarly, the value ranged from $4.82 \pm 1.1$mm (Sc1) to $10.38 \pm 2.1$mm (Sc17) in reproductive from in *S. chirayita*; however, the difference was significant ($p<0.05$) in both the forms. In terms of root diameter, Sc5 recorded the lowest value ($2.3 \pm 0.2$mm) while Sc21 recorded the highest value ($6.64 \pm 0.9$mm) in rosette form; the value was lowest for Sc22 ($4.22 \pm 0.8$) and highest for Sc17 ($10.98 \pm 3.6$mm) and the difference was significant ($p<0.05$) in both forms. In rosette form, the root length

ranged from 41.66 ± 6.7mm (Sc16) to 539.78 ± 154.7mm (Sc20); while in reproductive form, the root length ranged from 104.44 ± 15.9 (Sc4) to 358.89 ± 103.6mm (Sc1) and the difference was significant ($p<0.05$) in either from of *S. chirayita*. The number of leaves was significantly ($p<0.05$) high in Sc8 (15.56 ± 2.8) and Sc1 (15.0 ± 2.2) compared to lowest record for Sc12 (7.78 ± 2.0) in rosette form; similarly, in reproductive form, Sc6 recorded significantly (246.56 ± 151.2; $p<0.05$) highest number of leaves compared to all the populations except Sc17, for which the value was insignificant. The lowest value for the largest leaf length (31.05 ± 12.7mm) and leaf width (17.68 ± 1.8mm) recorded for Sc2 was significantly ($p<0.05$) lower than majority of the populations in rosette form; nevertheless, in reproductive form, the largest leaf length ranged from 55.19 ± 14.1mm (Sc22) to 189.53 ± 18.9mm (Sc11) and the largest leaf width ranged from 20.89 ± 2.2mm (Sc2) to 50.93 ± 6.8mm (Sc17) and the difference was significant ($p<0.05$) for both leaf length and width. In terms of plant height, Sc17 recorded the maximum plant height (134.1 ± 23.7cm) followed by Sc18 (121.0 ± 24.2cm), Sc5 (120.0 ± 5.7cm), and Sc20 (110.4 ± 17.7cm) and the value was significantly ($p<0.05$) greater compared to Sc11 (20.11 ± 1.7cm) and Sc 4 (39.56 ± 6.6cm), which recorded the minimum plant height than other populations (Table 6). Significant ($p<0.05$) difference in stem basal diameter was recorded between the highest record for Sc11 (7.58 + 1.9mm) and the lowest for Sc7 (2.69 + 0.2mm). In rosette form, in respect of total biomass, the highest value was recorded for Sc20 (4.8 + 1.9) and Sc17 (4.73 + 1.4); however the difference was insignificant compared to other populations (Figure 10). In reproductive form, significantly ($p<0.05$) highest total plant biomass (17.79 + 7.9gm) was recorded for Sc17 compared to all other populations (Figure 10); similarly, the high total plant biomass (10.66 + 12.2) recorded for Sc3 was significant ($p<0.05$) compared to majority of the populations.

In rosette form (Figure 11A, 112B), of the six morphological character recorded, most variations were observed in the root length with coefficient of variation ranging from 14.23% to 69.58% followed by root diameter (4.66% to 48.06%). Largest leaf length (3.62% to 40.89%) and largest leaf width (4.92% to 40.80%) subsequently followed the root diameter. The number of leaves showed lowest coefficient of variation value

(8.75% to 32.18%) followed by collar diameter with coefficient of variation value ranging from 7.07% to 37.37%. In reproductive form (Figure 12A, 12B, 12C), of the nine morphological characters identified, the number of branches recorded the highest coefficient of variation value ranging from 0% to 84.16% followed by number of leaves (8.59% to 83.65%) and root diameter (4.78% to 55.79%). The coefficient of variation value ranged from 3.24% to 43.30% (plant height), 3.45% to 49.95% (largest leaf length), 3.51% to 40.63% (largest leaf width) and 9.31% to 47.14% (root length). The lowest coefficient of variation value was recorded for the stem basal diameter (4.97% to 38.24%), followed by collar diameter (6.63% to 41.17%). In terms of total plant biomass, highest variation was recorded for Sc3 (reproductive) and Sc21 (rosette) while the lowest variation was observed in Sc18 (reproductive) and Sc10 (rosette) [Figure 13].

A cluster analysis, based on average linkage (between group) method and Pearson's correlation, divided the entire populations into three clusters in rosette (Figure 14A) and six clusters in reproductive form (Figure 14B) of *S. chirayita*. All the recorded morphological characters, except few, in both rosette (Table 9) and reproductive form (Table 10) in *S. chirayita* showed positive correlation with each other. In rosette form, the result of canonical discriminant analysis (CDA) indicated that, the morphological characters viz., leaf length; root length and collar diameter was the main discriminating character and were mainly found to be responsible for the total variation amongst the populations in *S. chirayita* (Table 11). The three morphological characters cumulatively accounted for 87.7% of the total variation. Leaf length alone accounted for 50.7% of the total variation; root length resulted for 20.7% variation and collar diameter accounted for 16.3% variation. Similarly, in reproductive form, the plant height, number of leaves, leaf width and stem basal diameter were mainly responsible for the total variations amongst the populations in *S. chirayita*, which cumulatively accounted for 83.6% variation (Table 12). On individual basis, the plant height, number of leaves, leaf width and stem basal diameter accounted for 42.6%, 19.6%, 13.0% and 8.5% variations.

## 5.4 Discussion

The microhabitat has direct influence on plant growth and vigour; at the same time soil pH and humus depth provides a good indication of the chemical status of soil and can be used to determine the plant growth potential. Increasing soil pH (Martensson, 2010) and altitude (Vare, 1997) decrease the soil nutrient. This might be the reason for positive correlation between the total plant biomass with increasing altitude and soil pH in the present study with *S. chirayita*. Several limiting factors, viz., altitude, competition amongst species for limited resources, disturbance, etc., regulate growth in plant species as observed in the present study. Plant height and altitude are negatively correlated (Kofidis et al., 2007); however, at the same time, the plant height and leaf number provide a measure of photosynthetic and transpiration area and are indicators of the plant quality (Ritchie, 1984). The statement is insufficiently suggestive for the present study because plant species modifies itself to adapt to the changing environment. Even though, in the present study, reduction in plant height and leaf number in *S. chirayita* was observed with increasing altitude; nevertheless, increase in the collar size, root size and leaf size (leaf length and width), which itself are the indicators of healthy plants, with altitude was recorded in both rosette and reproductive form. The positive correlation of the collar diameter and root diameter with below ground biomass further strengthens present findings that the plant quality does not merely depend on plant height and leaf number. Further, the decrease in the photosynthetic and transpiration area caused by reduction in plant height and number of leaves with altitude is nullified by increase in the leaf size (leaf length and width) with altitude.

In rosette form, population Sc5 which occupied bouldery habitat (refer chapter 4) recorded smallest value for collar diameter and root diameter; however, growth in terms of plant height was comparatively high in reproductive form. In adverse microhabitat conditions, plant species are prone to competition for limited available resources; however, the surviving plant species emerges as successful competitor and colonizer as it matures (refer chapter 6). This indicates that the *S. chirayita* is a successful competitor. At the same time, Sc21 which occupied forest-shrubbery

habitat recorded the high value for collar diameter and root diameter in both rosette and reproductive form; but, recorded smaller plant height revealing its sensitivity to habitat condition, as the denseness of the forest prohibited the *S. chirayita* plants from acquiring required amount of sunlight needed for photosynthesis and growth. As an adaptation to high radiation, lesser number of leaves were recorded in Sc12 followed by Sc3, which occupied completely open slope where the plants are directly exposed to the sunlight resulting in high water loss; whereas, Sc8 followed by Sc1 recorded the highest number of leaves, as these two population had occupied forest-shrubbery habitat where the shade of the nurse plant lessens rate of water loss. In reproductive form, the lowest number of leaf in Sc4 is the result of deficiency of the required nutrient and absence of sunlight as the population inhabited streamside and under dense canopy of *Cryptomeria japonica*; similarly, lower number of leaves in Sc13 is the result of disturbance in the form of frequent landslides in the study site.

Disturbance in any form inhibits plant growth and has been experienced in Sc2 with having smallest leaf size (leaf length and width) where fodder collection, trampling, water logging, *S. chirayita* collection (for domestic use) etc., were the major threats observed; similarly Sc22 recorded the smallest leaf size as the population was recovering from the disturbance caused by intense grazing in the past. As an adaptive mechanism to less soil nutrient, Sc20 followed by Sc17 recorded the longest root length because Sc20 occupied the habitat which was dominated by dense *Cryptomeria japonica* trees under which the survival of the plant species is almost impossible, and Sc17 occupied the land-slip naked slope contributing to the high total biomass. The smallest root length in Sc4 could be related to soil nutrient deficiency. In mountainous region, strong altitudinal gradient leads to dramatic variation in environmental conditions (Hong-Li *et al.*, 2009). With the increasing altitude, more environmental stresses, viz., extreme radiation, wind speed, etc., are created which greatly affects the plant growth and leaf size (Li *et al.*, 2008). Similar observations have been made in the present study where plant height decreased with altitude, as observed in other species (Bhadula and Purohit 1994; Bhadula *et al.*, 1996; Kofidis *et al.*, 2007). Nevertheless, the leaf size increased with increasing altitude; Sc11 located at highest altitude (2841m asl) of plant collection site recorded the lowest plant height in *S.*

*chirayita* appeared as an adaptation to extreme climatic conditions. Cumulatively, the vigorous growth and biomass was observed in Sc17 followed by Sc3 as both the populations occupied open habitat indicating this as the most suitable and preferred habitat by *S. chirayita.*. Nonetheless, less growth and biomass in Sc4 of all the population may be due to the reason that the population was located near to the stream and under dense canopy of *Cryptomeria japonica* forest creating stressful condition for *S. chirayita*. Similarly, the little growth and lower biomass in Sc9 and Sc10 was observed as both the population occupied highly disturbed area in the form of landslide, fuel-fodder collection, etc.

Cluster analysis revealed variability in morphological characters amongst the populations of *S. chirayita* in Sikkim which is further supported by difference in coefficient of variation in morphological characters within populations, indicating the role of topography, climate and microhabitat. The habitat colonized by *S. chirayita* differs from each other with regard to altitude, light, soil humus, soil moisture, slope and aspect as it is assumed that the ecological factors strongly affects genetic variation within and among populations (Nevo *et al.*, 1988; Nevo and Beiles, 1989; Kolliker *et al.*, 1998; Al-Saghir *et al.*, 2009). Location of the site may be responsible for non-clustering of Sc6 (reproductive form) and Sc16 (rosette form) with other populations. For example, Sc6 was located to near the human habitation which always faces the pressure to some extent in one or the other way. Disturbance in the form of forest litter collection for agricultural purpose, fuel and fodder collection results in trampling, which prohibits the plants from growth and development as different types of land-use practices contribute to the variation amongst populations (Poschlod and Jackel, 1993; Poschlod *et al.*, 2000). The altitude could be another factor as Sc6 occurred at the lowest average altitude (1583m) compared to other populations. Similarly, Sc16 was located at the landslide prone area where the plant completing its phenological cycle is merely by chance. In addition, fungal attack due to high moisture (Moles and Westoby, 2004ab) and plant litter (Xiong and Nilsson, 1999) under dense *Quercus lamellose* forest and the continuous flowing stream has negative impact on establishment of *S. chirayita* plants as it prefers the open dry slope, which is clear from the negative correlation of all the morphological characters with the

humus depth in either forms of *S. chirayita*, except for the number of leaves (reproductive form). The smallest root length in Sc16 further strengthens the impact of high soil nutrients on *S. chirayita* plants. Nonetheless, on the basis of cluster analysis, it is clear that when *S. chirayita* is in rosette stage, the plants marks more similarity; but, as when plant attains adult stage, the existing dissimilarity amongst populations becomes more prominent which emphasizes that reproductive stage is more important for exploring the existing morphological variations amongst populations of *S. chirayita*.

The present study revealed that, amongst different populations of *S. chirayita*, discriminating character responsible for total variation was different for the rosette and reproductive forms. The leaf length was the most discriminating character in the rosette form and the plant height emerged as the most discriminating character in reproductive form. However, it is suggested that, for identifying such character in *S. chirayita*, fully developed plant should be considered because in such plants, further development is not possible, however, as in rosette plants the growth process is continued. On the basis of above revelation, plant height emerged as the most appropriate indicator for identifying existing morphological variations amongst populations of *S. chirayita*. Further, the negative correlation between the plant height and the altitude of the site of plant collection revealed that the plant height vary with altitude supporting the above statement.

## 5.5 Conclusion

The present study concludes the following:

1. Several modifications undergoes in *S. chirayita* to adapt to the varying environmental conditions.
2. For evaluating the morphological variations amongst populations, fully matured plants rather than rosette individuals should be considered in *S. chirayita*.
3. Collar diameter is the good indicator for determining root biomass without undergoing destructive harvesting in *S. chirayita*.

4. Plant height is the most appropriate indicator that can be considered for identifying existing morphological variations amongst populations in *S. chirayita*.
5. Of several studied populations, Sc17 followed by Sc3 can be considered as the healthiest germplasms and can be used for their domestication *vis-a-vis* conservation of the species.
6. *S. chirayita* does not have rigid habitat preferences; however, the species performs well in open slope facing south-east, south-west, east and south direction.
7. The present findings should be considered as baseline for the future studies (recommended), by other researchers, at genetic level to determine extent of genetic variability amongst different populations in *S. chirayita* in Sikkim.

**Table 5.** Morphological differences amongst populations of *Swertia chirayita* (rosette) in Sikkim (Mean ± SD)

| Pop. | Collar diameter (mm) | Number of leaves | Root diameter (mm) | Root length (mm) | Largest leaf length (mm) | Largest leaf width (mm) |
|---|---|---|---|---|---|---|
| **Sc1** | 6.60 ± 1.5 | 15.00 ± 2.2 | 5.59 ± 1.4 | 271.89 ± 104.2 | 41.45 ± 8.1 | 19.70 ± 2.9 |
| **Sc2** | 5.28 ± 0.8 | 11.44 ± 1.9 | 4.07 ± 0.7 | 222.00 ± 84.9 | 31.05 ± 12.7 | 17.68 ± 1.8 |
| **Sc3** | 4.41 ± 1.2 | 8.89 ± 2.0 | 4.03 ± 1.4 | 226.67 ± 89.3 | 36.87 ± 9.1 | 19.72 ± 3.4 |
| **Sc4** | 3.96 ± 0.8 | 10.78 ± 2.0 | 3.19 ± 0.6 | 222.33 ± 154.7 | 31.66 ± 8.0 | 20.11 ± 2.8 |
| **Sc5** | 2.73 ± 0.4 | 11.22 ± 1.6 | 2.30 ± 0.2 | 188.56 ± 62.5 | 110.70 ± 12.0 | 31.31 ± 2.8 |
| **Sc6** | 4.33 ± 1.3 | 13.11 ± 2.7 | 3.25 ± 1.1 | 165.22 ± 32.5 | 143.38 ± 33.4 | 36.85 ± 4.5 |
| **Sc7** | 4.17 ± 1.6 | 13.67 ± 2.0 | 3.05 ± 1.0 | 184.00 ± 48.3 | 95.87 ± 23.3 | 31.25 ± 8.5 |
| **Sc8** | 5.22 ± 1.6 | 15.56 ± 2.8 | 3.88 ± 0.8 | 201.44 ± 60.3 | 136.96 ± 25.5 | 32.77 ± 11.2 |
| **Sc9** | 4.81 ± 1.3 | 12.67 ± 3.0 | 4.33 ± 1.3 | 205.56 ± 69.6 | 139.95 ± 28.2 | 30.50 ± 4.0 |
| **Sc10** | 3.76 ± 0.3 | 11.11 ± 1.8 | 3.33 ± 0.3 | 160.22 ± 28.8 | 85.26 ± 5.7 | 17.95 ± 0.9 |
| **Sc11** | 6.17 ± 1.8 | 12.67 ± 3.6 | 5.17 ± 2.5 | 200.67 ± 78.7 | 147.21 ± 31.6 | 32.43 ± 12.6 |
| **Sc12** | 3.99 ± 0.6 | 7.78 ± 2.0 | 3.23 ± 0.8 | 179.89 ± 65.1 | 115.35 ± 21.5 | 22.76 ± 3.1 |
| **Sc13** | 4.56 ± 1.0 | 11.11 ± 2.1 | 3.72 ± 0.8 | 230.11 ± 56.3 | 159.34 ± 45.7 | 30.34 ± 4.9 |
| **Sc14** | 4.42 ± 1.1 | 13.56 ± 3.0 | 3.83 ± 1.0 | 252.89 ± 99.5 | 168.33 ± 10.0 | 31.97 ± 4.6 |
| **Sc15** | 4.43 ± 1.3 | 11.56 ± 3.4 | 3.91 ± 1.4 | 223.33 ± 76.0 | 141.18 ± 28.1 | 29.31 ± 8.4 |
| **Sc16** | 4.73 ± 0.8 | 13.56 ± 4.4 | 4.32 ± 0.8 | 41.66 ± 6.7 | 132.97 ± 26.9 | 29.86 ± 6.8 |
| **Sc17** | 6.30 ± 1.3 | 14.00 ± 1.2 | 5.16 ± 1.1 | 214.00 ± 71.7 | 155.02 ± 45.3 | 37.91 ± 3.2 |
| **Sc18** | 5.37 ± 0.4 | 13.78 ± 2.0 | 4.18 ± 0.2 | 189.67 ± 32.8 | 124.41 ± 4.5 | 34.81 ± 2.59 |
| **Sc19** | 4.46 ± 1.0 | 12.67 ± 1.9 | 3.88 ± 1.0 | 145.22 ± 34.1 | 139.37 ± 38.2 | 35.19 ± 5.4 |
| **Sc20** | 5.93 ± 1.8 | 14.67 ± 2.5 | 6.10 ± 2.6 | 539.78 ± 154.7 | 175.60 ± 38.0 | 37.24 ± 6.1 |
| **Sc21** | 7.99 ± 1.1 | 14.78 ± 2.9 | 6.64 ± 1.5 | 274.33 ± 107.4 | 143.36 ± 48.8 | 37.58 ± 10.7 |
| **Sc22** | 7.18 ± 1.3 | 12.56 ± 2.5 | 5.64 ± 0.9 | 282.56 ± 40.2 | 43.43 ± 14.4 | 22.92 ± 9.4 |

**Table 6.** Morphological differences amongst populations of *S. chirayita* (reproductive) in Sikkim (Mean ± SD)

| Pop | Plant height (cm) | Stem basal diameter (mm) | Collar diameter (mm) | Number of branches | Number of leaves | Root diameter (mm) | Root length (mm) | Largest leaf length (mm) | Largest leaf width (mm) |
|---|---|---|---|---|---|---|---|---|---|
| **Sc1** | 93.44 ± 23.7 | 4.49 ± 1.1 | 4.82 ± 1.2 | 11.56 ± 5.5 | 91.56 ± 40.5 | 4.81 ± 1.3 | 358.89 ± 103.6 | 98.13 ± 15.3 | 23.88 ± 3.1 |
| **Sc2** | 99.56 ± 14.0 | 4.75 ± 0.7 | 6.74 ± 0.9 | 14.78 ± 5.5 | 56.44 ± 47.0 | 5.98 ± 0.8 | 308.89 ± 132.1 | 108.44 ± 12.4 | 20.89 ± 2.2 |
| **Sc3** | 99.78 ± 6.3 | 7.12 ± 2.7 | 9.65 ± 3.6 | 21.67 ± 1.9 | 58.33 ± 48.8 | 8.28 ± 3.2 | 225.56 ± 95.7 | 119.43 ± 24.7 | 21.98 ± 1.8 |
| **Sc4** | 39.56 ± 6.6 | 3.54 ± 0.3 | 4.94 ± 0.6 | --- | 10.33 ± 1.7 | 4.58 ± 0.5 | 104.44 ± 15.9 | 80.04 ± 5.5 | 24.01 ± 0.8 |
| **Sc5** | 120.00 ± 5.7 | 4.38 ± 0.9 | 6.44 ± 0.9 | 16.22 ± 4.4 | 141.44 ± 6.3 | 5.85 ± 1.0 | 313.33 ± 33.5 | 127.94 ± 8.0 | 21.47 ± 1.9 |
| **Sc6** | 92.78 ± 14.9 | 5.24 ± 0.7 | 7.78 ± 1.7 | 17.22 ± 7.1 | 246.56 ± 151.2 | 6.39 ± 1.6 | 251.11 ± 36.6 | 125.77 ± 7.7 | 21.06 ± 1.8 |
| **Sc7** | 61.22 ± 5.7 | 2.69 ± 0.2 | 5.38 ± 1.5 | 12.67 ± 6.0 | 119.67 ± 51.9 | 5.75 ± 1.6 | 272.22 ± 49.2 | 119.70 ± 6.1 | 20.53 ± 2.0 |
| **Sc8** | 64.33 ± 10.4 | 3.50 ± 0.8 | 6.22 ± 2.3 | 6.78 ± 4.2 | 71.89 ± 33.8 | 5.38 ± 1.2 | 272.22 ± 65.5 | 124.24 ± 5.1 | 21.24 ± 2.9 |
| **Sc9** | 79.56 ± 7.7 | 5.83 ± 0.6 | 6.53 ± 0.6 | 5.33 ± 2.3 | 33.78 ± 10.6 | 6.17 ± 0.6 | 173.33 ± 27.4 | 120.80 ± 6.4 | 26.77 ± 2.7 |
| **Sc10** | 72.33 ± 2.4 | 5.06 ± 0.4 | 5.53 ± 0.4 | --- | 18.67 ± 3.0 | 5.21 ± 0.3 | 154.44 ± 26.5 | 124.46 ± 4.3 | 26.00 ± 3.0 |
| **Sc11** | 20.11 ± 1.7 | 7.58 ± 1.9 | 8.15 ± 2.0 | --- | 15.89 ± 1.4 | 7.75 ± 3.0 | 326.67 ± 30.4 | 189.53 ± 18.9 | 44.03 ± 4.7 |
| **Sc12** | 95.22 ± 18.0 | 5.08 ± 1.6 | 7.51 ± 1.8 | 11.33 ± 6.0 | 20.44 ± 5.3 | 6.11 ± 2.3 | 218.89 ± 68.6 | 96.74 ± 22.2 | 28.06 ± 8.6 |
| **Sc13** | 54.22 ± 3.0 | 5.33 ± 1.2 | 7.18 ± 2.0 | --- | 13.78 ± 1.7 | 6.54 ± 1.7 | 207.78 ± 98.0 | 107.31 ± 35.9 | 27.52 ± 2.8 |
| **Sc14** | 81.44 ± 14.0 | 4.11 ± 1.2 | 5.74 ± 2.0 | 4.00 ± 1.7 | 31.56 ± 6.2 | 5.64 ± 1.8 | 237.78 ± 42.4 | 83.29 ± 20.1 | 39.90 ± 7.9 |
| **Sc15** | 80.44 ± 26.6 | 5.33 ± 1.2 | 8.34 ± 2.4 | 5.89 ± 4.38 | 37.22 ± 17.9 | 8.72 ± 3.5 | 241.11 ± 40.5 | 111.61 ± 34.3 | 35.31 ± 7.1 |
| **Sc16** | 72.67 ± 31.46 | 3.87 ± 1.1 | 5.25 ± 0.6 | 7.22 ± 6.1 | 66.44 ± 40.7 | 4.77 ± 0.7 | 285.56 ± 76.0 | 121.18 ± 60.5 | 32.67 ± 13.3 |
| **Sc17** | 134.10 ± 23.7 | 6.98 ± 1.2 | 10.38 ± 2.1 | 24.78 ± 3.8 | 172.56 ± 60.1 | 10.98 ± 3.6 | 326.67 ± 103.3 | 157.72 ± 8.1 | 50.93 ± 6.8 |
| **Sc18** | 121.00 ± 24.2 | 6.15 ± 2.4 | 8.28 ± 3.4 | 19.78 ± 9.1 | 78.56 ± 31.5 | 7.45 ± 2.3 | 327.78 ± 70.3 | 114.69 ± 23.4 | 34.05 ± 12.3 |
| **Sc19** | 92.89 ± 16.8 | 3.97 ± 0.5 | 5.65 ± 0.6 | 14.67 ± 6.4 | 41.67 ± 18.0 | 5.43 ± 0.7 | 224.44 ± 65.6 | 89.98 ± 23.0 | 27.30 ± 4.3 |
| **Sc20** | 110.44 ± 17.7 | 4.47 ± 1.2 | 6.13 ± 3.6 | 21.22 ± 9.7 | 140.33 ± 57.0 | 6.57 ± 3.7 | 278.89 ± 82.7 | 112.00 ± 32.9 | 32.23 ± 8.6 |
| **Sc21** | 89.33 ± 11.6 | 6.63 ± 0.6 | 9.69 ± 1.6 | 17.78 ± 3.0 | 22.67 ± 6.8 | 8.29 ± 1.5 | 332.22 ± 106.5 | 75.20 ± 10.1 | 33.79 ± 4.3 |
| **Sc22** | 69.44 ± 8.8 | 3.32 ± 0.4 | 5.15 ± 1.1 | --- | 20.22 ± 6.2 | 4.22 ± 0.8 | 198.89 ± 47.0 | 55.19 ± 14.1 | 22.01 ± 2.6 |

**Table 7.** Pearson's correlation coefficient ($r$) and significance of correlation (P) between the means of observed parameters of *S. chirayita* with altitude and humus depth in Sikkim

| | Vegetative (rosette form) | | | | | | Vegetative (Stock form) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Altitude (m) | | Humus depth (cm) | | Soil pH | | Altitude (m) | | Humus depth (cm) | | Soil pH | |
| **Parameters** | ***r*** | **P** | ***r*** | **P** | ***r*** | **P** | ***r*** | **P** | ***r*** | **P** | ***r*** | **P** |
| Plant height | --- | --- | --- | --- | --- | --- | -0.143 | ns | -0.054 | ns | 0.229 | ns |
| Stem basal diameter | --- | --- | --- | --- | --- | --- | 0.275 | <0.05 | -0.073 | ns | 0.129 | ns |
| Collar diameter | 0.309 | <0.05 | -0.158 | ns | 0.178 | ns | 0.084 | ns | -0.001 | ns | 0.015 | ns |
| Number of branches | --- | --- | --- | --- | --- | --- | -0.005 | ns | -0.003 | ns | 0.172 | ns |
| Number of leaves | -0.033 | ns | -0.280 | <0.05 | 0.117 | ns | -0.334 | <0.01 | 0.028 | ns | -0.154 | ns |
| Root diameter | 0.386 | <0.01 | -0.124 | ns | 0.066 | ns | 0.122 | ns | -0.017 | ns | 0.096 | ns |
| Root length | 0.192 | ns | -0.013 | ns | 0.133 | ns | 0.078 | ns | -0.087 | ns | 0.113 | ns |
| Largest leaf length | 0.027 | ns | -0.390 | <0.01 | -0.108 | ns | 0.092 | ns | -0.005 | ns | 0.260 | ns |
| Largest leaf width | 0.045 | ns | -0.314 | <0.01 | 0.132 | ns | 0.357 | <0.01 | -0.155 | ns | 0.241 | ns |
| Total dry biomass | 0.278 | <0.05 | -0.152 | ns | 0.152 | ns | 0.028 | ns | 0.108 | ns | 0.245 | ns |

ns: not significant

**Table 8.** Result of MANOVA showing morphological variation amongst populations of *S. chirayita* in Sikkim

| Dependent Variable | Rosette form | | | Reproductive form | | |
|---|---|---|---|---|---|---|
| | df | F | Sig. | df | F | Sig. |
| Plant height | --- | --- | --- | 21 | 25.098 | .000 |
| Stem basal diameter | --- | --- | --- | 21 | 10.796 | .000 |
| Collar diameter | 21 | 10.008 | .000 | 21 | 6.610 | .000 |
| Branch number | --- | --- | --- | 21 | 19.120 | .000 |
| Leaf number | 21 | 5.289 | .000 | 21 | 16.536 | .000 |
| Root diameter | 21 | 7.199 | .000 | 21 | 5.800 | .000 |
| Root length | 21 | 10.939 | .000 | 21 | 7.335 | .000 |
| Largest leaf length | 21 | 27.293 | .000 | 21 | 13.192 | .000 |
| Largest leaf width | 21 | 10.434 | .000 | 21 | 17.127 | .000 |
| Total plant dry biomass | 21 | 21.076 | .000 | 21 | 10.900 | .000 |

**Table 9.** Pearson's correlation matrix amongst populations of *S. chirayita* (rosette) in Sikkim

| | Collar diameter (mm) | No. of leaves | Root diameter (mm) | Root length (mm) | Largest leaf length (mm) | Largest leaf width (mm) | Above ground fresh wt. (g) | Below ground fresh wt. (g) | Above ground dry wt. (g) | Below ground dry wt. (g) | Total plant dry biomass (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Collar diameter (mm) | 1.000 | | | | | | | | | | |
| Number of leaves | 0.382$^{c}$ | 1.000 | | | | | | | | | |
| Root diameter (mm) | 0.851$^{c}$ | 0.401$^{c}$ | 1.000 | | | | | | | | |
| Root length (mm) | 0.365$^{c}$ | 0.167$^{a}$ | 0.372c | 1.000 | | | | | | | |
| Largest leaf length (mm) | 0.092$^{ns}$ | 0.249$^{c}$ | 0.180a | 0.076$^{ns}$ | 1.000 | | | | | | |
| Largest leaf width (mm) | 0.229$^{b}$ | 0.406$^{c}$ | 0.262c | 0.121$^{ns}$ | 0.722$^{c}$ | 1.000 | | | | | |
| Above ground fresh wt. (g) | 0.643$^{c}$ | 0.588$^{c}$ | 0.683c | 0.355$^{c}$ | 0.329$^{c}$ | 0.448$^{c}$ | 1.000 | | | | |
| Below ground fresh wt. (g) | 0.620$^{c}$ | 0.380$^{c}$ | 0.685c | 0.601$^{c}$ | 0.190$^{c}$ | 0.306$^{c}$ | 0.659$^{c}$ | 1.000 | | | |
| Above ground dry wt. (g) | 0.470$^{c}$ | 0.406$^{c}$ | 0.536c | 0.405$^{c}$ | 0.331$^{c}$ | 0.393$^{c}$ | 0.684$^{c}$ | 0.639$^{c}$ | 1.000 | | |
| Below ground dry wt. (g) | 0.552$^{c}$ | 0.329$^{c}$ | 0.610c | 0.418$^{c}$ | 0.300$^{c}$ | 0.391$^{c}$ | 0.540$^{c}$ | 0.765$^{c}$ | 0.733$^{c}$ | 1.000 | |
| Total plant dry biomass (g) | 0.532$^{c}$ | 0.404$^{c}$ | 0.599c | 0.437$^{c}$ | 0.342$^{c}$ | 0.418$^{c}$ | 0.677$^{c}$ | 0.728$^{c}$ | 0.968$^{c}$ | 0.880$^{c}$ | 1.000 |

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$; ns: not significant

Table 10: Pearson's correlation matrix amongst populations of *S. chirayita* (reproductive) in Sikkim

| | Plant height (cm) | Stem basal diameter (mm) | Collar diameter (mm) | Number of branches | Number of leaves | Root diameter (mm) | Root length (mm) | Largest leaf length (mm) | Largest leaf width (mm) | Above ground fresh wt. (g) | Below ground fresh wt. (g) | Above ground dry wt. (g) | Below ground dry wt. (g) | Total dry biomass (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant height (cm) | 1.000 | | | | | | | | | | | | | |
| Stem basal diameter (mm) | 0.309$^{c}$ | 1.000 | | | | | | | | | | | | |
| Collar diameter (mm) | 0.389$^{c}$ | 0.830$^{c}$ | 1.000 | | | | | | | | | | | |
| Number of branches | 0.738$^{c}$ | 0.384$^{c}$ | 0.487$^{c}$ | 1.000 | | | | | | | | | | |
| Number of leaves | 0.497$^{c}$ | 0.143$^{a}$ | 0.286$^{c}$ | 0.627$^{c}$ | 1.000 | | | | | | | | | |
| Root diameter (mm) | 0.398$^{c}$ | 0.763$^{c}$ | 0.906$^{c}$ | 0.474$^{c}$ | 0.317$^{c}$ | 1.000 | | | | | | | | |
| Root length (mm) | 0.376$^{c}$ | 0.329$^{c}$ | 0.379$^{c}$ | 0.473$^{c}$ | 0.342$^{c}$ | 0.384$^{c}$ | 1.000 | | | | | | | |
| Largest leaf length (mm) | 0.109$^{ns}$ | 0.462$^{c}$ | 0.329$^{c}$ | 0.232$^{b}$ | 0.300$^{c}$ | 0.369$^{c}$ | 0.319$^{c}$ | 1.000 | | | | | | |
| Largest leaf width (mm) | 0.167$^{a}$ | 0.458$^{c}$ | 0.417$^{c}$ | 0.119$^{ns}$ | 0.011$^{ns}$ | 0.480$^{c}$ | 0.270$^{c}$ | 0.425$^{c}$ | 1.000 | | | | | |
| Above ground fresh wt. (g) | 0.634$^{c}$ | 0.550$^{c}$ | 0.661$^{c}$ | 0.694$^{c}$ | 0.517$^{c}$ | 0.664$^{c}$ | 0.542$^{c}$ | 0.300$^{c}$ | 0.210$^{b}$ | 1.000 | | | | |
| Below ground fresh wt. (g) | 0.479$^{c}$ | 0.525$^{c}$ | 0.699$^{c}$ | 0.498$^{c}$ | 0.342$^{c}$ | 0.734$^{c}$ | 0.576$^{c}$ | 0.243$^{c}$ | 0.418$^{c}$ | 0.801$^{c}$ | 1.000 | | | |
| Above ground dry wt. (g) | 0.602$^{c}$ | 0.600$^{c}$ | 0.677$^{c}$ | 0.683$^{c}$ | 0.381$^{c}$ | 0.648$^{c}$ | 0.434$^{c}$ | 0.249$^{c}$ | 0.250$^{c}$ | 0.900$^{c}$ | 0.747$^{c}$ | 1.000 | | |
| Below ground dry wt. (g) | 0.515$^{c}$ | 0.554$^{c}$ | 0.669$^{c}$ | 0.561$^{c}$ | 0.364$^{c}$ | 0.726$^{c}$ | 0.421$^{c}$ | 0.277$^{c}$ | 0.456$^{c}$ | 0.820$^{c}$ | 0.880$^{c}$ | 0.852$^{c}$ | 1.000 | |
| Total dry biomass (g) | 0.599$^{c}$ | 0.605$^{c}$ | 0.692$^{c}$ | 0.675$^{c}$ | 0.387$^{c}$ | 0.679$^{c}$ | 0.442$^{c}$ | 0.260$^{c}$ | 0.298$^{c}$ | 0.905$^{c}$ | 0.792$^{c}$ | 0.994$^{c}$ | 0.903$^{c}$ | 1.000 |

a: $p<0.05$; b: $p<0.01$; c: $p<0.01$; ns: not significant

**Table 11.** Summary of Canonical Discriminant Function of the growth parameters among populations of *S. chirayita* (rosette) in Sikkim

| | Function | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** |
| Collar diameter | -0.161 | -0.084 | 1.108 | -0.210 | -0.206 | -1.108 |
| Number of leaves | 0.091 | 0.016 | 0.357 | 0.487 | 0.884 | 0.055 |
| Root diameter | -0.275 | 0.473 | -0.394 | -0.449 | -0.027 | 1.409 |
| Root length | 0.046 | 0.862 | -0.492 | 0.273 | -0.010 | -0.229 |
| Largest leaf length | 1.009 | 0.073 | -0.065 | -0.510 | 0.295 | -0.213 |
| Largest leaf width | 0.057 | -0.147 | 0.225 | 0.788 | -0.831 | 0.290 |
| Eigen value | 3.630 | 1.483 | 1.168 | 0.480 | 0.276 | 0.116 |
| % of Variance | 50.7 | 20.7 | 16.3 | 6.7 | 3.9 | 1.6 |
| Cumulative % | 50.7 | 71.5 | 87.8 | 94.5 | 98.4 | 100.0 |
| Canonical correlation | 0.885 | 0.773 | 0.734 | 0.570 | 0.465 | 0.323 |

**Table 12.** Summary of Canonical Discriminant Function of the growth parameters among populations of *S. chirayita* (reproductive) in Sikkim

| | Function | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Plant height | 1.048 | -0.045 | -0.161 | -0.024 | -0.682 | 0.022 | -0.446 | 0.283 | -0.010 |
| Stem basal diameter | -0.825 | -0.554 | -0.078 | 0.621 | -0.966 | 0.732 | 0.626 | -0.661 | 0.300 |
| Collar diameter | 0.359 | 0.260 | -0.919 | 0.318 | 0.600 | -0.608 | 0.498 | 1.765 | -1.202 |
| Branches number | 0.574 | -0.342 | 0.112 | 0.499 | 0.870 | -0.157 | 0.132 | -0.652 | -0.231 |
| Leaf number | -0.130 | 0.794 | 0.547 | -0.249 | -0.430 | -0.009 | 0.669 | 0.062 | 0.110 |
| Root diameter | -0.227 | -0.148 | 0.586 | -0.403 | 0.254 | -0.529 | -0.856 | -0.672 | 1.676 |
| Root length | -0.062 | 0.068 | 0.196 | -0.130 | 0.346 | 0.981 | -0.091 | 0.332 | 0.201 |
| Largest leaf length | -0.559 | 0.673 | 0.075 | 0.494 | 0.059 | -0.207 | -0.689 | 0.092 | -0.273 |
| Largest leaf width | -0.027 | -0.703 | 0.819 | -0.359 | -0.014 | -0.092 | 0.204 | -0.018 | -0.336 |
| Eigen value | 6.923 | 3.188 | 2.112 | 1.378 | 0.894 | 0.755 | 0.563 | 0.318 | 0.134 |
| % Variance | 42.6 | 19.6 | 13.0 | 8.5 | 5.5 | 4.6 | 3.5 | 2.0 | 0.8 |
| Cumulative % | 42.6 | 62.2 | 75.1 | 83.6 | 89.1 | 93.8 | 97.2 | 99.2 | 100.0 |
| Canonical correlation | 0.935 | 0.872 | 0.824 | 0.761 | 0.687 | 0.656 | 0.600 | 0.491 | 0.344 |

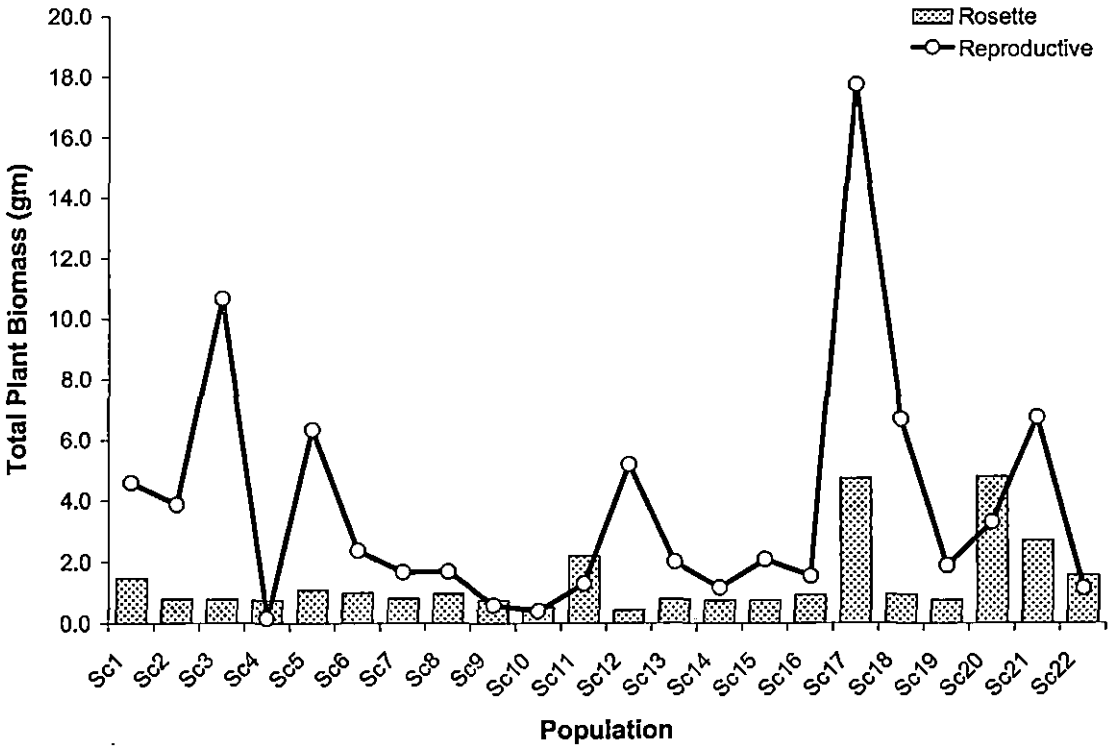



**Figure 10.** Difference in total plant dry biomass amongst populations of *Swertia chirayita* in Sikkim

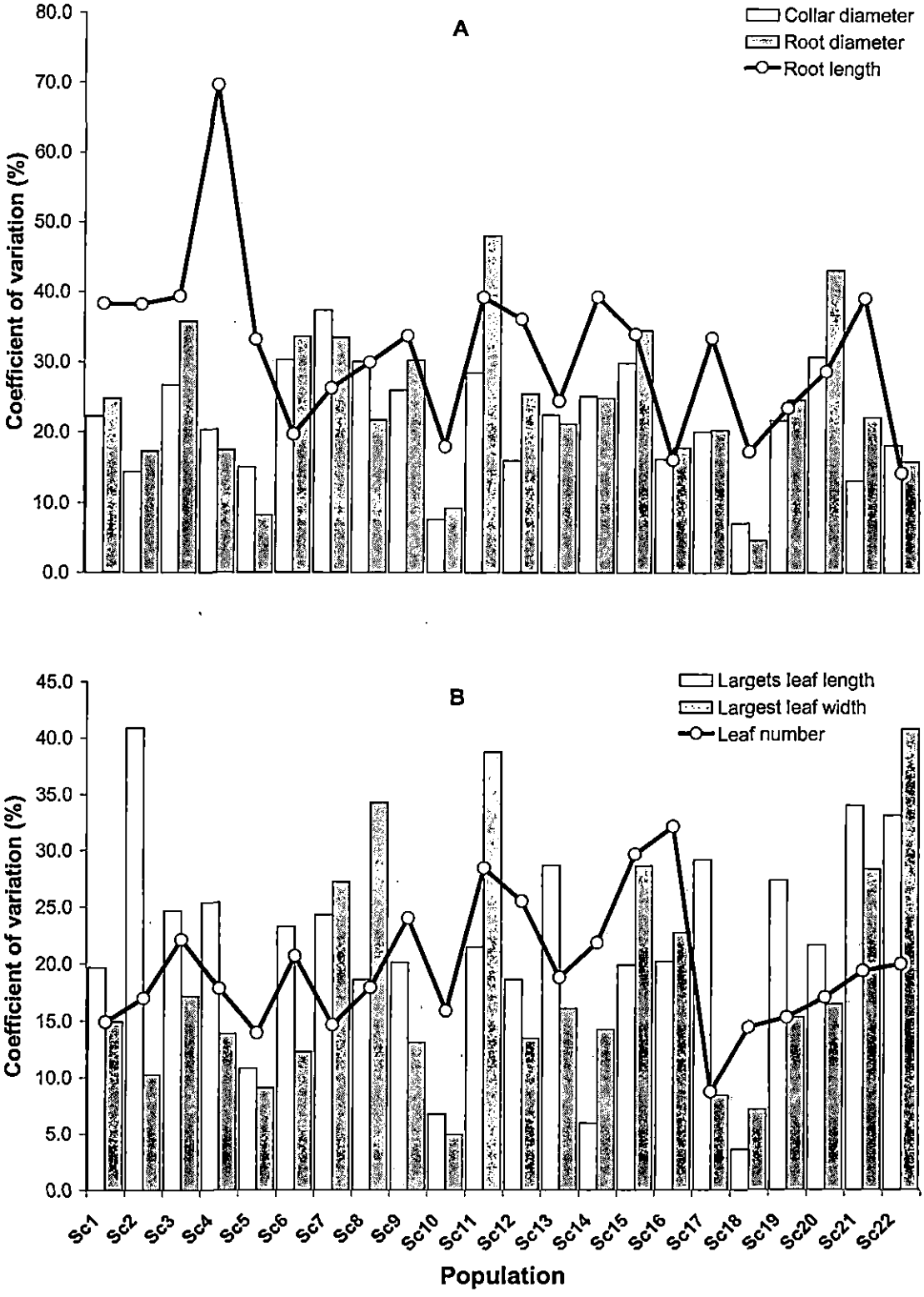



**Figure 11.** Coefficient of variation in morphological characters in populations of *Swertia chirayita* (rosette) in Sikkim

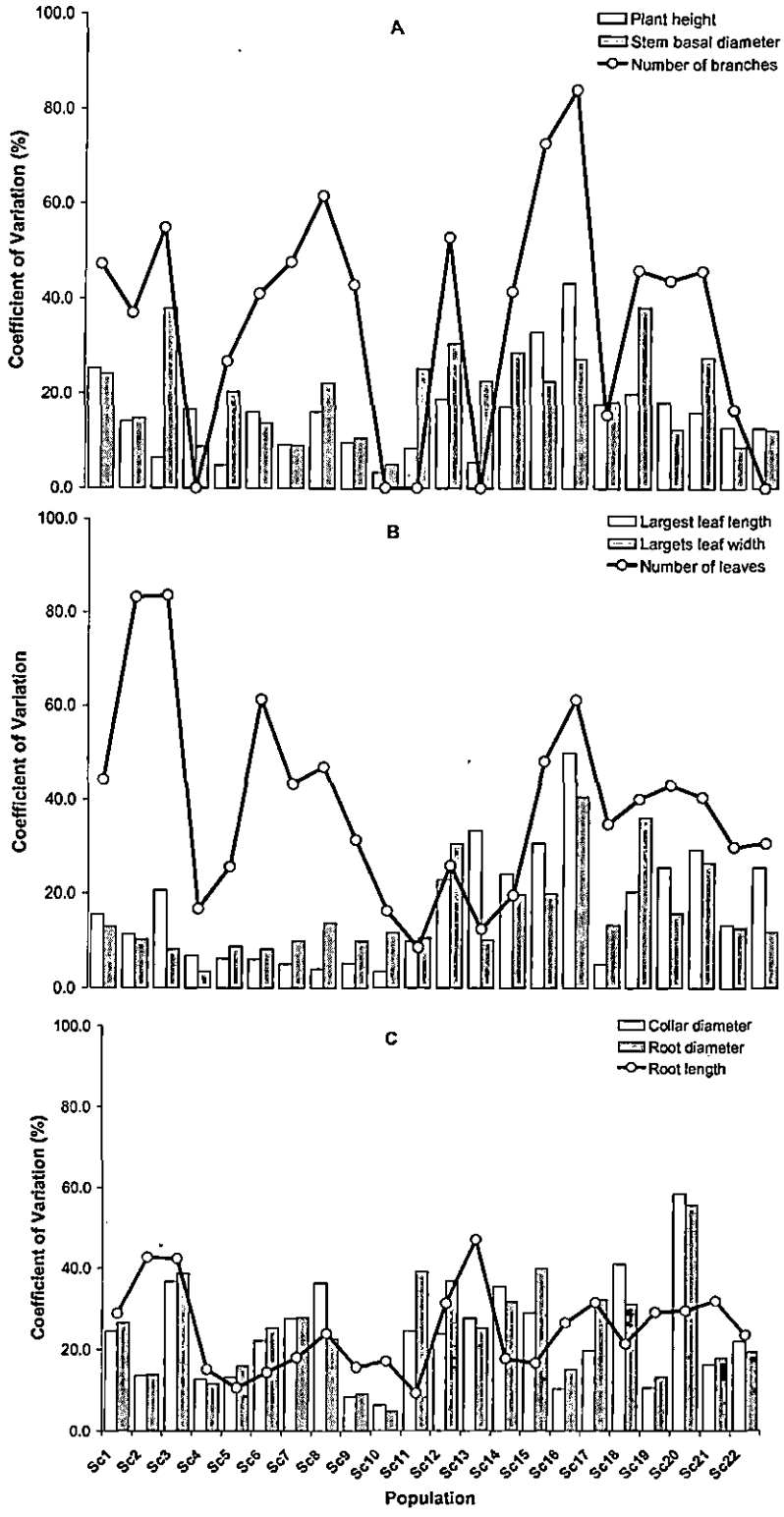



**Figure 12.** Variation in morphological characters in populations of *Swertia chirayita* (reproductive) in Sikkim

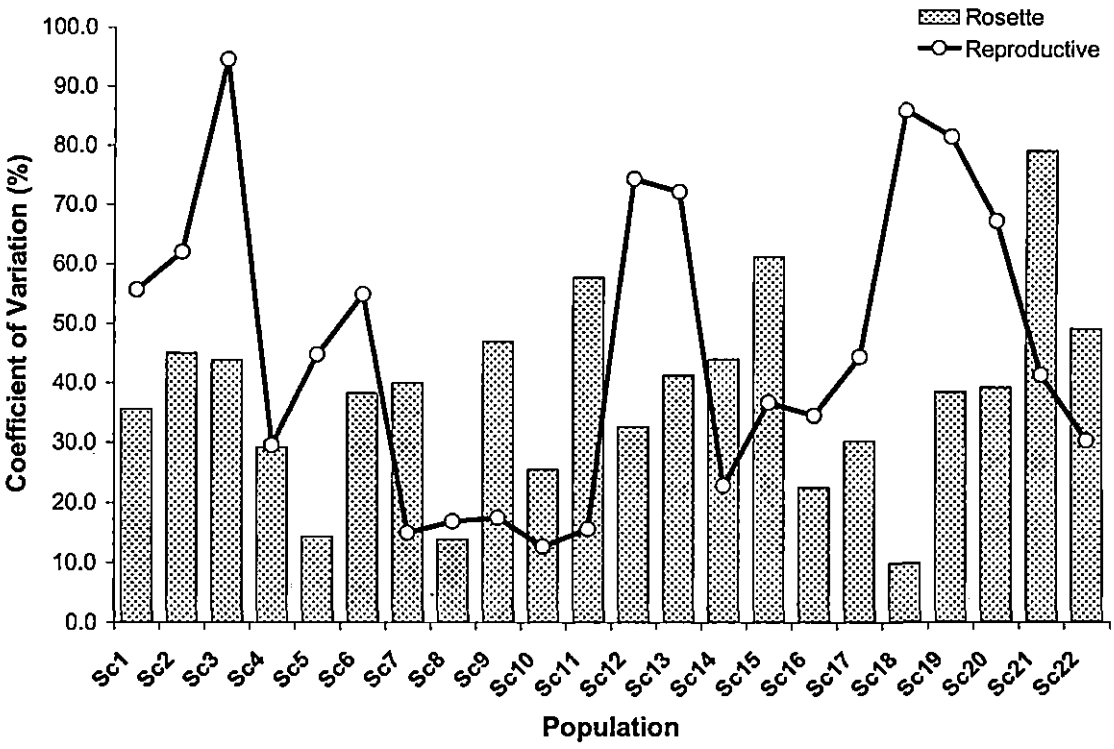



**Figure 13.** Variation in total plant biomass in population of *Swertia chirayita* in Sikkim

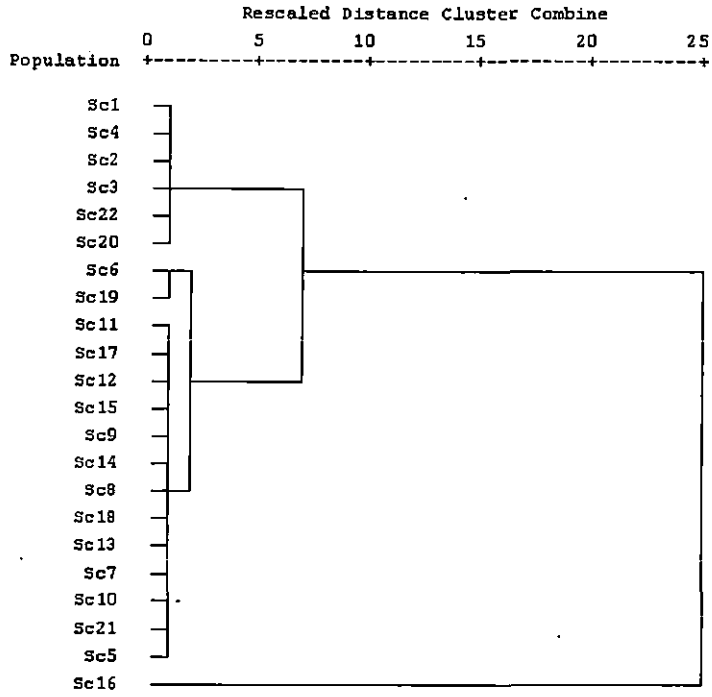



**Figure 14A.** Dendogram based on average linkage (between groups) method and Pearson correlation in rosette form of *Swertia chirayita* in Sikkim

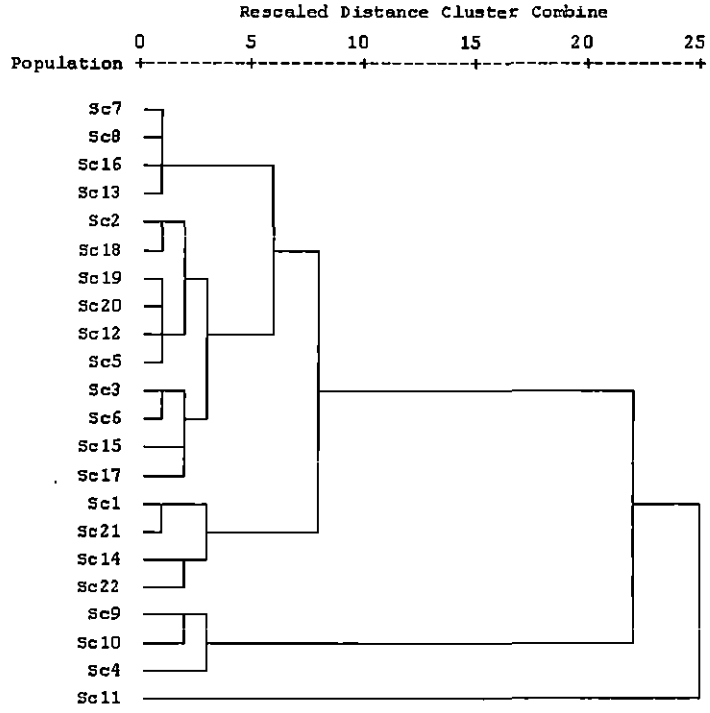



**Figure 14B.** Dendogram based on average linkage (between group) method and Pearson correlation in reproductive form of *Swertia chirayita* in Sikkim

CHAPTER 6

# Effect of Microhabitat, Light and Temperature on Seed Germination

## 6.1 Introduction

Seedling emergence and survival in plants differ greatly in their habitat preferences. Young seedlings on the forest floor are susceptible to many threats; recruitment of a species is possible in microhabitats having fewer threats. The characteristics of the microsite occupied by a seed may strongly influence its probability of germination and subsequent survival; species that live in highly specific habitats often produce seeds with highly specialized adaptations (Navarro and Guitian, 2003). Highly specific microhabitats may affect seed germination and subsequently seedling survival. Specific, though, congenial micro-habitats may offer high chances of establishing gregarious and massive populations even for a rare and endangered species (Badola and Pradhan, 2010).

Temperature regulates the seed germination in three ways (Bewley and Black, 1994), (i) by determining the capacity and percentage of germination; (ii) by eliminating primary and/or secondary dormancy; and (iii) by inducing secondary dormancy. The optimal germination temperature for most seeds which do not acquire dormancy is $25^0$C to $30^0$C. However, temperature range for germination depends largely on, (1) period of the year in which plants complete their life cycle, and (2) geographic origin of the species (Besnier, 1989).

In addition, light is also important for seed germination. In the case of light sensitive species, the seed will not germinate if it remains covered by litter or soil. Many species respond to the environment with optimal growth and development according to the light they receive (Maloof *et al.*, 2000). Some seeds germinate similarly in light and darkness (Baskin and Baskin, 2001), while others do it more readily either under light (Colbach *et al.*, 2002) or darkness conditions (Thanos *et al.*, 1989). Also, light requirements for germination can vary with temperature. It has been demonstrated

that some species need a constant temperature and light to germinate and others can germinate either under light or darkness conditions but need temperature fluctuations (Felippe, 1978).

*Swertia chirayita* Roxb. ex Fleming H. Kharst (Gentianaceae) is a perennial herb distributed in temperate Himalaya (1200m – 3000m asl) from Kashmir to Bhutan and in Khasia hills of Meghlaya, India (Chanda, 1976). In general, 1800-2300m altitude is a suitable range preferred by the species in Sikkim Himalaya (personal observation). Usually, species attains two forms in its 3 year life cycle; for the first two year, it remains in rosette form and during the third year, it gives out shoot, flower and fruit and becomes ready for harvest by the end of the season. However, it has been observed rarely that the plant completing its life cycle in two years which can be perceived as an abnormal behaviour (personal observation). Wind plays vital role in seed dispersal; the species is self pollinated (Chakroborty *et al.*, 2009), however, different pollinators have been observed during flowering season (personal observation).

Ethnomedicinal use of *S. chirayita* is known to exist prior to subsistence of modern health care system, for having numerous medicinal properties (Nadkarni, 1976; Biswas and Chopra, 1982; Kirtikar and Basu, 1984; Wawrosch *et al.*, 2005; Pradhan and Badola, 2008). It is a source of important chemical compounds such as, amarogentin (chirantin), gentianine, swerchirin and swertiamarin (Singh, 2008). Whole plant is known to possess anti-inflammatory, anti-platelet, anti-cancerous, anti-fungal and anti-malarial (Brahmachari *et al.*, 2004), anti-viral properties (Verma *et al.*, 2008). The raw material demand of *S. chirayita* is huge in both domestic and international market (Bhattarai and Acharaya, 1997; Badola and Pal, 2002, 2003; Badola and Aitken, 2003, Dutta, 2004, Olsen, 2005).

Due to indiscriminate extraction and habitat loss for several reasons, in many parts of Himalaya, *Swertia chirayita* has become critically endangered (Ved *et al.*, 2003a; IUCN, 2008) and is under the threats of extinction, and is a top priority species for its immediate conservation through *ex-situ* mechanism (Badola and Pal, 2002).

Conventional propagation of the species is possible only through seeds, and available literature reveals low percent germination in *S. chirayita* (Raina *et al.*, 1994; Basnet, 2001). There have been no previous studies available on the influence of microsites, as source, with light and temperatures, on seed germination in *S. chirayita.* Understanding germination behaviour of seeds from different microhabitats would help field managers and scientists to implement conservation strategies (Cervera *et al.*, 2007), particularly in case of species recovery programme of the species. The present study has been undertaken with the objectives to address these questions: (i) do seed germination variability exists amongst microhabitats in *Swertia chirayita*? (ii) what is the optimal temperature requirement for its seed germination?; and (iii) are the seeds light sensitive?

## 6.2 Materials and Methods

### *6.2.1 Seed germination test*

The study was carried out with the seeds of *Swertia chirayita* harvested during December from 6 different microhabitats, viz., tree base, stump base, boulder, under canopy, shrubberies and grassy slope, from the forests of East Sikkim, India, (ca. 2000m asl). Mature fruits/capsules were collected and brought to the laboratory. The seeds were separated from fruits. After removing available impurities, the seeds were room dried for 10 days, and soaked for 24 hrs in double distilled water separately for each microhabitat. The soaked seeds were surface sterilized by immersing in Sodium hypochlorite (available chlorine 4.0%, E. Merck Company, India) solution for 5 sec. and washed thoroughly with double distilled water before placing in petri plates (90mm) lined with single layer Whatmann no. 1 filter paper.

Three sets (set A, B and C) in alternating light and dark photoperiod (14/10 light/dark), and three sets (set A1, B1 and C1) in continuous darkness (petri plates wrapped with aluminum foil) for each microhabitat were placed in a germination chamber at constant temperature of $20^0$C, $25^0$C and $30^0$C, respectively. Each set of experiment had 3 replicates of 30 seeds each. Double distilled water was added whenever needed. The seeds were observed daily, and considered germinated upon radical emergence. On germination, the seeds were counted and removed. The

experiment was completed in 45 days. Set A1, B1 and C1, showing no germination or very poor germination in 45 days in continuous darkness, were then transferred to 14/10 hrs light/dark photoperiod individually at three continuous temperature regimes for another 45 days to determine the possibility of seed germination recovery in *S. chirayita*.

### 6.2.2 Statistical analysis

Statistical analysis was performed using SPSS 10.0 software for Windows. The data were submitted to Two-way ANOVA, to demonstrate the significance of main factors (seed source and temperatures) and their interaction in affecting the percent seed germination, germination rate, germination recovery percent and germination recovery rate. A Bonferroni test was used to determine if differences among means are significant ($p<0.05$). Mean germination rate ($r$) was calculated according to Labouriau (1972) as, $r = 1/\sum(xd)/\sum t$ where 'x' is number of newly emerged seedlings in each day d, and 't' is total number of seedling emerged at the end of the test. Percent germination recovery (GR) was determined from the expression, % GR = $[(b/n_i) - (a/N)] \times 100$ where "a" is number of seeds germinated in continuous darkness from the actual total seeds "N", "b" is number of seeds germinated after transferring the seeds to 14/10 hours light/dark photoperiod from the remaining seeds "$n_i$".

## 6.3 Results

Table 13 shows the effect of microclimatic conditions and the temperature on percent seed germination, germination rate, germination recovery percent and germination recovery rate. Over 70% seed germination was recorded in alternating light and dark photoperiod from all the seed sources and temperature conditions except grassy slope (68.89%; $20^0$C) [Figure 15A]. Seeds sourced from tree base, stump base and canopy resulted in highest of 82.22% germination at $20^0$C which was significantly higher than grassy slope (68.89±5.09; $p<0.05$) and non-significantly higher than shrubberies (71.11±1.92); similarly, at $25^0$C, stump base (97.78±3.85%) recorded highest germination value followed by boulder (94.44±5.09%), tree base (92.22±5.09%) and canopy (91.11±1.92%). These values were significantly higher than the values recorded for shrubberies (77.78±3.85%; $p<0.05$). Further, the highest value for stump

base was significantly higher compared to grassy slope (84.44+1.93%; $p<0.05$). At $30^0$C, the maximum seed germination recorded for boulder (87.78±1.92%) and canopy (86.67±3.33%) was significantly higher ($p<0.05$) than the lowest recorded for shrubberies (73.33±3.33%) and grassy slope (75.56±1.93%). Seeds sourced from tree base and stump base recorded 83.33±5.77% and 83.33±3.33% seed germination. When compared amongst the different temperature conditions, seeds incubated at $25^0$C resulted in highest seed germination followed by $30^0$C and $20^0$C (Figure 15A). In the case of seeds collected from stump base, boulder and grassy slope, significantly high ($p<0.05$) seed germination was observed at $25^0$C compared to $30^0$C incubation. Also, for seeds from stump base, the seed germination was significantly high ($p<0.05$) at $25^0$C compared to $20^0$C. Contrarily, in continuous darkness, the seeds either very poorly germinated or did not germinate under any of the three temperature regime (Figure 15B).

After 45 days, the non-germinated seeds when transferred from dark condition to 14/10 hours light/dark photoperiod significantly recovered in germination for all the microhabitat conditions and temperature regimes (Figure 15C). However, variation in germination recovery percent was observed. For example, at $20^0$C, significantly ($p<0.05$) highest seed germination recovery percent was observed in seeds from under canopy (81.68±3.9) compared to other microhabitat condition (tree base: 65.09±5.92; stump base: 61.11±1.92; shrubberies: 41.11±6.94; grassy slope: 59.89±5.26) except boulders (77.78±5.09) in which the difference in seed germination recovery percent was non-significant. Similarly, the lowest seed germination recovery percent recorded for shrubberies was significantly low ($p<0.05$) compared to all other microhabitat conditions. The seed germination recovery percent was significantly high ($p<0.05$) in boulders compared to seeds from stump base and grassy slope. Similarly at $25^0$C, highest seeds germination recovery percent recorded for seeds from under canopy (86.67±5.77) was significantly high ($p<0.05$) compared to seeds from tree base (70.0±6.67), stump base (65.56±1.93), shrubberies (42.22±5.09) and grassy slope (71.09±7.54), however, it was non-significant for seeds from boulders (83.98±3.94). The lowest value recorded for shrubberies was significantly low ($p<0.05$) compared to other microhabitat conditions. Seeds from stump base recorded significantly low

($p<0.05$) seed germination recovery percent compared to boulders. At $30^0$C, of all the microhabitats, seeds from shrubberies (45.56±1.93) recorded significantly low seed germination recovery percent than other microhabitat conditions (tree base: 68.29±1.39; stump base: 62.22±8.39; boulder: 74.44±1.93; canopy: 74.83±3.98; grassy slope: 60.0±5.77). Similarly, seeds from canopy and boulder recorded significantly high ($p<0.05$) germination recovery value than grassy slope. When compared amongst temperature, seeds incubated at $25^0$C recorded non-significantly high germination recovery value in seeds from all the microhabitat conditions except shrubberies where the value was higher at $30^0$C.

The mean germination rate (Figure 16A) and the germination recovery rate (after transferring seeds from complete darkness to light/dark photoperiod cycle) [Figure 16B] were significantly greater ($p<0.05$) at $25^0$C than at $20^0$C and $30^0$C, comparatively; amongst microhabitats, the variation in germination rate (Figure 16A) was non-significant while germination recovery rate was significant ($p<0.05$) [Figure 16B]. At all the three temperature conditions, seed sourced from shrubberies recorded significantly ($p<0.05$) lower germination recovery rate.

## 6.4 Discussion

Both internal and external environmental factors strongly influence the seed germination in nature (Baskin and Baskin, 2001). The ecological conditions prevailing in a given habitat affect seed germination (Uysal *et al.*, 2006) as evident from our study in which the variability in seed germination amongst the microhabitat conditions (sources) was observed. In nature, germination of seeds is often restricted to locations that meet specific environmental conditions (Vandelook *et al.*, 2008) and are often referred to as 'safe-sites' (Harper, 1977) or 'regeneration niches' (Grubb, 1977). Therefore, preservation of such safe-sites rather than restoration of habitat would be crucial for the conservation of the species. In present study, the seeds of *S. chirayita* when exposed to 14/10hours light/dark photoperiod under different temperature regime performed well, which coincide with day/night length in its natural environment. Plant species have a minimum temperature, an optimum temperature, and a maximum temperature requirement, above or below which their

seeds will not germinate (Cardoso, 1999; Yilmaz and Aksoy, 2007). In the present study, similar substantial effect on the percent seed germination was observed under different temperature conditions from $20^0$C to $30^0$C, and the seeds germination percent was higher at $25^0$C than $20^0$C and $30^0$C. The effectiveness of alternating day and night temperature in stimulating seed germination compared to constant temperature (Yilmaz and Aksoy, 2007) was not applicable with *S. chirayita*, as high seed germination percent was achieved in all the seed sources at different temperature regime at constant day and night temperature.

The germinating seeds and seedlings are more susceptible to disturbance (Sharma *et al.*, 2009) under canopy as it provides refuge to many herbivore species, which results in trampling and foraging on seedlings of many important plant species due to which the survival rate of the seedlings may turn very low. As an adaptive mechanism, the plants grown under canopy produce seeds which tend to be more viable than the seeds produced by plants grown in open spaces. Such adaptation became evident in the present study, where high seed germination was recorded in the seeds sourced from tree base, stump base and under canopy than shrubberies and grassy slope. At the same time, there is a minimum variation in daily air and soil surface temperatures under the canopy of nurse plants than outside the canopy (Cervera *et al.*, 2006). Thus, the seedlings under canopy become protected from extreme temperatures by the nurse plants thereby increasing seedling survival and extending the species' distributional boundaries (Steenbergh and Lowe, 1977; Nobel, 1980) resulting in productive and healthy plants producing maximum viable seeds. Further, in the present study, germination percent value for seeds collected from boulders exceeded the value obtained for shrubberies and grassy slope. This might be due to the fact that in the bouldery areas, the chances of large number of seeds gathered in depressions during seed dispersal may result in very less seedling survival due to over-crowding. Therefore, the seeds produced from the survived plants tend to be more viable and productive. Thus, understanding microhabitat – seed germination relation *vis- a-vis* information on the micro-climate is vital in the field studies and for developing conservation models as well as helping effective management plans for endangered plant species (Cervera *et al.*, 2007) like *Swertia chirayita*.

The importance of light in seed germination depends upon the size of the seeds, and life form (annual or perennial) and the habitat of the species (Uysal *et al.*, 2006). In continuous darkness, the minute seeds of *S. chirayita* failed to germinate or showed very poor germination which might be due to induction of secondary dormancy. Yilmaz and Aksoy (2007) reported the greatest response of *Rumax scutatus* seeds to alternating light/dark regimes than total darkness. Similar light requirements for germination in many small seeded species have been reported (Pons, 1992). In *Marathrum schiedeanum* and *M. rubrum*, seeds were unable to germinate both in complete darkness and under far red light (Ortega *et al.*, 2009). *Achmea nudalis* failed to germinate in total darkness but *Streptocalyx floribundus* showed little germination, and red light highly improved seed germination in both *A. nudalis* and *S. floribundus* from $15^0$C to $40^0$C (Pinheiro and Borghetti, 2003). However, *Phragmites australis* seeds germinated well in both continuous darkness and light over most of the temperature regime tested (Ekstam *et al.*, 1999). According to Walck *et al.*, (2000), even a green safe light is sufficient to stimulate germination in positively photoblastic seeds. Very low germination recorded under continuous darkness in seeds of *S. chirayita* from some microhabitats might be due to exposure of seeds to white light for little duration during periodical checking for germination (Baskin and Baskin, 2001) which confirms the photobalstic nature of seeds of *S. chirayita*.

For all the microhabitats, seeds after incubation in continuous darkness for 45 days under $20^0$C to $30^0$C temperature regimes, when exposed to 14/10 hours light/dark photoperiod in the same temperatures regimes showed over 50% germination recovery, except in case of shrubberies. However, some variation in germination recovery was noticed amongst microhabitat sources, which could be due to differences in the temperature regime to which they are exposed (Gul and Weber, 1999). These responses are similar to those seeds which were directly incubated at 14/10 hours light/dark photoperiod at $20^0$C to $30^0$C temperatures, indicating the light sensitivity of *S. chirayita*. This ensures that the seeds will germinate successfully on or near the soil surface when other conditions including light are favourable for seedling emergence, and if the seeds are buried at soil depths below in darkness, they may remain the part of persistent soil seed bank (Qu *et al.*, 2008). In *S. chirayita*, of

three temperature regimes, higher seed germination and germination recovery value were obtained at 25$^0$C. Similar results were reported in *Rumax scutatus* (Yilmaz and Aksoy, 2007).

Our data showed that seed germination and germination recovery percents were higher in all temperature regimes from 20$^0$C to 30$^0$C, however, the best results obtained at 25$^0$C with high germination, germination recovery value and also high mean germination rate, suggesting it as the optimal temperature for *S. chirayita* achieving better performance. Habitat loss and degradation ameliorates the stressful condition (Cervera *et al.*, 2006) for the plant species threatening its existence including the targeted taxa. Significant variation in percent germination amongst microhabitats indicated that the maintenance of natural habitats is the best option to conserve the genetic diversity of these species; however, suitable propagation protocols would be a simultaneous requirement strengthening *ex-situ* conservation mechanism and entrepreneurship (cultivation for trade) programme (Badola and Pal, 2002; Badola and Singh, 2003; Badola, 2009). The human participation will, however, be vital in the recovery of endangered species (Maschinski, 2004) like *S. chirayita*.

## 6.5 Conclusion

Present study concludes with conformity that, in *S. chirayita*, (i) there is an influence of micro-habitats on seed germination; (ii) 25$^0$C is the optimal temperature requirement for better performance; and (iii) the seeds are light sensitive.

**Table 13.** Results of two way ANOVA showing effect of temperature and microhabitats and their interaction on various germination parameters in *Swertia chirayita*

| Dependent variable | Independent variable | | |
|---|---|---|---|
| | Temperature (T) | Microhabitat (M) | T x M |
| Germination (%) | 45.50*** | 21.61*** | $1.46^{ns}$ |
| Germination rate | 146.74*** | $1.06^{ns}$ | $0.99^{ns}$ |
| Germination recovery (%) | 7.27* | 65.38*** | $1.27^{ns}$ |
| Germination recovery rate | 5.90** | 25.97*** | $0.78^{ns}$ |

* = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; ns = not-significant

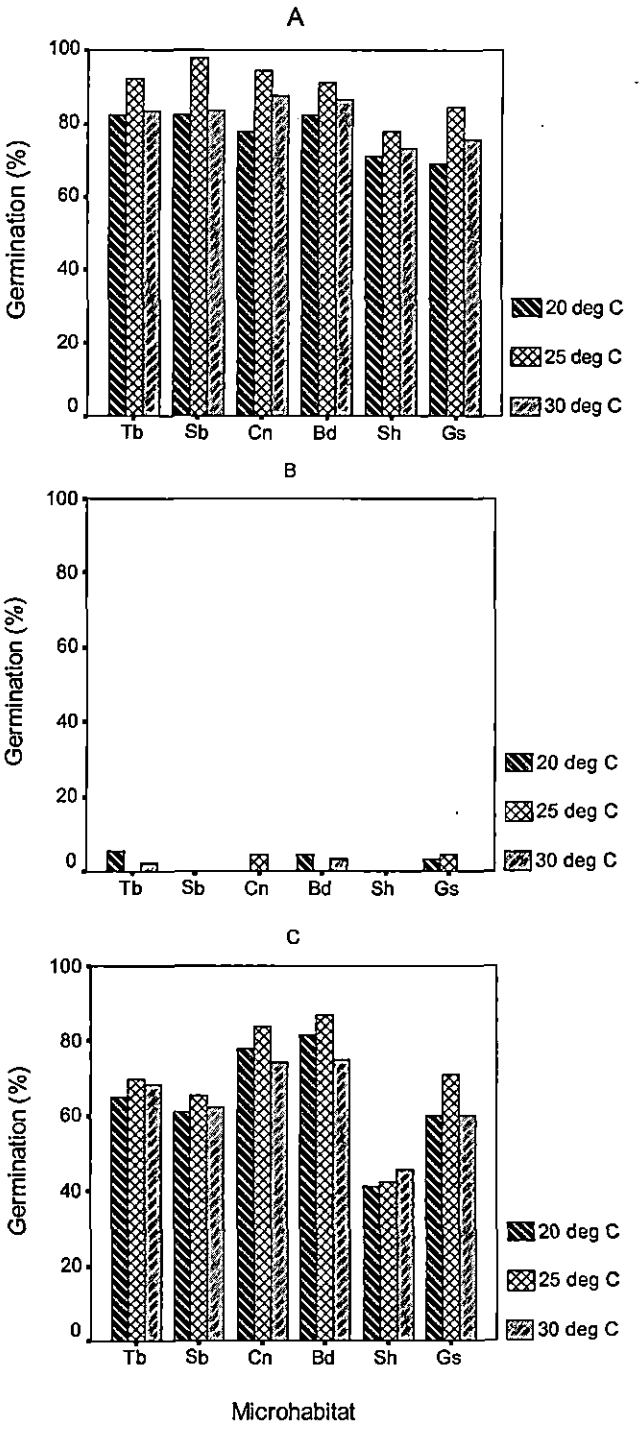



**Figure 15.** Seed germination response to various microhabitats and temperatures in *Swertia chirayita* (**A**: 14/10hrs. light/dark period, **B**: continuous darkness, **C**: transferred from continuous dark to 14/10hrs light/dark period after 45 days; Tb = tree base, Sb = stump base, Cn = canopy, Bd = boulder, Sh = shrubberies, Gs = grassy slope).

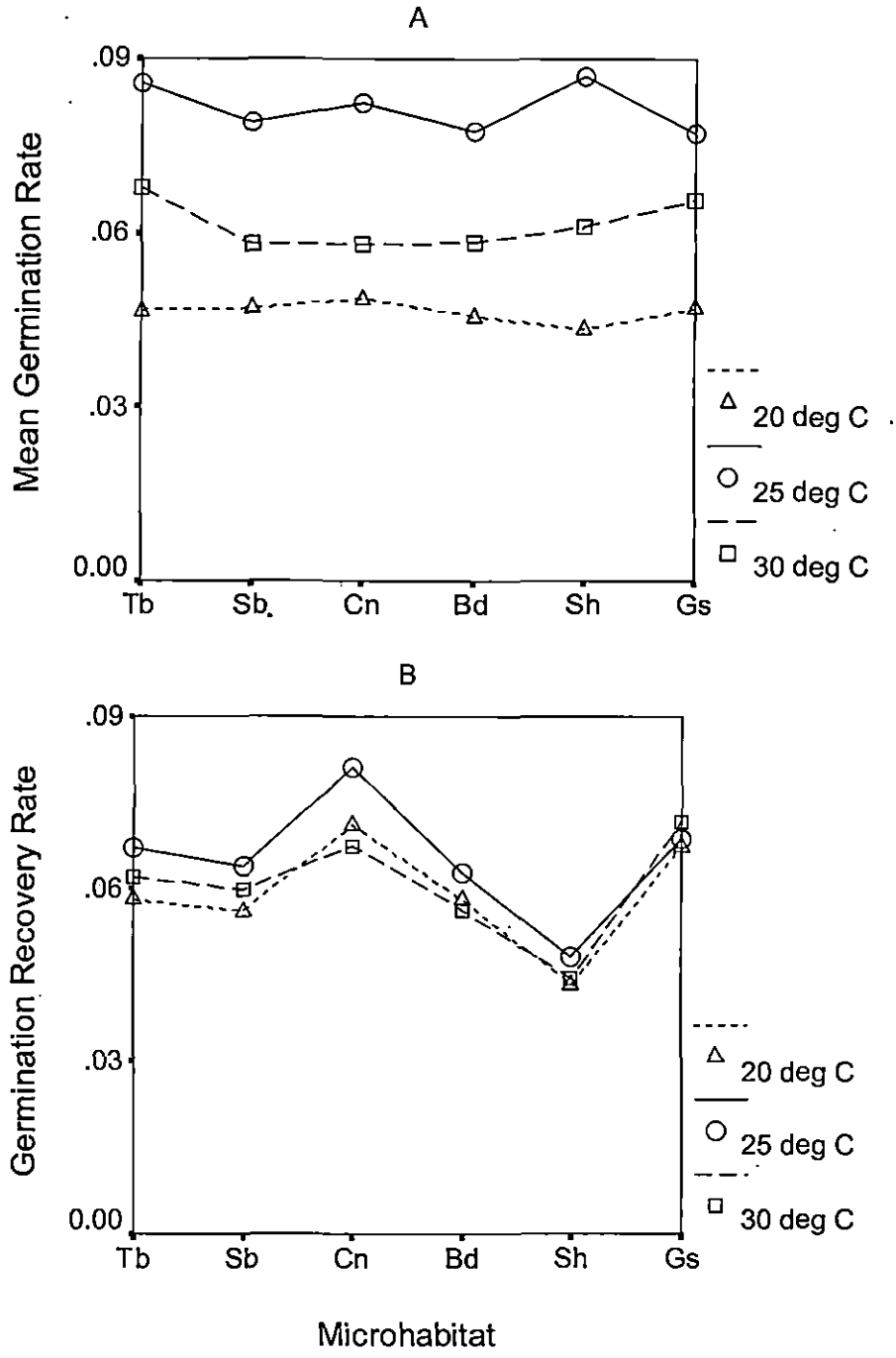



**Figure 16.** Microhabitat and temperature effect on germination rate and germination recovery rate in *Swertia chirayita* (**A**: Germination rate, **B**: Germination recovery rate; Tb = tree base, Sb = stump base, Cn = canopy, Bd = boulder, Sh = shrubberies, Gs = grassy slope).

CHAPTER 7

# Effect of Storage Period on Seed Germination

## 7.1 Introduction

Understanding response of different populations on seed germination provides helpful clues on genetic make-up of the species and its existence in the natural settings (Baskin and Baskin, 2001), essential to select elite seeds (Jayshanker *et al.*, 1999) for *ex-situ* conservation of gene resource. For the same, seed storage and its testing to monitor losses in germination rate, which might adversely affect nursery recovery rates, is considered as one of the most efficient methods (Gonzalez-Benito *et al.*, 1998, Williams *et al.*, 2007). However, poor seed germination in several cases is one of the limiting factors (Butola and Badola, 2004b, 2006a; Badola and Butola, 2005). Due to inappropriate storage, germination capacity of a species may decline during first few months after collection (Romanas, 1991); whereas, proper storage may be effective over a considerable period (Butola and Badola, 2004ab; Chen *et al.*, 2007).

*Swertia chirayita* (Roxb. ex Fleming) H. Karst, (Gentianaceae) grows mostly in temperate Himalaya (1200-3000m asl) from Afghanistan to Bhutan (Kirtikar and Basu, 1984), and used ethno-medicinally since centuries (Wawrosch *et al.*, 2005). Plant has numerous medicinal properties (Joshi and Dhawan, 2005), and is used as a liver stimulant and in bronchial asthma, dyspepsia, debility, fever, gastrointestinal infections, curing various skin problems and scorpion bite (Biswas and Chopra, 1982; Kirtikar and Basu, 1984; Nadkarni, 1976). For *Swertia chirayita*, the presently targeted species, very meager efforts are reported, such as, micro-propagation (Wawrosch *et al.*, 1999, Joshi and Dhawan, 2007) in the absence of successful domestication (Joshi and Dhawan, 2005). The current literature lacks any populations' based assessment of seed germination, especially related to storage in *S. chirayita*, a critically endangered species (Anonymous, 1997). The species has very high pharmaceutical value in national and international markets and is prioritized at top for ex-situ cultivation in an international experts' exercise (Badola and Pal, 2002). As the

species regenerates only through seeds, understanding response of different populations on seed germination behaviour, especially seed tolerance to periodical storage would be crucial for *ex-situ* conservation of the same. Low density of species in nature (Bhatt *et al.*, 2006) further necessitates such investigation.

The present study aimed to investigates *S. chirayita* for, (i) variability amongst populations for their seed characteristics and response on germination, (ii) trends of seed germination responses to storage periods amongst populations and individually for each population, to identify elite seed resources.

## 7.2 Materials and Methods

### *7.2.1 Seed collection*

Matured seeds of *S. chirayita* were collected from 13 populations (Table 14) of Sikkim Himalaya ($27^0$04'46" and $28^0$07'48" N lat. and $88^0$00'58" and $88^0$55'25" E. long.) during November-December 2005. At each population site, seeds were collected sustainably from 15 to 20 individuals randomly selected form the mid of the population without disturbing their natural spreading. The seeds for each population were mixed thoroughly to minimize effects of single parental plant on germination. The seeds were brought to the laboratory and cleaned thoroughly for impurities and dried for 10 days at room temperature.

### *7.2.2 Seed morphology*

To determine the moisture content, 50 seeds/replicate (3 replicates/population) were weighed and oven dried ($60^0$C; 48 hrs). To evaluate the number of seeds per fruit, seeds were counted using 10 healthy fruits per population. Seed size was measured using 30 seeds per population under microscope (10 seeds each of 3 replicates).

### *7.2.3 Seed germination*

Owing to minute seeds and finding difficulty in detaching embryo, the viability test using 2,3,5, Triphenyle tetrazolium chloride solution could not be executed. Seeds after room drying were tested for initial germination and remaining seeds were stored

population wise in proper sealed specimen tubes at $4^0$C, for periodical re-testing (6 months interval). For each test, 30 seeds/replicate (3 replicates/population) were disinfected (5 sec.) with sodium hypochlorite solution (4% w/v available Chlorine), washed thoroughly with double distilled water (DDW), and separately soaked in DDW for 24 hours, before placing in Petri-plates (90mm dia.) lined with single filter paper (Whattman No. 1) under incubator (average temperature $25^0$C $\pm$ 2.00; 14/10 hrs light/dark periods), maintaining randomized design and monitored daily (Photo Plate III). The filter papers were moistened daily using DDW. Seeds were considered germinated upon radical emergence and counted daily.

### *7.2.4 Statistical analysis*

Mean germination time (MGT) was calculated using equation, MGT = $\Sigma$(nd)/ $\Sigma$N; where n = number of newly germinated seeds after each incubation period in days d, and N = total number of seeds emerged at the end of the test (HartMann and Kester, 1989).Univariate and Multivariate ANOVA in general linear model (GLM) using SPSS 10.0 for Windows were used to determine the effects of population and storage period on seed germination and mean germination time. Bonferroni test was employed to determine the variation in means of seed germination and MGT.

## 7.3 Results

A variety of habitats supports populations of *Swertia chirayita*, which differed significantly for various seed characteristics (Table 14). Multivariate ANOVA revealed significant variation in mean germination percentage and mean germination time in *S. chirayita* amongst populations, increasing storage period and their interaction (Table 15).

During initial testing, 100% seed germination was recorded in majority of the populations (Figure 17). Testing of seeds of *S. chirayita* for their germination potential at initial ($r$ = -0.724; $p<0.05$), after 6 month ($r$ = -0.384) and 12 months ($r$ = -0.004) revealed negative correlation with altitude of the seed collection sites, while testing after 18 months and 24 months of storage showed positive correlation between seed germination and altitude. The duration of storage had significant effect on seed










**Photo Plate III.** Laboratory testing of seed germination potential in *Swertia chirayita*
A and B: Incubation of seeds in the germination chamber;
C: Petriplate containing germinated seeds

germination in *S. chirayita*; nevertheless, there was not much variation in seed germination percentage between initial and the 6 months storage in populations of *S. chirayita*. Across plant populations, mean seed germination in all the samples was significantly ($p<0.001$) higher than Sc10 during initial testing ($p<0.001$), and was significant at $p<0.05$ level after six month of storage except Sc11. However, Sc10 resulted in lower seed germination (70%) compared to 6 months (86.67%) and 12 months (81.11%). Further increasing the storage period to 12 months, Sc8 and Sc9 resulted in above 90% seed germination while other population retained above 60% germination; however, seed germination in P2 (52%) was significantly ($p<0.05$) low compared to other populations. Seed germination fell drastically in seeds collected from Sc4, Sc6, Sc7 and Sc11 after 18 months of storage; however populations viz., Sc1, Sc3, Sc5, Sc8 and Sc9 recorded above 50% seed germination (Figure 17). The seed germination remained maximum in P9 (79%), followed by P1 (74%) over other populations. Some of the populations such as Sc1, Sc8 and Sc9 resulted in above 50% seed germination even after 24 months of storage. The maximum seed germination percent was showed by P1 (59%) and P9 (58%). Of all the populations, Sc4 recorded fastest fall in seed germination percentage from initial (100%) to 24 months (8.89%) of storage (Figure 17). Collectively, for all the populations, seed germination percentage reduced significantly with increase in duration of storage (Figure 18).

The highest mean germination time (MGT) was recorded during initial test ($p<0.0001$) which ranged from 15.17 days (Sc3) to 59 days (Sc10) [Figure 19]. Cumulatively, six month storage recorded significantly ($p<0.0001$) lowest mean germination time in all the populations which showed increasing trend with increase in storage period upto 12 and 18 months but showed irregularity in MGT after 24 months storage (Figure 18). One way and Two way ANOVA revealed the main effect on seed germination in *S. chirayita* was the storage period followed by altitude of the seed collection site and their interaction (Table 15).

## 7.4 Discussion

The present experiment clearly indicates that 16% to 43% moisture content in seeds can retain seed viability for longer period in *S. chirayita* which is in conjunction with

our another study where 19% to 44% seed moisture resulted in longer seed viability in some of the populations in *S. chirayta* (refer chapter 10). Hampton and Hill (2002) also suggested 10% to 40% seed moisture content as a requirement for maintaining seed viability for longer period.

The variation in seed germination percentage amongst different populations in *S. chirayita* indicates the existence of species genetic diversity. Similar differences in germination response amongst plant population have been reported (Perez-Garcia *et al.*, 1995; Baskin and Baskin, 2001; Cerabolini *et al.*, 2004), which will provide valuable clues for selection of elite populations for seeds (Jayshanker *et al.*, 1999). 100% seed germination in majority of population during initial test, reveal the availability of potential gene bank of *S. chirayita* in Sikkim Himalàya. However, steady decline in germination from initial to final test (24 month storage) in *S. chirayita* indicates the seeds are destined to deteriorate over time, even under appropriate conventional storage. The increase in seed germination percentage from initial upto 12 months storage in Sc10 reveals that seeds of *S. chirayita* from high altitude (2841m asl) attains physiological dormancy, and dry storage at $4^0$C upto 6 to 12 months helps in breaking seed dormancy.

In *S. chirayita*, storage ($4^0$C) proved quite effective up to 12 months, suggesting valuable genetic material for propagation, retaining high seed longevity and providing an environment for minimum physiological and pathological deterioration (Williams, 2000). However, using of seeds after 6 month storage helps in reducing the germination time. Positive response of seeds after storage at $4^0$C responds after 6 months (Butola and Badola, 2004ab) to 12 months (Chen *et al.*, 2007) have been reported. This is important for scheduling spring/summer sowing in field, an appropriate time for many Himalayan herbs (Badola and Singh, 2003; Badola and Butola, 2003, 2005). In addition, the result of our study has broad implications for people who stores seed for longer period. In order to understand the germination potential of seeds of important plant species, it is imperative to test seed within a few days after the collection.

A majority of present populations of *S. chirayita* showed high seed germination, in general, irrespective of altitude or habitat differences indicating that a majority of sites in Sikkim offer suitable growing conditions for *S. chirayita*, which is an encouraging finding useful to restoration of this critically endangered species (Anonymous, 1997). Still, slow disappearance of this species from nature apart from anthropogenic pressure is a matter of concern, which may require further field studies on seedling growth and survival.

## 7.5 Conclusion

This study concludes that Sikkim Himalaya has high potential gene bank of *S. chirayita*. The seed of *S. chirayita* responds best if sown within 12 months after storage at $4^0$C and more suitably after 6 months and scheduling seed sowing in pre-monsoon for best results, instead of immediate sowing after collection.

**Table 14.** Seed characteristics of different populations of *Swertia chirayita* in Sikkim

| Name of Population | Altitude (m asl) | No. seeds/fruit | Seed weight (mg/50 seeds) | Seed length (µm) | Seed width (µm) | Moisture content (%) |
|---|---|---|---|---|---|---|
| P1-Luing (ES) | 2126 | 212 | 1.6 | 487.33 | 355.67 | 23.47 |
| P2 - Railgaon (ES) | 1948 | 302 | 1.5 | 534.83 | 381.83 | 20.54 |
| P3 – U Pangthang (ES) | 2176 | 316 | 1.6 | 503.00 | 381.50 | 27.15 |
| P4 - Guransey dara (ES) | 2107 | 230 | 1.1 | 457.30 | 356.17 | 24.39 |
| P5 - Jaunbari (SS) | 1651 | 221 | 1.3 | 452.33 | 376.67 | 35.16 |
| P6 - U Chamgaon (SS) | 1583 | 233 | 1.3 | 432.17 | 343.33 | 20.60 |
| P7 – Tiffin dara (SS) | 1667 | 227 | 1.2 | 435.83 | 335.83 | 16.24 |
| P8 – U Changrang (ES) | 2055 | 275 | 1.7 | 497.33 | 370.83 | 17.32 |
| P9 – Phinsyonala (NS) | 1761 | 233 | 1.2 | 446.00 | 347.00 | 38.23 |
| P10 – Zemathang-1 (NS) | 2841 | 249 | 1.3 | 461.67 | 333.17 | 42.61 |
| P11 – Khecheopalri (WS) | 1867 | 200 | 1.4 | 429.00 | 221.17 | 25.47 |
| **(P<0.05)** | | **81.84** | **0.16** | **29.53** | **38.25** | **7.01** |
| **F** | | **5.36** | **15.08** | **11.07** | **20.86** | **12.60** |

**Table 15.** Result of Multivariate ANOVA depicting the effect of population, storage period and their interaction on seed germination and mean germination time in *Swertia chirayita*

| | Population (P) | | Storage Period (SP) | | P x SP | |
|---|---|---|---|---|---|---|
| | **F value** | **Probability** | **F value** | **Probability** | **F value** | **Probability** |
| Germination (%) | 77.705 | .000 | 1169.434 | .000 | 18.802 | .000 |
| MGT | 22.367 | .000 | 218.493 | .000 | 21.231 | .000 |

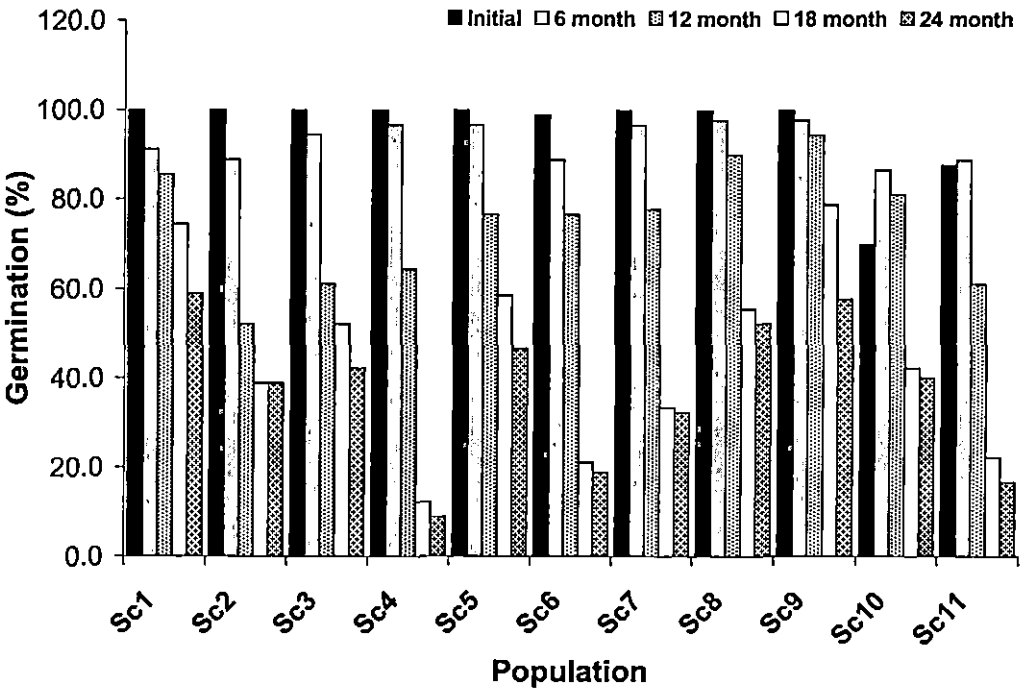



**Figure 27.** Effect of storage period on seed germination in populations of *Swertia chirayita*

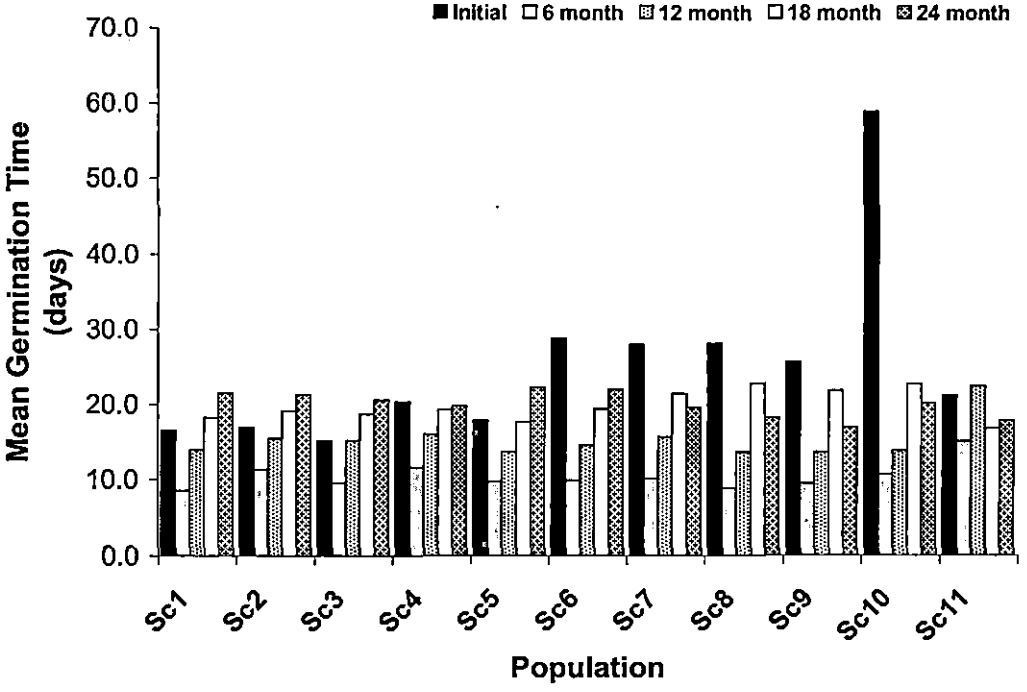



**Figure 18.** Effect of storage period on mean germination time in populations of *Swertia chirayita*

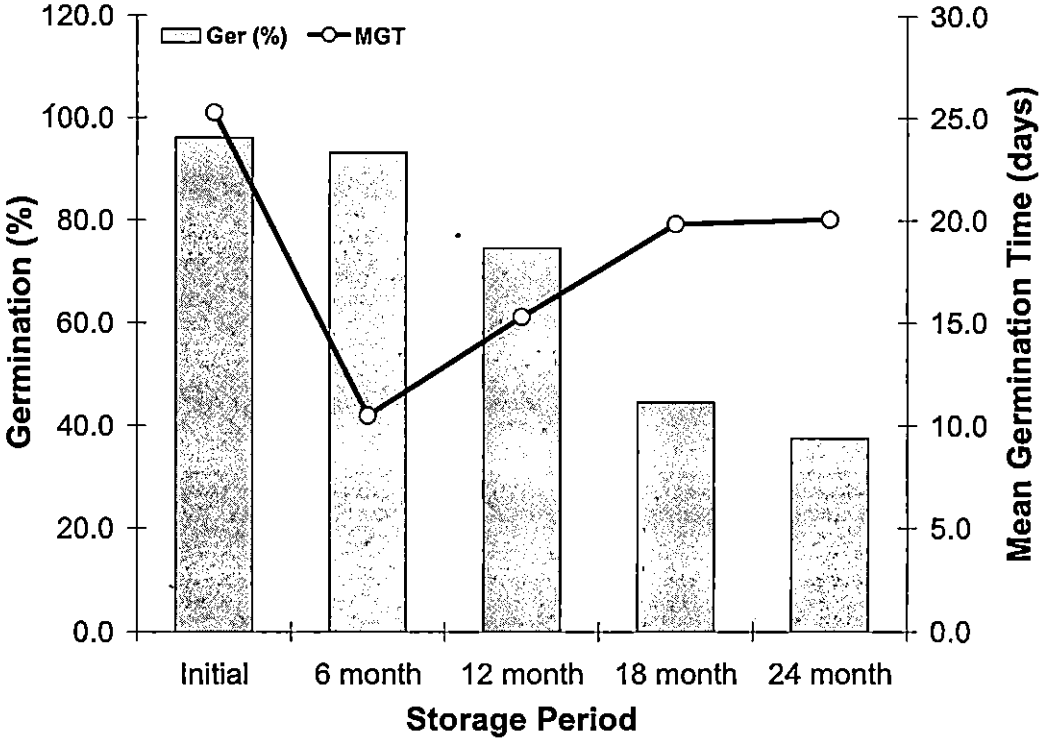



**Figure 19.** Effect of storage period on seed germination and mean germination Time in *Swertia chirayita*

CHAPTER 8

# Assessment of Seedling Emergence and Vigour for Quality Planting Material

## 8.1 Introduction

Indian Himalaya, an immensely rich reservoir of medicinal plant resources, has been targeted to harvest several high value species in meeting out ever-expanding raw material demand of the pharmaceuticals (Badola and Aitken, 2003). However, with growing herbal market in recent few decades, owing to over harvesting and unsustainable extraction practices several medicinal plant species have faced risk of their survival in natural habitats, and many assessed as threatened (Badola and Pal, 2002, 2003). Since, the raw material supply to herbal industry is a societal requirement, and conservation of genetic material is a vital concern towards its safeguard for long-term survival in nature. *Ex-situ* cultivation by developing suitable and easy to handle propagation and agro-technique packages for the farmers are the only answer to meet-out raw material supply of the pharmaceuticals, ensuring lessening harvesting pressures on natural habitats, thus helping *in-situ* conservation of threatened taxa (Nadeem *et al.*, 2000; Badola and Butola, 2005; Badola, 2009). Developing quality planting material is the primary requirement for successful cultivation of wild medicinal plants, especially Himalayan (Badola and Butola, 2003; Butola and Badola, 2006ac). The success of productive cropping will largely depend upon the appropriate choice of growing media or substrate combinations and in many Himalayan herbs, these substrates have proved promising to achieve improved seedling emergence, growth and crop productivity (Nautiyal *et al.*, 2001, 2003b; Pandey *et al.*, 2002; Badola and Butola, 2003; Butola and Badola, 2006b).

*Swertia chirayita* (Roxb. ex Fleming) H. Karst, a critically endangered herb, indigenous to temperate Himalaya, distributed along 1200 m – 3000 m asl is considered non toxic and safe in ethnomedicinal use (Nadkarni, 1998). The herb is exploited for bitter bioactive compounds including amarogentin, xanthones and iridoid glycosides (Jensen and Schripsema, 2002). The beverage industry has

discovered an alternative bitter product from *S. chirayita* (i.e. used in the liquor industries to impart bitter flavour to mouth). *S. chirayita* is valuable Ayurvedic medicinal herb, having high demand in Indian and international markets (Badola and Pal, 2002), and possess multiple use value in local medicine (Pradhan and Badola, 2008). Seeds are the only means of propagation in *S. chirayita* and poor seed germination and low seed viability in its natural environment has been considered as limitation to commercial cultivation of the species (Badola and Pal, 2002; Chaudhari *et al.*, 2007). The reason for poor natural regeneration in *S. chirayita* may be that the seed matures in dry period (November-December) where the availability of moisture in the natural environment is very low. The seed emerges out in late May prior to start of monsoon shower and the species does not get the required moisture for sufficient germination. The present authors field experiences reveals that the immature fruits of *S. chirayita* provides shelter and the necessary nutrients required for the growth and development of insect larvae which prohibits the fruit from further development and could be another factor resulting in poor natural regeneration in *S. chirayita.*

Micro propagation of *S chirayita* has been tried by several workers (Wawrosch *et al.*, 1999; Ahuja *et al.*, 2003; Chaudhari *et al.*, 2007, 2008, 2009; Joshi and Dhawan, 2007; Balaraju *et al.*, 2009b; Wang *et al.*, 2009). There are not many reports on studies relating to germination and viability of *S. chirayita* seeds. Raina *et al.*, (1994) reported 91% seed germination after chilling to $3^0$C for 15 days as compared to 3.4% germination in control seeds in Himachal population; Chaudhari *et al* (2007) reported difficulty in germination. However, the present study reports 100% seed germination, initially, under laboratory conditions, without any treatment in majority of the natural populations and also 5 populations retaining above 50% germination after 18 months of storage at $4^0$C in *S. chirayita* in Sikkim (refer chapter 7). Nevertheless, physiological dormancy was observed in seeds procured from domesticated plants, and that was broken by suitable chemical treatments (refer chapter 11). In commercial cultivation of the species, besides having slow growth rate (Basnet, 2001), the variability in commencement of seedling emergence has been identified as the major problem resulting in the production of non-uniform seedlings; therefore, the need of

the hour was to develop uniform and vigorous seedlings for successful and productive crop cultivation of the species.

In view of this, the present study aimed to (1) standardize effective substrate combination for obtaining uniform and vigorous seedlings, and (2) identify morphological traits for the assessment of healthy seedlings as quality planting material for cultivation in *Swertia chirayita*.

## 8.2 Materials and methods

### *8.2.1 Experimental design*

The matured fruits of *Swertia chirayita* were collected from thirteen populations (Table 16) distributed in different parts of Sikkim Himalaya (27°04′46″ and 28°07′48″ N lat. and 88°00′58″ and 88°55′25″E. long.) during the month of November – December. The collection of fruits/seeds was made sustainably in such a way that 15-20 robust plants per site were randomly selected from the middle of each population without disturbing the other plants keeping in view that the seeds are the only means of reproduction in endangered species *S. chirayita*; collection from the margins was avoided to prevent the natural spreading of the species and to limit the edge effect, if any. The collected fruits were brought to the laboratory and seeds were segregated by mashing up fruits which followed the thorough cleaning to remove impurities. The seeds were dried in ambient room temperature for ten days, and stored in refrigerator ($4^0$C) in properly sealed specimen tubes separately for different populations till nursery experiment initiated. Following good result at the initial test in the laboratory (refer chapter 11); the seeds were tested under nursery conditions in the end of February 2006 using garden soil [pH: 6.43±0.05; moisture (%): 42.00±2.00; nitrogen (%): 0.40±0.11; phosphorus (%): 0.41±0.05], forest humus [pH: 5.91±1.00: moisture (%): 42.67±2.31; nitrogen (%): 0.43±0.01; phosphorus (%): 0.29±0.04], farm yard manure [pH: 8.32±0.06; moisture (%): 46.39±0.17; nitrogen (%): 6.57±0.02; phosphorus (%): 7.29±1.25], and sand [pH: 7.17±0.02; moisture (%): 6.40±0.96; nitrogen (%): 0.25±0.03; phosphorus (%): 0.02±0.02]. At first, two substrate combinations of equal volume were prepared, substrate1 (pH: 6.5): Garden soil + Sand + Forest humus (1:1:1) and substrate 2 (pH: 7.08): Garden soil + Sand +

Farm yard manure (1:1:1), and substrate combinations were mixed well. These substrate combinations were filled in earthen trays. Trays filled with sand and Garden soil in 3:1 ratio (pH: 6.7) were considered as control. The seeds of *S. chirayita* were soaked overnight in double distilled water before sowing. Each set of experiment had three replicates of 30 seeds per replicate; the seeds were sown at 0.5cm depth with equal distance between seeds (Photo Plate IV). The experiment was conducted under an automatic temperature controlled green-house ($25^0C \pm 5^0C$). Mist irrigation was done twice a day (0930 hrs and 1630 hrs) till the completion of the seedling emergence which was reduced to once in a day (0930 hrs) after seedling emergence stopped providing optimal moisture to growing plants. Weeding was done at equal interval.

In another set of experiment, 100 seeds per population (Sc1, Sc2, Sc3 and Sc4) were sowed directly in the natural habitat viz., steep forest slope and mild forest slope, and were monitored for seedling emergence.

Seedlings after the completion of emergence (eight weeks) were thinned to mitigate competition, maintaining 8-10cm distances. After 24 weeks, the effect of different substrate combinations on seedling growth, different morphological traits were evaluated by harvesting five seedlings each of uniform size for all populations. During experiment, the seedlings remained in rosette form; the identified traits included collar diameter, largest leaf length, leaf width and number of roots and biomass. To obtain dry biomass, after recording the fresh weight, seedlings were oven dried at $60^0C$ to constant weight and reweighed and the value obtained was recorded.

### *8.2.2 Seedling survival*

For seedling survival test, fifteen seedlings, of more or less uniform size, per substrate (five each of three replicates), including control, were transplanted in 20 x 20 cm distance under a net shade in the Institute's experimental medicinal plant garden and monitored by the end of growing season. Populations were not taken into consideration for observing survival. Plant survival for each substrate combinations and control set was recorded.

### 8.2.3 Statistical analysis

SPSS 10.0 for Windows (SPSS Inc. 1989) was used for statistical analysis of the data. Mean emergence rate (*e*) was calculated following Labouriau (1970) as, $e = 1/\sum(xd)/\sum t$; where, x = number of newly emerged seedlings in each day d, and t = total number of seedling emerged at the end of the test. Multivariate analysis of Variance (MANOVA) was carried out to determine the effect of the substrate combinations concerning on the seedling emergence and seedling morphological traits. A Bonferroni test ($p<0.05$) was used to determine differences amongst the treatments. Pearson's correlation analyses were performed to analyze the relationship between different morphological traits for each substrate combinations including control. To identify the best substrate combinations, Seedling Vigour Index (V) was calculated following Butola and Badola (2004) as $V = (d/e)*100$; where, "*d*" is dry weight/seedling; "*e*" is mean emergence rate and 100 is constant.

## 8.3 Results

Direct sowing in the natural habitat resulted in very low seedling emergence in all the four populations in both the conditions; however, seedling emergence was higher in mild forest slope compared to steep forest slope (Figure 20).

Multivariate analysis of variance (MANOVA) revealed significant effect of populations, treatments and their interaction on various seedling emergence parameters and growth parameters in *S. chirayita* (Table 17 and Table 18).

### 8.3.1 Effect of substrate combinations on seedling onset, T50 and final emergence

Minimum of four weeks and maximum of five weeks were taken for the onset and completion of 50% seedling emergence ($T_{50}$), and minimum of five weeks and maximum of seven weeks were taken in completing final emergence in all the populations irrespective of substrate combinations, including control (Figure 21). However, seedling onset, $T_{50}$, and final emergence was significantly faster in Substrate 1 ($p<0.05$) than Substrate 2 and control.

### 8.3.2 Effect of different substrate combinations on seedling emergence, mean emergence time and emergence rate

Seedling emergence and altitude of populations (seed collection site) showed negative (non-significant) correlation with all the substrate combinations including control (substrate 1, $r$ = -0.304; substrate 2, $r$ = -0.092; control, $r$ = -0.163). Seedling emergence was significantly higher in substrate 2 ($p<0.05$) over substrate 1 and control. Similarly, Substrate 1 recorded higher seedling emergence over control ($P<0.05$). The lowest (Sc10; 2841m asl) and the highest (Sc11; 2000m asl) seedling emergence in control and substrate 1 and the lowest (Sc6; 1583m asl) and the highest (Sc13; 1867m asl) seedling emergence in substrate 2 were significant at $p<0.05$ level (Figure 22). Mean emergence time was significantly lower in substrate 1 over substrate 2 and control ($p<0.05$); likewise, it was significantly lower in substrate 2 compared to control ($p<0.05$). Comparatively, seedling emergence rate was significantly ($p<0.05$) higher in seeds sown in substrate 1 than substrate 2 and control (Figure 21).

### 8.3.3 Effect of different substrate combinations on seedling growth, biomass and vigour

Both substrate 1 and substrate 2 combinations were significantly ($p<0.05$) effective in improving all the seedling growth parameters over control in all the populations. However, on comparing between substrate 1 and substrate 2, the latter substrate combination was more effective ($p<0.05$) than former, but in the case of number of leaves the effectiveness was non-significant. Figure 23 and Figure 24 depict the effect of both the substrate combinations including control on various seedling growth parameters in *S. chirayita*. Significant ($p<0.05$) increase in total seedling biomass and SVI was obtained with substrate 1 and substrate 2 over control; however the best result achieved with substrate 2 ($p<0.05$) than substrate 1 (Figure 25). Of 13 populations assessed, seven (Sc1, 2, 3, 8, 9, 11, and 13) exhibited higher total seedling biomass and SVI using substrate 2, of which, Sc2 population followed by Sc8 recorded the highest values. For Sc6, the total seedling biomass and SVI was significantly greater with substrate 1 than substrate 2 ($p<0.05$). All the growth parameters were significantly ($p<0.0001$) correlated with each other (Table 19).

### 8.3.4 Effect of different substrate combinations on seedling survival

Seedlings developed using substrate 1 and substrate 2 combinations resulted in 77% and 83% survival, respectively, in nursery beds compared to control (63%). During the experimentation period, no insect infestation was observed in the seedlings.

## 8.5 Discussion

Difference in germination behaviour between the plant populations (Baskin and Baskin, 2001; Cerabolini *et al.*, 2004) has been indicated in earlier studies. Very low seedling emergence in *S. chirayita* sown directly in the natural habitat may be due to washing away of seeds or percolation of seeds to deep under ground due to heavy rain. The present study considered that appropriate substrate combinations are essential for the successful establishment of a species. Higher seedling emergence with substrate 1 and substrate 2 combinations compared to control in the present study further strengthens the above assumption. Of the two substrate combinations, substrate 2 resulted in higher seedling emergence in all the populations than substrate 1, which further significantly enhanced the seedling performance for different growth parameters and resulted in higher percent survival, as well, suggesting substrate 2 as the best substrate combination for *S. chirayita*. The present results are based on a large number of populations and mild use of manure, where all populations exhibited higher performance for both seedling emergence as well as seedling growth, especially greater seedling vigour in majority of populations. That means, the present substrate combination offers wider application for *S. chirayita* in cultivation programme. This is particularly applicable for developing quality planting material, a pre-requisite to successful cultivation of *S. chirayita*, this is also important, as the direct sowing in *S. chirayita* is not suggestive as in the case of *Heracleum candicans* (Badola and Butola, 2003) but the transplantation of one season old seedling performed well (refer chapter 10 and chapter 13). Butola and Badola (2006c) in their study recorded highest seedling emergence with the mixture of sandy loam and vermi-compost and sandy loam and forest humus (mixture in equal proportion, 1:1) in *Angelica glauca* and *Heracleum candicans*, respectively, suggesting that the species responds differently in their nutrient requirements.

The light requirement of the species for germination ensures that the seeds will germinate successfully on or near the soil surface, when other conditions are favourable for seedling emergence. In the present study, the seeds were sown at 0.5cm depth and showed positive result. Similar results have been earlier reported for Himalayan herbs (Butola and Badola, 2005, 2006c, 2007).

Germination of seeds may be inhibited if the amount of water is too low or if it is too much (Baskin and Baskin, 2001). In case of *Swertia chirayita*, healthy and robust form of the species in open slopes compared to flat lands in natural habitats suggested that the water requirement of the species is very low (*personal observation*). Therefore, beside appropriate substrate combinations, proper irrigation in terms of volume and timing is also essential to maximize production of high quality plant material and optimum percent survivability. Warren and Bilderback (2004) suggests an early morning hour (before 10.00 hrs) as suitable time to irrigate plants to reduce potential of wind blowing the irrigation water from the targeted area and to reduce evaporation of irrigation water. Irrigation in the form of mist has resulted in high percent emergence, as the seeds were protected from being swept away by the force of the water due to its minute size, thereby giving high survivability in *S. chirayita* in the present study.

Identifying appropriate morphological traits helps in assessing plant health and productivity (Butola and Badola, 2004b). For example, strong correlation of collar diameter to total seedling biomass suggested that these traits are of great use in pre-assessing crop productivity. The plant height could not be considered for identifying seedling vigour in *S. chirayita* as in the case of *Angelica glauca* (Butola and Badola, 2004b) because, for the first two years the *S. chirayita* plants remains in rosette form and merely exceeds 15 cm height. Instead, collar diameter provides a general idea about the seedling durability and has been considered as one of the best predictors of field survival and healthy growth (Thompson, 1984), which is supported by significantly strong and positive correlation between collar diameter and total seedling dry biomass ($r = 0.864$; $p<0.0001$) and between collar diameter and SVI ($r = 0.841$; $p<0.0001$) in case of *S. chirayita* in present study.

## 8.6 Conclusion

The present study concludes that substrate 2 (garden soil, sand, farm yard manure in equal proportion) is the most effective substrate combinations resulting in high percent emergence, uniform and vigorous seedlings and high field plant survival after transplantation. In addition, the collar size appeared to be the strongest morphological trait by which the seedling vigorousness could be identified, as an aid to successful *ex-situ* cultivation of high value medicinal crop, *Swertia chirayita.*




**Photo Plate IV.** A and B: Testing of seedling emergence with substrate combinations in *Swertia chirayita*

**Table 16.** Site characteristics of the populations of *Swertia chirayita* in Sikkim

| Site code | Site Name | Altitude (m) | Aspect | Slope (°) | GPS | |
|---|---|---|---|---|---|---|
| | | | | | Latitude (N) | Longitude (E) |
| Sc1 | Luing (ES) | 2126 | NE | 55 | 27°21'47.60" | 88°34'12.17" |
| Sc2 | Railgaon (ES) | 1948 | NE | 40 | 27°21'52.71" | 88°33'47.07" |
| Sc3 | U Pangthang (ES) | 2176 | SW | 45 | 27°21'13.54" | 88°33'53.37" |
| Sc4 | Guransey dara (ES) | 2107 | SE | 60 | 27°20'51.02" | 88°34'07.92" |
| Sc5 | Jaunbari (SS) | 1651 | SW | 50 | 27°11'47.20" | 88°23'33.60" |
| Sc6 | U Chamgaon (SS) | 1583 | NW | 55 | 27°11'03.80" | 88°22'26.20" |
| Sc7 | Tiffin dara1 (SS) | 1668 | W | 60 | 27°11'14.8" | 88°22'46.01" |
| Sc8 | U Changrang (ES) | 2055 | S | 65 | 27°21'51.97" | 88°35'20.31" |
| Sc9 | Phinsyonala (NS) | 1761 | NE | 55 | 27°37'01.6" | 88°37'26.2" |
| Sc10 | Zemathang 1 (NS) | 2841 | NE | 60 | 27°44'34.7" | 88°32'48.0" |
| Sc11 | L. Pangthang (ES) | 2000 | N | 40 | 27°21'49.52" | 88°33'32.15" |
| Sc12 | Kalipokhari (ES) | 2450 | N | 50 | 27°21'27.31" | 88°33'24.18" |
| Sc13 | Khecheopalri (WS) | 1867 | SW | 55 | 27°21'06.07" | 88°11'21.06" |

**Table 17.** Result of MANOVA characteristics by populations, treatments and their interaction on *Swertia chirayita*

| Dependent variables | Independent variables | | | | | |
|---|---|---|---|---|---|---|
| | Population (P) | | Treatment (T) | | P x T | |
| | F value | Probability | F value | Probability | F value | Probability |
| Seedling onset | 3.407 | 0.000 | 38.778 | 0.000 | 3.991 | 0.000 |
| $T_{50}$ | 2.111 | 0.025 | 19.030 | 0.000 | 2.058 | 0.009 |
| Final emergence | 5.086 | 0.000 | 13.000 | 0.000 | 4.363 | 0.000 |
| Emergence (%) | 8.587 | 0.000 | 34.595 | 0.000 | 3.566 | 0.000 |
| Mean emergence time | 3.808 | 0.000 | 40.388 | 0.000 | 4.786 | 0.000 |
| Emergence rate | 3.311 | 0.000 | 33.694 | 0.000 | 4.460 | 0.000 |

**Table 18.** Result of MANOVA characteristics by populations, treatments and their interaction on *Swertia chirayita*

| Dependent variable | Independent variables | | | | | |
|---|---|---|---|---|---|---|
| | Population (P) | | Treatment (T) | | P x T | |
| | F value | Probability | F value | Probability | F value | Probability |
| Collar diameter | 10.115 | 0.000 | 119.699 | 0.000 | 6.214 | 0.000 |
| Number of leaves | 7.354 | 0.000 | 384.623 | 0.000 | 8.772 | 0.000 |
| Largest leaf length | 31.524 | 0.000 | 880.279 | 0.000 | 18.160 | 0.000 |
| Largest leaf width | 9.576 | 0.000 | 437.101 | 0.000 | 8.233 | 0.000 |
| Number of roots | 10.922 | 0.000 | 182.709 | 0.000 | 6.502 | 0.000 |
| Total dry biomass | 12.394 | 0.000 | 241.057 | 0.000 | 0.668 | 0.000 |
| Seedling vigour index | 11.053 | 0.000 | 209.046 | 0.000 | 8.592 | 0.000 |

**Table 19.** Correlation matrix (Pearson Product-Moment Correlation Coefficient) between different growth parameters in *Swertia chirayita* after 24 weeks of sowing at *ca.* 2000m asl (n = 195; df = 193)

| | **Collar diameter (mm)** | **Number of leaves** | **Largest leaf length (mm)** | **Largest leaf width (mm)** | **Number of roots** | **Total seedling biomass (gm)** | **SVI** |
|---|---|---|---|---|---|---|---|
| Collar diameter (mm) | | | | | | | |
| Number of leaves | 0.668 | | | | | | |
| Largest leaf length (mm) | 0.824 | 0.776 | | | | | |
| Largest leaf width (mm) | 0.773 | 0.798 | 0.908 | | | | |
| Number of roots | 0.741 | 0.610 | 0.832 | 0.737 | | | |
| Total seedling biomass (gm) | 0.864 | 0.674 | 0.916 | 0.804 | 0.843 | | |
| SVI | 0.841 | 0.695 | 0.914 | 0.806 | 0.815 | 0.980 | |

*All seedling growth parameters significant at $p < 0.0001$

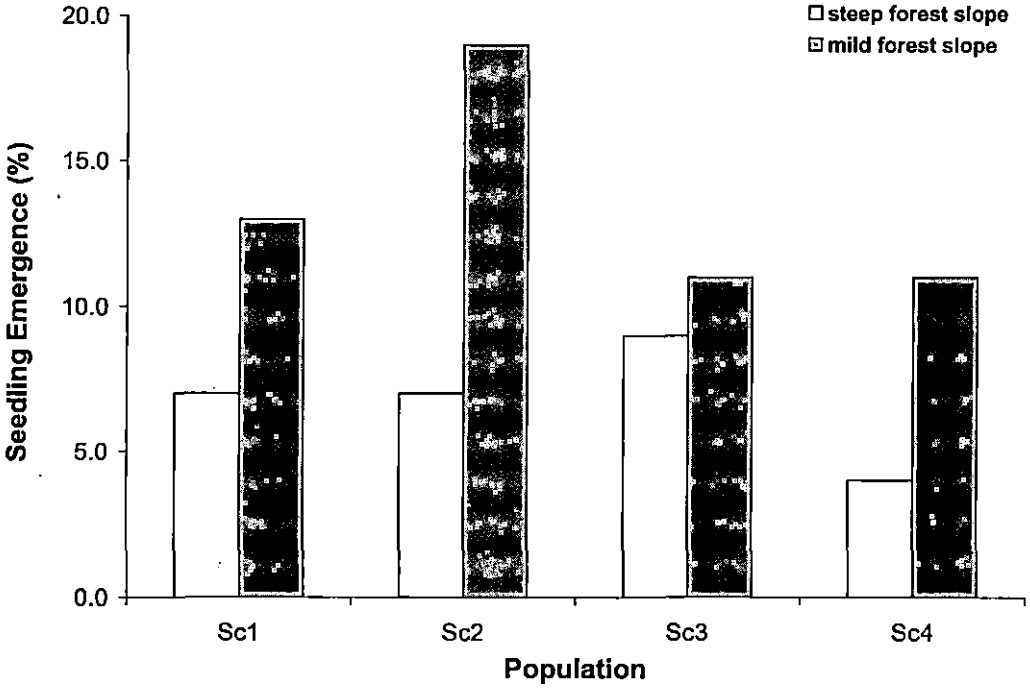



**Figure 20.** Seedling emergence *Swertia chirayita* as a result of direct sowing in natural habitat

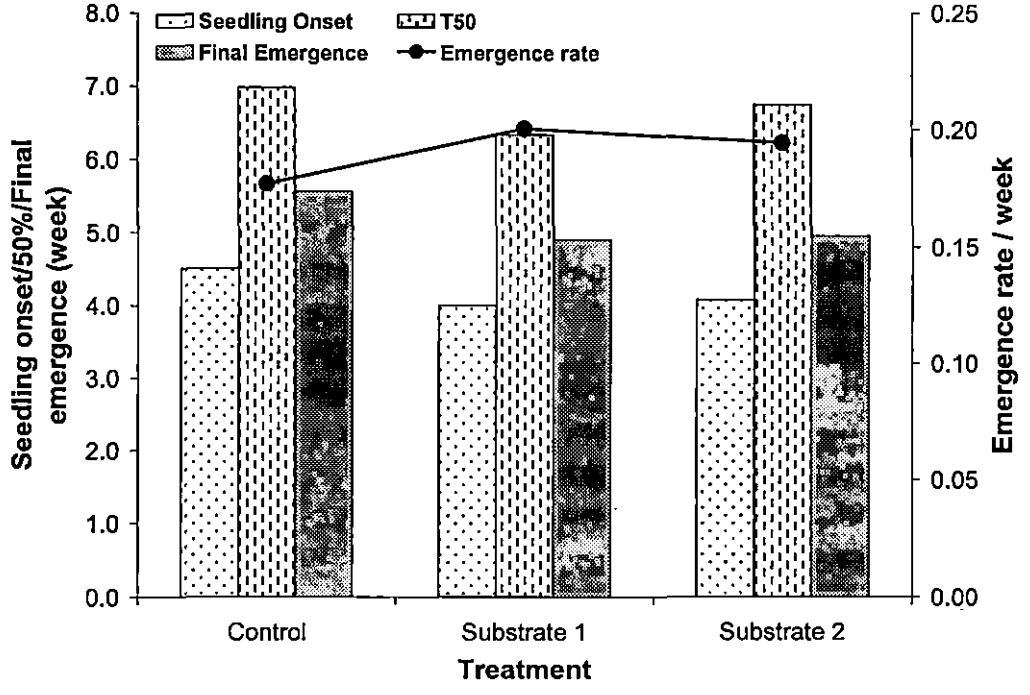



**Figure 21.** Effect of different substrate combinations on seedling onset, $T_{50}$, final emergence and emergence rate in *Swertia chirayita*

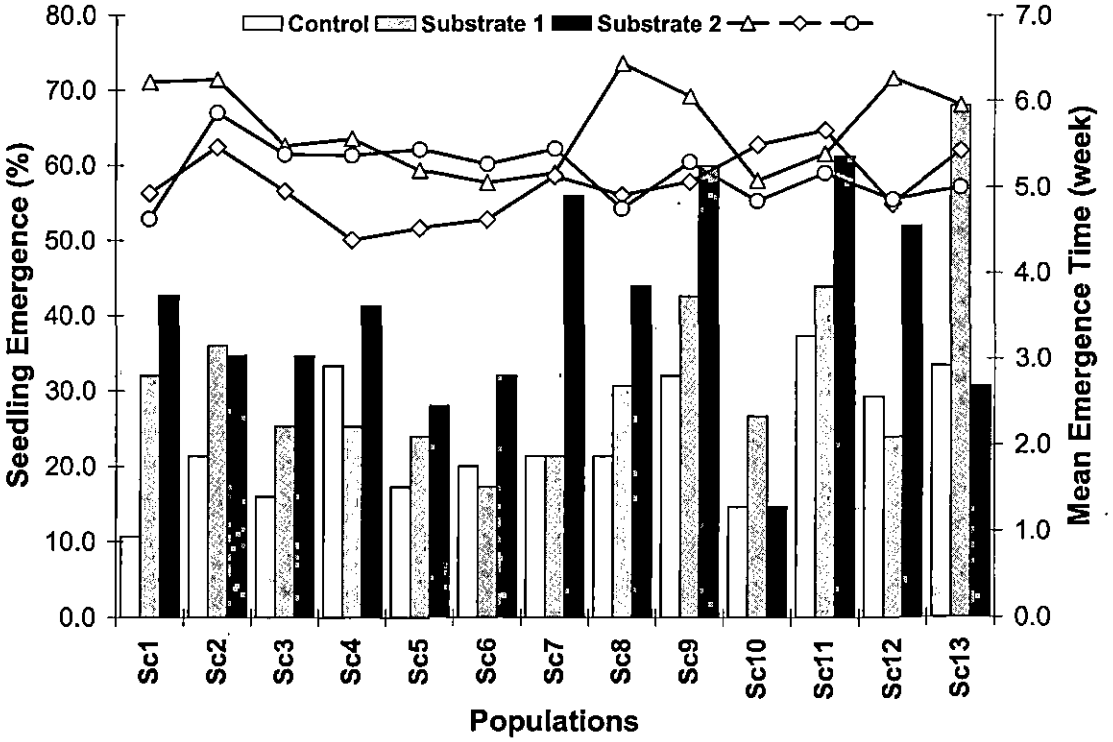



**Figure 22.** Effect of different substrate combinations on seedling emergence and mean emergence time in populations of *Swertia chirayita*

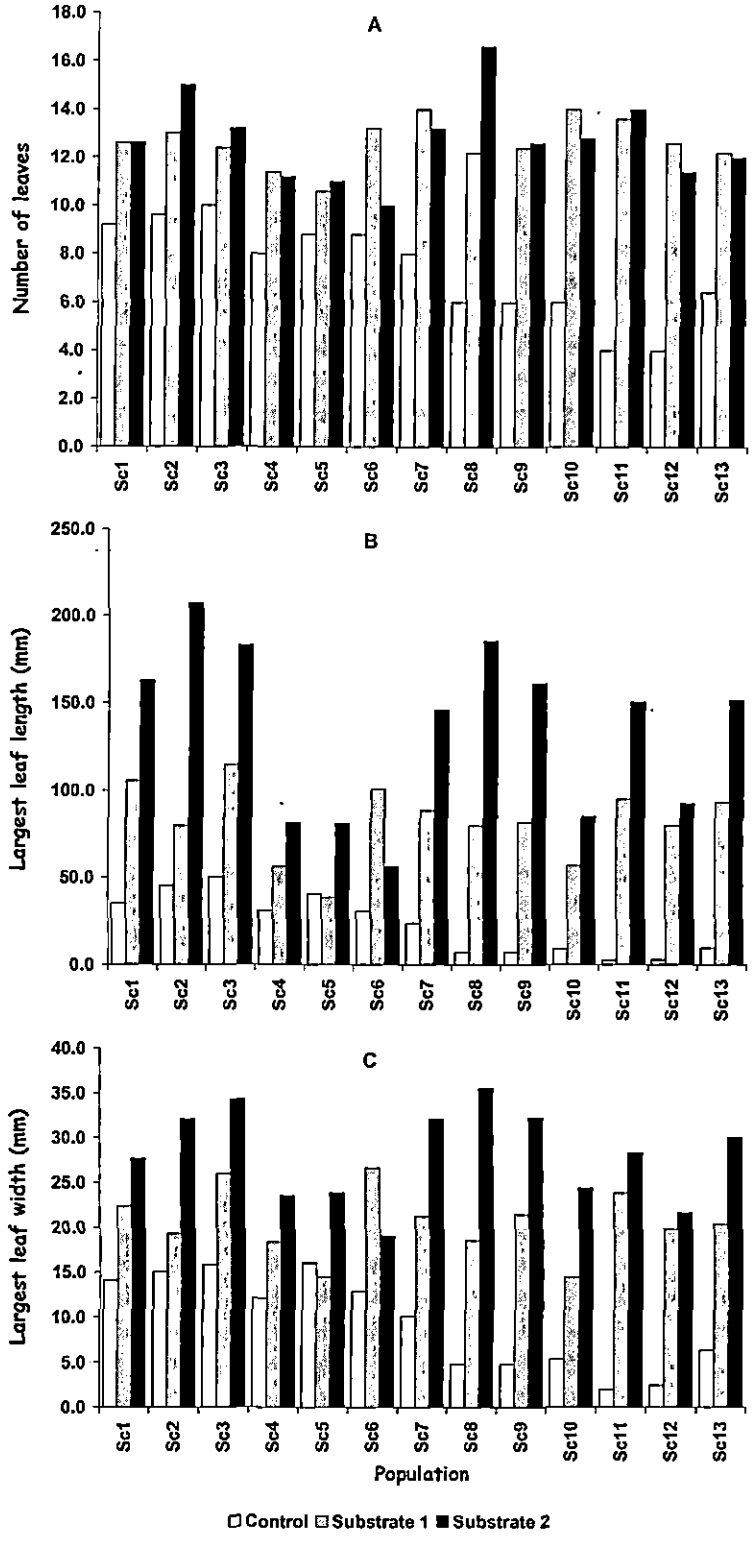



**Figure 23.** Effect of different substrate combinations on number of leaves, largest leaf length and leaf width in *Swertia chirayita*

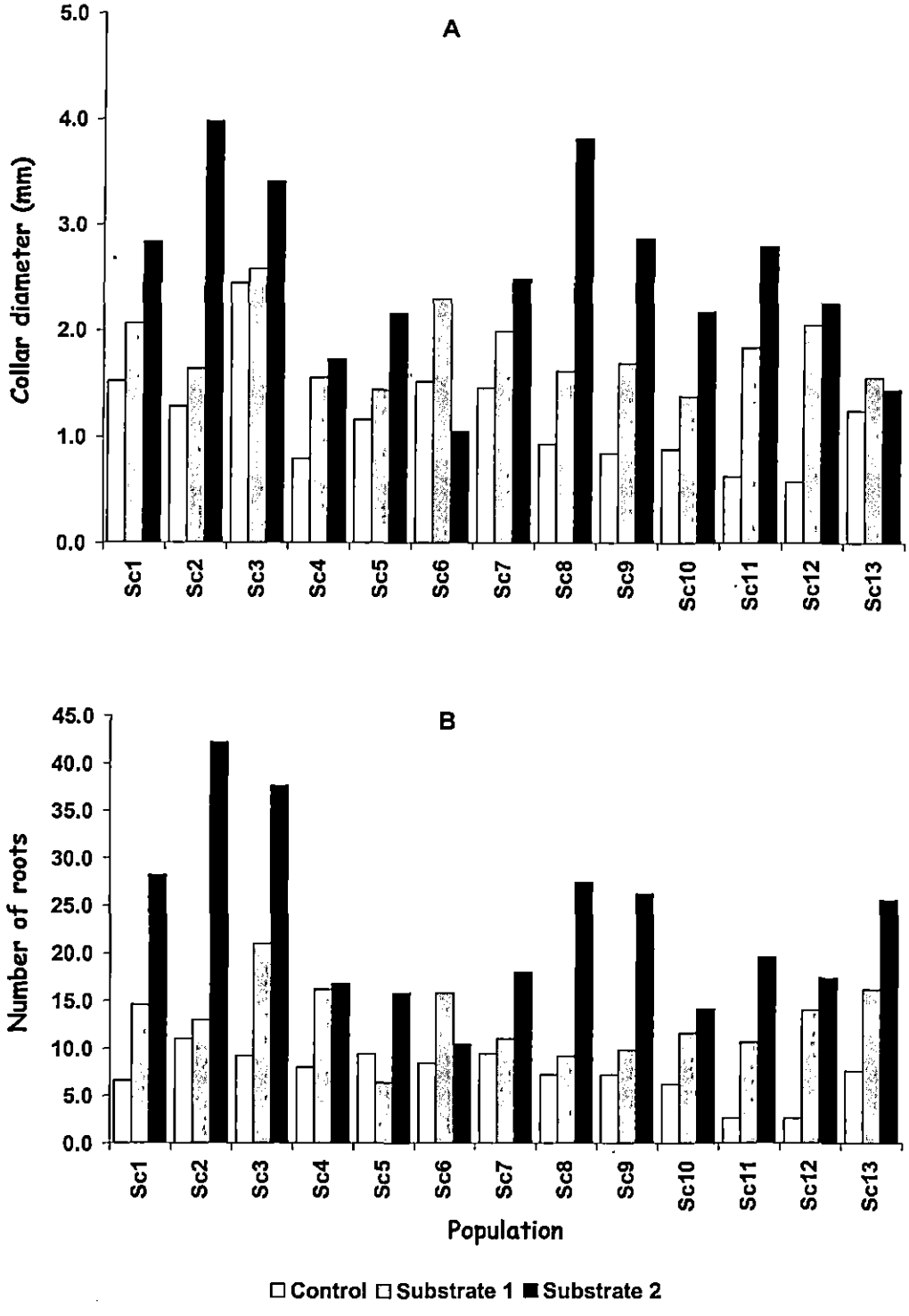



**Figure 24.** Effect of different substrate combinations on collar diameter and number of roots in *Swertia chirayita*

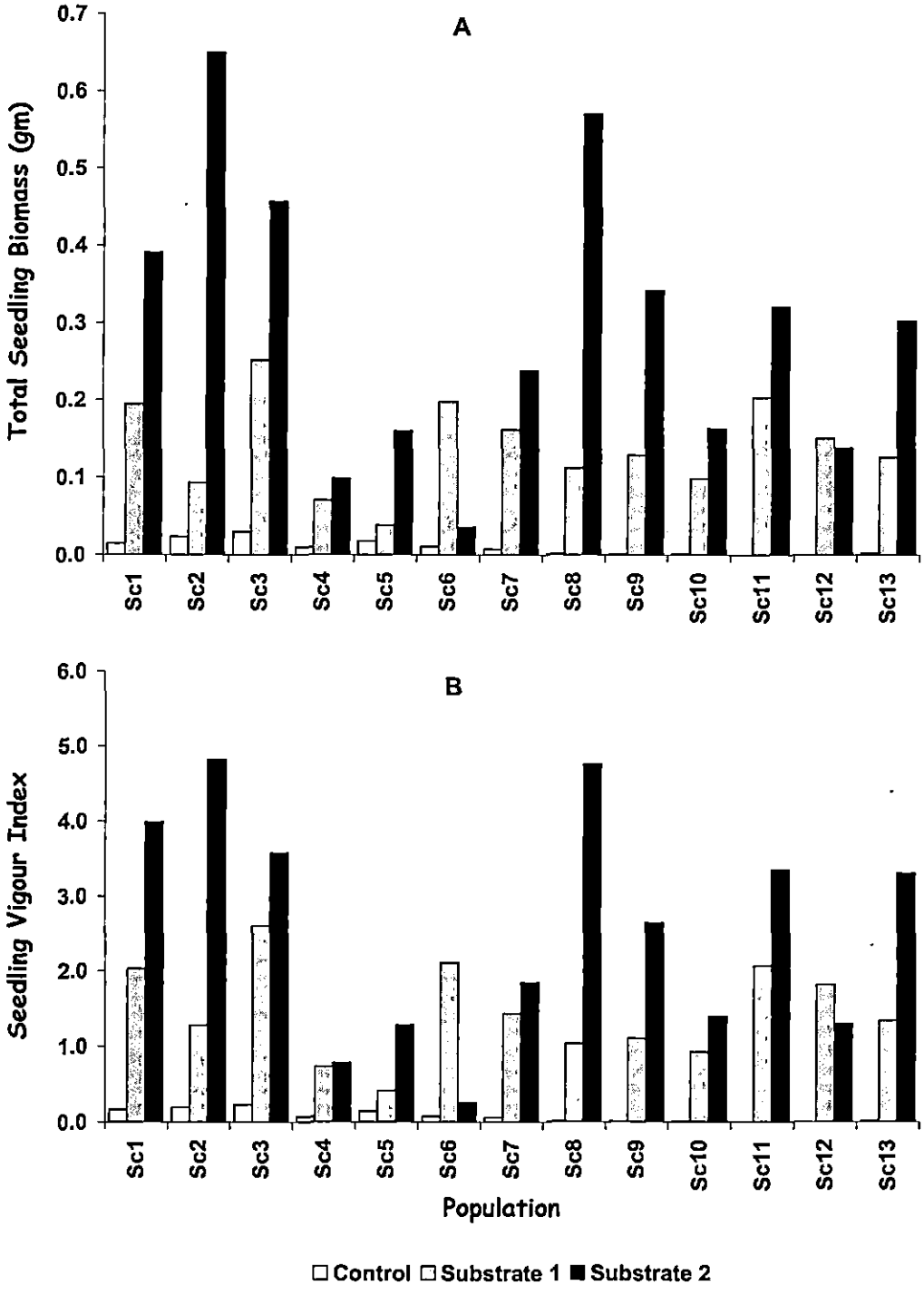



**Figure 25.** Effect of different substrate combinations on seedling biomass and seedling vigour in *Swertia chirayita*

CHAPTER 9

# Effect of Storage Conditions and Storage Periods on Seed Germination

## 9.1 Introduction

Storage of seeds as *ex situ* germplasm is an essential step for the long term conservation of plant genetic resources. Maintaining seed viability for longer period is very essential to preserve the genetic integrity in stored samples. Since very early days, simple techniques have been adopted to maintain the seed viability in both domesticated and wild sources (Ellis *et al.*, 1991b, 1992; Onyekwelua and Fayose, 2007; Roberts, 1973, 1975; Vertucci and Roos, 1990, 1991). Inappropriate storage medium (Hezewijk *et al.*, 1993; Muller *et al.*, 2011) such as room temperature storage often results in low seed germination, seed deterioration and loss of viability, which is a natural phenomenon during storage (Nasreen *et al.*, 2000; Schmidt, 2002). Several factors, viz., temperature, nature of the seeds, seed moisture content, relative humidity, etc., influences seed longevity during storage (Roberts, 1972; Bonner, 1990; Gordon, 1992; Copeland and McDonald, 1994; Bradbeer, 1998; Takos, 1999; Butola and Badola, 2004ab; Yang *et al.*, 2005; Onyekwelua and Fayose, 2007). There is a close relationship between the loss of seed viability during storage and the accumulation of genetic damage in the surviving seeds, (Ellis and Roberts, 1981; Roos, 1982; Rao *et al.*, 1987). Seed moisture content, temperature and storage periods are among the main factors affecting above relationship (Roberts, 1988). Slight increase in temperature and moisture may promote fungal growth (Roberts, 1972) and insect development in seeds (Christensen, 1971). According to Harrington (1973), 1% rise in moisture content and $5^0$C in temperature reduces the half storage life of seeds. Depending on the duration and method adopted, drying and long term storage may lead to considerable reduction in germination or to eventual death of the seeds. Before storage, if the seeds are not properly dried, the high moisture content may reduce the seed viability by promoting fungal growth. Such deterioration could further results in decline of seed germination capacity (Romanas, 1991). Proper storage conditions however, may effectively retain substantial viability in seeds over a considerable

storage period (Butola and Badola, 2004ab; Chen *et al.*, 2007). Such approaches are especially crucial in case of endangered species, where judicial use of seeds as valuable genetic material through standardizing proper storage mechanism is a precondition to strengthen species conservation programme.

*Swertia chirayita* (Roxb. ex Fleming) H. Karst (Gentianaceae) is one of the highly marketed (national and international) endangered medicinal herbs of Himalaya and prioritized at the top for the conservation through *ex-situ* cultivation by an international experts' exercise (Badola and Pal, 2002). Critically endangered standing of *S. chirayita* (Ved *et al.*, 2003b) further necessitates its propagation and mass multiplication, for which a protocol targeting an appropriate and relatively longer period of storage of seeds would be vital and urgent need of the time. This will strengthen both *in-situ* conservation as well as *ex-situ* cultivation of the species. We assessed the seeds of different populations of *S. chirayita* under long term storage, as an initial effort, but the germination testing was limited to only $4^0$C storage (refer chapter 7). As an erect, about 3-5 ft, biannual or triennial herb S. *chirayita* is locally known as Chirowto or Pothi Chirowto or Kalo Chirowto, and distributed in temperate Himalaya from Kashmir, Nepal, Bhutan along 1200-3000m asl (Kirtikar and Basu, 1984). The stems are robust, branching; the leaves are broadly lanceolate; the flowers occur in large panicles and are lurid greenish yellow tinged with purple; the capsules are egg-shaped, many-sided, and sharp pointed; the seeds are smooth and many-angled. The breeding in *S. chirayita* is through self pollination (Chakroborty *et al.*, 2009); however, cross pollination in the species has also been reported (Khoshoo and Tendon, 1963). S. *chirayita* bears huge pharmacological importance (see chapter 1); whole plant is used in traditional medicine; however the root is mentioned to be the most effective and bitter part. Plant has anti-inflammatory (Chowdhary *et al.*, 1995; Banerjee *et al.*, 2000), hypoglycemic (Bajpei *et al.*, 1991; Banerjee *et al.*, 1994; Chandrasekhar *et al.*, 1990), anti-pyretic (Bhargava *et al.*, 2009), anti-fungal, anti-bacterial, anti-malarial (Bhat and Surolia, 2001) and anti-oxidant (Scartzzini and Speroni, 2000) properties, and considered to be a bitter tonic, febrifuge and laxative and is used in fever, burning of body, intestinal worms and skin diseases etc. *S. chirayita* is used in all kinds of fever in a variety of forms and in combination with

other medicines (Dutt and King, 1877). Entire plant contains gentiamine alkaloids, and xanthones is found in aerial part (Sharma, 1982). Community people in Sikkim use the juice, obtained through boiling the entire plant, to cure fever, cold, cough, diarrhea, and stomach-ache (Pradhan and Badola, 2008). Also, plant is used in mental disorders (Chowdhary *et al.*, 1995), effective in curing gastric ulcers (Rafatullah *et al.*, 1993), liver diseases (Krishnaraju *et al.*, 2005), and possess anti-carcinogenic properties (Lin *et al.*, 1996; Das *et al.*, 2004). Further, the species is practiced in the preparation of herbal drug, Diabecon (Kohli *et al.*, 2004), D-400 (Sundaram *et al.*, 1996), and Himoliv (Bhattacharya *et al.*, 2003).

The present study was undertaken to test the hypotheses: (1) seed germination varies amongst the population, (2) storage conditions and storage period affects seed germination in *S. chirayita.* The study was conducted on eleven populations from different parts of Sikkim, India. Till present reporting, there is no publication available on the effect of long term storage on seed germination of *S. chirayita*, and first time covering different storage conditions.

## 9.2 Materials and Methods

### *9.2.1 Experimental design*

During November-December, freshly ripened seeds of *Swertia chirayita* were collected from 11 populations located along 1600m asl to 2700m asl in different parts of Sikkim Himalaya, India ($27^0$04' – $28^0$07' North and $88^0$00' – $88^0$55' East). Seeds from four of the above populations (Sc1 to Sc4), earlier tested from 2005 collection for storage at $4^0$C (refer chapter 7), were recollected after one year time in 2006. Seeds from all populations were tested for three storage conditions over time. During seed collection, 15 – 20 plants/site were selected randomly from the middle of each population, to avoid edge effect if any and not to disturb natural spread of the population, practicing sustainable harvesting. The seeds procured were pooled separately for each population and brought to the laboratory, cleaned thoroughly for impurities, and dried in room temperature for 15 days. Seed moisture content was determined by oven drying ($60^0$C; 48 hrs) 50 seeds in 3 replicates from each

population. For each population 10 healthy fruits were considered for seed counting. Seed size was measured using 30 seeds per population under microscope (10 seeds each in 3 replicates). Seed viability test using 2,3,5, triphenyle tetrazolium chloride solution could not be conducted because of the minute seed size and the difficulty in finding the detached embryo. For each population, immediately after room drying, seeds were tested for their initial germination potential. The remaining seed lots were stored in three different experimental conditions, viz., room temperature (25 $\pm$ 5$^0$C), and in refrigerator at 4$^0$C and at -15$^0$C, in properly sealed specimen tubes. The seeds were periodically tested for their germination viability at six months interval for next 24 months.

For each germination test, seeds were at first disinfected with Sodium hypochlorite solution (4% w/v available Chlorine) for 5 sec. to reduce the incidence of fungal attack. Disinfected seeds were washed thoroughly with double distilled water (DDW) and soaked in DDW for 24 hours. The soaked seeds were placed in Petri-plates (90mm dia.) lined with single layer filter paper (Whatman No. 1) saturated with DDW. For each population, three replicates of 30 seeds each for each of three storage condition were used; and Petri-plates then placed in a seed germination chamber at 25$^0$C $\pm$ 2$^0$C; with alternating light (14/10 hrs. photoperiod). The experiment was performed in a randomized design. The seeds were kept moist (DDW) and checked every day. Visible protrusion of the radical was the criterion to score seed germination. The germinated seeds were counted and removed. Seeds were observed daily until constant reading obtained. The germination experiment was observed up to 45 days in each of the testing at six months interval.

### *9.2.2 Statistical analysis*

MANOVA (multivariate analysis of variance) was performed using General Linear Model in SPSS 10.0 for windows (SPSS Inc. 1989) to determine the effect of populations, storage conditions and storage period and their interaction on seed germination percent and mean germination time (MGT). MGT was calculated using equation, $MGT = \sum(nd)/\sum N$; where n = number of newly germinated seeds after each incubation period in days d, and N = total number of seeds germinated at the end

of the test (Hartmann and Kester, 1989). Bonferroni test ($p<0.0001$) was employed to determine the variation in means of seed germination and MGT. The present experiment was designed to determine aging rates of seeds of *S. chirayita* stored in different storage conditions. Percent germination value obtained for the different populations prior to storage served as control value and a way to evaluate the variability in percent germination for periodical storage period. By recording changes in percentage germination and the time required for seeds to germinate, deterioration was evaluated.

## 9.3 Results

Populations of *Swertia chirayita* differed significantly for various seeds characteristics (Table 20). MANOVA revealed significant variations in seed germination percent and mean germination time amongst populations, storage conditions and storage period and their interaction (Table 21). At the initial test, seed germination percent ranged from 87.78% (Sc5) to 100% (Sc2) and showed negative correlation with altitude of the collection sites ($r$ = -0.087). Minimum 12 to 16 days (average: 13 days) was required for onset and 20 to 40 days (average: 31 days) for the completion of seed germination in most of the populations for all the three storage conditions and storage period (Figure 26). Collectively, significant reduction in germination percent was observed with the elapse of storage period compared to initial test ($p<0.0001$) in populations of *S. chirayita*. Cumulatively for all the populations, seeds stored at $4^0$C showed significantly ($p<0.0001$) higher germination percent than $-15^0$C and room temperature stored seeds. Similarly, the seed germination was significantly higher in $-15^0$C stored seeds over room temperature stored seeds ($p<0.0001$). For the seeds stored at $4^0$C, 73% (8 populations) of the total populations recorded over 90% seed germination after 6 months; 64% (7 populations) of the total populations recorded over 80% germination after 12 months; 91% (10 populations) of the total populations recorded over 60% germination after 18 months and 70% (8 populations) of the total populations recorded over 50% seed germination after 24 months of storage (Figure 27A). For the seeds stored at $-15^0$C, Sc3 recorded highest and Sc10 recorded the lowest germination after 6 months, Sc1 and Sc10 recorded the highest and the lowest seed germination after 12 and 18 months of

storage, respectively; however, after 24 months of storage, Sc2 recorded the highest and Sc6 recorded the lowest seed germination (Figure 27B). Room temperature storage resulted in above 80% seed germination in 82% (9 populations) of the total populations after 6 months. Here, the percent seed germination ranged between 48.89% (Sc9) to 73.33% (Sc3) after 12 months; 45% of the total populations recorded above 50% germination after 18 months, and the seed germination recorded below 33% in 82% of the total populations after 24 months of storage (Figure 27C).

Significant variation ($p<0.0001$) in mean germination time (MGT) was observed amongst the storage conditions compared to the initial test. For example, seeds stored for 12 months at $4^0$C recorded lowest MGT in all the populations (Figure 28A); similarly, 64% of the total populations recorded lowest MGT after 6 months of storage at $-15^0$C (Figure 28B) and 12 months of storage in room temperature (Figure 28C). Significant fall ($p<0.01$) in seed germination was observed in all the three storage conditions with the increase in storage time (Figure 29). The rate of fall in germination percent per 6 month was higher in room temperature (15.86%) followed by deep freeze (13.26%) and $4^0$C (10.89%) stored seeds. At this rate of fall, seed looses its viability completely by approximately 36 months in $4^0$C stored seeds which is slower compared to room temperature and deep freeze (Figure 29).

## 9.4 Discussion

To retain good seed longevity, 10% to 40% seed moisture content is desirable (Hampton and Hill, 2002). Improper drying of seeds results in rapid loses of viability during storage owing to high moisture content (Siddique and Wright, 2003). According to McDonald (2004), seed loses viability and vigor during processing and storage mainly because of high seed moisture content, i.e. greater than 18%. In our present study, however, seeds of *S. chirayita* from all 11 populations maintaining with 19% to 44% moisture content after drying resulted in high percent germination for the freshly collected seeds, which helped retaining substantial viability under storage for longer period. We recorded 100% germination in freshly collected seeds of *S. chirayita* for majority of populations with 16 to 42% moisture content (refer Chapter 7). However, Bhatt *et al.*, (2005b) recorded low seed germination with the moisture

content of 22% to 29% in freshly collected seeds of *Swertia angustifolia*. In our study, even after maintaining the moisture content of 15% to 21% in the domesticated seeds of *S. chirayita*, low germination at the initial testing was observed (refer chapter 11). This indicates species specific requirement of seeds for desirable moisture content for long term viability.

The length of time for which the seeds remain viable varies greatly by species and storage conditions (Siddique and Wright, 2003). Our present investigation, testing seeds for long term storage (at $4^0$C, $-15^0$C and room temperature) confirm that the storage temperature significantly affects the seed germination capacity as stated by (Bradbeer, 1988). Our study indicates that the seed deterioration rates may vary depending on the storage conditions, however, the germination percent and/or seed viability gradually declines with increase in storage period (Yilmaz and Aksoy, 2007) irrespective of different storing conditions. The reported variability in percent seed germination for the seeds collected from different populations at same time or from same population at different times even if they are provided with the same treatments or test conditions (Perez-Garcia *et al.*, 1995; Baskin and Baskin, 2001) is not applicable to *S. chirayita* in Sikkim Himalaya. Our present study with *S. chirayita* revealed that seeds collected from the same location for two different years in case of four populations, i.e. Sc1, Sc2, Sc3 and Sc4 after a year gap resulted in 98% to 100% germination (roughly the similar trend) in comparison to the earlier result of 100% germination for the same populations (refer chapter 7). This is an indication that the initial germination potential may remain similar over different times if right collection strategy is maintained, especially the time of collection and selection of same spots.

In our present study, six month storage did not show much variation in percent germination among the storage conditions suggesting the suitability of all three conditions for short-term storage, which is often practiced in several Himalayan herbs (Butola and Badola, 2004ab, 2006a). For $4^0$C storage, all the population showed higher percent germination after 12 months, comparing to $-15^o$C storage. In general, above 60%, 70% and 80% germination after 12 month storage in room temperature, at $-15^0$C, and at $4^0$C, respectively, suggest that up to 12 months, seed storage in room

temperature can also be opted provided due care be taken in respect of moisture content level prior to storage. After 18 months, abrupt fall in percent seed germination below 48% in 55% populations indicates the room temperature as non appropriate condition of storing seeds for longer period in *S. chirayita*. Seed storage at -15$^0$C maintained > 60% germination with majority of the populations (7 populations) after 18 months of storage. Continued storage for 24 months at -15$^0$C resulted in decrease in seed germination (below 50%) for nine populations, which suggests this as unsuitable condition for long term seed storage.

Comparatively, seeds stored at 4$^0$C resulted in higher percent germination than the seeds stored at room temperature and at -15$^0$C, on subsequent testing. For all three storage conditions, progressive and significant reductions in percent germination were observed with increasing duration of storage. On an average basis, loss of seed viability was much faster at room temperature followed by -15$^0$C stored seeds but gradual at 4$^0$C suggesting it as most appropriate condition for the long term storage of *S. chirayita* seeds. Chauhan and Nautiyal (2007) reported much faster loss of seed viability at room temperature (10-35$^0$C) and retaining of seed viability for more than two years (storage at 0-4$^0$C in refrigerator) in *Nardostachys jatamansi*. Onyekwelua and Fayose (2007) stated that the seeds cannot be stored at sub-zero temperature probably due to freezing injury resulting from ice formation, which can alternatively be controlled by placing them in air tight containers. Our present study suggests that storing seeds of *S. chirayita* at -15$^0$C can be a second option after 4$^0$C; however, here the seeds loose their viability early compared to 4$^0$C even if they are placed in air tight containers. Many studies reported that the seed storage at 4$^0$C was effective for germination after 6 months (Butola and Badola 2004b) to 12 months (Chen *et al.*, 2007; refer chapter 7). In several other species, loss of seed viability is observed within a few months of storage at room temperature (Douglas, 1995; Verma *et al.*, 1996). Seed moisture content is adjusted as per the relative humidity of the surrounding air which changes with the air temperature and seeds differ in the way they adjust their moisture content to humidity. In refrigerator (4$^0$C), both temperature and the relative humidity are properly maintained thereby retaining the seed viability for longer period, relatively.

For *Swertia chirayita*, in our study (see chapter 7), an increasing trend in MGT was recorded after six months of storage at $4^0$C, on subsequent testing for 18 months. Whereas, in our current study with four re-tested populations (Sc1 to Sc4) in second year and the remaining sets of populations, the case little differed as MGT declined till 12 months in majority of the populations, and then MGT enhanced subsequently when provided with similar storage condition. Similar trend in MGT reduction was observed for the seeds stored in room temperature. Seed moisture content plays a critical role in the seed germination process. The increase or decrease in the moisture content during the storage period of seed may be responsible for such variation in the MGT value which requires further detailed investigation for *S. chirayita*. For the seeds of *Kochia prostrate* stored in laboratory and under shed, mean germination time decreased as storage time increased, and varied unpredictably for cold room-stored seed, and remained unchanged for freezer-stored seed (Kitchen and Monsen, 2001), is not applicable to *S. chirayita*. Non-uniformity in MGT observed in the seeds on subsequent tests is rather confusing in the case of -$15^0$C stored seeds in *S. chirayita*. However for the seeds stored in room temperature and at $4^0$C, the MGT first decreased up to 12 months of storage compared to initial test, which increased with the prolonged storage period.

## 9.5 Conclusion

Our present study confirms that various populations of *Swertia chirayita* in Sikkim Himalaya possess high germination potential. It also showed that the seed germination (1) varies amongst the populations; (2) is highly affected by the storage condition and the storage period. It further confirms that the $4^0$C is the best/effective storage condition for the seeds of *S. chirayita* that retains seed viability for the longer period than other storage conditions. Also, for the shorter period up to one year, the other storage conditions may be opted, provided due care taken during storage. Equally, important is the recommendations on sustainable harvesting taking care of precise time and exact spot in the site, where earlier collection were made in case of using potential populations fro conservation programmes in case of endangered species.

**Table 20.** Seed characteristics of different plant population of *Swertia chirayita* collected in Sikkim

| Name of Populations | Altitude (m asl) | No. Seeds/fruit | Seed weight (mg/50 seeds) | Seed length (µm) | Seed width (µm) | Moisture content (%) |
|---|---|---|---|---|---|---|
| Sc1 - Luing (ES) | 2126 | 209 | 1.5 | 486 | 355 | 25.8 |
| Sc2 - Railgaon (ES) | 1948 | 297 | 1.6 | 532 | 383 | 22.4 |
| Sc3 - U Pangthang (ES) | 2176 | 309 | 1.6 | 502 | 380 | 29.1 |
| Sc4 - Jaunbari (SS) | 1651 | 222 | 1.2 | 454 | 374 | 39.1 |
| Sc5 - Tiffin dara 2 (SS) | 1744 | 269 | 1.4 | 406 | 362 | 26.7 |
| Sc6 - Dhupi Dara (ES) | 2124 | 258 | 1.8 | 418 | 378 | 18.8 |
| Sc7 - Deewani Taar (WS) | 2055 | 294 | 1.2 | 358 | 325 | 36.5 |
| Sc8 - Gumpa Dara (WS) | 1987 | 260 | 1.2 | 372 | 351 | 29.7 |
| Sc9 - Hilley (WS) | 2697 | 260 | 1.4 | 389 | 369 | 44.3 |
| Sc10 - Tendong (SS) | 2099 | 230 | 1.6 | 390 | 372 | 27.0 |
| Sc11 - Raavngla (SS) | 2160 | 256 | 1.3 | 320 | 297 | 25.6 |
| (P<0.05) | | 38.49 | 0.14 | 21.53 | 21.14 | 10.54 |
| F value | | 5.23 | 15.95 | 76.93 | 12.94 | 4.23 |

ES: East Sikkim; WS: West Sikkim; NS: North Sikkim; SS: South Sikkim

**Table 21.** Result of Three way ANOVA showing the effect of populations, storage conditions and storage periods and their interaction on seed germination and mean germination time in *Swertia chirayita*

| Source | F value | Sig. | F value | Sig. |
|---|---|---|---|---|
| | Germination (%) | | Mean Germination Time | |
| Population (P) | 14.443 | .000 | 42.392 | .000 |
| Storage Condition (SC) | 279.387 | .000 | 716.784 | .000 |
| Storage Period (SP) | 1038.901 | .000 | 519.862 | .000 |
| P x SC | 8.914 | .000 | 9.144 | .000 |
| P x SP | 3.993 | .000 | 5.728 | .000 |
| SC x SP | 6.789 | .000 | 147.827 | .000 |
| P x SC x SP | 1.810 | .001 | 4.878 | .000 |

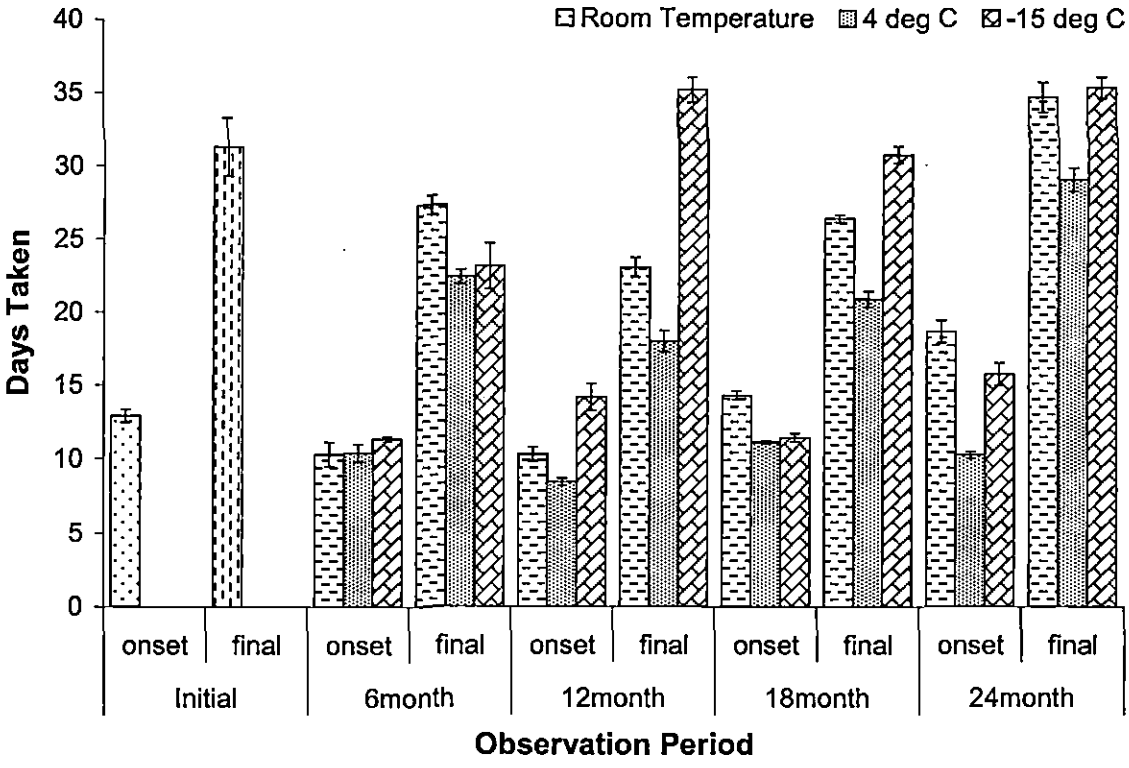



**Figure 26.** Storage effect on onset and final seed germination in *Swertia chirayita*

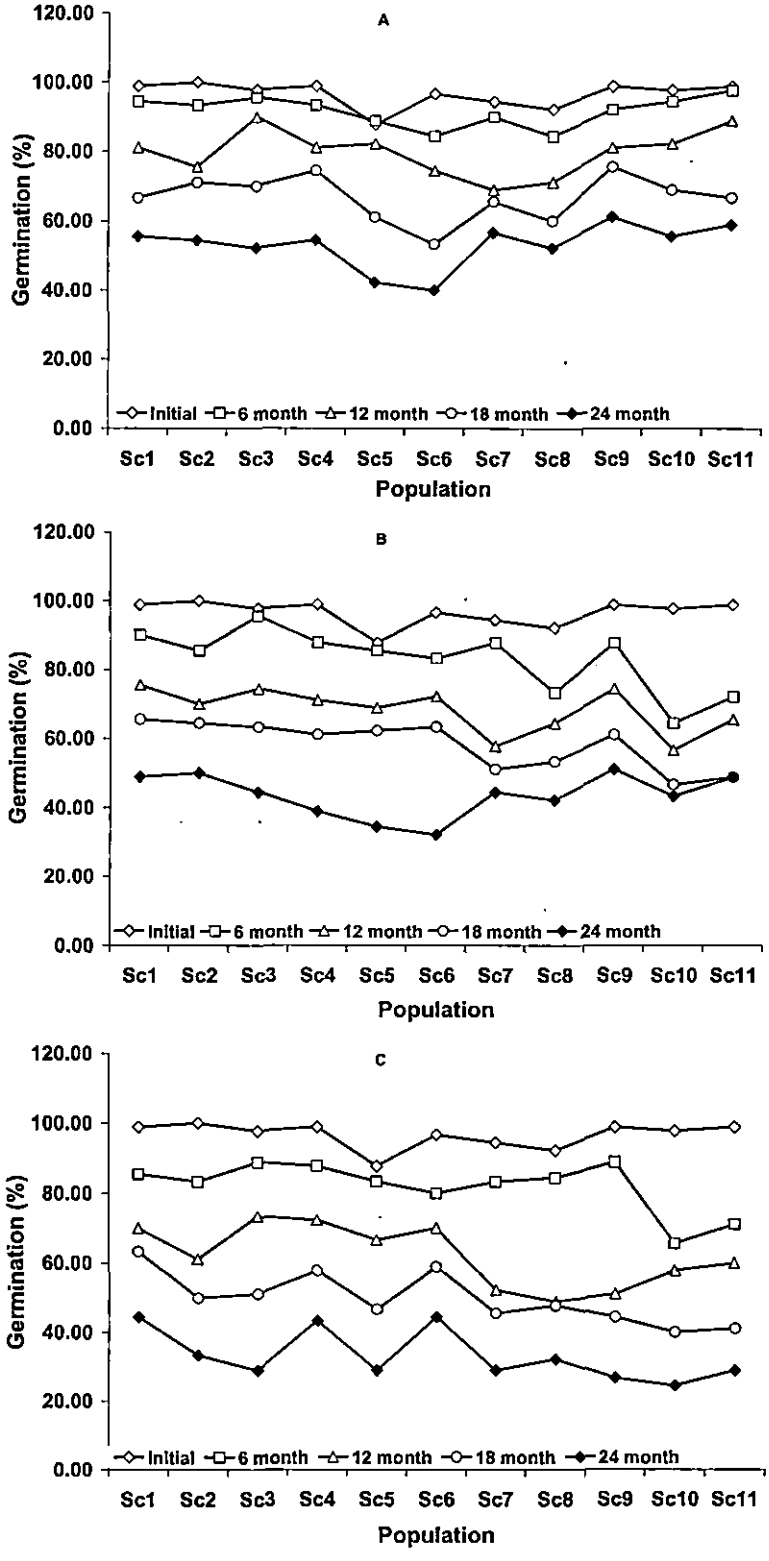



**Figure 37.** Effect of storage conditions and storage period on seed germination in populations of *Swertia chirayita*; A: $4^0$C; B: $-15^0$C; C: Room temperature

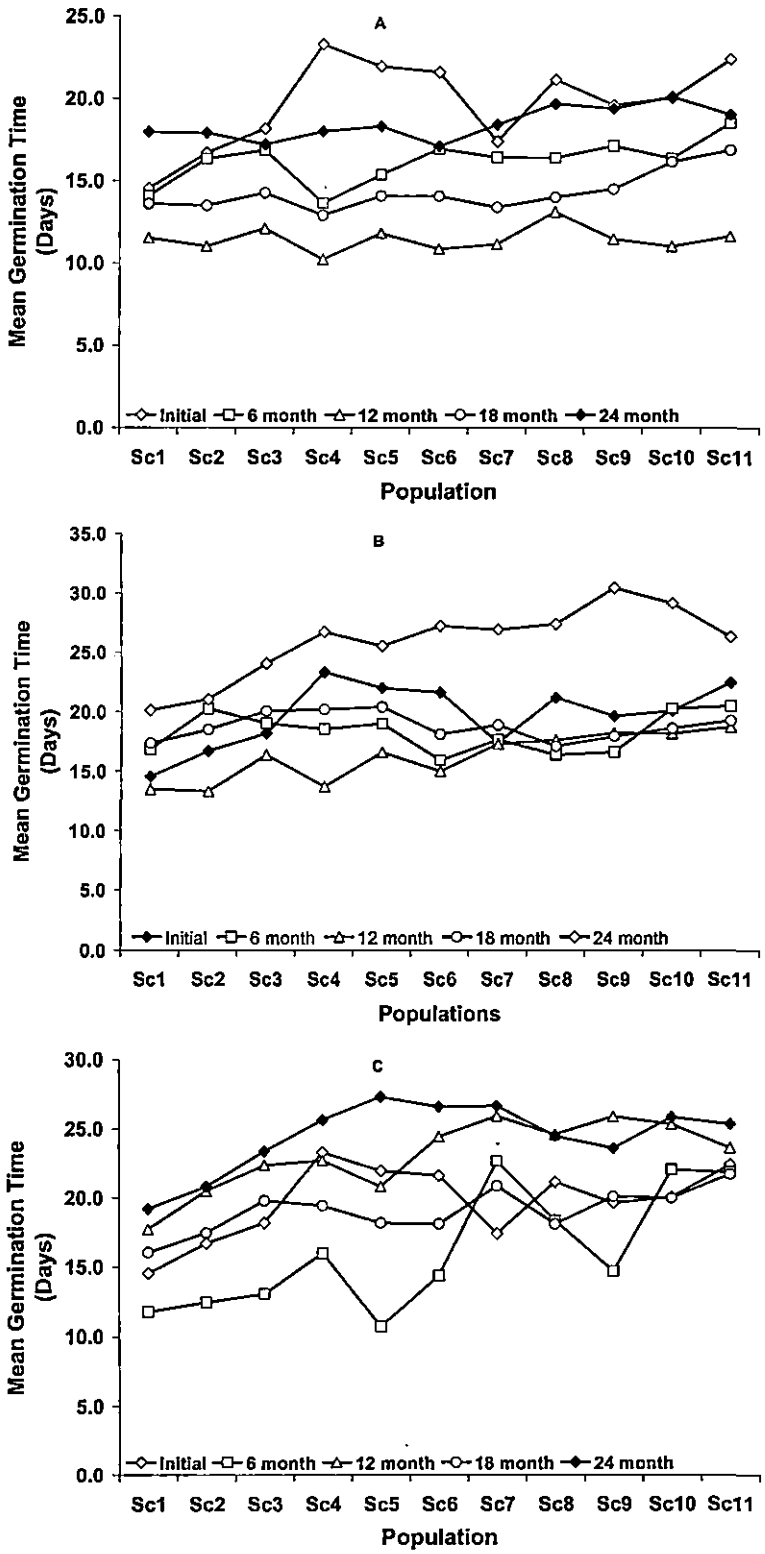



**Figure 28.** Effect of storage conditions and storage period on mean germination time in populations of *Swertia chirayita*; A: $4^0$C; B: $-15^0$C; C: Room temperature

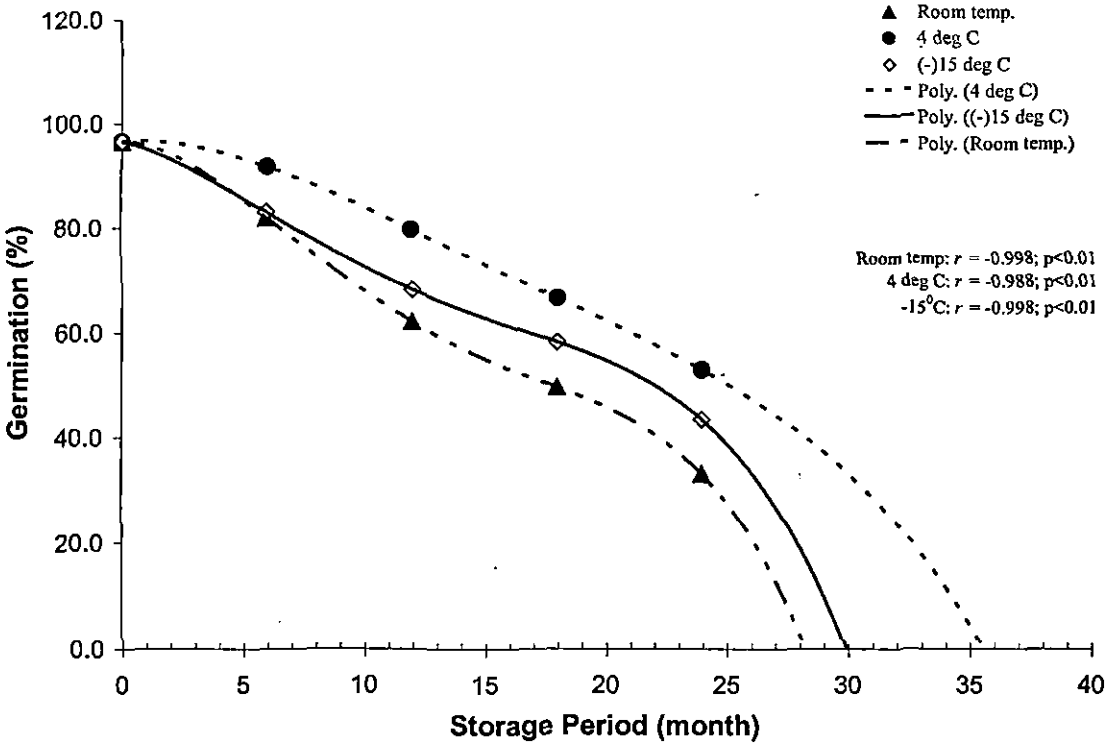



**Figure 29.** Seed viability as affected by storage period and storage condition in *Swertia chirayita*

CHAPTER 10

# Phenology, Growth and Development

## 10.1 Introduction

The phenological recording in plants is very useful as this provides information about different growth phases and their periodicity, length of the growing seasons and of the dormancy (Badola, 1994; Jeffrey *et al.*, 2009) and its knowledge is crucial to understanding of community function and diversity (Fenner, 1998). These phenological phases are influenced by the seasonal environmental factors (Badola, 1994, 2010; Jeffrey *et al.*, 2009); therefore, in order to assess the vulnerability of species to phenological changes, it would be imperative to know the environmental factors that control its development (Wipf, 2010). Seasonal timing events can be critical for the survival of life and reproduction (Kilic *et al.*, 2006). Phenological studies impart knowledge on the morphological and functional traits offering clues about adaptability mechanisms of the plants under varied and periodically changing climatic conditions in which they grow, and thus help in developing management and conservation plans for valuable plant species (Badola, 1994; Martinez-Calvo *et al.*, 1999; Baumgartner and Hartmann, 2000; Bhat and Murali, 2001; Sanz-Cortes *et al.*, 2002; Hamann, 2004; Tebar *et al.*, 2004; Badola, 2010). Plant phenology also provides the basic understanding of the functional rhythms (Rawal *et al.*, 1991) and seasonal periodicity in growth and reproduction (Hoffmann *et al.*, 1992; Badola, 1994) of communities; however, for which, continuous monitoring is necessary. Studies have shown the climatic factors, viz., temperature (Van Schaik, 1986; Badola *et al.*, 1992), rainfall (Foster, 1982; Bullock and Solis-Magallanes, 1990), water availability (Bullock and Solis-Magallanes, 1990), photoperiodism (Daubenmire, 1972), duration of dry spell (Leigh Jr. and Windsor, 1982), change in day length and temperature (Bhat, 1992) etc., are to be mainly responsible for the vegetative and reproductive phenology, at both community and species level (Bhat and Murali, 2001; Badola, 1994, 2010).

The microclimate of the growing condition significantly affects phenology (Bliss, 1966), growth and development in plants. Growth and survival of the plants greatly depend on the genetic adaptation to the temperature regimens (Criddle *et al.*, 1997) and moisture and water availability (Nautiyal *et al.*, 1994; Bhat and Murali, 2001) within given or native environments. The specific environment of a growing medium enables the survival of those plant species which could withstand the severity of that environment. Extreme environmental fluctuations occurring at the soil surface affect seedling survival as they may be less tolerant to such conditions (Dodd and Donovan 1999); however, the presence of nurse plants often reduces the rate of seedling mortality because such environmental fluctuations are less occur under the canopy of nurse plants than outside the canopy (Cervera *et al.*, 2006), thereby increasing the seedling survival and extending the distributional boundaries of the species (Steenbergh and Lowe 1977; Nobel 1980). Also, the transplanted genotypes perform better at their site of origin than at foreign sites (Van 1998; Hufford and Mazer 2003), provided the availability of suitable substrates is ensured which plays a significant role in better performance of the species (refer chapter 8). However, different species may behave differently to a given growing environment especially in the case of herbs (Butola and Badola, 2008).

*Swertia chirayita* (Roxb. ex Fleming) H. Karst (Gentianaceae), triennial herb is known globally for its use in traditional and modern medicine extensively (Pradhan and Badola, 2008). Plant is used as a liver stimulant and in bronchial asthma, dyspepsia, debility, fever, gastrointestinal infections, curing various skin problems and scorpion bite (Biswas and Chopra, 1982; Kirtikar and Basu, 1984). Owing to high raw material demand at national and international markets (Badola and Pal, 2002), the species has been considered critically endangered (IUCN), endangered (Indian Red Data Book), critically rare and virtually endemic to Himalaya (Samant *et al.*, 1998), and vulnerable to Sikkim (Ved *et al.*, 2003); and offers immense scope for *ex-situ* cultivation (refer chapter 8). The species has also been prioritized at the top for its conservation through cultivation (Badola and Pal, 2002). Nevertheless, the problem with *S. chirayita* is the low seed germination as reported in earlier studies (Raina *et al.*, 1994; Bhatt *et al.*, 2007). However, an understanding of the phenology is a

prerequisite tool in developing and standardizing agro-technology for the targeted species (Badola, 2002), which provides important clues towards possible domestication of the species (Butola and Badola, 2006). The present study was designed to perform comparative study on phenology, growth and development in *Swertia chirayita,* transplanted under different natural and nursery conditions, periodically and to assess whether the plants following transplantation under both natural and nursery conditions behave similarly to adapt varied growing conditions. A perusal of literature revealed that no such study have been undertaken, so far, on *S. chirayita* and is first of its kind in Himalaya.

## 10.2 Materials and Methods

### *10.2.1 Study site*

The study was carried out at Pangthang (Lat: 27$^0$21'51"N and Long: 088$^0$34'10"E), Sikkim (India), situated at an altitude of *ca.* 2000m asl. The area falls under temperate climatic zone with maximum temperature reaching up to 20.1$^0$C (August) and minimum temperature up to 6.42$^0$C (January) [Figure 30]; maximum and minimum relative humidity range between 98.4% (July) and 70.3% (December) with occasional snowfall (January). The area receives heavy rainfall from May to September; and experiences highest rainfall of 4619.56 mm (August) and lowest of 51.04 mm (January). The soil is clay loam type and slightly acidic in nature (pH: 6.43). Meteorological data viz., temperature and rainfall of the study area were collected daily using thermo-hygro-clock (Mextech; Model No. J412CTH) and manual rain gauze.

### *10.2.2 Experimental design*

Eight months' old (completing one growing season), relatively uniform size, seedlings of *Swertia chirayita*, developed under green house, were randomly transplanted to six experimental plots (35 seedlings each), four in natural habitat of the species viz., shrubberies, forest slope, open slope and tree canopy; and two in nursery conditions viz., temperature controlled green house (25$^0$C $\pm$ 5) and open net-shade, maintaining a distance of 50 cm between individuals (Photo Plate V) during March. Of 35 seedlings

transplanted/plot, 15 seedlings/plot (as 15 replicates) were tagged for the long term periodical monitoring. The morphological data on growth and development such as the largest leaf length, largest leaf width (in both rosette plant and stem plant), plant height, number of nodes in main stem, number of shoots/plant, number of branches/plant, number of fruits/ plant etc., (stem plant) in *S. chirayita* were recorded on monthly basis (for 22 months) till the whole plant reached to senescence stage by the end of the third growing season. Also, the weekly observations on the parameters such as, shoot initiation/development, floral bud appearance, bud sprouting, flower blooming, fruiting initiation, fruit maturation and fruit ripening etc., were made till the whole process completed at the end of the third growing season. After the plants undergone complete senescence in all six growing conditions, the seeds were collected and the plants harvested to estimate the differences in total plant biomass.

### *10.2.3 Statistical analysis*

One way multivariate analysis of variance (MANOVA) using general linear model (GLM) was carried out to check the effect of growing conditions on different growth parameters using SPSS 10.0 software for windows (SPSS Inc. 1989). If the results indicated significant effects, multiple comparisons were performed to test the differences by applying Bonferroni test. Pearson's correlation test was conducted amongst different growth parameters; however, the growing conditions were not taken into consideration. Pearson's correlation test was also performed to see the effect of temperature and rainfall on different growth parameters in plants transplanted to various growing conditions; nonetheless, green house was excluded from the test because plants under green house were maintained under temperature controlled environment.

## 10.3 Results

### *10.3.1 Survivability in different growing conditions*

*Swertia chirayita* completed its first growing season in the green house. Immediately after transplantation in the second growing season, older leaves in the plants were replaced by the newer ones in all the six growing conditions. Final plant survival




**Photo Plate V.** Phenological observation in different natural and nursery conditions in *Swertia chirayita*

A: Shrubberies; B: Forest slope; C: Open slope; D: Tree canopy;
E: Flowering in temperature controlled green house; F: Flowering in open net-shade

varied amongst the different growing conditions, viz., 71% each (shrubberies and tree-canopy), 54% (forest-slope), 22% (open-slope), 43% (net-shade), 88% (green-house). Due to the very low survival rate in open slope (7 of the 35 plants), 7 replicates were only considered for the final calculations in all the growing conditions for growth parameters.

### *10.3.2 Phenological events in different growing conditions*

In the 2nd growing season, vegetative (rosette form) plant growth in terms of leaf length and leaf width initiated immediately after transplantation of plants in all the six growing conditions, which coincided with beginning of the rise in temperature and the early spring shower (Figure 30). The leaf growth continued till September for the majority of the growing conditions (Figure 31a and 31b) when the average temperature and total rainfall was still higher; however, growth stopped with the abrupt fall in temperature as well as decrease in total rainfall during dry periods (November to January). The rosette leaves continued growing with the onset of the spring in the third growing season (till March under green house and open net-shade, and till May in natural habitat) even after the shoot developed, and completely disappeared by the late April (green house), July (shrubberies, forest slope, open slope, tree canopy) and August (open net-shade) [Figure 31a and 31b]. The shoot development of too coincided with the beginning of the rise in temperature and the early spring shower in the 3rd growing season (Figure 30); whereas, the phenomenon initiated 2 weeks earlier under the green house and open net-shade (February end) compared to the plants transplanted to natural habitats (March 2nd week; Figure 32). The active growth period in terms of plant height was February to July (green house and open net shade) and March to September in other growing conditions, when the average temperature and total rainfall was substantially high; however minor growth in plant height was observed during October in plants in forest slope, open slope and tree canopy (Figure 32). Vegetative growth (stem form) in terms of leaf size (leaf length and width) also initiated in all the six growing conditions with the onset of spring and continued till August-September (Figure 33a and Figure 33b). There was not much variation recorded in leaf size in plants growing in open net-shade throughout the third growing season from March to December. In all the six growing

conditions, the branches developed during summer (May-June-July). However, under green house and open net-shade, branches appeared at the onset of the summer (May 2nd week) and during mid-summer in shrubberies (June 3rd week) and under canopy (June end); while branches emerged during summer end in case of forest slope and open slope (July 3rd week). Over 70% of *S. chirayita* plants grown in open net-shade recorded 3 whorled leaves in each node, while plants grown in other conditions did not exhibited such character.

In *S. chirayita*, floral buds appeared during peak monsoon (July-August), when the average temperature and total rainfall was ranging between 17°C – 19°C and 3000mm to 4000mm, respectively, with longer day length (Figure 34). Green house grown plants recorded the earlier floral bud appearance (Figure 35e); however, in the remaining five growing sites exposed to natural climatic conditions, floral bud emerged earlier under open net-shade (May end; Figure 35b), the month recorded the highest weekly total rainfall and above 18°C average weekly temperature (Figure 34). Buds were sprouted one week later in all the growing conditions except shrubberies, where sprouting delayed by 3 weeks (Figure 35a-f). Similarly, the flowers opened fully a week later in the plants in all the growing conditions; however, flowers initiated 2 weeks later in shrubberies and under tree canopy. The longest flowering period was recorded for the green house and shortest for the open slope grown plants (Figure 35a-f). Fruiting initiated by July 2nd week (open net-shade), 4th week (green house) and September in plants transplanted to the natural habitat plots (Figure 35a-f).

Fruits started maturing during the October in natural habitats and coincided with decrease in weekly total rainfall while the air temperature was still much higher (approximately 18°C); however, the fruit started maturing by August (3rd week) and September (1st week) in open net-shade and green house, respectively. In general, the longest fruiting period was recorded for the green house, followed by open net-shade. In all the growing conditions, the aerial part of *S. chirayita* started drying by October with the maturation of seeds indicating the senescence stage, which completed by December; however, the plant senescence started by November and completed by January in green house grown plants. Seed shedding initiated as the total plant dried

up in all the growing conditions. It was observed that in all the growing conditions, the plant senescence began from the top and moved down slowly.

### *10.3.3 Effect of growing conditions on growth and development*

One way MANOVA indicated the significant ($p<0.0001$) effect of various growing conditions on different plant growth parameters and biomass (Table 22). Plants performed better under the green house and in open net-shade. For example, the maximum plant height under green house (175.01 $\pm$ 41.21 cm) was significantly greater at $p<0.001$ level compared to shrubberies, forest-slope and open-slope; and at $p<0.01$ level compared to tree canopy (Figure 32). Similarly, the plants grown in open net-shade produced significantly taller plants than those grown in forest slope ($p<0.01$); open slope and tree canopy ($p<0.001$). The highest number of nodes/plant (20.29 $\pm$ 2.50) under green house was significantly greater than those from open slope ($p<0.001$), forest slope ($p<0.01$) and tree canopy ($p<0.05$). Similarly, the number of nodes/plants in shrubberies and open net-shade were significantly ($p<0.05$) greater than open slope, which recorded the lowest number of nodes/plant (Figure 36). Only single shoot/plant was developed in all four natural habitats; nevertheless, the multiple shoot/plant was recorded in open net-shade (6.86 $\pm$ 0.69) and under green house (3.0 $\pm$ 2.31) [Figure 36]. The number of branches/plant was significantly ($p<0.001$) more in open net-shade (39.57 $\pm$ 5.19) than in other growing conditions (Figure 36). Similarly, the number of branches/plant under green-house (23.14 $\pm$ 3.39) was significantly greater ($p<0.001$) compared to forest-slope and open slope; and compared to tree-canopy ($p<0.01$). Amongst the four natural conditions, the number of branches/plant was high in shrubberies compared to forest slope ($p<0.01$), open slope and tree canopy grown plants (Figure 36). The number of fruits/plant (96.43 $\pm$ 21.94) was significantly ($p<0.001$) low in forest slope compared to other growing conditions except open slope (Figure 36). The biggest stem diameter was recorded for the plants grown under green house (19.57 $\pm$ 2.95) and open net-shade (15.0 $\pm$ 5.92) was significantly greater at $p<0.001$ level compared to other natural growing conditions; however, the difference was significant at $p<0.01$ level when compared between open net-shade and the forest slope (Figure 37).

The maximum average total plant biomass recorded for the open net-shade (44.16 ± 6.7), and green house (41.3 ± 4.61) appeared significantly ($p<0.001$) greater compared to the plants grown in four natural habitats; however, the difference in the mean plant biomass amongst the four natural habitats as well as between open net-shade and green house was non-significant (Figure 37). Plant growth parameters showed significant positive correlation with each other at $p<0.01$, except for the number of nodes and number of shoots which was significant at $p<0.05$ level (Table 23). Temperature and rainfall showed insignificant positive correlation with the plant height in natural habitats, while the plant height under open net-shade ($r = 0.703$; $p<0.05$) was significantly correlated with the temperature.

## 10.4 Discussion

The seedlings after transplantation need time to adapt to the microclimate of the site, as they are less tolerant to the extreme environmental fluctuations occurring at the soil surface of the site which strongly affects the survival rate of plants (Dodd and Donovan 1999), as seen in *Swertia chirayita*, where the highest seedling mortality occurred in the plants transplanted to open slope. This might be due to the early delicate nature of *S. chirayita* seedling produced under the green house to tolerate open exposure to direct sunlight at the site. However, seedlings transplanted to shrubberies, forest slope and under tree canopy were provided with some sort of shade and protection by the nurse plants/associated species, thereby resulting in greater survival, comparatively.

The temperature (Holway and Ward, 1965; Van Schaik, 1986; Badola *et al.*, 1992; Badola 1994) as well as rainfall (Foster, 1982; Bullock and Solis-Magallanes, 1990; Badola *et al.*, 1992) is important factor in different phenological phases. As the temperature begins to rise, the metabolic activity inside the plant intensifies (Badola, 1994; 2010) which could be manifested externally, initially through new leaf emergence, increase in leaf number and size as well as plant height. Plant requires tremendous amount of water for the growth and development and the rain is the main source of water, to major extent. Water acts as a solvent for the minerals and carbohydrates moving through the plants; and, also serves as a medium through which

nutrients and other solutes move in to the plant from the soil. In *S. chirayita* the plants maintained under controlled temperature in green house; the growth activities such as new leaf emergence, increase in leaf size, plant height, etc. were initiated during February-March. This trend was more or less similar to open net shade grown plants. This indicates that, the temperature and water alone is not responsible for the plant growth; however, there are other factors such as changing photoperiod etc., as organism adjusts itself seasonally in response to changing photoperiod (Chapin (III) *et al.*, 2002). With the elongation in day length, the photosynthesis activity increases in plants thereby resulting in plant growth and the synchronized leaf emergence; for photosynthesis, leaf maturation at high temperature and longer days are favourable (Salisbury, 1974). This suggests that the phenological event occurs as a result of combined effect of several environmental factors. Further, irrespective of growing conditions, the plants follow a defined seasonal pattern of growth (Badola, 2010). Beside seasons, plant growth also depend on the reserve of food acquired in the previous season and that largely depends on the leaf size, as it forms the net source of carbon for plants (Chapin (III) *et al.*, 2002). Plant growth generally occurs when the light availability is greater (Bazzaz, 1996). Under green house and open net-shade, absence of competitor species as well as frequent removal of the weed species created high light availability and required nutrients which promoted early growth in *S. chirayita* plants compared to those transplanted to natural habitat plots.

When *S. chirayita* is in its rosette form, the horizontal growth (leaf size) is more prominent than vertical growth (plant height). In addition, the growth in the form of new leaf emergence results in clustering of leaves in rosette form in *S. chirayita*, as a result of which, the larger and older leaves at the base (close to soil) get piled up prohibiting air circulation and moisture trapping between the leaves resulting in decomposition of the older leaves, which caused monthly variability in the number of leaves under all six growing conditions. The seedlings surviving after transplanting to their natural habitats are capable to withstand the harshness of the microclimate of the site, which means that they may behave similar to those plants naturally established. This has been observed in the present study where the phenological events in terms of new leaf emergence, shoot development, flowering, fruiting, senescence etc., in *S.*

*chirayita* plants transplanted to natural habitats coincided with the plants of *S. chirayita* already established in nature.

Both, rainfall and temperature play significant role in the floral initiation (Omoloja *et al.*, 2009); nonetheless, there will be some variation in temperature under the shade and open condition (Cervera *et al.*, 2006); and the plants respond differently when there is temperature differences (Bergqvist *et al.*, 2001), thereby resulting in some variation in the flowering time. At the same time, variations in rainfall, photoperiod and temperature govern the timing of flowering (Daubenmire, 1972; Bullock and Solis-magalanes, 1976; Foster, 1982; Leigh Jr. and Windsor, 1982; Schaik, 1986; Badola, 2010). Warm climate and increasing rainfall promote flowering (Omoloja *et al.*, 2009) as observed in the present study where constant high rainfall and rise in temperature in July-August during third growing season promoted flowering in *S. chirayita* transplanted to natural habitat plots. Similar effect of rainfall and temperature on flowering intensity has been observed by Alvim (1984) and Omolaja *et al.*, (2009). An early flowering initiation, floral bud sprouting and blooming under the green house is attributed to the constant high temperature ($25 \pm 5^{O}C$) and frequent watering; similarly, direct influence of sunlight/high air temperature and rainfall promoted the early flowering, floral bud sprouting and blooming in plants under open net-shade.

The fruiting time is another important phenological event for which the species needs adaptation to climatic conditions prevailing at the site (Bhat and Murali, 2001). Likewise in floral development, rainfall, temperature and photoperiod play significant role in fruit development and maturation. In *S. chirayita,* fruit development initiated one month earlier in open net-shade (July $2^{nd}$ week) and green house (July end); while it started in September in natural habitat plots. However, in either situation, the temperature and rainfall was at maximum and day length was also high comparatively. In natural habitat plots, fruit ripening initiated when there was considerable decrease in rainfall during October, having maximum sunny days and minimum rainy days, indicating the importance of direct sunlight exposure and high temperature in fruit ripening in *S. chirayita*. However, early fruit development

resulted in earlier fruit ripening in *S. chirayita* in open net-shade and green house. Comparatively, the shortest fruiting period was recorded in the plants grown in open slope, because here, fruiting initiated much later; nevertheless, being exposed to direct sunlight, the fruit maturation completed within short time span, as an adaptive advantage to cope with any possible disturbances in natural open conditions during, if a longer period prevails.

Sunlight regulates the photosynthesis, photo-morphogenesis and provides radiant energy (Bergqvist *et al.*, 2001) that heat up the plant tissues and the direct sunlight heats plant tissues more efficiently than diffuse light (Smart and Sinclair, 1965). In *S. chirayita* plant senescence began during October except under green house, when the rainfall as well as day length decreased substantially, while the temperature was still high. The present study further revealed that in *S. chirayita*, plant senescence is basipetal (begins from the top and moved down slowly to the bottom) and is much faster in the sites where plants are directly exposed to the sunlight, like open net-shade and open slope. This might be due to the earlier opening of terminal flower than the lateral flowers as a consequence of faster exposure to the sunlight (Goebel, 1924; Endress, 2010) in case of raceme or spike inflorescence (Goebel, 1924; Troll, 1964) as in *S. chirayita*. Cumulatively, especially in outdoors, the intensification in major growth activities i.e., leaf number and size, shoot growth, branch development, flowering, fruiting, etc., was observed during summer and rainy season, where the temperature and rainfall recorded high and the days were longer as well, convincing that the both temperature and rainfall to be equally important for the plant growth and development in addition to photoperiod in *S. chirayita*.

The development of multiple shoots in plants grown under both nursery conditions compared to single shoot development in plants grown in natural conditions, appeared as one of the most interesting observations in the present study. The higher number of shoots in *S. chirayita* had substantially produced greater number of branches in open net-shade than the plants grown under green house as well as other natural conditions, leading to higher plant biomass. Number of branches in a plant also determines the plant growth behaviour (Maqsood *et al.*, 1999). Study on the morphological

characters of *S. chirayita* for 22 natural populations in Sikkim Himalaya did not record the development of multiple shoots in any of the populations (authors' unpublished results). Generally in *S. chirayita*, each node bears two opposite leaves, but in the plants grown in open net-shade in the present study, three whorled leaves in each node in majority of the sample plants as well as others, were observed, which also played significant role in increasing the biomass of the plant; however, the other growing conditions including green house did not exhibit such morphological manifestation. Development of multiple shoots *vis-à-vis* higher number of branches, greater plant height etc., in plants in nursery conditions (green house and open net-shade) resulted in greater biomass per plant; whereas the production of single shoot, small plant height, lesser number of branches etc., in plants grown in four natural conditions resulted to lower biomass, relatively in *S. chirayita* in the present study.

## 10.5 Findings

The present study concludes with the following findings:

1. The third growing season is the most important phase in the life cycle of *S. chirayita*, because major phenological events right from shoot initiation to plant senescence occur in this particular phase.
2. Development of multiple shoot in *S. chirayita* under nursery conditions is a new and an important finding of the present study.
3. Irrespective of growing condition, the *S. chirayita* plants follow defined seasonal pattern of growth even if they are maintained under controlled environment.
4. The seedlings/plants of *S. chirayita* once adapted to microclimate of the site after transplantation behaves similar to those naturally established in in wild habitats, in terms of phenology, growth and development.
5. In *S. chirayita*, plant senescence starts from the top and moves down slowly to the bottom (basipetal) and the process is much faster in the sites where the plants are directly exposed to sunlight, like open net-shade and open slope.
6. Development of three whorled leaves in plants under open net-shade is a new and interesting finding of the study.

The populations of *S. chirayita* have declined considerably in the Himalaya due to habitat degradation, over exploitation, unsustainable/untimely harvesting, etc. The present study forms the baseline for both *in situ* and *ex-situ* conservation of the species. Further, on the basis of the above phenological information, harvesting time for *S. chirayita* can be determined which will impart opportunity to the species to recover from the disturbance in the nature thus providing sustainable income generation option to the medicinal plant collectors in the Himalayan region and largely from the potentially possible *ex-situ* cultivation.

**Table 22.** Summary of one way MANOVA depicting the effect of different growing conditions on the growth and biomass of *Swertia chirayita*

| Parameters | F value | Sig. | Eta squared |
|---|---|---|---|
| Plant height (cm) | 19.59 | .000 | 0.731 |
| Stem diameter (mm) | 24.32 | .000 | 0.772 |
| Number of nodes | 07.23 | .000 | 0.501 |
| Number of shoot | 40.51 | .000 | 0.849 |
| Number of branches | 39.54 | .000 | 0.846 |
| Number of fruits | 26.17 | .000 | 0.784 |
| Total plant biomass (gm) | 35.99 | .000 | 0.833 |

**Table 23.** Pearson's correlation coefficient amongst different growth parameters in *Swertia chirayita*

| | Plant height (cm) | Stem diameter (mm) | No. nodes | No. shoot | No. branches | No. fruits | Tot. plant biomass (gm) |
|---|---|---|---|---|---|---|---|
| Plant height (cm) | 1.000 | | | | | | |
| Stem diameter (mm) | 0.683** | 1.000 | | | | | |
| Number of nodes | 0.809** | 0.514** | 1.000 | | | | |
| Number of shoot | 0.537** | 0.543** | 0.336* | 1.000 | | | |
| Number of branches | 0.607** | 0.645** | 0.518** | 0.809** | 1.000 | | |
| Number of fruits | 0.651** | 0.540** | 0.677** | 0.281$^{ns}$ | 0.532** | 1.000 | |
| Tot. plant biomass (gm) | 0.796** | 0.816** | 0.637** | 0.767** | 0.860** | 0.631** | 1.000 |

** p<0.01; * p< 0.05; ns: not significant

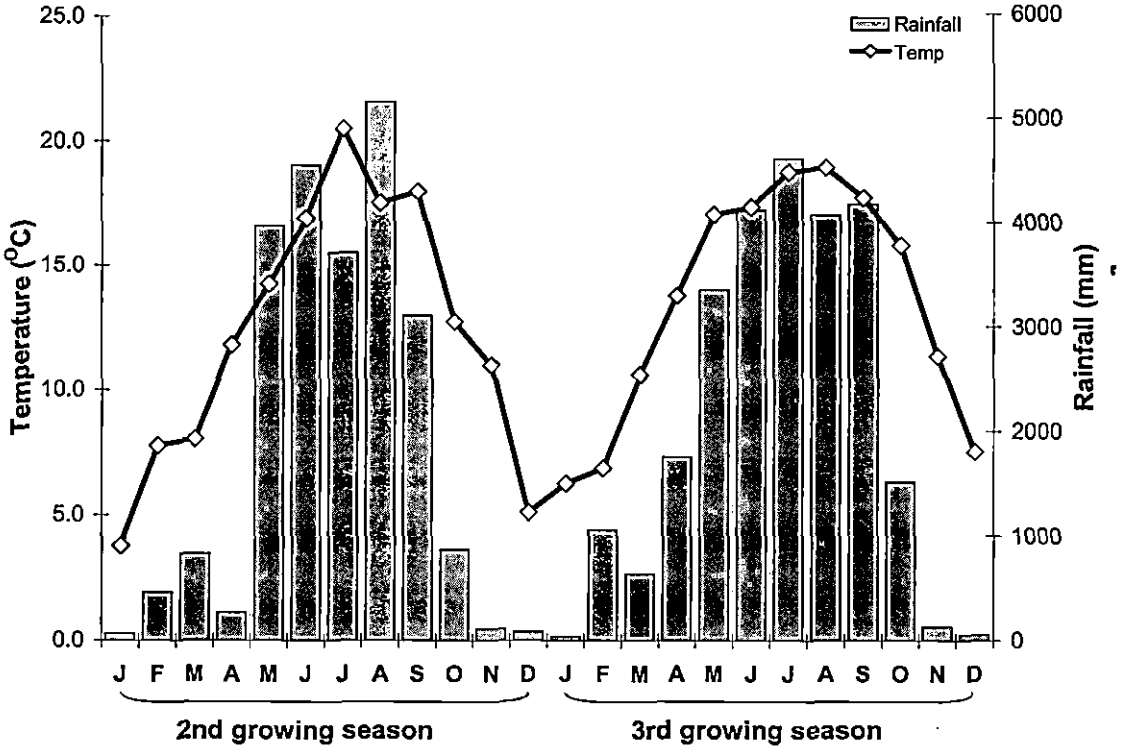



**Figure 30.** Climatic variations at Pangthang (Sikkim) during the study period

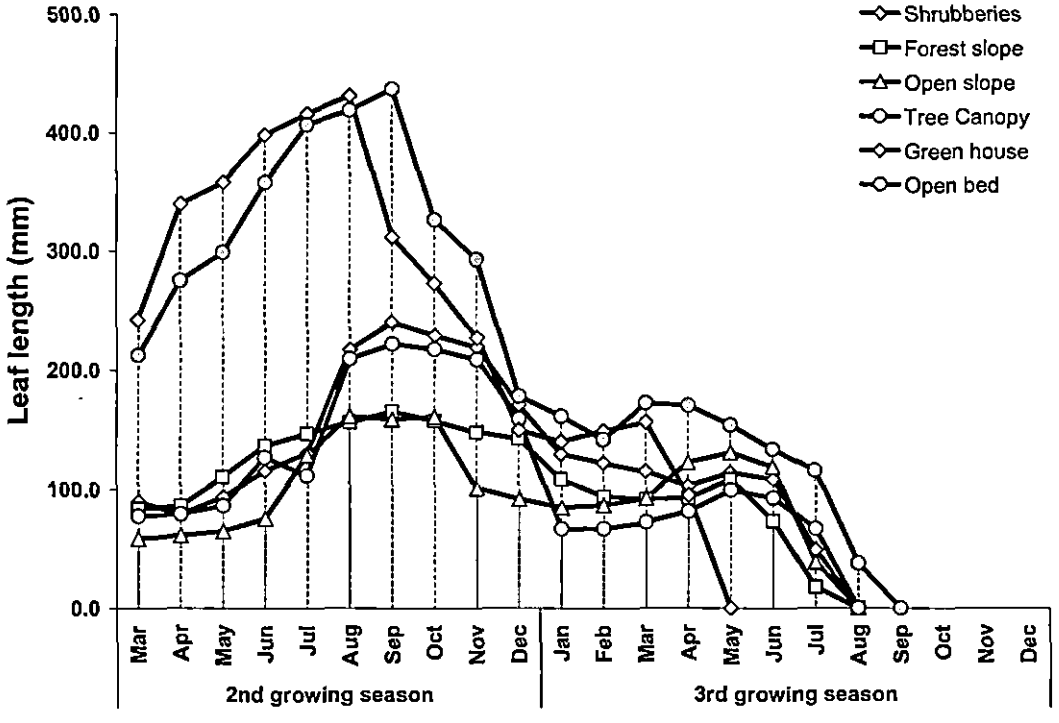



**Figure 31a.** Effect of growing conditions on average leaf length in *Swertia chirayita* (rosette form)

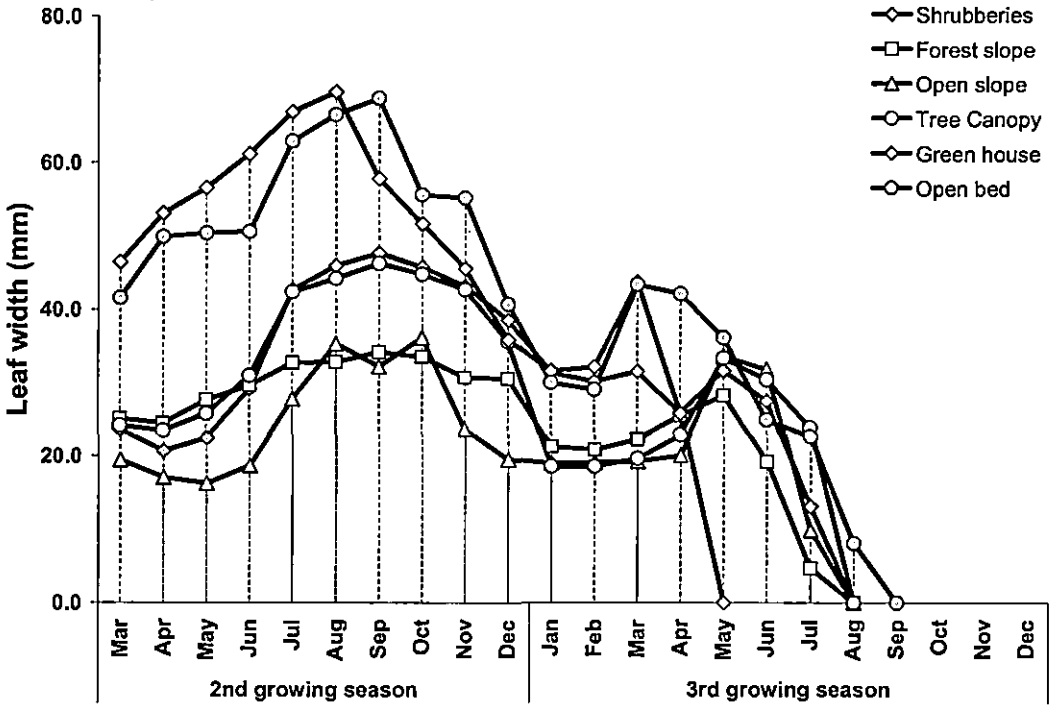



**Figure 31b.** Effect of growing conditions on average leaf width in *Swertia chirayita* (rosette form)

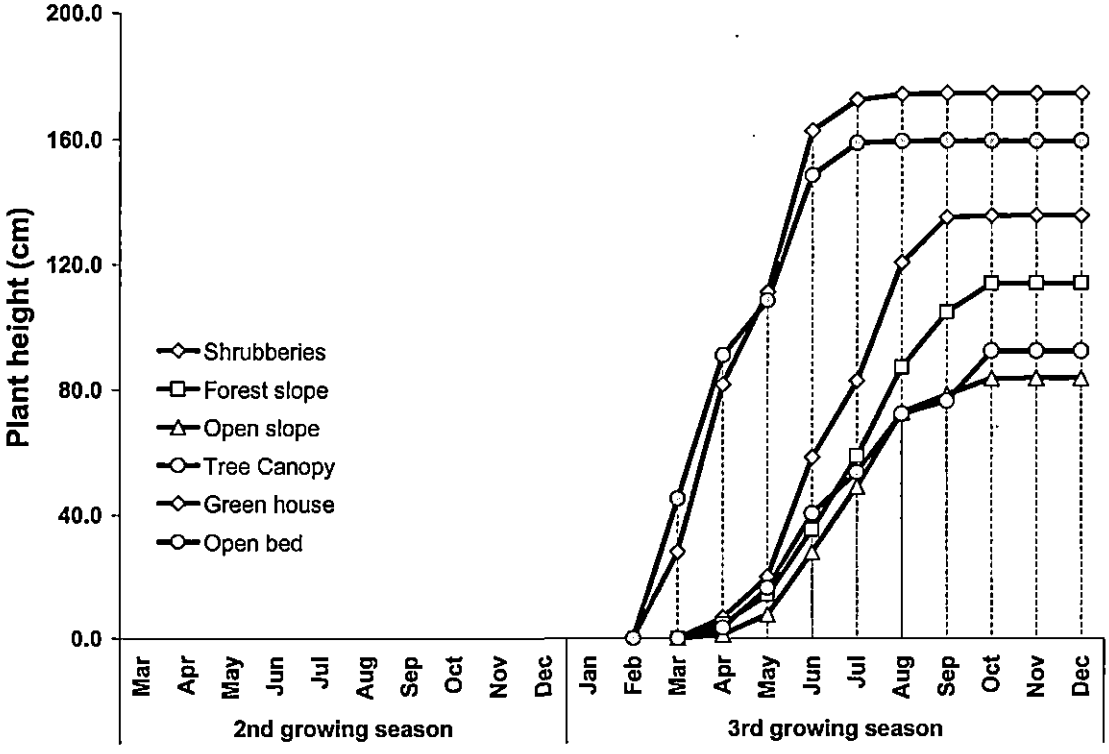



**Figure 32.** Effect of growing conditions on plant height in *Swertia chirayita*

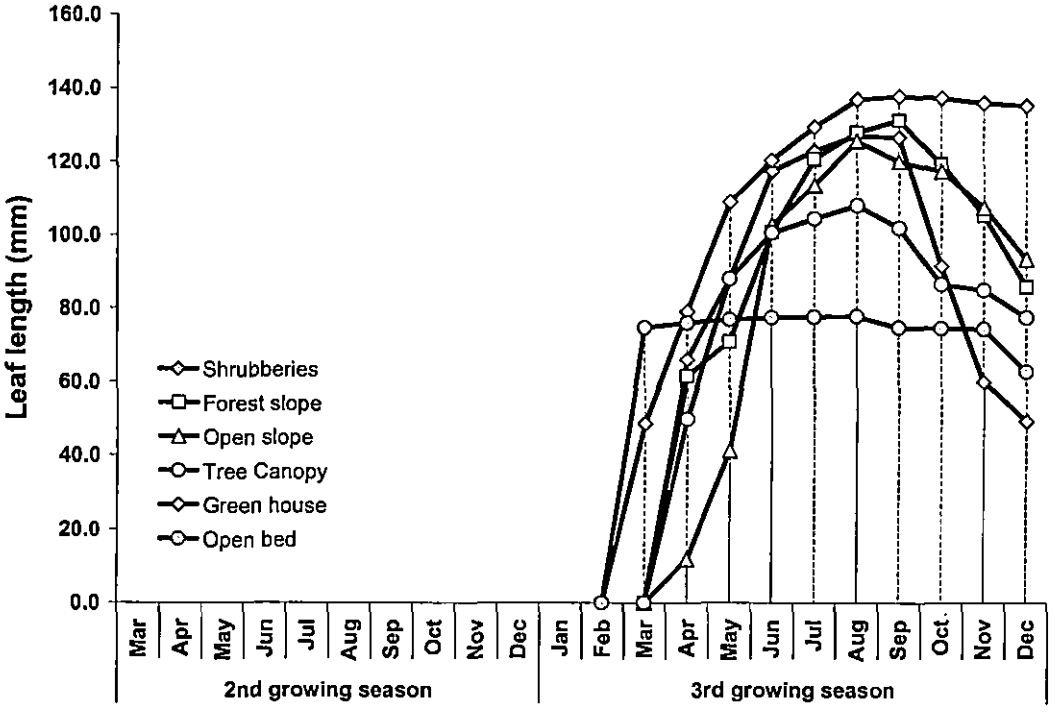



**Figure 33a.** Effect of growing conditions on average leaf length in *Swertia chirayita* (vegetative stem form)

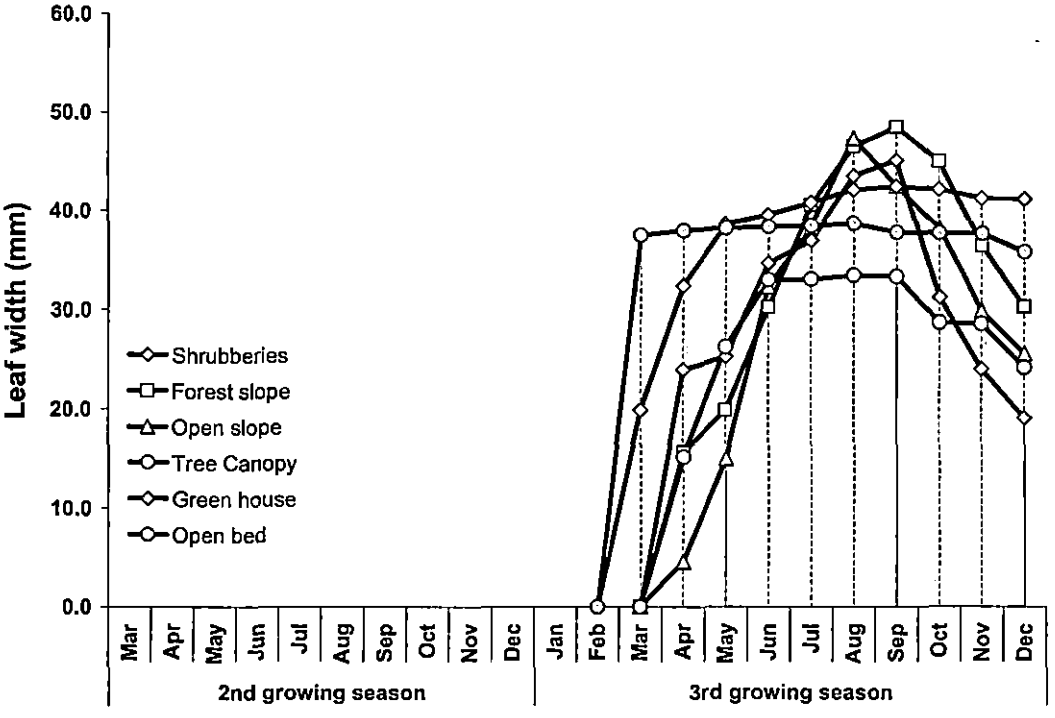



**Figure 33b.** Effect of growing conditions on average leaf width in *Swertia chirayita* (vegetative stem form)

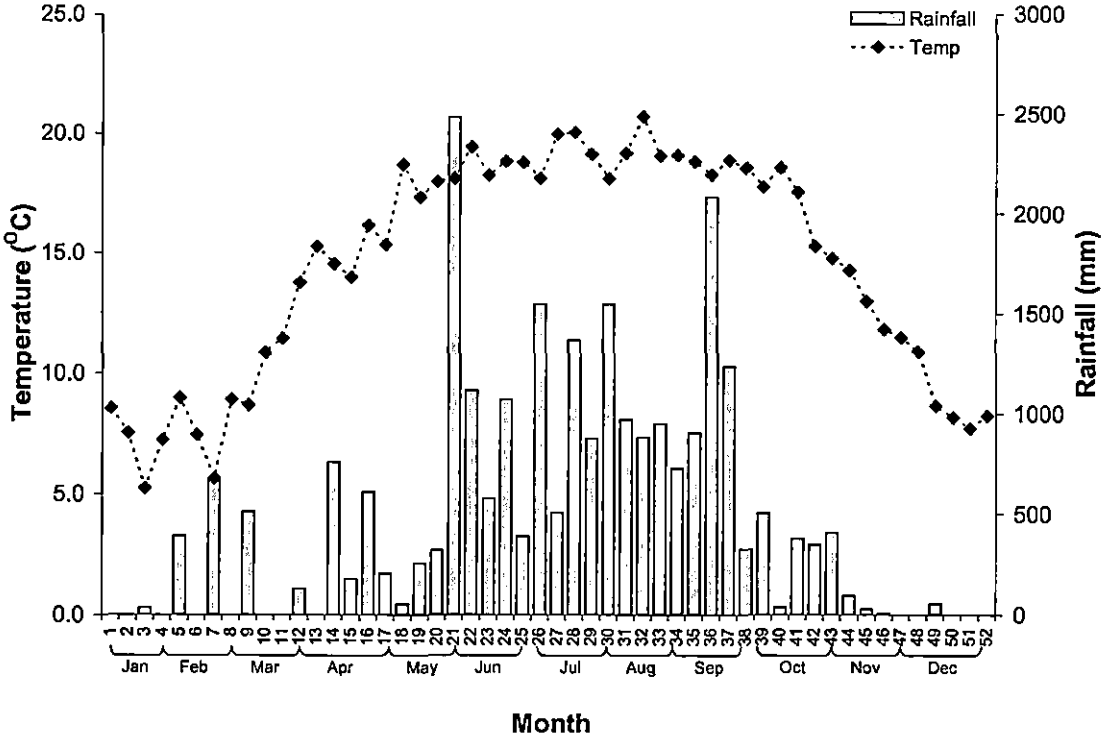



**Figure 34.** Weekly climatic properties at Pangthang (Sikkim) during the 3rd growing season

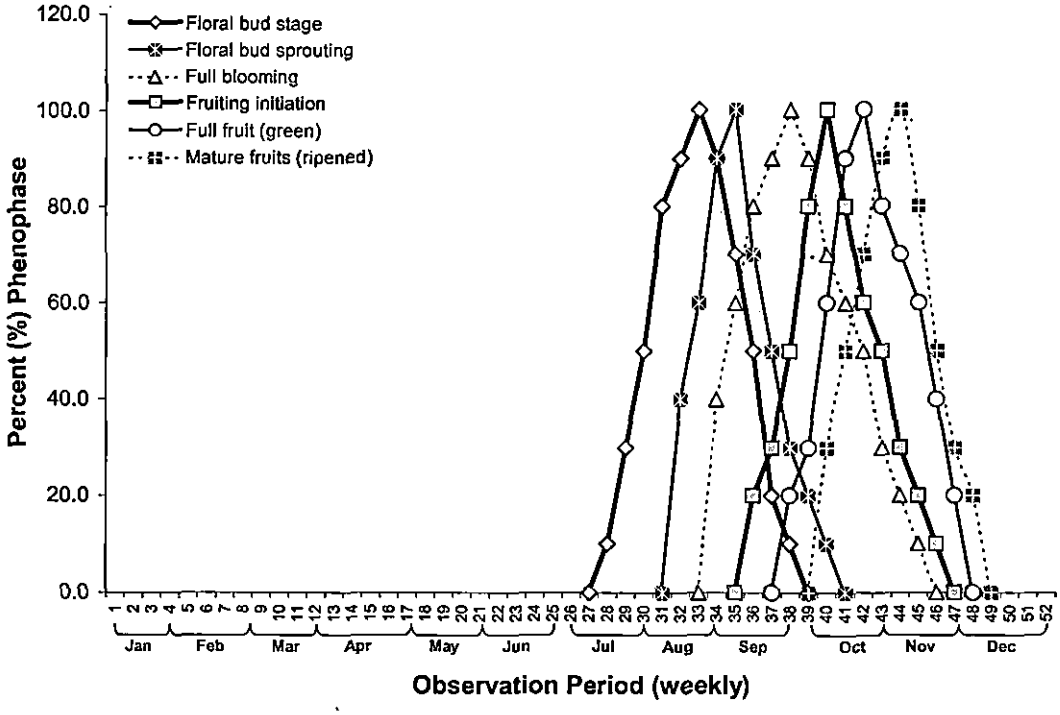



**Figure 35a.** Phenological observations (third year) in *Swertia chirayita* grown in shrubberies

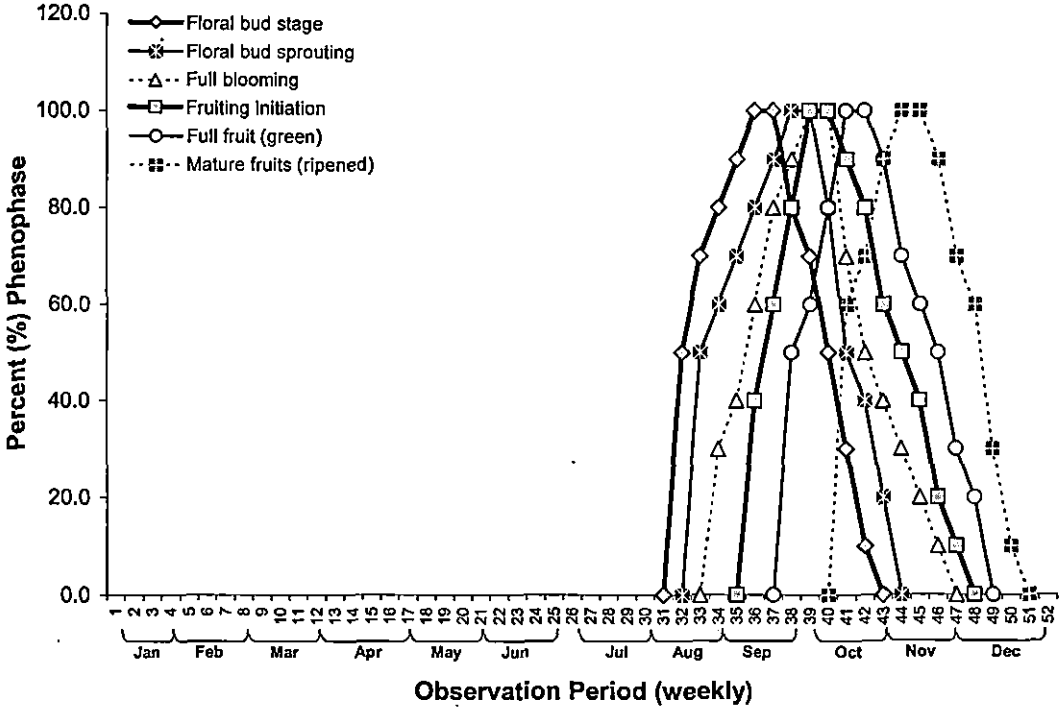



**Figure 35b.** Phenological observations (third year) in *Swertia chirayita* grown in forest slope

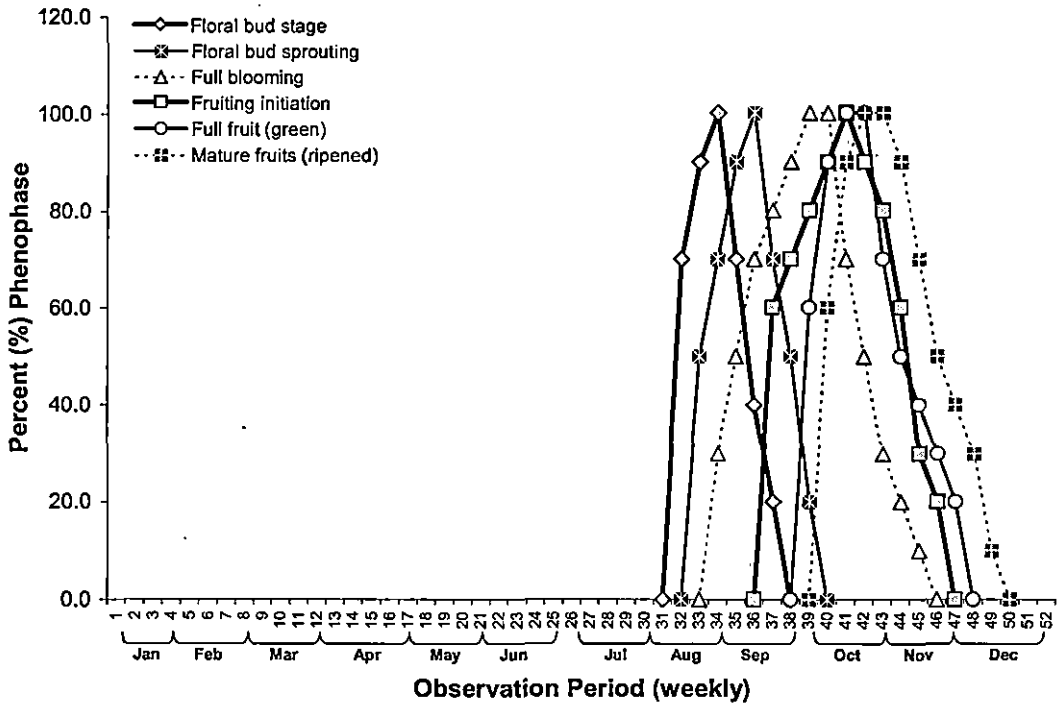



**Figure 35c.** Phenological observations (third year) in *Swertia chirayita* grown in open slope

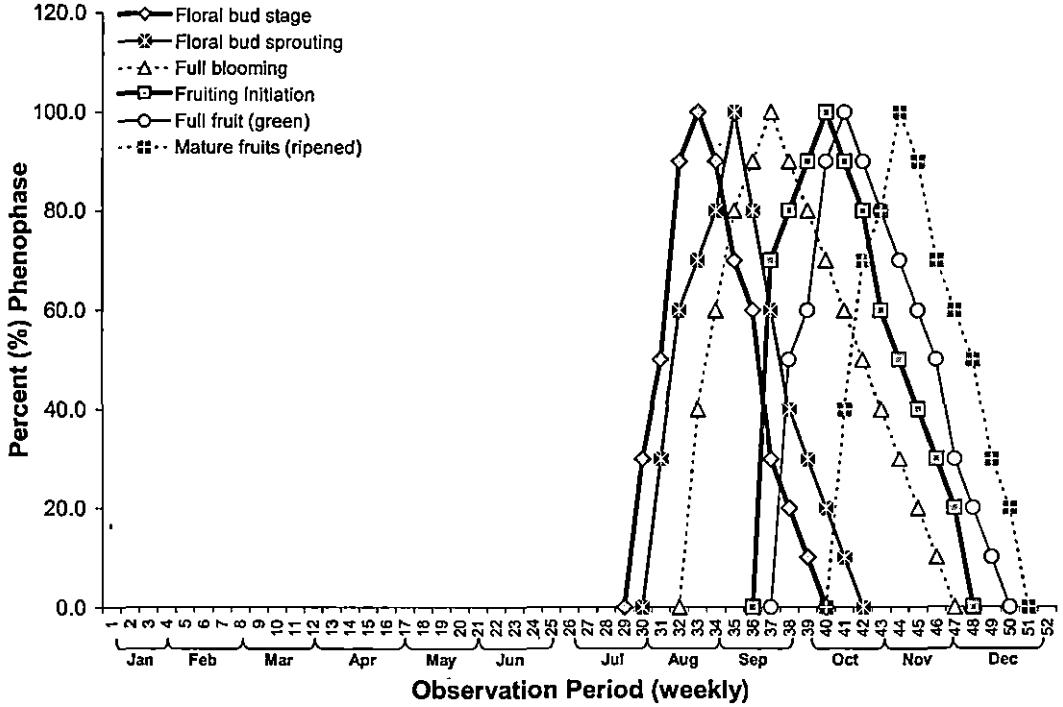



**Figure 35d.** Phenological observations (third year) in *Swertia chirayita* grown under tree canopy

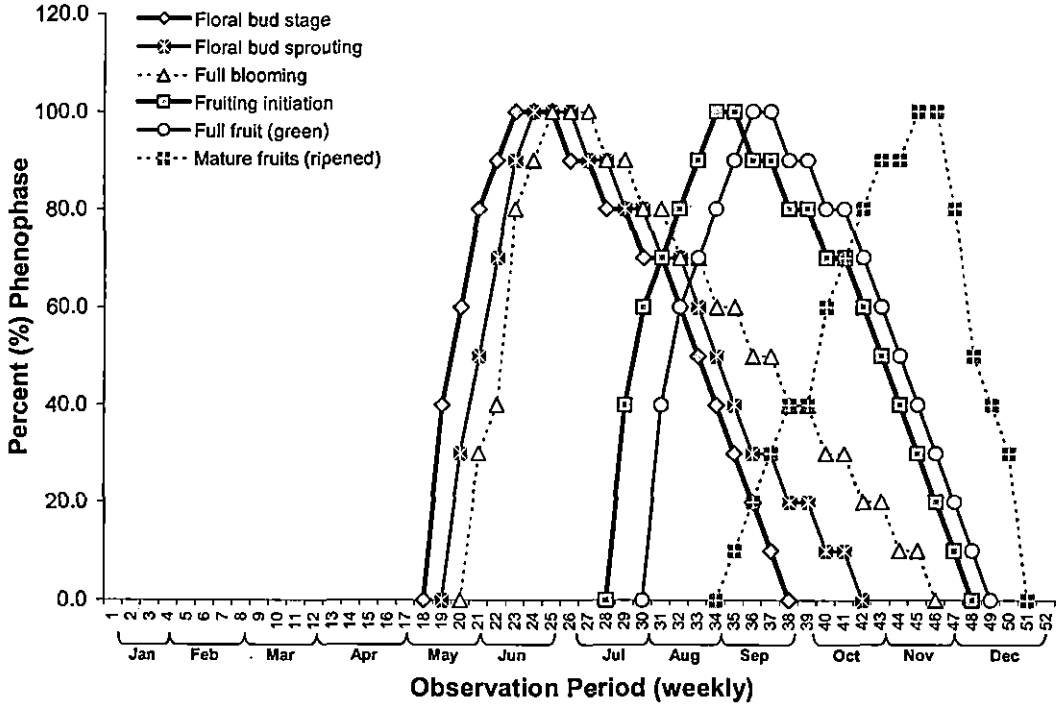



**Figure 35e.** Phenological observations (third year) in *Swertia chirayita* grown under temperature controlled green house

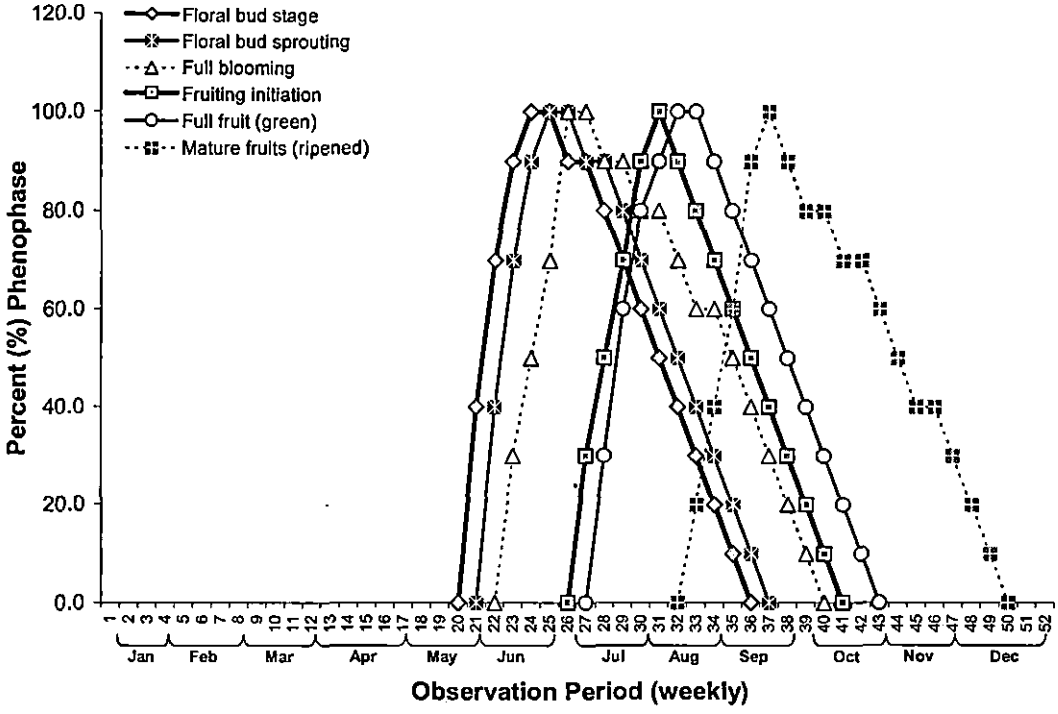



**Figure 35f.** Phenological observations (third year) in *Swertia chirayita* grown in open bed

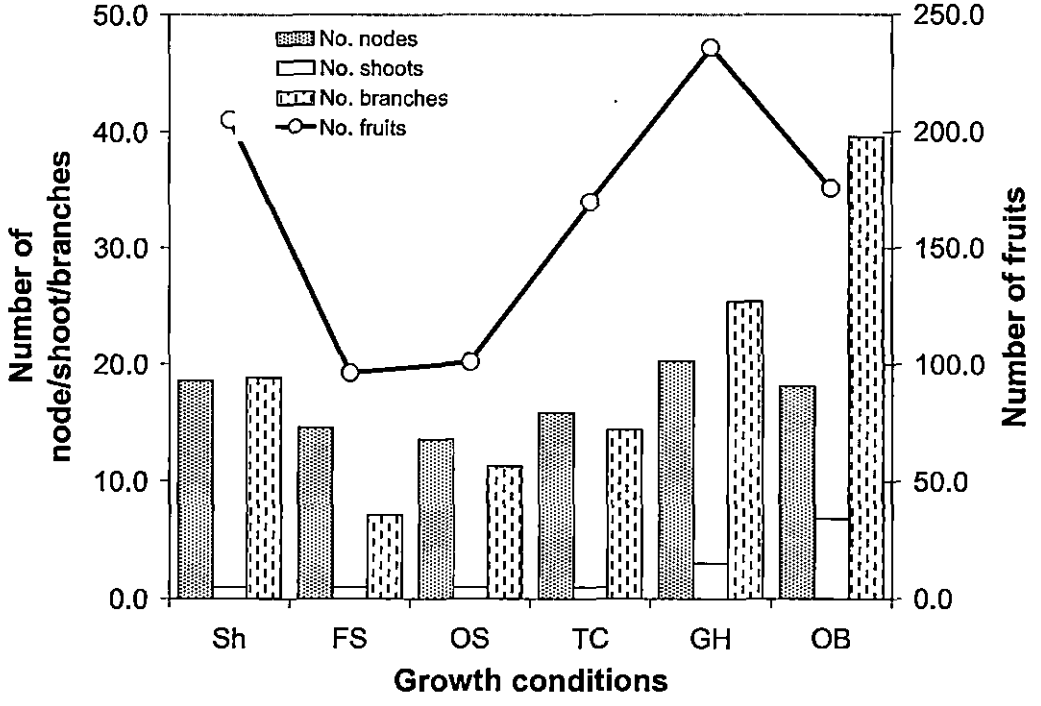



**Figure 36.** Effect of growing conditions on different growth parameters in *Swertia chirayita*; Sh: shrubberies, FS: forest slope, OS: open slope, TC: tree canopy, GH: green house, OB: open bed

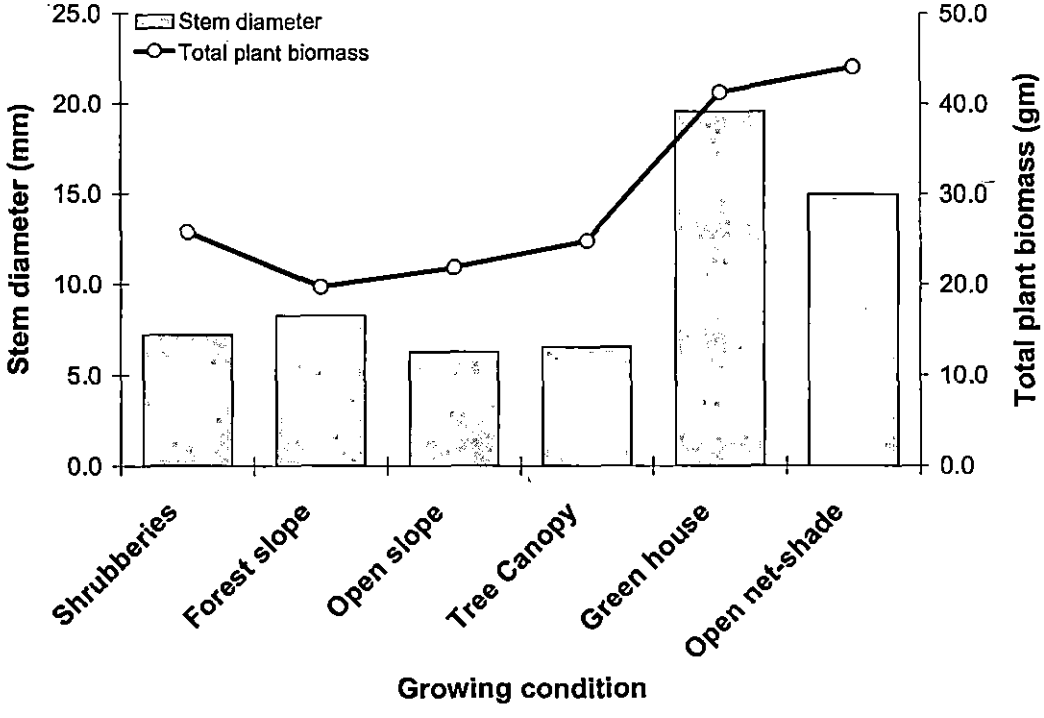



**Figure 37.** Growth and biomass in *Swertia chirayita* as affected by growing condition

CHAPTER 11

# Chemical Stimulation of Seed Germination

## 11.1 Introduction

One of the richest pools of biological diversity in the world, Indian Himalaya, has been experiencing unreasonable extraction of wild medicinal plants due to market demand, endangering many of its high-value gene stock (Badola and Pal, 2002, 2003; Badola and Aitken, 2003; Butola and Badola, 2008a) including *S. chirayita* (Bhattarai and Acharya, 1997; Badola and Pal, 2002, 2003; Dutta, 2004; Olsen, 2005). *Ex-situ* cultivation is considered as a possible solution to the conservation of endangered taxa and at the same time to meet out commercial raw material demand (Badola and Pal, 2002; Badola and Aitken, 2003; Badola and Singh, 2003; Heywood and Iriondo, 2003; Badola and Butola, 2003, 2005). It has been realized that to sustain availability of continuous stock of planting material for commercial cultivation as well as for species recovery, it is crucial to have in hand an appropriate multiplication technique to build a massive reserve of *ex-situ* plants (Badola and Butola, 2003, 2005; Butola and Badola, 2007, 2008b). Additionally, the selection of species specific appropriate propagation technology (Fay and Muir, 1990; Butola and Badola, 2004ab, 2006ab) and ideal propagation conditions for mass multiplication (Butola and Badola, 2008b) would minimize the waste of propagule resource. However, poor seed germination of viable seeds in several Himalayan plant species is experienced as a limiting factor in large scale plant multiplication (Nadeem *et al.*, 2000; Butola and Badola, 2004ab, 2006a, 2007, 2008ab). Pre-sowing chemical treatments are used to enhance seed germination of wild sources of several Himalayan medicinal plants (Nadeem *et al.*, 2000; Pandey *et al.*, 2000; Joshi and Dhar, 2003; Manjkhola *et al.*, 2003; Butola and Badola, 2004ab, 2006b, 2007; Shivkumar *et al.*, 2006) and in plants of other regions (Plummer and Bell, 1995; Yamaguchi *et al.*, 2000; Ghimire *et al.*, 2006; Kulkarni *et al.*, 2007; Vandelook *et al.*, 2007; Kaur *et al.*, 2009). The above studies were mostly confined to test seeds from wild. Seeds of only a few endangered Himalayan medicinal herbs have been evaluated, following establishment of *ex-situ* set-ups, for

their germination potential assessment, especially using various chemical stimulants and growing conditions (Butola and Badola, 2006b, 2007).

A critically endangered Himalayan herb (Ved *et al.*, 2003a) and prioritized on top for conservation through *ex-situ* cultivation by the international experts (Badola and Pal, 2002), *Swertia chirayita* (Roxb. ex Fleming) H. Karst was targeted for the current study. The genus *Swertia* (Gentianaceae) comprises of over 170 species globally, of which 79, 27 and 40 species are distributed in China, Nepal and India, respectively. Sikkim alone, a north-eastern state of India, harbours 13-14 species of *Swertia*. Amongst these, *S. chirayita*, which grows mostly in temperate Himalaya (1200m – 3000m asl) from Kashmir to Bhutan and in Khasia hills of Meghlaya, India (Chanda, 1976) in open pastures, grassy slopes and forests, is of great medicinal importance. Distribution of *S. chirayita* depends upon the altitude and slope and is not uniform. The species prefers to grow on north facing slopes and descends below 1500m, while on south facing slope the plants are found between 1500m and 3000m. In general, 2000m altitude is a suitable range preferred by the species in Nepal (Bhattarai and Shrestha, 1996) and 1800-2300m in Sikkim Himalaya. Over centuries, *S. chirayita* is used ethno-medicinally (Wawrosch *et al.*, 2005; Pradhan and Badola, 2008) for having numerous medicinal properties (Nadkarni, 1976; Biswas and Chopra, 1982; Kirtikar and Basu, 1984). In *S. chirayita*, seed is the only viable solution to the reproduction of the plant. Very limited publications are available on seed germination of *S. chirayita*, all limited to wild seed resources (Raina *et al.*, 1994).

The objectives of the present study on *S. chirayita* were, (i) germination assessment of seeds from six *ex-situ* produced sources; and (ii) enhancement of seed germination, in *ex-situ* produced seeds, using various pre-sowing chemical treatments. It is for the first time the present study makes a move beyond wild stock and targets further the descendent seeds as a stock to put on viability of *ex-situ* cultivation technology on *S. chirayita* for conservation. The current study makes a break from the ground and attempts to make a crucial step to understand process of domestication of species to attain predictability. The study targets to compare the *ex-situ* produced seeds of *S.*

*chirayita* from six sources and make various disclosers on the germination behaviour of seeds.

## 11.2 Materials and Methods

### *11.2.1 Propagule collection*

In December 2007, mature seeds were harvested from six *ex-situ* conditions i.e., shrubberies, forest slope, open slope, tree canopy, open net shade and temperature controlled green house (refer chapter 10), and mixed well individually for each source. To determine moisture content, 100 seeds/replicate (3 replicates/seed source) were weighed and oven dried ($60^0$C; 48 hrs). Seeds were counted for 10 healthy fruits per source. Seed size was measured for 30 seeds per sample (10 seeds each of 3 replicates) under a microscope. The remaining seeds were air dried in room temperature for 15 days. The seed viability test could not be performed due to minute seed size. In January 2008, seed germination potential using distilled water was tested (6 replicates; 30 seeds each) in a seed germinator (*experimental details given below*), which yielded poor germination. The remaining seeds were stored in air tight specimen tubes in refrigerator ($4^0$C) till the initiation of the pre-sowing chemical treatment experiment which was performed soon after the initial test was over.

### *11.2.2 Seed germination test*

In February, air dried seeds were surface sterilized by dipping in 0.04% aqueous solution of mercuric chloride ($HgCl_2$) for 10 sec to discourage fungal infection and washed thoroughly with double distilled water in all cases. The disinfected seeds were soaked in beakers containing various freshly prepared test solutions, viz., gibberellic acid ($GA_3$: 50, 150, 250, 350 µM); potassium nitrate ($KNO_3$: 50, 150, 250 mM) for 24 hrs and sodium hypochlorite ($NaHClO_3$, 5% available chlorine) for 5, 10 and 15 minutes. Treated seeds were washed 2-3 times with double distilled water and placed in petri-plates (90 mm) lined with single layer of Whattman No. 1 filter paper moistened with double distilled water. Control sets were maintained using double distilled water. Each treatment had 06 replicates of 30 seeds each, which were incubated for a maximum of 45 days in germination chamber (temperature: $25^0$C $\pm$

$2^0$C; photo period: 14/10 hrs light/dark), following a complete randomized design. Seeds were checked daily and moistened with double distilled water as and when required, and recorded as germinated on radical emergence. Germinated seedlings were counted and removed.

### *11.2.3 Statistical analysis*

The data were analyzed for mean and standard deviation (SD). Mean germination time (MGT) was calculated using equation, $MGT = \sum(nd)/\sum N$ ; where n = number of newly germinated seeds after each incubation period in days d, and N = total number of seeds emerged at the end of the test (Hartmann and Kester, 1989). Analysis of variance (ANOVA) was conducted on different parameters. The difference among the means was compared by least significant difference (LSD) test (Snedecor and Cochran, 1967).

## 11.3 Results

Before fruit harvest in December 2007, the final plant survival varied in different growing conditions, viz. 71% (Shrubberies), 54% (forest-slope), 22% (open-slope), 71% (tree-canopy), 43% (net-shade) and 88% (green-house) [refer chapter 10]. At attaining full maturity of plants, fruits harvested in December 2007 showed significant differences amongst sources. Number of seeds per fruit was significantly higher for shrubberies, which further varied amongst plot sources ($p<0.01$). Seed moisture content ranged from 14 to 22%. Significant differences ($p<0.05$) were obtained for seed weight, seed size and the moisture content amongst sources (Table 24). In initial control test, very low seed germination was recorded for all sources, viz., shrubberies: 31%; forest-slope: 12%; open-slope: 20%; tree-canopy: 21%; net-shade: 23%; green-house: 19%. Seeds produced in shrubberies showed significantly higher germination ($p<0.001$) compared to other sources, except net-shade, which differed at $p<0.01$.

In pre-sowing chemical stimulation experiment, in control, the seeds from forest-slope showed lowest germination (9.44%) comparing to other plots; this followed more or less same trend to that of earlier initial control test. Pre-sowing chemical treatments

significantly stimulated seed germination in *S. chirayita* over control, which varied amongst sources (Table 25). In general, $GA_3$ was most effective in stimulating seed germination over other treatments. Across seed sources, compared to control, $GA_3$ treatments improved the percent germination significantly ($p<0.001$). Seed germination value ranged between 70% (*minimum*; net-shade) and 97% (*maximum*; forest-slope and tree-canopy) using different $GA_3$ concentrations. Amongst them, $GA_3$ 250 µM followed by $GA_3$ 350 µM effectively stimulated seed germination ($p<0.001$). Seed sources did not show uniformity of germination specifically to a particular concentration of $GA_3$. For example, seeds from shrubberies responded best with 50µM $GA_3$; from open slope best germination was obtained for 250 and 350 µM $GA_3$, whereas seeds from tree-canopy, net-shade, and green-house responded best to $GA_3$ 250 µM (Table 25). Seeds from forest-slope showed an exceptional uniformity in germination of about 96% with all $GA_3$ concentrations except $GA_3$ 50 µM (89%). Comparing to control, $KNO_3$ treatments proved beneficial in improving seed germination in majority of cases; in which, $KNO_3$ 100 mM proved equally effective to stimulate seed germination in all sources ($p<0.001$). Individually, maximum seed germination (72%; $p<0.001$) was achieved with $KNO_3$ 50 mM for shrubberies. However, $KNO_3$ 150 mM was detrimental in case of three seed sources (forest-slope, open-slope and tree-canopy). Soaking in $NaHClO_3$ (5 minutes) had significantly ($p<0.001$) stimulated seed germination (48%: *open-slope* to 56%: *shrubberies*). Increased soaking time in $NaHClO_3$ (15 min) appeared harmful to the delicate seeds of *S. chirayita.*

Significant decline ($p<0.05$) in germination delay (number of days taken for 1st seed germination) was recorded with different concentrations of $GA_3$ over control, with lowest of 10 days for $GA_3$ 50, 150, and 350 µM, which varied amongst seed sources from experimental plots. The $KNO_3$ and $NaHClO_3$were effective in reducing germination delay only on the seeds collected from shrubberies and forest slope. The half of the maximum seed germination time (days taken to reach 50% of final germination) of 26 days was recorded in control for the forest-slope, and a minimum of 13 days in $GA_3$ 150 µM (shrubberies and tree-canopy), $GA_3$ 250 µM (tree-canopy) and $GA_3$ 350 µM (shrubberies and open-slope). Gibberllic acid significantly reduced

($p<0.05$) mean germination time (MGT) over control compared to other treatments (Table 26). After soaking in different concentration of $KNO_3$ and for different period in $NaHClO_3$, significant reduction ($p<0.05$) in MGT was achieved for the seeds collected from shrubberies, forest-slope and net-shade. Individually, significant reduction in MGT ($p<0.05$) was recorded for tree-canopy in $NaHClO_3$ (5 and 10min) and for green-house in $NaHClO_3$ (10min). In general, among all treatments, $GA_3$ had greatly improved percent germination (Table 25) and lowered the MGT (Table 26).

## 11.4 Discussion

In the present study, *ex-situ* produced seeds of *S. chirayita* harvested from different growing conditions showed very low germination, initially, under control. Likewise in many Himalayan herbs (Pandey *et al.*, 2000; Butola and Badola, 2004ab, 2006b, 2007; Shivkumar *et al.*, 2006), pre-sowing chemical treatments significantly improved germination percentage, onset and final germination of *S. chirayita* in present study. Here, gibberellic acid most effectively stimulated seed germination and minimized the difference in onset and final germination which can be attributed to increased activity of hydrolytic enzymes (Al-Helal, 1996; Joshi and Dhar, 2003; Manjkhola *et al.*, 2003) or mobilization of nutrients in dormant seeds (Kumar and Purohit, 1986; Hartmann and Kester, 1989). Further, gibberellic acid is known to promote germination by breaking dormancy in wide range of seeds (Vandelook *et al.*, 2007; Perez-Garcia, 2008). In *S. chirayita*, $GA_3$ in all concentrations was highly stimulatory to seed germination. Further, except for two sources, i.e. tree-canopy and net-shade, the difference amongst $GA_3$ treatments was insignificant in other four sources. That suggested effectiveness of all used concentrations of $GA_3$ in stimulating seed germination as well as reducing mean germination time in *S. chirayita* as gibberellic acid is known to affect physiological as well as metabolic activities of seeds resulting in early germination (Tipirdamaz and Gomurgen, 2000; Chuanren *et al.*, 2004). With the increase in $GA_3$ concentrations, increase in percent germination was observed (Abdel-Hady *et al.*, 2009; Keshtkar *et al.*, 2008). However, present study indicated non-uniformity in germination with different concentrations of $GA_3$ amongst the seed sources as reported by Bhatt *et al.*, (2005b) for *Swertia angustifolia*. However,

exogenous application of $GA_3$ had no effect on the germination of seeds in *Ferula assafoetida* (Otroshy *et al.*, 2009)

Sodium hypochlorite overcomes seed dormancy by either increasing permeability of the seed coat to oxygen through the removal of phenolics (Hurley *et al.*, 1989) or by scarification or modification of the seed coat (Hsaio, 1979; Butola and Badola, 2004ab, 2007) which might have resulted in early seedling onset and increased percent germination in seeds treated with $NaHClO_3$ (5 and 10 minutes) compared to control for all tested sources in the present experiment. The $NaHClO_3$ (10 min) could significantly stimulated seed germination for only two sources (forest-slope and green-house) at $P<0.05$. However, the time period for soaking in $NaHClO_3$ needs to be standardized from species to species. For instance, seed germination was enhanced by soaking for 5 sec to 60 sec in *Amaranthus powellii* but declined as the soaking period exceeded 60 sec in annual weed (Tommaso and Nurse, 2004). Similarly, seed germination was increased from 2% to 19% in *Zizania palustris* when soaked in $NaHClO_3$ for 2 hours (Oelke and Albrecht, 1985). A range of soaking time in $NaHClO_3$ (15 - 45 minutes) effectively used in many Himalayan herbs (Butola and Badola, 2004ab, 2006a, 2007). Since, the seeds are susceptible to fungal infection during germination, but, treating with $NaHClO_3$ further reduces the chances of fungal infection to seeds (Butola and Badola, 2007) thereby resulting in improved germination as in the present study.

In present study, $KNO_3$ 50 and 100 mM proved significantly effective for all sources used, lesser stimulatory comparing to $GA_3$. Whereas, increased concentration of $KNO_3$ (150 mM) was beneficial significantly only in case of shrubberies and net-shade. Contrarily, in *Angelica glauca* (with large sized seed, compared to minute seeds in *S. chirayita*), $KNO_3$ (150 mM) was found effective in stimulating seed germination (Butola and Badola, 2004b), may relate to the seed size as an important factor, need to be considered while standardizing level of concentration. Effectiveness of $KNO_3$ in stimulating seed germination may be possibly due to oxidized form of nitrogen causing a shift in respiratory metabolism to the pentose phosphate pathway (Roberts and Smith, 1977). Nitrogenous compounds in various forms, particularly

nitrates (e.g. $KNO_3$) have been used to stimulate germination (Choudhary *et al.*, 1996; McIntyre *et al.*, 1996). They play a critical role in increasing the physiological efficiency (Bhargava and Banerjee, 1994) and influence germination through change in water relationship (Nikolaeva, 1977). The responses of seeds to a particular concentration of $KNO_3$ may vary with species. In Gentianaceae family, in general, physiological dormancy is reported (Nikolaeva, 1977). Contrarily, the assessment on 13 wild populations of *S. chirayita* (Gentianaceae) did not show any seed dormancy (refer chapter 7). However, in the present study, *ex-situ* produced seeds were found with high dormancy. The results with improved germination rates in current study under $GA_3$ and $KNO_3$ treatments further correspond well with these generalizations, as both these substances are considered best for breaking physiological dormancy (Nikolaeva, 1977). The present study confirms that the seeds of *S. chirayita* from *ex-situ* grown sources, initially using wild seed stock, had physiological dormancy, which was broken by pre-sowing chemical treatments.

## 11.5 Conclusion

Over 70% (minimum) seed germination in eleven wild populations of *S. chirayita*, which gradually declined over time and further differed amongst sources under storage conditions was achieved (refer chapter 7). Seed germination potential differences in the same species/genus have been reported (Celiklar *et al.*, 2006; Pezzeni and Monatana, 2006). Such differences may be due to the environmental variations in the light and temperature (Karlsson and Milberg, 2007; Vandelook *et al.*, 2007), and climate present in the native habitats of the species (Celiklar *et al.*, 2006), but if proper strategies in respect of seed collection time etc. is maintained, such difference can be minimized to some extent. The present results suggest, there might be various environmental factors, the plants grown in or transplanted from green-house to natural habitats had to encounter during acclimatization and adaptation over two more growing seasons, might have induced greater physiological seed dormancy in second generation, which was later broken by chemical treatments. This can be viewed as a part of defence mechanism of a plant's survival tactics in response to a sudden change in its' eco-climatic condition. More detailed investigations are recommended to assess such eco-physiological changes occur from a sudden snap

from wild species adapted environmentally over ages. The study further recommends $GA_3$ (50 to 350 μM) as the best treatment for breaking seed dormancy and greatly improving germination in *ex-situ* produced seeds in *S. chirayita*. Good survival in natural conditions following transplantation of plants developed in green-house, using above pre-sowing treatments, has appeared as a technological package for strengthening conservation as well *ex-situ* cultivation of targeted endangered taxa, *S. chirayita*, in Himalaya.

**Table 24.** Seed characteristics of *Swertia chirayita* grown under different conditions at *ca.* 2000m asl

| Seed source | Shrubberies | Forest-slope | Open-slope | Tree-canopy | Net-shade | Green-house | P<0.05 | F |
|---|---|---|---|---|---|---|---|---|
| **Site conditions** | Slope: $20^0$; Dominant species: *Symploccos theifolia, S. glomerata* | Slope: $40^0$; Dominant species: *Bucklundia populnea* | Slope: $10^0$; Dominant species: *Osbeckia* sp. | Slope: $20^0$; Dominant species: *Castanopsis tribuloides* | Flat beds (soil amended with forest humus) | Flat beds (soil amended with forest humus) | | |
| **No. seeds per fruit** | 316 | 230 | 184 | 146 | 172 | 200 | 60.42 | 26.04 |
| **Seed weight (mg/100 seeds)** | 3.20 | 2.93 | 2.67 | 2.73 | 2.90 | 3.03 | 0.34 | 2.93 |
| **Seed length (µm)** | 362.20 | 394.07 | 480.17 | 379.83 | 417.77 | 361.90 | 1.96 | 40.60 |
| **Seed Width (µm)** | 315.27 | 310.40 | 355.70 | 306.10 | 345.77 | 293.63 | 2.15 | 11.28 |
| **Moisture content (%)** | 14.62 | 14.81 | 17.47 | 21.91 | 17.38 | 20.89 | 4.45 | 4.10 |

**Table 25.** Effect of different chemical treatments on seed germination in *Swertia Chirayita,* sourced from six *ex-situ* conditions

| % Germination | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Shrubberies** | **Forest-slope** | **Open-slope** | **Tree-canopy** | **Net-shade** | **Green-house** | **p<0.05** | **p<0.01** | **p<0.001** | **F** |
| Control | 28.33 | 9.44 | 20.56 | 19.44 | 21.11 | 17.78 | 5.91 | 7.93 | 10.46 | 8.77 |
| $GA_3$ (50µM) | 91.67 | 88.89 | 83.33 | 85.00 | 70.00 | 82.78 | 10.61 | 14.23 | 18.77 | 4.11 |
| $GA_3$ (150µM) | 83.33 | 96.67 | 83.89 | 92.78 | 73.33 | 82.22 | 9.75 | 13.09 | 17.25 | 5.95 |
| $GA_3$ (250µM) | 86.11 | 95.56 | 91.67 | 96.67 | 89.44 | 90.56 | 7.39 | 9.92 | 13.07 | 2.33 |
| $GA_3$ (350µM) | 85.56 | 96.11 | 91.11 | 91.11 | 85.56 | 82.22 | 6.75 | 9.05 | 11.94 | 4.64 |
| $KNO_3$ (50mM) | 72.22 | 23.89 | 20.00 | 31.67 | 48.89 | 27.22 | 8.61 | 11.56 | 15.24 | 43.69 |
| $KNO_3$ (100mM) | 59.44 | 35.00 | 42.22 | 37.78 | 45.00 | 37.78 | 9.30 | 12.47 | 16.44 | 7.51 |
| $KNO_3$ (150mM) | 45.56 | 07.22 | 06.11 | 05.00 | 32.78 | 18.89 | 6.41 | 8.60 | 11.33 | 56.05 |
| $NaHClO_3$ (5min) | 56.11 | 53.33 | 48.33 | 52.22 | 48.89 | 54.44 | 9.84 | 13.20 | 17.41 | 0.81 |
| $NaHClO_3$ (10min) | 24.44 | 20.56 | 31.67 | 23.89 | 21.67 | 42.22 | 9.61 | 12.89 | 16.99 | 6.05 |
| $NaHClO_3$ (15min) | 12.22 | 5.00 | 23.33 | 13.33 | 7.78 | 10.00 | 7.07 | 9.48 | 12.50 | 6.64 |
| **p<0.05** | 9.50 | 6.87 | 9.09 | 7.98 | 7.95 | 8.16 | | | | |
| **p<0.01** | 12.63 | 9.13 | 12.08 | 10.60 | 10.56 | 10.84 | | | | |
| **p<0.001** | 16.40 | 11.86 | 15.69 | 13.77 | 13.72 | 14.08 | | | | |
| **F** | 68.78 | 255.83 | 101.21 | 155.37 | 94.73 | 109.64 | | | | |

**Table 26.** Effect of different chemical treatments on Mean Germination Time in *Swertia Chirayita,* sourced from six *ex-situ* conditions

| Mean Germination Time | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Shrubberies** | **Forest-slope** | **Open-slope** | **Tree-canopy** | **Net-shade** | **Green-house** | **p<0.05** | **F** |
| Control | 23.55 | 27.26 | 17.53 | 20.46 | 22.88 | 20.20 | 2.69 | 12.82 |
| $GA_3$ (50µM) | 15.51 | 17.85 | 16.22 | 15.97 | 18.38 | 16.94 | 1.87 | 2.97 |
| $GA_3$ (150µM) | 14.27 | 15.66 | 15.29 | 13.74 | 16.41 | 14.65 | 1.18 | 5.63 |
| $GA_3$ (250µM) | 15.44 | 17.58 | 14.92 | 14.96 | 15.73 | 15.33 | 0.91 | 9.74 |
| $GA_3$ (350µM) | 14.16 | 16.72 | 14.52 | 15.21 | 15.24 | 15.37 | 0.95 | 7.07 |
| $KNO_3$ (50mM) | 19.17 | 22.64 | 20.45 | 21.08 | 21.14 | 20.08 | 1.61 | 4.37 |
| $KNO_3$ (100mM) | 20.24 | 22.19 | 19.04 | 22.81 | 20.46 | 22.18 | 1.53 | 7.54 |
| $KNO_3$ (150mM) | 23.86 | 24.25 | 23.33 | 20.00 | 23.83 | 20.66 | 2.60 | 4.12 |
| $NaHClO_3$ (5min) | 19.77 | 18.62 | 18.11 | 17.79 | 19.13 | 18.45 | 2.67 | 0.59 |
| $NaHClO_3$ (10min) | 19.46 | 23.18 | 19.62 | 17.76 | 18.53 | 17.56 | 3.07 | 3.69 |
| $NaHClO_3$ (15min) | 17.93 | 20.06 | 17.56 | 20.89 | 20.43 | 18.28 | 3.35 | 1.51 |
| **p<0.05** | 2.20 | 2.27 | 1.97 | 2.28 | 2.23 | 2.03 | | |
| **F** | 19.02 | 20.29 | 14.44 | 13.83 | 12.61 | 11.70 | | |

CHAPTER 12

# Chemical Treatments and Its Impact on Seedling Emergence and Vigour

## 12.1 Introduction

*In-situ* conservation within protected habitats must remain the primary means to conserve plant species. Plants are very sensitive to the environmental changes around them. The habitat disturbance and change in habitat conditions, ultimately leading to habitat loss, due to undesirable growth of unwanted plant species over important plant species, devoid them of required ecological conditions. This would result in fragmentation of the population thereby restricting its distribution to smaller pockets and finally wiping the species from the entire area. At this crucial point, *ex-situ* mechanism plays an important role in conserving endangered plant species (Badola and Pal, 2002) through their recovery, habitat rehabilitation and restoration. *Ex situ* conservation helps to provide the flexibility in responding to unforeseen environmental changes. The risk of extinction of threatened species in nature can be reduced by reinforcing the *ex-situ* raised individuals (Bowes, 1999). Expensive techniques may not be essential for successful seed germination and developing healthy individuals for some species, but identifying suitable technique, based on species requirements, would minimize the wasting of the resources (Fay, 1994; Benson *et al.*, 2000; Butola and Badola, 2004b, 2007). For raising mass planting material in species conservation programme, especially for Himalayan herbs, the best option would be the production of uniform and vigorous seedlings using identified pre-sowing chemical treatments (Butola and Badola, 2004b; 2006a, 2007). Also, high and rapid germination determine good stand establishment which results in higher yields. Therefore, fast and uniform germination is equally important for superior crop production (Afzal *et al.*, 2002).

The genus *Swertia* (Gentianaceae) is represented by over 170 species globally, out of which over 40 species occur in Indian Himalayan region and 13-14 species are recorded in Sikkim Himalaya (India). Amongst them, *Swertia chirayita* is of great

importance for its several biologically important compounds (Singh, 2008) and its multiple utilities in local medicine (Pradhan and Badola, 2008). Species bears high pharmaceutical value in national and international markets (Badola and Pal, 2002). Plant possesses anti-inflammatory, hypoglycemic, anti-fungal, anti-bacterial, anti-malarial, anti-oxidant (Joshi and Dhawan, 2005) and anti-pyretic (Bhargava *et al.*, 2009) properties; and can be used as natural larvicide (Balaraju *et al.*, 2009). *S. chirayita* is considered to be a bitter tonic, febrifuge and laxative and is used in fever, burning of body, intestinal worms and skin diseases etc., in a variety of forms and in combination with other medicines. In ancient Indian literature, *Madhavachikitsa* text, chapter *Jvarachikitsa*, one of the most important post *Caraka Sushruta Samhita* (*Caraka Sushruta* era: between $5^{th}$-$10^{th}$ century) mentions effectiveness of *S. chirayita* in *Jvara* or fever (Mishra, 2009). As entire plant possesses medicinal properties, the *S. chirayita* is uprooted unsustainably in wild at very early stage of development or before seeds are fully matured. Such human pressures have led to its endangerment in nature and has been assessed as critically endangered (Ved *et al.*, 2003b; IUCN, 2008). Amongst the prioritized species, *S. chirayita* further recommended at the top for its conservation through *ex-situ* cultivation by an international forum of experts (Badola and Pal, 2002). For both strengthening *in-situ* conservation and promoting *ex-situ* cultivation, availability of standardized propagation techniques and quality planting material of targeted species is a pre-requisite (Butola and Badola, 2004b, 2006a, 2007). To maintain sustainability of genetic stock (seeds), it would be equally important to use seeds from *ex-situ* sources (Butola and Badola, 2007)

The present investigation was carried out with the aim to fulfill ever-increasing demand of the pharmaceuticals and to strengthen *in-situ* species recovery programme by developing and standardizing suitable technique to improve seedling emergence and raise vigorous and mass scale planting material for *Swertia chirayita*. The objective of the present study were, (i) to examine the effect of earlier laboratory tested and identified best pre-sowing chemical treatments on nursery seedling emergence, emergence rate, growth and biomass; (ii) to examine variability amongst seed sources on above parameters in nursery conditions, and (iii) to identify appropriate morphological traits in screening vigorous seedlings in *S. chirayita*.

In the present work, we chose two earlier tested best chemical treatments in improving seed germination potential in a seed germinator, using the same *ex-situ* sources for obtaining seeds, as a continuum (second phase) of earlier work (refer chapter 11) in nursery condition with addition of assessment on seedling growth and vigour in *S. chirayita*.

## 12.2 Materials and methods

### *12.2.1 Seed collection*

Mature seeds of *Swertia chirayita* (Roxb. ex Fleming) H. Karst.were harvested during December from six experimental set ups viz., four in natural habitats (1. shrubberies, 2. forest-slope, 3. open-slope and 4. tree-canopy) and two in nursery conditions, on soil amended beds using garden soil and forest humus (1. open net-shade, and 2. temperature controlled green-house; $25^0C \pm 5^0C$) [refer chapter 11]. Seeds were mixed well individually for each source, room dried for 15 days and stored in air tight specimen tubes at $4^0C$ until the experimentation.

### *12.2.2 Experimental design*

During May 2008, seeds from all the six sources were provided with the best chemical treatments, which we had earlier tested and identified in laboratory on germination in *S. chirayita* seeds collected from the same plots (refer chapter 11). In the previous experiment, all four concentrations of gibberellic acid resulted in high percent seed germination and $GA_3$ (250 µM) most effectively stimulated seed germination for majority of the sources (open slope, tree canopy, net shade, green house); and amongst three concentrations used for $NaHClO_3$, the 5minutes soaking time gave best results; while, all the three concentrations of $KNO_3$ were less effective in stimulating seed germination, except for shrubberies, due to which $KNO_3$ was not considered in the current experiment. The seed lots from each source were divided into three sets containing 150seeds/set/source. The first set of seeds was soaked in $GA_3$ (250µM) for 24hrs and the second set soaked in $NaHClO_3$ for 5minutes. The third set was soaked in double distilled water (DDW) for 24 hrs which served as control. The soaked seeds for each set were washed thoroughly with DDW and sown

(0.5cm depth in rows; maintaining 5cm distance between each seed in three replicates; 50seeds each per replicate) in nursery trays containing a mixture of garden soil, sand and crushed farm yard manure (pH: 7.02) in equal volume. The experiment was performed under the net-house (Temperature, max: 25.04$^0$C, min: 13.22$^0$C; Relative humidity, max: 91%, min: 65%) located in the Institute campus at an altitude of *ca* 2000m, in a completely randomized design. Prior to seed sowing, the soil mixture was wetted with water in order to avoid washing away of seeds from the trays. Taking in account the importance of soil moisture in seedling emergence, careful watering was done as per the necessity.

Daily observation on seedling emergence was made using a powerful hand lens for 60 days. To avoid competition, thinning was done after 16 weeks of seed sowing, maintaining maximum distances between each seedling (10-12 cm). After 24 weeks of seed sowing, 5 seedlings for each treatment per seed source from experimental trays were randomly harvested and assessed for growth parameters, including the total dry biomass. As the plant remains in the rosette form for consecutive two year, the plant height was not taken into consideration. The root length was determined from the root collar to the tip of the primary root. The collar diameter was measured using a digital calliper (Mitutoyo, Japan). The dry biomass was determined by oven-drying seedlings to constant weight at 70$^0$C.

### *12.2.3 Statistical analysis*

Multivariate analysis of variance (MANOVA; $\alpha = 0.05$) in general linear model (GLM) was carried out to determine the effect of seed sources, treatments and their interaction on seedling emergence variables and morphological traits using SPSS 10.0 software (SPSS Inc. 1989). A Bonferroni test ($p<0.05$) was used to determine the differences amongst mean values for different treatments. Mean days for emergence ($M_d$) was calculated according to the equation of Edmond and Drapala (1958) as: $M_d = \sum nd/\sum N$, where, "n" is number of newly emerged seedlings in each day "d", and "N" is total number of seedlings emerged at the end of the test. Seedling emergence rate was calculated using modified Timson index of germination velocity by Khan and Ungar (1984) as: $T = \sum G/t$, where "G" is percentage of seeds emerged after 2

days interval and "t" is the total emergence period. To identify the most effective treatment for producing healthy seedlings, seedling vigour index (SVI) was calculated following Butola and Badola (2004b), SVI = $(d/M_d)$*100, where, "$d$" is dry weight/seedling; "$M_d$" is seedling mean days of emergence and 100 is constant value. In order to analyze the relationship between different morphological traits and to identify convenient morphological traits to select vigorous seedlings, Pearson's correlation ($r$) analyses were performed by pooling data from all the treatments and the relationship amongst different seedling traits was examined.

## 12.3 Results

### *12.3.1 Seedling emergence*

Table 27 depicts the result of multivariate analysis of variance (MANOVA) showing effect of treatments, seed sources and their interaction on different seedling emergence variables. Different treatments showed significant effect on all the seedling emergence parameters ($p<0.0001$).

Seedling emergence in all the sources and treatments including control started within 18 to 22 days of seed sowing and maximum number of seedlings emerged within 56 days of sowing. Both $GA_3$ (250μM) and $NaHClO_3$ (5 minutes) highly stimulated the seedling emergence in all seed sources over control; however $GA_3$ was more effective than $NaHClO_3$ (Figure 38). Over 59% seedling emergence was recorded with $GA_3$ in all the seed sources and the maximum latitude in stimulation was recorded in seeds sourced from forest slope, where seedling emergence was promoted from 6% (control) to 69% ($GA_3$). Comparatively, 40% seedling emergence was the maximum value recorded in seeds (green house) treated with $NaHClO_3$.

Seedling emergence rate was faster in $GA_3$ followed by $NaHClO_3$ in all the seed sources (Table 28). With $GA_3$, amongst six sources, the seedling emergence was faster for green house (11.53±0.47) and slower for net-shade (9.51±2.39), comparatively. With $NaHClO_3$, the seedling emergence was faster for tree canopy (4.52 ± 1.35) and shrubberies (4.51 ±0.93) compared to other sources.

### 12.3.2 Seedling growth and biomass

MANOVA revealed significant ($p<0.0001$) effect of seed sources and treatments on all the seedling growth parameters; however, the effect of their interaction was non-significant for number of leaves and root diameter (Table 29). Amongst the seed sources, forest slope resulted in significantly ($p<0.05$) higher values for root length, total plant dry biomass and seedling vigour index compared to other sources (Figure 39, 40 and 41). Significantly ($p<0.05$) minimum seedling growth was achieved for shrubberies. Significantly ($p<0.05$) lesser number of leaves was recorded for the seed sourced from open slope compared to other sources. $GA_3$ and $NaHClO_3$ treatment significantly ($p<0.05$) enhanced all the seedling growth parameters over control; however the effect of $NaHClO_3$ was in-significant for number of leaves. On comparision, $GA_3$ appeared significantly ($p<0.05$) more effective over $NaHClO_3$ treatment for all the growth parameters including total plant dry biomass and seedling vigour index. All the plant growth parameters showed significant correlation with each other except number of leaves (Table 30).

## 12.4 Discussion

In a species, variation in germination behaviour between sites (Baskin and Baskin, 1998; Ceraboloni *et al.*, 2004) is applicable not only to the natural populations but also to the *ex-situ* produced seeds under different conditions (refer chapter 10). Present study, with *ex-situ* produced seeds in *S. chirayita,* indicates the germination variability amongst the seed sources in addition to existence of physiological dormancy (refer chapter 10).

Both $GA_3$ (Mehanna *et al.*, 1985; Tipirdamaz and Gomurgen, 2000; Butola and Badola, 2004ab, 2006a; Chandra *et al.*, 2006) and $NaHClO_3$ (Fieldhouse and Sasser 1975; Hsiao 1979; Drew and Brocklehurst 1984; Bewely and Black, 1994; Butola and Badola, 2004ab, 2006a, 2007) are known for their stimulatory action on seed germination, breaking seed dormancy and increasing germination rate. In the present study, both $GA_3$ (250μM) and $NaHClO_3$ (5minutes) treatments were stimulatory to seedling emergence and over all seedling growth for all six *ex-situ* seed sources. The

commencement of seedling emergence was significantly faster ($p<0.05$) in $NaHClO_3$ treatment followed by $GA_3$ over control. Such differences in seedling emergence between treated and untreated seeds might be due to alteration of physiological process by the liberated enzymes inside the embryo (Kattimani *et al.*, 1999). Although, the mean days of seedling emergence were higher in $GA_3$ than $NaHClO_3$, the faster seedling emergence rates for $GA_3$ indicated it as more effective treatment than $NaHClO_3$ (Table 28).

$GA_3$ proved to be more effective than $NaHClO_3$ in stimulating seedling emergence in *S. chirayita* in all six *ex-situ* seed sources in the present study, however, higher effectiveness of $NaHClO_3$ over $GA_3$ was observed in case of *Angelica glauca* and *Heracleun candicans* (Butola and Badola, 2004b, 2006a). In present study, greatly stimulated seedling emergence by using higher concentration of $GA_3$, i.e., 250µM is comparable to *Picrorhiza kurrooa,* where Chandra *et al.*, (2006) achieved marked improvement in seed germination (78.3% compared to 1.7% in control). The inhibitory effect of higher soaking period in $NaHClO_3$ (10 and 15 minutes) was observed in *S. chirayita* having smaller seed size (refer chapter 10), whereas, increased soaking period resulted in higher seed germination in the case of *A. glauca* and *H. candicans* with large seeds (Butola and Badola, 2004b, 2006a). This has indicated that the seed size might play a vital role in deciding concentration of chemicals or growth regulators to be used for achieving best results. Such variability in Himalayan herbs indicates species specific nature of seeds. As in *S. chirayita*, $GA_3$ stimulated seed germination and germination rate in many plant species is known (Nicolas *et al.*, 1996; Koyuncu, 2005; Cetinbas and Koyuncu, 2006). Similar stimulatory effect of $NaHClO_3$ in seed germination has been reported (Clevering, 1995; Yildiz and Celal, 2002; Butola and Badola, 2007).

Higher seedling emergence in *S. chirayita* with $GA_3$ in the present study might be due to amylase synthesis in seeds (Amen, 1968; Galtson and Davies, 1969). $GA_3$ stimulates hydrolytic enzymes that are needed for the degradation of the cells surrounding the radical and thus speeds up germination by promoting seedling elongation (Karssen *et al.*, 1989; Rood *et al.*, 1990; Silva *et al.*, 2004). $GA_3$ also

regulates hypocotyl growth altering the extent of hypocotyl cell elongation (Cowling *et al.*, 1999) and plants response to external environment (Chakrabarti and Mukherji, 2003). However, Chen and Chang (1972) argued against the contention that gibberellic acid stimulates seed germination via amylase synthesis, taking case of dormant wild oat *(Avena fatua)* seeds, which confirmed that amylase activity increases following post germination growth.

Scarification or bleaching of seed coat by $NaHClO_3$ (Vujanovic *et al.*, 2000; Bohm, 2003) or other modification in seed coat (Hsaio, 1979) may not be the cause of stimulation of seedling emergence in *S. chirayita*, as the delicate seeds are enclosed in very thin seed coat which do not need extra physical effort in promoting germination. It may be due to either increase permeability of seed coat to oxygen through the removal of phenolics (Hurley *et al.*, 1989) or release of oxygen with decomposition of $NaHClO_3$, thus enhancing oxidative respiration, thereby, promoting seed germination (Bewely and Black, 1994; Vujanovic *et al.*, 2000).

Other factor which might be responsible for stimulation of seedling emergence by $NaHClO_3$ are decomposition of germination inhibitors (Mackinnon and Alderton, 2000), overcoming dormancy by lowering of pH thereby promoting oxygen uptake (Bohm, 2003), or promoting an enhancement of α-amylase activity (Kanecko and Morohashi, 2003). Soaking seeds in $NaHClO_3$ for lesser time (5 minutes) appears relatively useful to achieve successful recruitment of *S. chirayita*. $NaHClO_3$ not only protects the seeds from pathogens in nursery conditions but also facilitates leaching of toxic compounds and improves the uptake of water and oxygen contributing to increase seed germination or seedling emergence.

In the present study, $GA_3$ application resulted to higher seedling dry biomass and seedling vigour index. This may be due to the fact that the action of $GA_3$ applied before sowing could have sustained itself till the plants reached vegetative stage. Further, this could be related to an elaboration of an endo-membrane system and regulated synthesis of proteins required for germination, which in turn could have contributed additionally to the amino acid rescue and protein turnover during active

metabolism, later in plant life (Shah, 2007). $NaHClO_3$ is applied as a suitable disinfectant for seed surface sterilization (Bewley and Black, 1994; Kaneko and Morohashi, 2003) and also known to promote seed germination and overcomes seed dormancy (Bewely and Black, 1994). However, $NaHClO_3$ effect on seedling growth (Fieldhouse and Sasser, 1975; Chun *et al.*, 1997; Butola and Badola, 2004b, 2006a, 2007) and its mechanism has rarely been reported.

All the seedling growth parameters were positively correlated with each other but the correlation was non-significant for some of the growth parameters. Both collar diameter and root diameter though showed significant correlation with all the growth parameters except number of leaves; collar diameter is considered strongest morphological trait for identifying seedling vigourousness in *S. chirayita* because root being present below ground surface is not conveniently visible. Further, the collar diameter is always larger than the root diameter (*personal observation*) which does not necessitates going further step to identify vigorous seedlings.

## 12.5 Conclusion

The present study suggests that stimulatory effect of $GA_3$ (250μM) (preferably) and $NaHClO_3$ (5 minutes) on seedling emergence and seedling vigour may solve the problem of increasing pressure on the wild population of species for raw material, as a tool to strengthen *ex-situ* cultivation of *S. chirayita*. Higher seedling emergence rate obtained with $GA_3$ (250μM) may lead to early establishment of the seedlings, which bear the ability to compete and withstand the rough climate. This could be advantageous during the process of species reinforcement in the nature using individuals raised by *ex-situ* means. While reinforcing the species into the natural habitats, collar diameter should be used for identifying the vigourous seedlings which would not only save time but also minimize the wastage of the resources (Fay, 1994; Benson *et al.*, 2000; Butola and Badola, 2004b). Such step will definitely reduce the risk of extinction of endangered species (Bowes, 1999) like *S. chirayita* in Himalaya.

**Table 27.** Results of MANOVA showing effect of different seed sources, treatments and their interaction on seedling emergence variables in *Swertia chirayita*.

| Dependent variable | Independent variable | | | | | |
|---|---|---|---|---|---|---|
| | Source (S) | | Treatment (T) | | S x T | |
| | F | Sig. | F | Sig. | F | Sig. |
| Seedling onset time (days) | 6.902 | .000 | 9.581 | .000 | 2.633 | .016 |
| 50% emergence time (days) | 6.784 | .000 | 17.562 | .000 | 3.887 | .001 |
| Final emergence time (days) | 10.176 | .000 | 456.074 | .000 | 8.152 | .000 |
| Seedling emergence (%) | 2.526 | .046 | 689.359 | .000 | 2.622 | .017 |
| Mean days of emergence (days) | 9.231 | .000 | 103.197 | .000 | 9.558 | .000 |
| Emergence rate | 0.954 | .458 | 378.929 | .000 | 1.026 | .442 |

**Table 28.** Index of emergence rate in *Swertia chirayita*, using a modified Timson Index of germination velocity.

| Source | Treatment | | |
|---|---|---|---|
| | Control | $GA_3$ (250µM) | $NaHClO_3$ (5 minutes) |
| Shrubberies | $1.27 \pm 0.35^{a}$ | $10.20 \pm 0.75^{a}$ | $4.51 \pm 0.93^{a}$ |
| Forest slope | $0.20 \pm 0.17^{b}$ | $10.34 \pm 1.38^{a}$ | $4.43 \pm 0.47^{a}$ |
| Open slope | $0.48 \pm 0.13^{bc}$ | $11.33 \pm 1.49^{a}$ | $3.27 \pm 0.96^{a}$ |
| Tree canopy | $0.69 \pm 0.19^{ab}$ | $11.08 \pm 2.53^{a}$ | $4.52 \pm 1.35^{a}$ |
| Net shade | $1.02 \pm 0.13^{ac}$ | $09.51 \pm 2.39^{a}$ | $2.99 \pm 0.69^{a}$ |
| Green house | $0.54 \pm 0.23^{bc}$ | $11.53 \pm 0.47^{a}$ | $4.28 \pm 0.65^{a}$ |

Values in each column with the same superscript are not significantly different at $p>0.05$, Bonferroni test ($\pm$: standard deviation).

**Table 29.** Results of MANOVA showing effect of seed sources, treatments and their interaction on different growth parameters in *Swertia chirayita.*

| Dependent variable | Independent variable | | | | | |
|---|---|---|---|---|---|---|
| | Source (S) | | Treatment (T) | | S x T | |
| | F | Sig. | F | Sig. | F | Sig. |
| Collar diameter | 40.429 | .000 | 99.950 | .000 | 3.839 | .000 |
| Leaf number | 9.842 | .000 | 11.358 | .000 | 0.626 | .787 |
| Largest leaf length | 78.377 | .000 | 95.652 | .000 | 7.250 | .000 |
| Largest leaf width | 24.729 | .000 | 26.920 | .000 | 4.089 | .000 |
| Root diameter | 13.780 | .000 | 12.200 | .000 | 0.662 | .755 |
| Root length | 138.446 | .000 | 182.174 | .000 | 4.929 | .000 |
| Seedling dry biomass | 311.877 | .000 | 262.684 | .000 | 43.899 | .000 |
| Seedling Vigour Index | 328.761 | .000 | 148.523 | .000 | 33.030 | .000 |

**Table 30.** Correlation matrix (Pearson Product-Moment Correlation Coefficient) between different growth parameters in *Swertia chirayita*.

| | Number of leaves | Collar diameter (mm) | Largest leaf length (mm) | Largest leaf width (mm) | Root diameter (mm) | Root length (mm) | Seedling dry biomass (gm) | Seedling Vigour Index |
|---|---|---|---|---|---|---|---|---|
| Number of leaves | 1.000 | | | | | | | |
| Collar diameter (mm) | 0.149$^{ns}$ | 1.000 | | | | | | |
| Largest leaf length (mm) | 0.304** | 0.717** | 1.000 | | | | | |
| Largest leaf width (mm) | 0.113$^{ns}$ | 0.693** | 0.887** | 1.000 | | | | |
| Root diameter (mm) | 0.202$^{ns}$ | 0.694** | 0.709** | 0.654** | 1.000 | | | |
| Root length (mm) | 0.233* | 0.677** | 0.707** | 0.607** | 0.620** | 1.000 | | |
| Seedling dry biomass (gm) | 0.127$^{ns}$ | 0.591** | 0.753** | 0.730** | 0.485** | 0.697** | 1.000 | |
| Seedling Vigour Index | 0.094$^{ns}$ | 0.558** | 0.718** | 0.705** | 0.456** | 0.678** | 0.989** | 1.000 |

** $p<0.01$, * $p<0.05$, ns: non significant

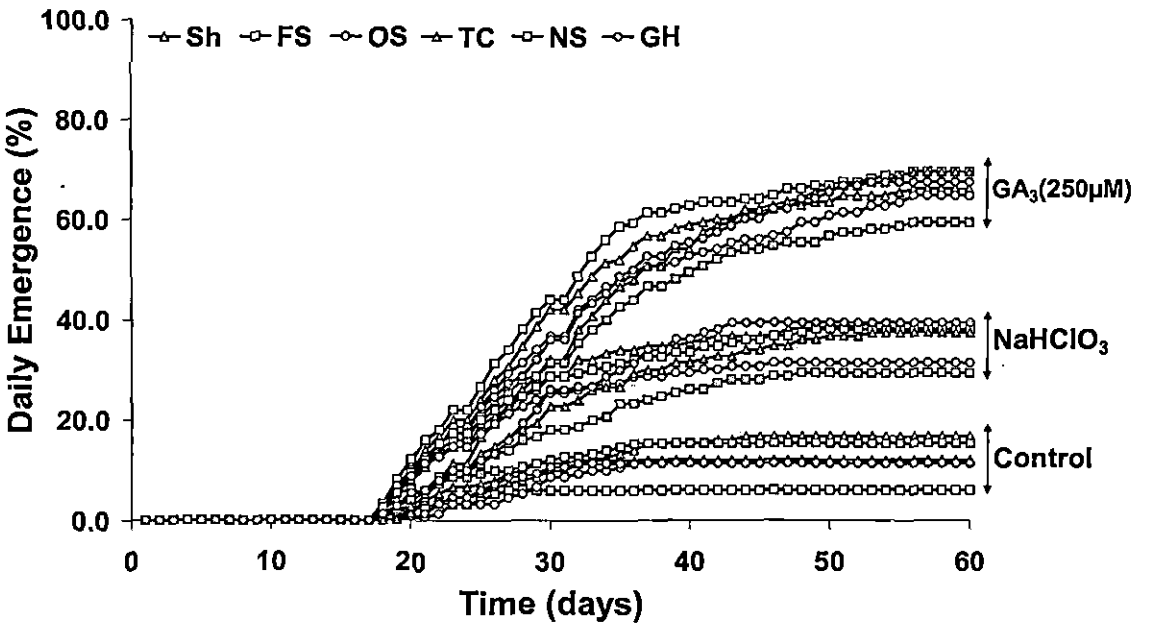



**Figure 38.** Effect of different seed sources and pre-sowing chemical treatments on seedling emergence in *Swertia chirayita*; Sh: shrubberies, FS: forest slope, OS: open slope; TC: tree canopy; NS: net shade; GH: green house

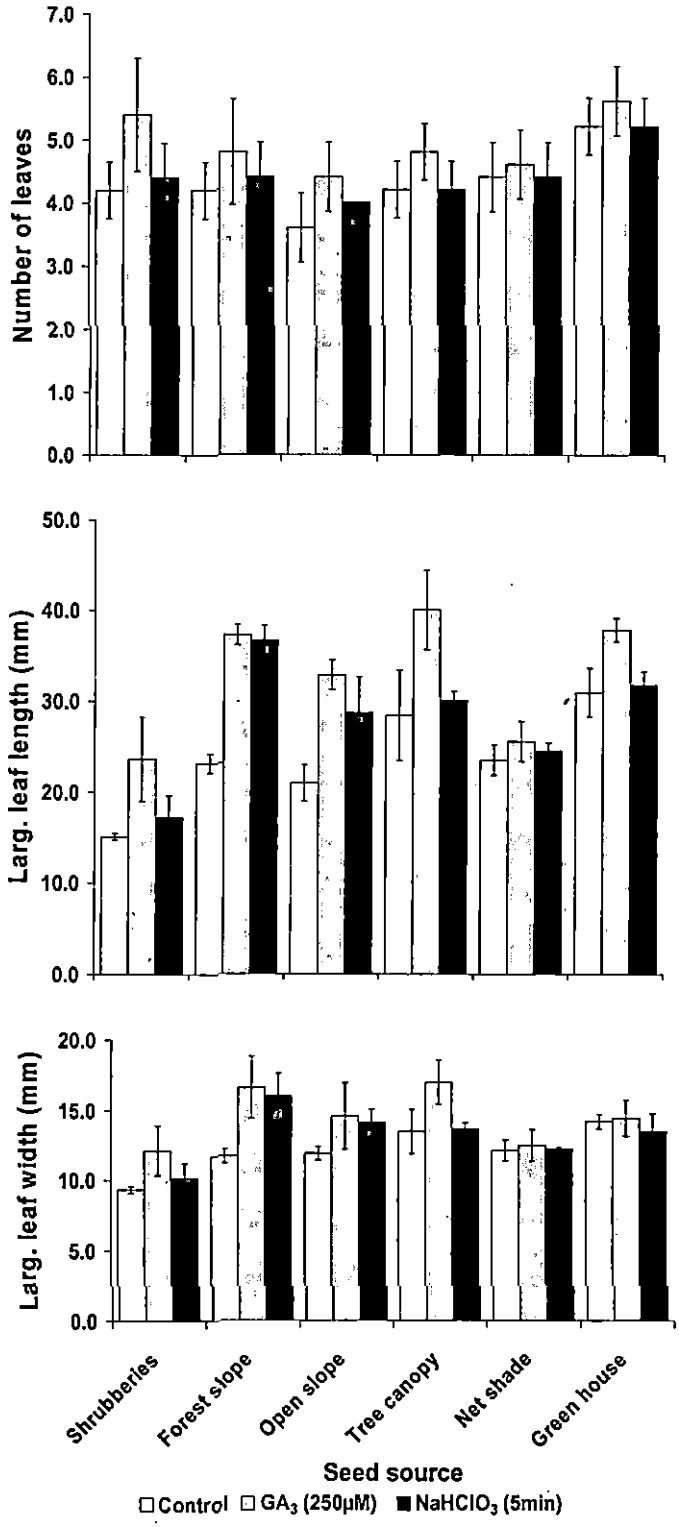



**Figure 39.** Effect of pre sowing chemical treatments on number of leaves, largest leaf length and width in *Swertia chirayita* (error bars: standard deviations)

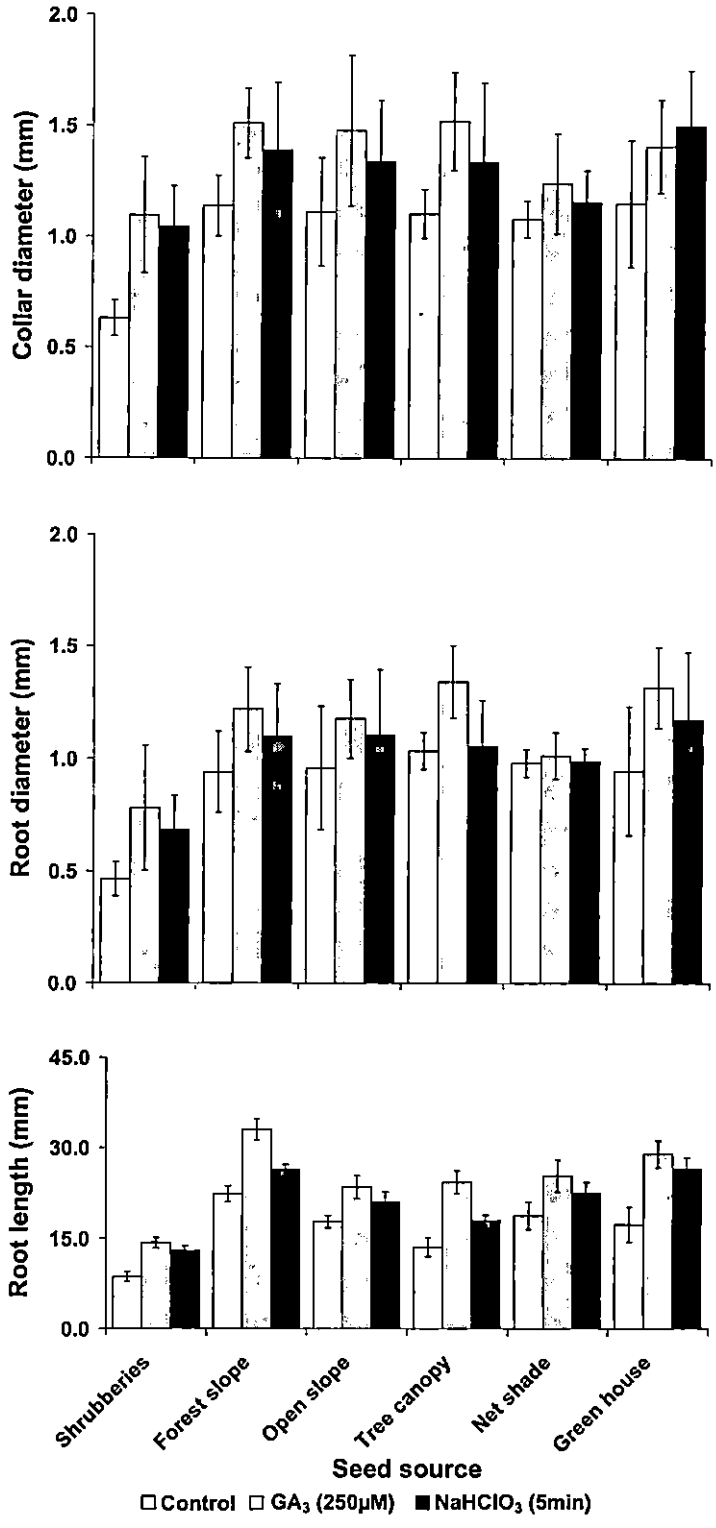



**Figure 40.** Effect of pre sowing chemical treatments on collar diameter, root diameter, root length in *Swertia chirayita* (error bars: standard deviations)

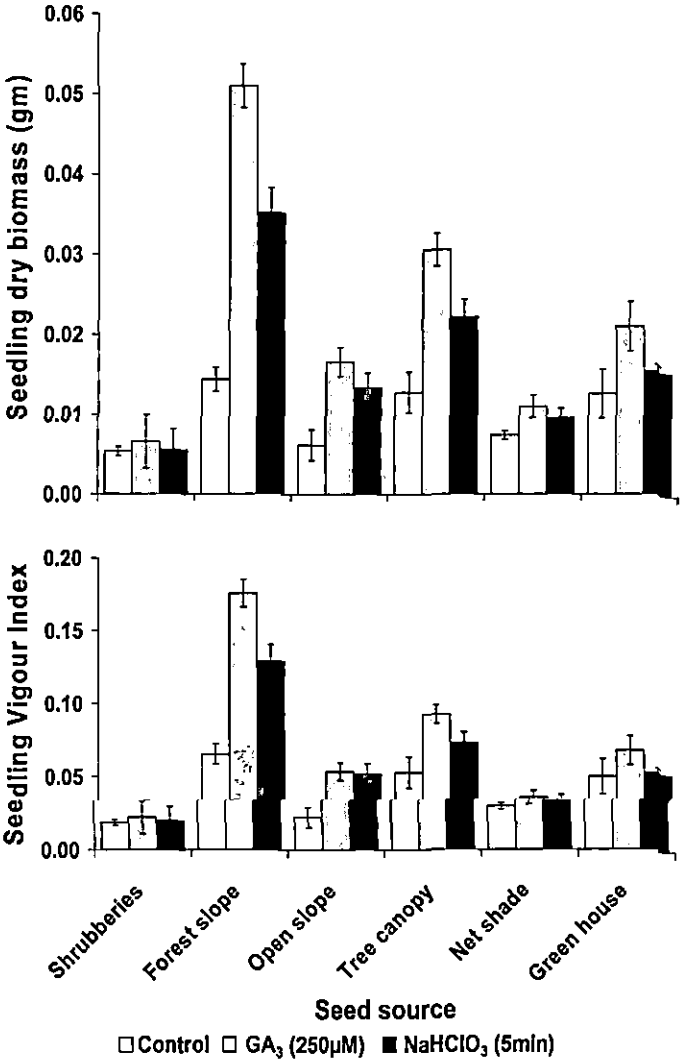



**Figure 41.** Effect of pre sowing chemical treatments on seedling dry biomass and seedling vigour in *Swertia chirayita* (error bars: standard deviations)

CHAPTER 13

# *Ex-situ* Cultivation Trials and Sustainable Harvesting Techniques

## 13.1 Introduction

Over 80% of the global population (6.1 billion) largely depends on the traditional medicine for primary health care. In different medical practices, natural populations of medicinal plants are the major source of required raw materials. Global increase in human population will significantly lead to amplified demand of medicines *vis-à-vis* increasing pressure on the natural populations of medicinal plants, which have been the main source of raw materials to pharmaceutical sectors worldwide (Butola and Badola, 2006c; Sher *et al.*, 2010a) till date. In India, 7800 existing drug manufacturing units consume about 2000 tons of medicinal herbs annually, and about 2000 drugs in use are of plant origin (Dikshit, 1999). In addition, India is the second largest volume exporter of raw herbal drugs with the exports of over 32,000 tons per annum (US$ 27.06 million) after China with an export of over 120,000 tons per annum (US$ 264.5 million) [Wakdikar, 2004].

To overcome the problems of adulteration, incorrect identification, and non-availability of the raw materials throughout the year, the pharmaceuticals units are now looking towards the possibility of raw material supply from cultivated sources because of the expected authentication, reliability and continuous material availability to ensure long term sustainability. As well, the *ex-situ* cultivation is regarded as an appropriate tool for *in-situ* conservation of the endangered species (Badola and Pal, 2002). Cultivated source offers pharmacological advantages and other benefits over wild-collection. For example, (i) the variation in quality and composition leading to uncertainty of the therapeutic benefits due to environmental and genetic differences in wild collected plants are much reduced in cultivated plants; (ii) the production and profits of cultivation are largely controlled by both producers and purchaser, as an advantage; (iii) the yield can be increased in the cultivated plants by using better manure which offer more concentrated financial returns to the cultivators (Badola and

Butola, 2003, 2005; Butola and Badola, 2006c). However, the major constraints hindering the commercialization of cultivation are non-availability of quality planting material, poor development and extension support in the cultivation and processing, and unorganized markets, etc.

*Swertia chirayita* [(Roxb. ex Fleming) H. Karst] or East Indian Balmony (locally called 'Chirowto'; Family: Gentianaceae) is a triennial herb (refer chapter 11); native to temperate Himalaya prevailing between 1200m and 3000m asl, and contains important molecules, namely iridoids, xanthones, mangiferin and C-glucofla-vones (Joshi and Dhawan, 2005). The entire plant is used in traditional medicine (Pradhan and Badola, 2008). Due to extensive habitat loss, non-systematic harvesting practices and excessive collection in wild, etc., during the past few decades, *S chirayita* has been considered critically endangered in the Himalaya and identified as a top-priority species for conservation through cultivation (Badola and Pal, 2002). The problems with *S. chirayita* are the low seed viability, slow growth, long gestation period, etc., which appeared as the major constraints to the successful domestication using conventional technique (Badola and Pal, 2002). Interestingly, the natural populations of *S. chirayita* from Sikkim (eastern Himalaya) were tested to be highly viable (refer chapter 6) compared to those from central and north-western Himalaya (Bhatt *et al.*, 2007); however, the seeds obtained from domesticated sources showed problems in germination (refer chapter 11).

Despite having an established market, both in India and at international level (Badola and Pal, 2002; Joshi and Dhawan, 2005), *S. chirayita* is still collected from the wild and rarely cultivated, and negligible efforts have been made for the development of suitable agro-techniques for its domestication (Joshi and Dhawan, 2005). In natural habitats, *S. chirayit*a plants are uprooted before the seeds get matured, without plant-age consideration, which reduces the chances of regeneration in wild leading to increased danger of its extinction (Bhattarai and Shrestha, 1996). Keeping in view the high pharmaceutical demand of *S. chirayita* and its consequent depletion in the natural environment, the cultivation of the species is mandatory (Badola abd Pal, 2002). Such initiatives will help local entrepreneurs to improve their livelihood

options and get opportunities for self employment and income generation (Badola and Butola, 2003, 2005; Butola and Badola, 2006c). Further, this will help in conservation of the species by reducing the disturbances to its natural populations (Badola and Pal, 2002). In recent years, *ex-situ* cultivation efforts have been reported for other Himalayan species (Badola and Butola, 2003, 2005; Butola and Badola, 2006c) but lacked for *S. chirayita*. In this pursuit, appropriate substrate combinations have been identified to raise quality planting material for cultivation in *S. chirayita* (refer chapter 8). Considering the multifaceted medicinal utility and the high market demand of *S. chirayita*, it is high time to testify its probability of successful cultivation and assess the economic viability of cultivation under different growing conditions. The present study aimed to, (1) evaluate the cultivation and domestication prospects, and (2) assess economic feasibility of cultivation in *S. chirayita*, and (3) to develop simple, reliable and sustainable harvesting techniques for *Swertia chirayita*.

## 13.2 Materials and Methods

Cultivation trials in *Swertia chirayita* were carried out at Pangthang (2000m asl; Lat: $27^0$21'51"N and Long: $088^0$34'10"E), Sikkim, India under three different nursery growing conditions. The area falls under temperate climatic zone with annual maximum temperature reaching upto $20.1^0$C (August) and minimum temperature upto $6.42^0$C (January); average maximum and minimum relative humidity ranging between 98.4% (July) and 70.3% (December) with occasional snowfall. The area receives highest of 4619.56 mm (August) and lowest of 51.04 mm (January) average rainfall. The soil is clay loam type and slightly acidic in nature (pH: 6.43).

In order to provide suitable environmental conditions to raise sufficient planting materials for cultivation, seedlings were developed under green house, using soil, sand and farm yard manure (1:1:1 ratio). In each of the three growing conditions viz., (i) open beds ($Temp_{max}$: $20.1^0$C, $Temp_{min}$: $6.42^0$C; $RH_{max}$: 98.4%, $RH_{min}$: 70.3%), (ii) poly house ($Temp_{max}$: $35.05^0$C, $Temp_{min}$: $17.49^0$C; $RH_{max}$: 68%, $RH_{min}$: 47%), and (iii) temperature controlled green house (Temp: $25^0 \pm 5^0$C; $RH_{max}$: 98%; $RH_{min}$: 87%), three beds of 1m x 1m were prepared by digging soil. The farm yard manure (FYM) was applied at the rate of $1kg/m^2$ in all nine raised beds. Healthy seedlings (8 months

old) of *S. chirayita* were transplanted in each bed in all the three growing conditions at a distance of 25 cm each in rows and the distance between each bed was 60 cm (Photo Plate VI and Photo Plate VII). Watering and weeding was done as per the necessity. By the end of the third growing season, survival and yield (in terms of seeds and plant biomass) were observed under three growing conditions at the time of plant harvesting, and the values then converted on per hectare basis.

Total production of the seeds and dry biomass was estimated on the basis of three replicates (1m x 1m beds) for each of the three nursery growing conditions. Total number of the seedlings was estimated to be approximately 1,60,000 for one hectare of land. For the estimation of projected seed production and yield per hectare, average seed production and yield per plant in each of the three growing conditions were calculated and multiplied with total number of plants in a hectare land. Cost benefit analysis was made on the basis of the total output in the form of cash, and total investment estimated for one hectare land for the site development, seedling preparation, labour charge and manure cost for three years. Net profit analysis was performed on the basis of projected percent survival (100%, 75%, and 50%), as well as for the actual survival and prevailing minimum market price.

To assess the plant morphological parameters, 10 plants (as 10 replicates) were harvested randomly from each of three growing conditions. For each plant harvested, growth parameters viz., plant height and root length were measured using measuring tape; and the collar diameter and root diameter were measured using vernier caliper (Model no.: CD-8"CS, Mituyoto, Japan). After proper removal of the adhered soil particles, the plants were room-dried for 10 days; average total biomass per plant was obtained by weighing 10 plants each (10 replicates) per growing condition. For assessing the seed production per plant in each growing condition, number of seeds per 10 fruit per plant was counted to obtain the average number of seeds per fruit, which was then multiplied with the total number of the fruits recorded per plant. There after, average weight of a single seed was assessed by weighing 100 seeds each in 10 replicates which was then multiplied with the total number of seeds per plant.




**Photo Plate VI.** Cultivation trial of *Swertia chirayita* under different nursery conditions;
A: Development of planting material; B: Plants (3rd year) in temperature control green house; C: Plants (3rd year) in open bed; D: Plants ($2^{nd}$ year) in poly house

Analysis of variance was conducted using SPSS 10.0 software for Windows (SPSS Inc. 1989) and the difference in means amongst growing conditions were determined using Bonferroni test ($p<0.05$). To assess the commercial viability of *S. chirayita* cultivation, total investment [Indian Rupee (Rs); 1 US$ = 44.68 INR; 1 EUR = 62.89 INR], including the expenditures made on land preparation, labour and manure charge and post harvesting costs, was calculated on per hectare basis. Total cost of the seeds (0.500kg @ Rs 2000/kg) to raise planting materials was estimated to be Rs 1000, manure cost Rs 15,000 for three years and miscellaneous expenses Rs 22,500 for three years. Total labour charge for 158, 180 and 257 days in 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ year, respectively, @ Rs 130 per day was estimated to be Rs 77,384. Total input value came to Rs. 1,15,884 in one hectare of land for three years (Table 31). This expenditure includes land preparation, seedling transplantation, irrigation, harvesting, plant processing and packing, etc. Net economic benefit was analysed on the basis of total investment made during cultivation and present market rate (seeds: Rs 2000/kg; plant biomass: Rs 200/kg) for the maximum production for projected survival (100%, 75%, 50%) as well as for actual survival, as, Net benefit = total income (seed + biomass) – total investment.

In the entire calculations, the cost of basic cultivation infrastructures such as automatic green house and large poly house facilities were not included. As, these are the permanent and high cost one time investments, and could not be included for year basis productivity and income estimation; also, these will largely depend upon the economic status and willingness of the investors.

## 13.3 Results

### *13.3.1 Seedling survival and plant growth under three growing conditions*

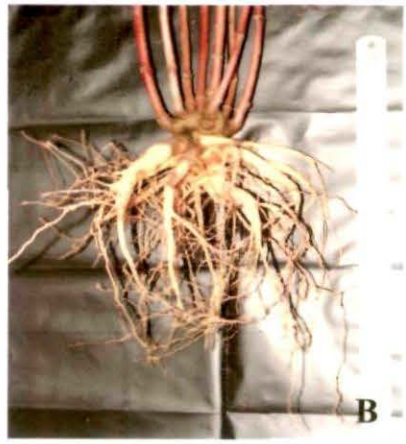
Seedling survival percentage varied significantly ($F$ = 13.36; $p<0.05$) amongst different growing conditions. Maximum seedling survival was observed under green house (92%), followed by poly house (77%) and open beds (63%) [Figure 42]. ANOVA indicated significant ($p<0.0001$) effect of the growing conditions on some plant morphological parameters (Table 32). The plants grown in green house (167.91

± 13.08 cm) were significantly ($p<0.05$) taller compared to other two conditions. Whereas, the plants grown in open beds had significantly ($p<0.05$) higher number of shoots (7.10 ± 0.99) and branches (42.90 ± 8.57) compared to those grown in other conditions (Table 32). Fruit production per plant insignificantly differed for three growing conditions ($F = 0.25$); however, the maximum number of the fruits per plant were produced in open beds (379.50 ± 94.52), followed by green house (360.20 ± 88.89) and poly house (351.10 ± 92.42). Number of seeds per fruit was significantly ($F = 5.97$; $p<0.05$) greater in the poly house (209.50 ± 20.91) and green house (203.60 ± 28.99), compared to the open bed (176.60 ± 16.43). Highest seed weight/100 seeds under the green house (3.04 ± 0.16 gm) grown plants was non-significantly different ($F = 0.93$) from the seeds obtained from the plants grown in open bed (2.96 ± 0.15 gm) and poly house (2.96 ± 0.13 gm). Stem diameter and collar diameter insignificantly differed amongst growing conditions. However, the root length was significantly ($p<0.05$) longer in open beds (Table 32).

### 13.3.2 Account of yield (seed and biomass) under three growing conditions

The seed weight per plant was maximum in green house (2.23 gm/plant) followed by poly house (2.18 gm/plant) and open beds (1.18 gm/plant). However, the maximum plant yield (biomass) in open beds (42.60 gm/plant) was significantly ($F = 4.59$; $p<0.05$) greater than poly house (31.52 gm/plant) grown plants (Figure 42). Maximum projected seed production and biomass on assuming 100% plant survival was estimated to be 3.56 quintal/hectare, 3.49 quintal/hectare and 3.17 quintal/hectare and 60.46 quintal/hectare, 50.43 quintal/hectare and 68.16 quintal/hectare, in green house, poly house and open beds, respectively (Table 33). Table 33 also depicts the total out put in terms of seed production and plant yield (biomass) on the basis of 75% and 50% plant survival. To estimate the actual production of the seeds and plant biomass (after three year growing period), mortality of transplanted seedlings was also accounted. With 92%, 77% and 63% plant survival, in actual, total production of the seeds was estimated to be 3.28 quintal/hectare, 2.68 quintal/hectare and 2.0 quintal/hectare, respectively, and estimated biomass as 55.61 quintal/hectare, 38.83













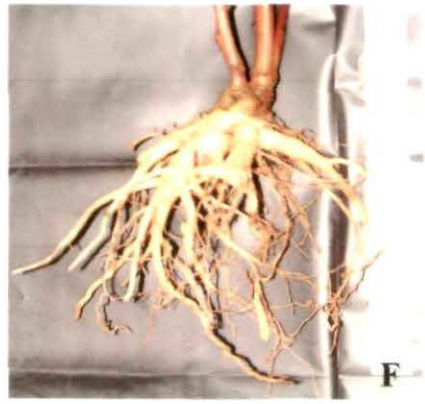



**Photo Plate VII.** Growth and below ground biomass production under different nursery growing conditions in *Swertia chirayita*
A and B: Open bed; C and D: Temperature controlled green house;
E and F: Poly house

quintal/hectare and 42.94 quintal/hectare, respectively, in green house, poly house and open beds (Table 33).

### 13.3.3 Commercial viability of cultivation under three growing conditions

Under different growing conditions, the income estimation differed for the seed and plant biomass based production (Table 34). With projected plant survival of 100%, 75% and 50%, highest income through seeds was calculated for the green house grown plants; while, in terms of plant biomass, the highest income was calculated with open bed grown plants, followed by green house and poly house grown plants for a hectare land (Table 34). The net economic benefit was higher for open beds followed by the green house and poly house on the basis of projected plant survival of 100%, 75% and 50%.

Based on the actual plant survival under different growing conditions i.e., 92% (green house), 77% (poly house) and 63% (open beds), the maximum income from the seeds was estimated from the green house followed by poly house and open beds. However, in terms of plant biomass, the maximum income was estimated for green house, followed by open beds and poly house, for a hectare land (Table 34). Similarly, on actual plant survival basis, the net profit from cultivation of *S. chirayita* was highest for the green house followed by poly house and open beds (Table 34).

## 13.3 Discussion

Appropriate growing media and conditions play a significant role in the vegetative growth (Al-Menaie *et al.*, 2008; Badola and Butola, 2003; Butola and Badola, 2006c) and species prefer distinct growth environments, as in *S. chirayita* (present study), where taller plant height, large stem diameter, as well as, collar diameter were recorded in the green house. Plants attain maximum growth when exposed to the high day temperature due to the increase in photosynthesis rate thereby affecting the entire plant phenology; at the same time, high temperature reduces the growth of tubers/roots (Lafta and Lorenzen, 1995). This has been experienced in the present study, where plants of *S. chirayita* grown under the poly house recorded little higher

plant height compared to the open beds, but had shorter root length and overall low productivity, comparatively. Compared to indoor plants (poly house and green house), the out door plants (open beds) are exposed to harsh, near to natural climatic conditions which affects growth and flowering/fruiting pattern (Al-Menaie *et al.*, 2008), as seen in the present study where plants did have shorter height, but robustness with more number of shoots and branches and fruits *vis-a-vis* longer roots per plant, compared to the other growing conditions. In open beds, the available soil nutrients percolates or gets washed away by the rain water. Therefore, the root elongates in search of required nutrients for better growth resulting in longer root length. However, under the temperature controlled green house, due to the availability of required soil nutrients in the vicinity, shoot rather than root elongates thereby resulting in higher shoot length. The robustness of the plants may be attributed to the nature of this species, as the *S. chirayita* is adapted better to open condition in its natural habitat slopes. Hence, overall, the plant productivity (biomass) on the basis of projected plant survival was higher in the open beds compared to the green house and poly house condition.

In green house, the survival rate and seed production were much higher, compared to the poly house and open beds, because green house experienced very limited fluctuation in temperature (temperature: $25^0C \pm 5^0C$). In addition, temperature is the most important factor in different phenological phases (Badola, 2010) which greatly affects the growth and productivity as recorded in *S. chirayita* under different growing conditions. Susceptibility to the pests and aphid infestation due to prevailing high humidity is a common problem in the indoor plants (Al-Menaie *et al.*, 2008; Badola abd Butola, 2005). Similar aphid infestations have been observed under green house in some of non-experimental *S. chirayita* plants in reproductive stage mostly affecting the tender apical parts, flowers, etc. Relatively, low air circulation in the enclosed green house could be the other reason of such infestation; however, cow urine (15%) appeared as best remedy for such infestation. In poly house, high temperature increases metabolic activities and causes little stress to plants which results in relatively weaker plant development thereby affecting the productivity, as observed in

the present study. Therefore, the open beds are the most suitable conditions for the large scale cultivation of *S. chirayita*.

Maximum projected benefit from 100%, 75%, and 50% plant survival was calculated for the open beds followed by green house and poly house, respectively. On the basis of actual plant survival, net benefit was calculated maximum for the green house (Rs 16,51,988) followed by poly house (Rs 11,97,716) and open beds (Rs 11,42,203), respectively. These outputs may offer encouraging possibilities of domestication (Butola and Badola, 2006bc) of *S. chirayita* compared to the plants collected from wild, where 4.37 quintal/hectare of estimated average biomass (air dried) could be obtained (Anonymous, 2001) with monetary value reaching only Rs 87,401.

In general, all three growing conditions appeared to be suitable for the cultivation of *S. chirayita*; but, the infrastructure development cost would be much higher for the green house and polyhouse, which is almost zero when the cultivation is carried out in open beds. In the present study, seedlings were prepared under temperature controlled green house; however, its cost of construction was excluded from the total investment part, as it being a relatively permanent asset which need not be developed annually. At the same time, seedlings can be prepared annually under the same structure with time to time maintenance. Further, it is not justifiable to quote the construction cost of the green house and polyhouse especially in the mountainous region because the same depends largely on the area of the land to be cultivated, quality of the material used for construction, the location of the land where the structure has to be constructed, economic status of a farmer, etc. Therefore, the present study suggests that, for a hill farmer, open beds with minor precautionary measures will be more viable for large scale cultivation of *S. chirayita*, as lucrative option for the income generation. However, for the large scale industrial entrepreneurship, the green house with control environment could possibly be feasible; it is recommended, they should be technically designed with proper ventilation, etc. In addition, it would be suggestive to maintain reasonable distance (roughly, the double than the open beds) between plants to avoid over crowding, thus allowing ample air circulation, minimizing possibility of pests and aphid infestation, inside the green house. This may perhaps give low benefit than

estimated one in cases. For poor farmers, it is recommended that the seedlings may be prepared under low cost poly houses instead of open beds as the minute seeds of *S. chirayita* can not withstand harsh environmental conditions; however, for the cultivation, seedlings should be transplanted in the open beds, as a follow-up. It can be presumed that the cultivated plants are somewhat different in their properties from those collected from the wild; however, certain values in plants can be deliberately enhanced under controlled conditions of the cultivation (Palevitch, 1991).

The estimated good economic return from *S. chirayita* cultivated at *ca.* 2000m asl in the present study suggests suitability of this elevation for the large scale farming, as, in natural condition in Sikkim, *S. chirayita* is found along 1200m to 3000m asl, so, species can be cultivated within these elevation range, but the total production and net profit may vary with the elevation, which need more cultivation trials. In addition, efforts are also needed to decrease the seedling mortality after transplantation, with further standardization of agro-technology over few cropping cycles over time.

## 13.5 Recommended cultivation techniques

On the basis of present study, the following recommendations are made:

1. Direct seed sowing in the field is not suitable owing to minute seed size which gets easily washed away by the rain water or irrigation water. The planting materials should be developed under low cost green house or poly house with proper ventilation facility.
2. The appropriate soil composition for the seedling preparation should be soil, sand and farm yard manure (1:1:1 ratio).
3. The best time for the seed sowing is April-May and seedling transplantation is March-April after the soil receives the first few early spring showers for open bed cultivation.
4. For cultivation, 10-15 cm raised and deeply ploughed beds (bed size: 1 – 1.5 m x 9-10 m; depending on the land availability) with mild slope (15-20$^0$) and well drainage facilities would be an additional advantage.

5. Minimum distance of 50-60 cm should be maintained between each bed; and the seedlings should be planted at a minimum distance of 25 cm, which helps in gaining enough profit even if the mortality rate is up to 50%.
6. Adequate irrigation is not recommended in *S. chirayita,* as sloppy soil condition results in decaying of roots subsequently resulting in higher mortality rate; however, during winters, irrigation once in a week is necessary.
7. Uses of chemical fertilizer are strongly prohibited; maximum production can be achieved by using farm yard manure, which, is available at low cost for farmers.
8. To prevent plants from the aphid infestation, if any, inside green house (not necessary in the case of open beds), cow urine (15-20%) can be applied from time to time.
9. The appropriate time for the plant harvest is December.
10. The most recommended feasible cultivation condition is open condition.

## 13.6 Sustainable harvesting, drying and storing techniques

On the basis of the present study, following harvesting, drying and storing techniques are recommended:

1. The best time for harvesting of *Swertia chirayita* is December – January (at 2000m asl) when the above ground part fully dries up and the capsules are about to dehisce. Regular monitoring is required in order to determine the exact harvesting period which may vary with the altitude of the region.
2. Seed is the only mode of reproduction in *S. chirayita.* Therefore, seeds should be collected along with the capsules before harvesting of the plants for next year plant development. Segregate the seeds from the capsules by mashing with hand and remove the impurities and dry the seeds at room temperature for at least 10-15 days. After drying, store the seeds in hermetic plastic bags or air tight plastic bottles and keep it in refrigerator at $4^0$C for long term storage. The seeds to be used for plant development in the next season can be stored at room temperature.
3. Harvesting of the plants should be done in the morning or the evening time when the intensity of heat is poor. Harvesting during the day leads to breakage of the plant or its parts due to high intensity of the sunlight.

4. Loosen the soil and remove other weed species before uprooting the plants to avoid breakage of the underground parts. Use small hand fork for digging to prevent the underground part from getting damaged.
5. The rubber gloves should be used while harvesting the plants which will prevent the material from getting moistened by the sweat.
6. Do not use pressure while uprooting the plants, hold at the bottom of the plant and pull it slowly thus reducing the chances of the breakage.
7. The adhered soil should be removed with hard paint brush instead of hitting the harvested material on the ground or cleaning with water.
8. The harvested material should be kept under the shade at least for 15 days for proper drying; however, drying under the poly-house should be avoided because the vapor formed due to heat cannot escape out form the poly-house and turns into water droplet. The drying material will absorb that water when it falls on the ground thereby resulting in decomposition and infestation of the materials and finally degrading the quality.
9. The material should not be dried directly under the sunlight because root part takes more time to dry out. However, high intensity of sunlight results in over drying and breaking of the shoot part which may get blown away by the wind resulting in reduction in the quantity of the material.
10. While drying, the plant material should be spread directly on the ground or on the bamboo mats. The drying material should not be spread on the plastic sheet because formation of water droplet due to evaporation gets accumulated on the sheet maximizing the chances of fungal attack or decomposition of the material.
11. While drying, ample circulation of air should be allowed which helps in faster drying of the trapped moisture.
12. Overturn the material regularly for proper drying and preventing the material from fungal attack.
13. Once the material dries up completely, tie it in bundles and store under the shade with proper ventilation facility till the material is supplied to the vendors or to the wholesalers.
14. While storing the dried material, one should take care of avoiding direct contact of the material with the soil or the ground.

## 13.7 Conclusion

The study clearly indicates that the *ex-situ* cultivation could easily meet out the present market demand of *Swertia chirayita,* if, proper scientific and technical guidelines are followed, which will not only check the extraction of *S. chirayita* from its natural habitats but at the same time, this will help in the conservation of this species *in-situ*. Further, the local entrepreneurs can generate substantial income and empowerment through *ex-situ* cultivation of *S. chirayita*.

**Table 31.** Total investment (in Indian Rupee, Rs*) per hectare on the basis of present market rate for the cultivation of *Swertia chirayita* at Pangthang (*ca.* 2000m asl), Sikkim (India)

| | 1st year | 2nd year | 3rd year | Total |
|---|---|---|---|---|
| Seed material (0.500 kg @ Rs 2000/kg) | 1000.0 | | | 1000.0 |
| Farm Yard Manure (10.0 tonnes/year @ Rs 0.50/kg) | 5000.0 | 5000.0 | 5000.0 | 15000.0 |
| Manpower (158, 180, 257 days @ Rs 130/day) | 20550.0 | 23440.0 | 33394.0 | 77384.0 |
| Miscellaneous expenses (Rs) | 7500.0 | 7500.0 | 7500.0 | 22500.0 |
| Total (Rs) | 34050.0 | 35940.0 | 45894.0 | |
| Grand Total (Rs) | | | | **115884.0** |

*1 US$ = 44.68 Indian Rupee; 1 EUR = 62.89 Indian Rupee

**Table 32.** Result of ANOVA characterized by the effect of growing conditions in *Swertia chirayita* cultivated at Pangthang (*ca.* 2000m asl), Sikkim (India)

| Parameter | Growing condition | | | F | Sig. |
|---|---|---|---|---|---|
| | Open bed | Poly house | Green house | | |
| Plant height (cm) | 142.98[a] | 143.23[a] | 167.91[b] | 18.28 | 0.000 |
| | (6.29) | (11.22) | (13.08) | | |
| Number of shoots | 7.10[a] | 4.20[b] | 4.40[b] | 28.45 | 0.000 |
| | (0.99) | (1.23) | (0.52) | | |
| Number of branches | 42.90[a] | 20.70[b] | 24.40[b] | 37.84 | 0.000 |
| | (8.57) | (5.19) | (3.44) | | |
| Stem diameter (mm) | 14.84[a] | 15.17[a] | 17.38[a] | 0.83 | 0.447 |
| | (5.03) | (2.34) | (6.18) | | |
| Collar diameter (mm) | 15.19[a] | 15.50[a] | 17.82[a] | 0.89 | 0.424 |
| | (4.94) | (2.31) | (6.32) | | |
| Root length (mm) | 103.50[a] | 85.80[b] | 87.20[b] | 17.13 | 0.000 |
| | (11.22) | (4.08) | (5.20) | | |

Values in parentheses indicates standard deviation (SD); values amongst the growing conditions with same letters are not significant (Bonferroni test, $p<0.05$)

**Table 33.** Total production (per hectare) at the end of third year of cultivation of *Swertia chirayita* on the basis of projected and actual plant survival percent, at Pangthang (*ca.* 2000m asl), Sikkim (India)

| Survival (%) | Seed production (quintal/hectare) | | | Total biomass production (quintal/hectare) | | |
|---|---|---|---|---|---|---|
| | Open bed | Poly house | Green house | Open bed | Poly house | Green house |
| 100 | 3.17 | 3.49 | 3.56 | 68.16 | 50.43 | 60.46 |
| 75 | 2.38 | 2.61 | 2.67 | 51.12 | 37.82 | 45.34 |
| 50 | 1.58 | 1.74 | 1.78 | 34.08 | 25.21 | 30.23 |
| **Actual survival (%)** | | | | | | |
| 92 | ---- | ---- | 3.28 | ---- | ---- | 55.61 |
| 77 | ---- | 2.68 | ---- | ---- | 38.83 | ---- |
| 63 | 2.00 | ---- | ---- | 42.94 | ---- | ---- |

**Table 34.** Economic viability (per hectare) at the end of third year of cultivation of *Swertia chirayita* on the basis of projected and actual plant survival percent, at Pangthang (*ca.* 2000m asl), Sikkim (India)

| Total investment/hectare in three years (Rs) | Survival (%) | Total income/hectare from seed [@ Rs 2000/kg] (Rs) | | | Total income/hectare from biomass [@ Rs 200/kg] (Rs) | | | Net profit/hectare (Rs) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Open bed | Poly house | Green house | Open bed | Poly House | Green house | Open bed | Poly house | Green house |
| 115884 | 100 | 633738 | 697423 | 712481 | 1363226 | 1008550 | 1363226 | 1881080 | 1590089 | 1959823 |
| | 75 | 475304 | 523068 | 534361 | 1022419 | 756413 | 906840 | 1381839 | 1163597 | 1325317 |
| | 50 | 316869 | 348712 | 356240 | 681613 | 504275 | 604560 | 882598 | 737103 | 844916 |
| | Actual survival (%) | | | | | | | | | |
| | 92 | ---- | ---- | 655482 | ---- | ---- | 1112390 | ---- | ---- | 1651988 |
| | 77 | ---- | 537016 | ---- | ---- | 776584 | ---- | ---- | 1197716 | ---- |
| | 63 | 399255 | ---- | ---- | 858832 | ---- | ---- | 1142203 | ---- | ---- |

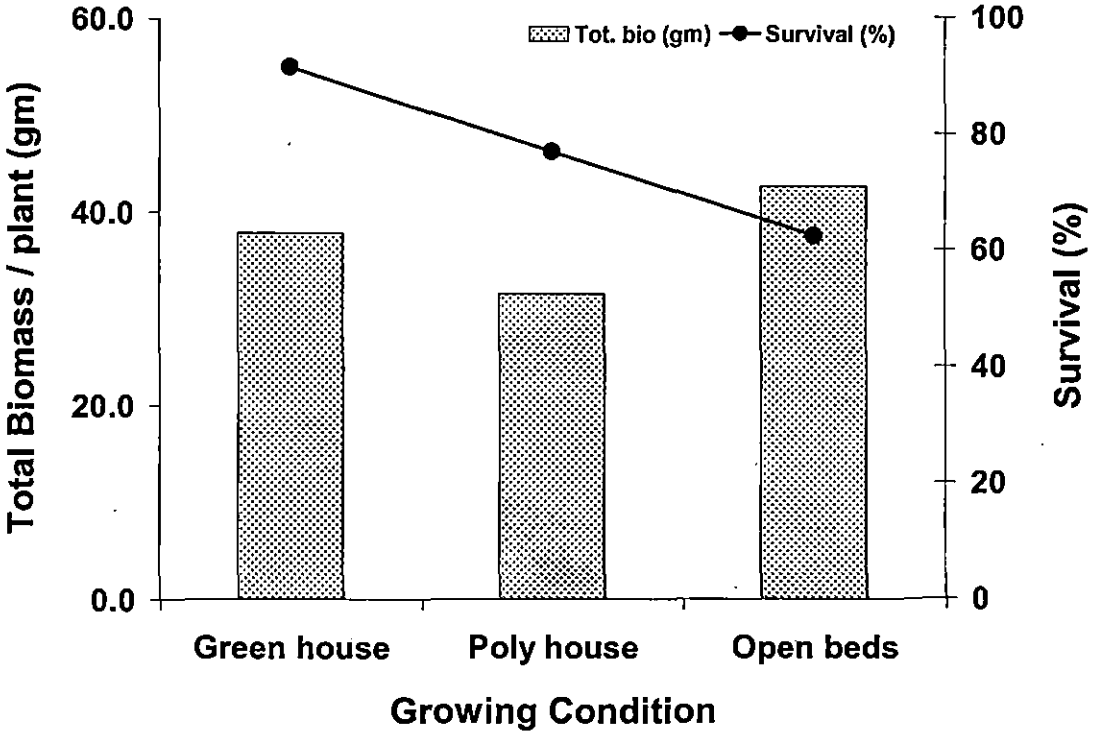



**Figure 42.** Effect of growing conditions on the total biomass/plant and survival in *Swertia chirayita* cultivated at Pangthang (*ca.* 2000m asl), Sikkim (India)

CHAPTER 14

# Conservation Plan for Swertia chirayita

## 14.1 Introduction

There has been global concern about the decreasing populations, loss of genetic diversity, local extinction of the important medicinal plants, etc (Badola and Aitken, 2003, 2010; Canter *et al.*, 2005). There are several reasons associated with the problems viz., unsustainable harvesting, habitat degradation, overgrazing, pathogens, herbivores, etc. In order to conserve the threatened medicinal plants, there is a need for both *in-situ* and *ex-situ* actions, such as, habitat conservation, community participation in framing policies and programmes, information on trade, development of sustainable harvesting techniques, trainings to the harvesters, development and standardization of propagation and cultivation technologies, etc, addressing targeted taxa.

The importance of *Swertia chirayita* is globally acknowledged; however, no efforts have been undertaken to replenish its source due to which the species has become critically endangered (IUCN), endangered (Indian Red Data Book), critically rare and virtually endemic to Himalaya (Samant *et al.*, 1998), and vulnerable to Sikkim (Ved *et al.*, 2003a). At this juncture, if no serious conservation initiatives are taken, the species would become extinct from many parts of Himalayan region. Therefore, on the basis of the present study, following conservation plans have been framed out for *Swertia chirayita*, which should be applicable not only in Sikkim but in the wider areas in Himalaya.

## 14.2 Habitat Conservation

Habitat destruction, degradation and fragmentation are the driving forces behind decline in species and biodiversity. Several anthropogenic activities can have significant negative impacts on the habitats such as clearing of forests for agriculture or township or industries, construction of roads, etc. Apart from these, the natural

disturbances such as landslides, forest fires, etc., also cause habitat degradation or fragmentation. Similar problem of habitat destruction or modification have been observed threatening the existence of *Swertia chirayita* in Sikkim. Therefore, the first step in the species conservation is the conservation management of its habitat, taking micro-habitat niches as important clues. The availability of *S. chirayita* near to the human habitation or in areas where human interference existed or in open areas created due to other reasons indicates adaptability of species to minor disturbances and its habitat preferences. It was further noticed that due to the absence of human intervention in the forest areas in Sikkim, several shrub species have taken over the forest gaps and open spaces or abandoned areas resulting habitat modifications and the fragmentary distribution of the species. Therefore, the modification in government policy is required thus allowing minor and controlled human intervention under constant monitoring in the forest areas. This will help check the encroaching shrub and other unpalatable and or invasive species causing threat to the existence of *S. chirayita*. Local communities can be mobilized and imparted with responsibility to conserve the habitats of *S. chirayita* in their surroundings. They are directly associated with the nature, so their traditional knowledge and skills can be used for the preservation of the habitats.

## 14.3 Conservation through legalization of the wild harvesting

*S. chirayita* has three year life cycle. After dispersal of the seeds in the nature in third year, the plant material can be collected instead of wasting the resources, which can be an important source of income for the rural people of the mountains. Therefore, collection of *S. chirayita* can be legalized through licensing to specific number of people under defined rules and constant monitoring by the area guards of the related department, which will also help in preserving its habitats through human movement at the same time; however, only the mature plants after seed shedding should be allowed to collect which can commence from December end. Depending upon the projection of seed setting, the area managers of the government should declare the starting dates for collection and ensure that the plant materials are not collected before

the seed dispersal. Further, the late collection after January should not be allowed in order to avoid damage to the new seedlings.

Strict regulations should be framed out where it should be clearly mentioned regarding the punishment such as seizure of the license, fines, etc., if the harvester goes out of their way. Additionally, the guidelines prohibiting the direct sale or purchase of the plant material should be framed. To avoid such actions, there have to be a dumping centre for the collected material from where the companies can directly purchase the material under the guidance of concern land department. This will keep check on the collection of the immature plants as well as the middle man. The harvesters should provide information on volume of plant material they collected. This will help in designing proper management plans if the harvesting records reveal decline in volume of collected plant material. Further, the sustainable and careful harvesting method should be standardized as well as the harvesters should be trained, to prevent damage to the immature plants and change to the microclimate of the area. Conservation agencies should undertake occasional surveys in the areas where the plants are being planned for harvested or during harvesting. Local communities should be involved in designing and planning of the harvesting techniques under scientific guidance from local research organizations. In each of the village or communities, stakeholder such as village panchayat or NGO should be involved in training the harvesters; their involvement enhances people's participation.

## 14.4 Conservation through re-introduction

Several factors, such as, washing away of seeds or percolation of seeds to deep under ground due to heavy rain, decay of seeds due to fungal attack, seed predation, etc., affects seedling emergence resulting in low availability of *S. chirayita* in its natural habitats. In addition, untimely harvesting also leads to low availability of *S. chirayita* in its natural habitats. In any of the situation, the seedlings can be developed in the nursery, near to the population site, from the seeds collected from the nature (from the similar populations), which can be transplanted back to the nature in areas experiencing diminishing populations; however, population has to be considered while transplanting, for preservation of its genetic diversity and genetic erosion. Only

trained personal and qualified staffs should be permitted to collect seeds for propagation purpose; the scientific guidance and advisories of related organizations/individuals would be beneficial.

## 14.5 Conservation through *ex-situ* cultivation

*Ex-situ* cultivation of targeted high value medicinal plants is the only means by which the increasing demand of the pharmaceuticals can be meet out and the motives of *in-situ* conservation of the species can be achieved. Further, the local entrepreneurs can generate substantial income and empowerment through *ex-situ* cultivation of *S. chirayita*. Local entrepreneurs can gain 10-15 times more profit from cultivation compared to collection from the wild if proper scientific and technical guidelines are followed, as developed in the present study. There are many other advantages of cultivation over wild harvesting. Nevertheless, economic feasibility is one of the main issues for deciding to undertake cultivation of the medicinal plants (Badola and Pal, 2002; Schippmann *et al.*, 2002, Badola and Butola, 2005; Badola, 2009). In this context, it is essential to generate awareness amongst the stakeholders, basically farmers, or the entrepreneurs from time to time regarding the market potential and market related issues of *S. chirayita* to increase their involvement in its cultivation. In addition, the government should assure market and better price for their quality material, and assistance in technical and infrastructural development, provision for the purchase of their materials, etc.

## 14.6 *Circa-situ* conservation

*Circa-situ* (Hawkes *et al.*, 2001) conservation falls between *in-situ* and *ex-situ* conservation mechanisms. This includes the deliberate encouragement and the retention of *S. chirayita* in and around the agricultural field or the home gardens during the land clearing or weed removal process for agricultural purpose or the gardening purposes. Such type of conservation practices can also be encouraged along or near to the road side, where *S. chirayita* was found to be growing well in the present study, during road cleaning or clearing process.

## 14.7 Conservation through seed bank development

Seed banks are the best form of *ex-situ* conservation of plants. However, at present, it is considered only for the agricultural crops. The important medicinal plants like *S. chirayita* can be stored in seed banks for re-introducing the species back to the natural habitats in future when the species faces extinction risk or becomes endangered. Conventional storage of *S. chirayita* at $4^0$C has been proved beneficial at least for over 24 months under present study; this should be taken as a clue to strengthen big gene bank programmes in the state. However, the preservation of the species in the seed bank and re-introduction of the species to its natural habitat is of very limited use unless the factors that cause the threat of endangerment or extinction are eliminated largely.

## 14.8 Conservation through communication and co-operation

Public support is vital in the conservation of the threatened medicinal plants. Prior to the initiation or implementation of the conservation efforts, confidence building between the government agencies and local stakeholders is a must, which can be done by establishing a communication strategy and through information and education programmes. Also, the community participation is an important ingredient in *in-situ* conservation of the threatened medicinal plants because it is not easier for the government officials or the associated agencies alone to keep track on the compliance of the rules and regulations by the harvesters.

The present study in totality offers essential clues and guidelines, both scientific and practical to ground level, for effective and feasible scientific based conservation and management efforts of the targeted species, *Swertia chirayita*, in particular and broadly to other threatened plant species in Himalaya and other mountainous regions of the world.

# Summary

1. *Swertia chirayita* (Roxb. ex Fleming) H. Karst (family Gentianaceae) is a high value critically endangered and internationally prioritized medicinal herb for immediate conservation in Himalaya. Presnet study explored and assessed different populations of *S. chirayita* to identify potential gene stock for their variability amongst them and for habitats, and community associations, and to strengthen both *in-situ* and *ex-situ* conservation mechanisms in Sikkim using conventional approaches. Further, the study sets up a strong ground offering practically feasible recommendations and provides possible guidelines for an effective conservation and management plan for the species.

2. Quantum availability, community association and threat status of *S. chirayita* were investigated by exploring 22 natural populations in Sikkim Himalaya, India. Eleven major communities for *S. chirayita* were identified; however in 5 populations, *S. chirayita* formed pure community with smaller share of other associates. *S. chirayita* density ranged between 1.63 $\pm$ 0.69 ind/$m^2$ and 21.67 $\pm$ 8.62 ind/$m^2$, whereas, the relative density emerged high (8.27% to 78.38%) compared to overall population density in majority of the cases.

3. Of total, 13 populations showed 100% frequency of occurrence. On the basis of abundance to frequency ratio, *S. chirayita* exhibited contagious/clustered distribution in majority of the populations except two, which had random distribution. Study identified 14 microhabitats for *S. chirayita*, suggesting greater species pliability towards different microhabitat niches. The open grassy slope appeared as the most favourable microhabitat for *S. chirayita*, and marshy grassy slope as least suitable.

4. One way multivariate analysis of variance (MANOVA) revealed a significant ($p<0.0001$) variation in the recorded morphological characters including total plant dry biomass amongst populations in both rosette and reproductive forms in *S. chirayita*. In rosette form, of the six morphological character recorded, most

variations were observed in the root length with coefficient of variation ranging from 14.23% to 69.58% followed by root diameter (4.66% to 48.06%). In reproductive form, of the nine morphological characters identified, the number of branches recorded the highest coefficient of variation value ranging from 0% to 84.16% followed by number of leaves (8.59% to 83.65%) and root diameter (4.78% to 55.79%).

5. The result of canonical discriminant analysis (CDA) indicated leaf length; root length and collar diameter as the main discriminating characters and responsible for the total variation amongst the populations in rosette form of *S. chirayita*; whereas the plant height, number of leaves, leaf width and stem basal diameter were mainly responsible for the total variations amongst the populations in reproductive form, cumulatively accounting to 83.6% variation.

6. The germinability in seeds of *S. chirayita* collected from six microhabitats was determined at constant temperatures of $20^0$C, $25^0$C and $30^0$C under both 14/10 hours light/dark photoperiod and continuous darkness. Germination was negligible in continuous darkness under all the three temperature regimes. Two way ANOVA indicated significant effects of microhabitats and temperatures on percent seed germination, germination rate, germination recovery percent and germination recovery rate.

7. In general, seed sourced from under canopy micro-habitats showed significantly ($p<0.05$) higher germination over open habitats when directly incubated in 14/10 hours light/dark photoperiod at $20^0$C, $25^0$C and $30^0$C. When transferred after incubating for 45 days in completely dark to 14/10 hrs light/dark photoperiod, seeds from under canopy recorded significantly ($p<0.05$) highest seed germination recovery at $20^0$C and $25^0$C than other microhabitats (tree base, stump base, shrubberies, grassy slope) except boulder where the value was non-significant in both the temperatures. Seed germination percent, seed germination recovery percent, mean germination rate and germination recovery rate was significantly

($p<0.05$) greater at $25^0$C than $20^0$C and $30^0$C comparatively. . Amongst microhabitats, the variation in germination recovery rate was significant ($p<0.05$).

8. Multivariate ANOVA revealed significant variation in mean germination percentage and mean germination time in *S. chirayita* amongst populations, increasing storage period and their interaction. During initial testing, 100% seed germination was recorded in majority of the populations. The duration of storage had significant effect on seed germination; nevertheless, there was not much variation in seed germination percentage between initial and the 6 months storage in populations. The highest mean germination time (MGT) was recorded during initial test ($p<0.0001$) which ranged from 15.17 days (Sc3) to 59 days (Sc10) and six month storage recorded significantly ($p<0.0001$) lowest mean germination time.

9. The effect of two substrate combination (substrate 1: garden soil, sand, forest humus; substrate 2: garden soil, sand, farm yard manure; 1:1:1) and control (sand and garden soil; 3:1) on seedling emergence, growth, biomass and vigour were tested with the seeds from 13 wild populations in Sikkim. Seedling emergence was significantly higher in substrate 2 ($p<0.05$) over others. Substrate 2 significantly ($p<0.05$) improved all the seedling growth parameters except number of leaves. Of 13 populations, seven (Sc1, 2, 3, 8, 9, 11, and 13) exhibited higher seedling biomass and SVI using substrate 2.

10. Effect of different storage conditions (room temperature, $4^0$C, and $-15^0$C) and different storage periods over 24 months on seed germination in *Swertia chirayita* collected from different altitudes was determined. Multivariate ANOVA revealed significant ($p<0.0001$) effect of storage condition and storage period on seed germination and mean germination time. Seed germination percentage significantly ($p<0.01$) varied between 87.78% (Sc5) to 100% (Sc2) during initial testing. Comparatively, high seed germination, low mean germination time and low rate of fall in seed germination percentage in seeds stored at $4^0$C over different storage period were recorded.

11. Periodical phenology, growth and development in *Swertia chirayita*, an endangered high value medicinal Himalayan herb, were observed for 22 months under six growing conditions, viz., four natural (shrubberies, forest slope, open slope, tree canopy) and two nursery (temperature controlled green house and open net-shade), following the transplantation of green house produced eight month old seedlings. The green house helped in early commencement of floral bud initiation, bud sprouting, and flowering, whereas, the senescence begun quite late, comparatively. Robust plants were produced under green house and open net-shade. Study recorded the single shoot development in plants grown under all natural habitats. However, in open net-shade and green house, plants produced multiple shoots, resulting in significantly ($p<0.001$) higher total plant biomass. Under open net-shade the plant height significantly correlated with the temperature ($r = 0.703$; $p<0.05$).

12. The seeds procured from 6 *ex-situ* set ups, shrubberies, forest-slope, open-slope, tree-canopy; and green-house and net-shade, showing poor germination at initial testing (12% to 31%) were subjected to 11 pre-sowing chemical treatments. Among the pre-sowing treatments, gibberellic acid (50 to 350 µM) most effectively stimulated seed germination (96.7%, maximum; $p<0.001$) and reduced mean germination time ($p<0.05$), followed by potassium nitrate (100mM) and sodium hypochlorite (5 minutes) ($p<0.001$). Study confirms *ex-situ* produced seeds attained physiological dormancy, which was broken by pre-sowing treatments, as a tool to *ex-situ* species conservation.

13. The effects of identified pre-sowing chemical treatments on seedling emergence and vigour using seeds from *ex-situ* raised plants under natural conditions (4 sources) and nursery conditions (2 sources) were tested. Seedling emergence was promoted from 6% (control) to 69% ($GA_3$ 250µM). Seedling emergence rate was faster in $GA_3$ (250µM) followed by $NaHClO_3$ (5minutes) in all the seed sources. MANOVA indicated significant effect ($p<0.0001$) of treatments on all seedling emergence variables; seed sources and treatments had significant ($p<0.0001$) effect on different seedling growth parameters. Collar diameter appeared as the

strongest morphological trait, which can be used to identify vigorous seedlings for plantation.

14. Seedlings, raised under green house, were transplanted under three growing conditions, viz. temperature controlled green house, poly house and open beds, using soil, sand and farm yard manure (1:1:1). Highest plant survival (92%; $p<0.05$) was recorded for the green house. Maximum plant biomass per plant (42.60 gm/plant) was recorded in open bed, over green house (37.79 gm/plant) and poly house (31.52 gm/plant; $p<0.05$). Overall, the high productivity (seeds and plant biomass) and greater profit (economic) was gained in open beds for the projected 100%, 75% and 50% survival; but on the basis of actual survival (green house: 92%; poly house: 77%; open beds: 63%), net economic benefit was higher under green house. Compare to expensive infrastructure requirement for green house and poly house, the study suggests that open beds, with due care maintaining better survival, are much feasible and profitable condition for large scale cultivation of *S. chirayita*, which may offer good economic returns to the cultivators, especially poor farmers.

15. The conservation requires both, *in-situ* and *ex-situ* actions, such as habitat conservation, community participation in framing policies and programmes, information on trade, development of sustainable harvesting techniques, trainings to the harvesters, development and standardization of propagation and cultivation technologies. The present study in totality offers essential clues and guidelines, both scientific and practical to ground level, for effective and feasible scientific based conservation and management efforts of the targeted species, *Swertia chirayita*, in particular and broadly to other threatened plant species in Himalaya and other mountainous regions of the world.

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# Seed Germination Response of Populations of *Swertia chirayita* [(Roxb. ex Fleming) H. Karst] Following Periodical Storage

Bharat K. Pradhan and Hemant K. Badola*

## ABSTRACT

*Swertia chirayita*, a critically endangered and high value Himalayan medicinal herb was investigated to understand response of different populations on initial seed germination, and germination after storage at 4 °C for 18 months. Populations differed significantly for seed characteristics, indicating considerable genetic diversity. Of 13 populations tested, eight exhibited 100% germination immediately after collection, which was negatively correlated with altitude of the sites where plants were growing. The results suggested an acceptable range of 16 to 42% seed moisture content, before storage, as suitable criteria for *S. chirayita* populations in long term gene resource conservation. Seed from 10 plant populations showed a steady decline in germination over the storage period. Varied germination responses offer a possibility of selecting elite populations as a seed resource in *S. chirayita*. The Mean Germination Time (MGT) significantly declined after six months, but increased after 12 and 18 months of seed storage for the majority of plant populations. The best germination and lowest MGT occurred for seed of *S. chirayita* that was sown six months after collection, followed by slightly lower levels at 12 months. Several populations tested offer promise for the conservation of this species as potential gene bank in Sikkim Himalaya.

## EXPERIMENTAL TECHNIQUES

Understanding the response of different plant populations on seed germination provides helpful clues on the genetic make-up of the species and its existence in the natural settings (Baskin and Baskin, 2001), which is essential to select elite seeds (Jayshanker et al., 1999) for *ex-situ* conservation of gene resources. Seed storage, and frequent testing to monitor losses in germination rate, which might adversely affect nursery recovery rates, is considered as one of the most efficient methods (Gonzalez-Benito et al., 1998; Williams et al., 2007) to select elite populations. However, poor seed germination has been shown to be one of the limiting factors (Butola and Badola, 2004a; Badola and Butola, 2005; Butola and Badola, 2006). Due to inappropriate storage, germination capacity of a species may decline during the first few months after collection (Romanas, 1991); whereas, proper storage may be effective over a considerable storage period (Butola and Badola, 2004a, 2004b; Chen et al., 2007).

Little information has been reported for *Swertia chirayita* (Roxb. ex Fleming) H. Karst, a critically endangered species regarding micro-propagation (Wawrosch

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et al., 1999; Joshi and Dhavan, 2007) in the absence of successful domestication (Joshi and Dhawan, 2005). The current literature lacks any populations' based assessment of seed germination, especially related to storage in *S. chirayita*. The species has very high pharmaceutical value in national and international markets and is prioritized at the top for ex-situ cultivation in an international experts' report (Badola and Pal, 2002). Since the species regenerates only through seeds, understanding the response of different populations on seed germination behaviour, especially seed tolerance to periodical storage would be crucial for ex-situ conservation. Low density of this species in nature (Bhatt et al., 2006) further necessitates such an investigation.

*Swertia chirayita* (Gentianaceae) grows mostly in temperate Himalaya (1200–3000 m asl) from Afghanistan to Bhutan (Kirtikar and Basu, 1984), and has been used ethno-medicinally for centuries (Wawrosch et al., 2005). The plant has numerous medicinal properties (Joshi and Dhawan, 2005), and is used as a liver stimulant and in bronchial asthma, dyspepsia, debility, fever, gastro-intestinal infections, curing various skin problems and scorpion bite (Biswas and Chopra, 1982; Kirtikar and Basu, 1984; Nadkarni, 1976). The objectives of this study were to investigate *S. chirayita* for, (i) variability among plant populations for their seed characteristics and germination response, (ii) seed germination responses following storage of various plant populations and individually for each population in an attempt to identify elite seed resources.

Matured seeds of *S. chirayita* were collected from 13 populations (Table 1) of Sikkim Himalaya (27°04′46″ and 28°07′48″ N lat. and 88°00′58″ and 88°55′25″ E. long.) during November–December 2005 and dried for 10 d at room temperature. To determine seed moisture content, 50 seeds/replicate (three replicates/population) for each population were oven dried (60 °C; 48 h). Seeds were counted using 10 healthy fruits per population. Seed size was measured using 30 seeds per population under microscope (10 seeds each for three replicates). Because of the minute seed size and the difficulty in finding the detached embryo, the viability test using 2,3,5, tri-phenyl tetrazolium chloride solution could not be conducted. After drying, seeds were tested for initial germination and the remaining seeds in each population were stored in sealed specimen tubes at 4 °C, for periodical re-testing at six month intervals for 18 months. For each germination test, 30 seeds/replicate (three replicates/population) were disinfected (5 s) with sodium hypochlorite solution (4% w/v available Chlorine), washed thoroughly with double distilled water (DW), and separately soaked in DW for 24 h, before placing in Petri-plates (90 mm dia.) lined with single filter paper (Whattman No. 1). The Petri-plates were placed in an incubator at 25 °C (± 2 °C); with alternating light (14/10 h light/dark periods) for a maximum period of 90 d. Three replicates/population were tested in a randomized design. The filter papers were moistened daily using DW. Seeds were counted daily and were considered germinated when radicles had emerged. Germination was complete when a constant reading was obtained. Mean germination time (MGT) was calculated using equation, $MGT = \Sigma(nd)/\Sigma N$; where n = number of newly germinated seedlings after each incubation period in days d, and N = total number of seeds emerged at the end of the test (Hartmann and

**TABLE 1. General features and seed characteristics of different plant populations of *Swertia chirayita* collected in Sikkim**

| Name of Population | Altitude | No. seeds/ fruit | Seed weight | Seed length | Seed width | Moisture content | Habitat |
|---|---|---|---|---|---|---|---|
| | (m asl) | | (mg/50 seeds) | (μm) | (μm) | (%) | |
| P1 – Luing (ES) | 2126 | 212 | 1.6 | 487 | 356 | 23.5 | Ridge, moist grassy slope with *Cryptomeria japonica* and *Castanopsis tribuloides* |
| P2 – Railgaon (ES) | 1948 | 302 | 1.5 | 535 | 382 | 20.5 | Shrubberies with *C. japonica* and *C. tribuloides* |
| P3 – U Pangthang (ES) | 2176 | 316 | 1.6 | 503 | 381 | 27.1 | Shrubberies, moist grassy slope with *Quercus lamellosa* and *C. tribuloides* |
| P4 – Guransey dara (ES) | 2107 | 230 | 1.1 | 457 | 356 | 24.4 | Moist mixed Forest with *C. japonica* and *Rhododendron* sp. |
| P5 – Jaunbari (SS) | 1651 | 221 | 1.3 | 452 | 377 | 35.2 | Moist grassy slope with *Alnus nepaulensis* and *Michelia lanuigilosa* |
| P6 – U Chamgaon (SS) | 1583 | 233 | 1.3 | 432 | 343 | 20.6 | Dry grassy slope with *A. nepaulensis* and *Schima wallichi* |
| P7 – Tiffin dara (SS) | 1667 | 227 | 1.2 | 436 | 336 | 16.2 | Moist grassy slope with *C. japonica* and *A. nepaulensis* |
| P8 – U Changrang (ES) | 2055 | 275 | 1.7 | 497 | 371 | 17.3 | Moist grassy slope, shrubberies with *Machilus* sp. and *C. tribuloides* |
| P9 – Phinsyonala (NS) | 1761 | 233 | 1.2 | 446 | 347 | 38.2 | Marshy grassy slope with *A. nepaulensis* |
| P10 – Zemathang-1 (NS) | 2841 | 249 | 1.3 | 462 | 333 | 42.6 | Rocky steep slope with *Selenium* sp and *Artemesis* sp. |
| P11 – L Pangthang (ES) | 2000 | 210 | 1.3 | 468 | 248 | 28.2 | Grassy slope with *Symingtonia populnea* |
| P12 – Kali Pokhari (ES) | 2450 | 284 | 1.5 | 444 | 238 | 17.7 | Shrubberies with *Osbeckia* sp. |
| P13 – Khecheopalri (WS) | 1867 | 200 | 1.4 | 429 | 221 | 25.5 | Mixed temperate forest with *Castanopsis tribuloides, Quercus lamellosa, Q. glauca, Michelia excelsa.* |
| (P < 0.05) | | 75.85 | 0.17 | 27.20 | 19.60 | 7.13 | |
| F | | 6.24 | 10.50 | 11.19 | 19.60 | 11.02 | |

†ES: East Sikkim; WS: West Sikkim; SS: South Sikkim; NS: North Sikkim

Kester, 1989). Analysis of variance was conducted on different parameters and least significant difference was used to determine differences among treatments (Snedecor and Cochran, 1968).

## RESULTS AND DISCUSSION

A variety of habitats support populations of *Swertia chirayita*, which differed significantly for various seed characteristics (Table 1), indicating species genetic diversity. Seed moisture content showed a weak negative correlation with altitude of the populations sites ($r = -0.509$). Across plant populations, mean seed germination in all samples was significantly higher ($p < 0.05$) than P-10 in the initial test and after six months' storage time (Table 2). Eight populations showed 100% germination when tested initially, which indicated an availability of potential gene bank of *S. chirayita* in Sikkim Himalaya. Germination was negatively correlated with altitude ($r = -0.700$; $P < 0.02$), but remained non-significant in post-storage testing. Seed germination variability at various altitudes has previously been reported (Baskin and Baskin, 2001). After 12 months of storage, seed germination in P9 was 94%, which was the maximum level and significantly ($p < 0.05$) higher, than P2 (the lowest level, 52%) and several other populations. After 18 months, germination was at maximum levels (79%) for P9, followed by P1 (74%) and both were significantly higher than all other populations. Five populations remained above 50% germination even after 18 months of storage, which is promising for conservation of *S. chirayita*. However, a steady decline in germination from the initial to final test in 10 populations indicates that the seeds are destined to deteriorate over time, even under appropriate conventional storage. It has previously been reported that a desirable range in seed moisture for seed longevity in storage is 10 to 40% (Hampton and Hill, 2002). The seeds collected in this study ranged from 16 to 42 % seed moisture which generally fit into that range. In many cases, seed storage at 4 °C responds effectively after six (Butola and Badola, 2004a, 2004b) to 12 months (Chen et al., 2007) storage. In *S. chirayita*, storage at 4 °C proved quite effective up to 12 months, suggesting this as a valuable method for propagation to retain high seed longevity and provide an environment for minimum physiological and pathological deterioration (Williams, 2000). This is important for scheduling spring/summer sowing in field, an appropriate time for many Himalayan herbs (Badola and Singh, 2003; Badola and Butola, 2003, 2005). The MGT significantly differed among populations (Table 2). At the initial test, MGT ranged from 14 d (P11) to 59 d (P10). After six months MGT declined in all populations, indicating that the seeds need some after ripening period before actual germination starts in a natural environment, in summer (personal observations, unpublished), for survival, overcoming winter frost and chilling. However, MGT significantly increased in third and fourth test during storage (Table 2).

The present study confirms that the populations of a species differ in their germination responses (Perez-Garcia et al., 1995), which may offer valuable clues for selection of elite populations for seeds (Jayshanker et al., 1999). A majority of present populations of *S. chirayita* showed high seed germination, in general,

TABLE 2. Seed germination and mean germination time for various plant populations of *Swertia chirayita* following storage periods at 4 °C

| Name of Population | Mean Germination (%)t | | | | | | Mean Germination Time (d) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | 6 month | 12 month | 18 month | P (<0.05) | F | Initial | 6 month | 12 month | 18 month | P (<0.05) | F |
| P1 – Luing (ES) | 100 | 91 | 86 | 74 | 5.18 | 41.18 | 16 | 9 | 14 | 18 | 2.30 | 32.35 |
| P2 – Railgaon (ES) | 100 | 89 | 52 | 39 | 8.98 | 101.62 | 17 | 11 | 15 | 19 | 1.37 | 55.93 |
| P3 – Upper Pangthang (ES) | 100 | 94 | 61 | 52 | 6.69 | 122.37 | 15 | 10 | 15 | 19 | 3.99 | 8.66 |
| P4 – Guransey dara (ES) | 100 | 97 | 64 | 12 | 7.13 | 315.68 | 20 | 12 | 16 | 19 | 8.10 | 2.24 |
| P5 – Jaunbari (SS) | 100 | 97 | 77 | 59 | 3.45 | 296.25 | 18 | 10 | 14 | 18 | 2.38 | 25.45 |
| P6 – Upper Chamgaon (SS) | 99 | 89 | 77 | 21 | 4.23 | 651.27 | 29 | 10 | 15 | 19 | 3.98 | 39.54 |
| P7 – Tiffin dara (SS) | 100 | 97 | 78 | 33 | 8.64 | 121.96 | 28 | 10 | 16 | 21 | 2.43 | 95.69 |
| P8 – Upper Changrang (ES) | 100 | 98 | 90 | 56 | 8.29 | 59.98 | 28 | 9 | 14 | 23 | 3.92 | 47.55 |
| P9 – Phinsyonala (NS) | 100 | 98 | 94 | 79 | 6.69 | 19.64 | 26 | 9 | 14 | 22 | 1.63 | 199.29 |
| P10 – Zemathang-1 (NS) | 70 | 87 | 81 | 42 | 11.98 | 26.47 | 59 | 11 | 14 | 23 | 4.08 | 285.21 |
| P11 – Lower Pangthang (ES) | 99 | 93 | 61 | 24 | 7.92 | 181.65 | 14 | 12 | 17 | 18 | 6.47 | 1.94 |
| P12 – Kali Pokhari (ES) | 93 | 97 | 71 | 40 | 8.11 | 100.40 | 20 | 9 | 16 | 20 | 5.78 | 6.47 |
| P13 – Khecheopalri (WS) | 88 | 89 | 61 | 22 | 5.46 | 316.93 | 21 | 15 | 22 | 17 | 6.69 | 2.65 |
| P (<0.05) | 3.52 | 7.53 | 7.22 | 8.18 | | | 4.57 | 1.03 | 3.54 | 5.71 | | |
| F | 55.70 | 2.32 | 25.57 | 51.96 | | | 53.43 | 23.35 | 3.58 | 0.94 | | |

irrespective of altitude or habitat differences indicating that a majority of sites in Sikkim offer suitable growing conditions for *S. chirayita*, which is an encouraging finding useful to restoration of this critically endangered species. Still, slow disappearance of this species from nature apart from anthropogenic pressure is a matter of concern, which may require further field studies on seedling growth and survival. This study concludes that the seed of *S. chirayita* responds best if sown within 12 months after storage at 4 °C and more suitably after six months and scheduling seed sowing in pre-monsoon (May–June) for best results, instead of immediate sowing after collection.

## CONCLUSIONS

- Populations of *Swertia chirayita* offer considerable diversity for seed characteristics and seed germination potential.
- The range of 16 to 42% seed moisture content, before storage, proved suitable for long term species gene resource conservation.
- Seed storage at 4 °C is recommended to maintain high germination for six to 12 months and to retain moderate germination potential up to 18 months. High seed germination in a large number of populations provides excellent promise for the conservation of critically endangered species, *S. chirayita* and a potential gene bank in Sikkim Himalaya.

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# Chemical Stimulation of Seed Germination in *ex situ* Produced Seeds in *Swertia chirayita*, A Critically Endangered Medicinal Herb

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**Abstract:** Present study on *S. chirayita* was taken to (1) assess the germination potential of seeds procured form *ex situ* source and (2) enhance seed germination in *ex situ* produced seeds, using various pre-sowing chemical treatments. In *Swertia chirayita*, a critically endangered and high value medicinal plant, the seeds procured from 6 *ex situ* set ups, shrubberies, forest-slope, open-slope, tree-canopy; and green-house and net-shade, showing poor germination at initial testing (12 to 31%) were subjected to 11 pre-sowing chemical treatments. Among the pre-sowing treatments, gibberellic acid (50 to 350 μM) most effectively stimulated seed germination (96.7%, maximum; $p<0.001$) and reduced mean germination time ($p<0.05$), followed by potassium nitrate (100 mM) and sodium hypochlorite (5 min) ($p<0.001$). Study confirms *ex situ* produced seeds attained physiological dormancy, which was broken by pre-sowing treatments, as a tool to *ex situ* species conservation.

**Key words:** Critically endangered, *ex situ*, Himalayan, seed germination, *Swertia chirayita*

## INTRODUCTION

One of the richest pools of biological diversity in the world, Indian Himalaya, has been experiencing unreasonable extraction of wild medicinal plants due to market demand, endangering many of its high-value gene stock (Badola and Pal, 2002, 2003; Badola and Aitken, 2003; Butola and Badola, 2008a) including *S. chirayita* (Bhattarai and Acharya, 1997; Badola and Pal, 2002, 2003; Dutta, 2004; Olsen, 2005; Pradhan and Badola, 2008a). *Ex situ* cultivation is considered as a possible solution to the conservation of endangered taxa and at the same time to meet out commercial raw material demand (Badola and Pal, 2002; Badola and Aitken, 2003; Badola and Singh, 2003; Heywood and Iriondo, 2003; Badola and Butola, 2003, 2005). It has been realized that to sustain availability of continuous stock of planting material for commercial cultivation as well as for species recovery, it is crucial to have in hand an appropriate multiplication technique to build a massive reserve of *ex situ* plants (Badola and Butola, 2003, 2005; Butola and Badola, 2007, 2008b). Additionally, the selection of species specific appropriate propagation technology (Fay and Muir, 1990; Butola and Badola, 2004a, b, 2006a, b) and ideal propagation conditions for mass multiplication (Butola and Badola, 2008b) would minimize the waste of propagule resource.

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However, poor seed germination of viable seeds in several Himalayan plant species is experienced as a limiting factor in large scale plant multiplication (Nadeem *et al.*, 2000; Butola and Badola, 2004a, b, 2006b, 2007, 2008a, b; Pradhan and Badola, 2008a). Pre-sowing chemical treatments are used to enhance seed germination of wild sources of several Himalayan medicinal plants (Nadeem *et al.*, 2000; Pandey *et al.*, 2000; Joshi and Dhar, 2003; Manjkhola *et al.*, 2003; Butola and Badola, 2004ab, 2006a, 2007; Shivkumar *et al.*, 2006) and in plants of other regions (Plummer and Bell, 1995; Yamaguchi and Kamiya, 2001; Ghimire *et al.*, 2006; Kulkarni *et al.*, 2007; Vandelook *et al.*, 2007; Kaur *et al.*, 2009). The above studies were mostly confined to test seeds from wild. Seeds of only a few endangered Himalayan medicinal herbs have been evaluated, following establishment of *ex situ* set-ups, for their germination potential assessment, especially using various chemical stimulants and growing conditions (Butola and Badola, 2006a, 2007).

A critically endangered Himalayan herb (Ved *et al.*, 2003) and prioritized on top for conservation through *ex situ* cultivation by the international experts (Badola and Pal, 2002), *Swertia chirayita* (Roxb. ex Fleming) H. Karst was targeted for the current study. The genus *Swertia* (Gentianaceae) comprises of over 170 species globally, of which 79, 27 and 40 species are distributed in China, Nepal and India, respectively. Sikkim alone, a North-Eastern state of India, harbours 13-14 species of *Swertia*. Amongst these, *S. chirayita*, which grows mostly in temperate Himalaya (1200-3000 m.a.s.l.) from Kashmir to Bhutan and in Khasia hills of Meghlaya, India (Chadha, 1976) in open pastures, grassy slopes and forests, is of great medicinal importance. Distribution of *S. chirayita* depends upon the altitude and slope and is not uniform. The species prefers to grow on north facing slopes and descends below 1500 m, while on south facing slope the plants are found between 1500 and 3000 m. In general, 2000 m altitude is a suitable range preferred by the species in Nepal (Bhattarai and Shrestha, 1996) and 1800-2300 m in Sikkim Himalaya (present authors; *unpublished*). Over centuries, *S. chirayita* is used ethno-medicinally (Wawrosch *et al.*, 2005; Pradhan and Badola, 2008b) for having numerous medicinal properties (Nadkarni, 1976; Biswas and Chopra, 1982; Kirtikar and Basu, 1984). In *S. chirayita*, seed is the only viable solution to the reproduction of the plant. Very limited publications are available on seed germination of *S. chirayita*, all limited to wild seed resources (Raina *et al.*, 1994; Pradhan and Badola, 2008a).

The objectives of the present study on *S. chirayita* were, (1) germination assessment of seeds from six *ex situ* produced sources and (2) enhancement of seed germination, in *ex situ* produced seeds, using various pre-sowing chemical treatments. So, far the authors' knowledge is concerned, it is for the first time the present study makes a move beyond wild stock and targets further the descendent seeds as a stock to put on viability of *ex situ* cultivation technology on *S. chirayita* for conservation. The current study makes a break from the ground and attempts to make a crucial step to understand process of domestication of species to attain predictability. The study targets to compare the *ex situ* produced seeds of *S. chirayita* from six sources and make various disclosers on the germination behavior of seeds.

## MATERIALS AND METHODS

In March 2006, nursery raised (at, ca 2000 m altitude) 8 month old healthy seedlings of *Swertia chirayita*, initially developed through mass sowing (under biodiversity conservation programme) of wild seeds collected from the forests in East Sikkim, India (2050 m), were transplanted (140 seedlings; 35 plants/condition), maintaining 50 cm distance between plants, under four natural habitats of the species (shrubberies, forest-slope, open-slope and

tree-canopy) and two nursery conditions (70 seedlings; 35 plants/condition), on soil amended beds using garden soil and forest humus (open net-shade and temperature controlled green-house; 25°C±5), located at Pangthang-Gangtok, Sikkim, India (ca 2000 m altitude; N 27°21'51.6"; E 088°34'10.1"). Average minimum and maximum temperature of the area ranged between 6.42°C (January) and 20.1°C (August) and average minimum and maximum relative humidity varied between 70.3% (December) and 98.4% (July). Average monthly rainfall for nearest station, Gangtok, varied between 8.0 mm (minimum: December) and 691.1 mm (maximum: July), which remained high in August (617.8 mm). The percent survival of all transplanted plants was recorded. In December 2007, mature seeds were harvested from above six *ex situ* conditions and mixed well individually for each source. To determine moisture content, 100 seeds/replicate (3 replicates/seed source) were weighed and oven dried (60°C; 48 h). Seeds were counted for 10 healthy fruits per source. Seed size was measured for 30 seeds per sample (10 seeds each of 3 replicates) under a microscope. The remaining seeds were air dried in room temperature for 15 days. The seed viability test could not be performed due to minute seed size (Pradhan and Badola, 2008a). In January 2008, seed germination potential using distilled water was tested (6 replicates; 30 seeds each) in a seed germinator, which yielded poor germination. The remaining seeds were stored in air tight specimen tubes in refrigerator (4°C) till the initiation of the pre-sowing chemical treatment experiment which was performed soon after the initial test was over.

**Seed Germination Test**

In February 2008, air dried seeds were surface sterilized by dipping in 0.04% aqueous solution of Mercuric chloride ($HgCl_2$) for 10 sec to discourage fungal infection and washed thoroughly with double distilled water in all cases. The disinfected seeds were soaked in beakers containing various freshly prepared test solutions, viz., gibberellic acid ($GA_3$: 50, 150, 250, 350 µM); potassium nitrate ($KNO_3$: 50, 150, 250 mM) for 24 h and sodium hypochlorite (NaOCl; 5% available chlorine) for 5, 10 and 15 min. Treated seeds were washed 2-3 times with double distilled water and placed in petri-plates (90 mm) lined with single layer of Whattman No. 1 filter paper moistened with double distilled water. Control sets were maintained using double distilled water. Each treatment had 06 replicates of 30 seeds each, which were incubated for a maximum of 45 days in germination chamber (temperature: 25± 2°C; photo period: 14/10 h light/dark), following a complete randomized design. Seeds were checked daily and moistened with double distilled water as and when required and recorded as germinated on radical emergence. Germinated seedlings were counted and removed.

**Statistical Analysis**

The data were analyzed for mean and standard deviation (SD). Mean germination time (MGT) was calculated using equation:

$$MGT = \frac{\sum(nd)}{\sum N}$$

where, n is number of newly germinated seeds after each incubation period in days d and N is total number of seeds emerged at the end of the test (Hartmann and Kester, 1989). Analysis of variance was conducted on different parameters. The difference among the means was compared by Least Significant Difference (LSD) test (Snedecor and Cochran, 1967).

## RESULTS

Before fruit harvest in December 2007, the final plant survival varied in different growing conditions, *viz.*, 71% (Shrubberies), 54% (forest-slope), 22% (open-slope), 71% (tree-canopy), 43% (net-shade) and 88% (green-house). At attaining full maturity of plants, fruits harvested in December 2007 showed significant differences amongst sources. Number of seeds per fruit was significantly higher for shrubberies, which further varied amongst plot sources ($p<0.01$). Seed moisture content ranged from 14 to 22%. Significant differences ($p<0.05$) were obtained for seed weight, seed size and the moisture content amongst sources (Table 1). In initial control test, very low seed germination was recorded for all sources, viz., shrubberies: 31%; forest-slope: 12%; open-slope: 20%; tree-canopy: 21%; net-shade: 23%; green-house: 19%. Seeds produced in shrubberies showed significantly higher germination ($p<0.001$) compared to other sources, except net-shade, which differed at $p<0.01$.

In pre-sowing chemical stimulation experiment, in control, the seeds from forest-slope showed lowest germination (9.44%) comparing to other plots; this followed more or less same trend to that of earlier initial control test. Pre-sowing chemical treatments significantly stimulated seed germination in *S. chirayita* over control, which varied amongst sources (Table 2). In general, $GA_3$ was most effective in stimulating seed germination over other treatments. Across seed sources, compared to control, $GA_3$ treatments improved the percent germination significantly ($p<0.001$). Seed germination value ranged between 70% (minimum; net-shade) and 97% (maximum; forest-slope and tree-canopy) using different $GA_3$ concentrations. Amongst them, $GA_3$ 250 μM followed by $GA_3$ 350 μM effectively stimulated seed germination ($p<0.001$). Seed sources did not show uniformity of germination specifically to a particular concentration of $GA_3$. For example, seeds from shrubberies responded best with 50 μM $GA_3$; from open slope best germination was obtained for 250 and 350 μM $GA_3$.

Table 1: Seed characteristics of *Swertia chirayita* grown under different conditions at ca. 2000 m a.s.l.

| Seed source | Shrubberies | Forest-slope | Open-slope | Tree-canopy | Net-shade | Green-house | p<0.05 | F-value |
|---|---|---|---|---|---|---|---|---|
| Site conditions | Slope: 20°, Dominant species *Symplocos theifolia, S. glomerata* | Slope: 40°, Dominant species: *Buckhundia papadnea* | Slope: 10°, Dominant species: *Osbeckia* sp. | Slope: 20°, Dominant species: *Castanopsis tribuloides* | Flat beds (soil amended with forest humus) | Flat beds (soil amended with forest humus) | | |
| No. seeds per fruit | 316.00 | 230.00 | 184.00 | 146.00 | 172.00 | 200.00 | 60.42 | 26.04 |
| Seed weight (mg $100^{-1}$ seeds) | 3.20 | 2.93 | 2.67 | 2.73 | 2.90 | 3.03 | 0.34 | 2.93 |
| Seed length (μm) | 362.20 | 394.07 | 480.17 | 379.83 | 417.77 | 361.90 | 1.96 | 40.60 |
| Seed Width (μm) | 315.27 | 310.40 | 355.70 | 306.10 | 345.77 | 293.63 | 2.15 | 11.28 |
| Moisture content (%) | 14.62 | 14.81 | 17.47 | 21.91 | 17.38 | 20.89 | 4.45 | 4.10 |

Table 2: Effect of different chemical treatments on seed germination in *Swertia Chirayita*, sourced from six *ex situ* conditions

| | Germination (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | Shrubberies | Forest-slope | Open-slope | Tree-canopy | Net-shade | Green-house | p<0.05 | p<0.01 | p<0.001 | F-value |
| *Control* | *28.33* | *9.44* | *20.56* | *19.44* | *21.11* | *17.78* | *5.91* | *7.93* | *10.46* | *8.77* |
| $GA_3$ (50 μM) | 91.67 | 88.89 | 83.33 | 85.00 | 70.00 | 82.78 | 10.61 | 14.23 | 18.77 | 4.11 |
| $GA_3$ (150 μM) | 83.33 | 96.67 | 83.89 | 92.78 | 73.33 | 82.22 | 9.75 | 13.09 | 17.25 | 5.95 |
| $GA_3$ (250 μM) | 86.11 | 95.56 | 91.67 | 96.67 | 89.44 | 90.56 | 7.39 | 9.92 | 13.07 | 2.33 |
| $GA_3$ (350 μM) | 85.56 | 96.11 | 91.11 | 91.11 | 85.56 | 82.22 | 6.75 | 9.05 | 11.94 | 4.64 |
| $KNO_3$ (50 mM) | 72.22 | 23.89 | 20.00 | 31.67 | 48.89 | 27.22 | 8.61 | 11.56 | 15.24 | 43.69 |
| $KNO_3$ (100 mM) | 59.44 | 35.00 | 42.22 | 37.78 | 45.00 | 37.78 | 9.30 | 12.47 | 16.44 | 7.51 |
| $KNO_3$ (150 mM) | 45.56 | 07.22 | 06.11 | 05.00 | 32.78 | 18.89 | 6.41 | 8.60 | 11.33 | 56.05 |
| $NaHClO_3$ (5 min) | 56.11 | 53.33 | 48.33 | 52.22 | 48.89 | 54.44 | 9.84 | 13.20 | 17.41 | 0.81 |
| $NaHClO_3$ (10 min) | 24.44 | 20.56 | 31.67 | 23.89 | 21.67 | 42.22 | 9.61 | 12.89 | 16.99 | 6.05 |
| $NaHClO_3$ (15 min) | 12.22 | *5.00* | *23.33* | *13.33* | *7.78* | *10.00* | *7.07* | *9.48* | *12.50* | *6.64* |
| **Statistical analysis** | | | | | | | | | | |
| p<0.05 | 9.50 | 6.87 | 9.09 | 7.98 | 7.95 | 8.16 | | | | |
| p<0.01 | 12.63 | 9.13 | 12.08 | 10.60 | 10.56 | 10.84 | | | | |
| p<0.001 | 16.40 | 11.86 | 15.69 | 13.77 | 13.72 | 14.08 | | | | |
| F-value | 68.78 | 255.83 | 101.21 | 155.37 | 94.73 | 109.64 | | | | |

Table 3: Effect of different chemical treatments on mean germination time in *Swertia Chirayita*, sourced from six *ex situ* conditions

| | Mean germination time | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatments | Shrubberies | Forest-slope | Open-slope | Tree-canopy | Net-shade | Green-house | p<0.05 | F-value |
| Control | 23.55 | 27.26 | 17.53 | 20.46 | 22.88 | 20.20 | 2.69 | 12.82 |
| $GA_3$ (50 µM) | 15.51 | 17.85 | 16.22 | 15.97 | 18.38 | 16.94 | 1.87 | 2.97 |
| $GA_3$ (150 µM) | 14.27 | 15.66 | 15.29 | 13.74 | 16.41 | 14.65 | 1.18 | 5.63 |
| $GA_3$ (250 µM) | 15.44 | 17.58 | 14.92 | 14.96 | 15.73 | 15.33 | 0.91 | 9.74 |
| $GA_3$ (350 µM) | 14.16 | 16.72 | 14.52 | 15.21 | 15.24 | 15.37 | 0.95 | 7.07 |
| $KNO_3$ (50 mM) | 19.17 | 22.64 | 20.45 | 21.08 | 21.14 | 20.08 | 1.61 | 4.37 |
| $KNO_3$ (100 mM) | 20.24 | 22.19 | 19.04 | 22.81 | 20.46 | 22.18 | 1.53 | 7.54 |
| $KNO_3$ (150 mM) | 23.86 | 24.25 | 23.33 | 20.00 | 23.83 | 20.66 | 2.60 | 4.12 |
| $NaHClO_3$ (5 min) | 19.77 | 18.62 | 18.11 | 17.79 | 19.13 | 18.45 | 2.67 | 0.59 |
| $NaHClO_3$ (10 min) | 19.46 | 23.18 | 19.62 | 17.76 | 18.53 | 17.56 | 3.07 | 3.69 |
| $NaHClO_3$ (15 min) | 17.93 | 20.06 | 17.56 | 20.89 | 20.43 | 18.28 | 3.35 | 1.51 |
| **Statistical analysis** | | | | | | | | |
| p<0.05 | 2.20 | 2.27 | 1.97 | 2.28 | 2.23 | 2.03 | | |
| F-value | 19.02 | 20.29 | 14.44 | 13.83 | 12.61 | 11.70 | | |

whereas seeds from tree-canopy, net-shade and green-house responded best to $GA_3$ 250 µM (Table 2). Seeds from forest-slope showed an exceptional uniformity in germination of about 96% with all $GA_3$ concentrations except $GA_3$ 50 µM (89%). Comparing to control, $KNO_3$ treatments proved beneficial in improving seed germination in majority of cases; in which, $KNO_3$ 100 mM proved equally effective to stimulate seed germination in all sources ($p<0.001$). Individually, maximum seed germination (72%; $p<0.001$) was achieved with $KNO_3$ 50 mM for shrubberies. However, $KNO_3$ 150 mM was detrimental in case of three seed sources (forest-slope, open-slope and tree-canopy). Soaking in NaOCl (5 min) had significantly ($p<0.001$) stimulated seed germination (48%: open-slope to 56%: shrubberies). Increased soaking time in NaOCl (15 min) appeared harmful to the delicate seeds of *S. chirayita*.

Significant decline ($p<0.05$) in germination delay (number of days taken for 1st seed germination) was recorded with different concentrations of $GA_3$ over control, with lowest of 10 days for $GA_3$ 50, 150 and 350 µM, which varied amongst seed sources from experimental plots. The $KNO_3$ and NaOCl were effective in reducing germination delay only on the seeds collected from shrubberies and forest slope. The half of the maximum seed germination time (days taken to reach 50% of final germination) of 26 days was recorded in control for the forest-slope and a minimum of 13 days in $GA_3$ 150 µM (shrubberies and tree-canopy), $GA_3$ 250 µM (tree-canopy) and $GA_3$ 350 µM (shrubberies and open-slope). Gibberllic acid significantly reduced ($p<0.05$) Mean Germination Time (MGT) over control compared to other treatments (Table 3). After soaking in different concentration of $KNO_3$ and for different period in NaOCl, significant reduction ($p<0.05$) in MGT was achieved for the seeds collected from shrubberies, forest-slope and net-shade. Individually, significant reduction in MGT ($p<0.05$) was recorded for tree-canopy in NaOCl (5 and 10 min) and for green-house in NaOCl (10 min). In general, among all treatments, $GA_3$ had greatly improved percent germination (Table 2) and lowered the MGT (Table 3).

## DISCUSSION

In the present study, *ex situ* produced seeds of *S. chirayita* harvested from different growing conditions showed very low germination, initially, under control. Likewise in many Himalayan herbs (Pandey *et al.*, 2000; Butola and Badola, 2004a, b, 2006a, 2007; Shivkumar *et al.*, 2006), pre-sowing chemical treatments significantly improved germination percentage, onset and final germination of *S. chirayita* in present study. Here, gibberellic

acid most effectively stimulated seed germination and minimized the difference in onset and final germination which can be attributed to increased activity of hydrolytic enzymes (Al-Helal, 1996; Joshi and Dhar, 2003; Manjkhola *et al.*, 2003) or mobilization of nutrients in dormant seeds (Kumar and Purohit, 1986; Hartmann and Kester, 1989). Further, gibberellic acid is known to promote germination by breaking dormancy in wide range of seeds (Vandelook *et al.*, 2007; Perez-Gracia, 2008). In *S. chirayita*, $GA_3$ in all concentrations was highly stimulatory to seed germination. Further, except for two sources, i.e., tree-canopy and net-shade, the difference amongst $GA_3$ treatments was insignificant in other four sources. That suggested effectiveness of all used concentrations of $GA_3$ in stimulating seed germination as well as reducing mean germination time in *S. chirayita* as gibberellic acid is known to affect physiological as well as metabolic activities of seeds resulting in early germination (Tipirdamaz and Ve-Gömürgen, 2000; Chuanren *et al.*, 2004). With the increase in $GA_3$ concentrations, increase in percent germination was observed (Abdel-Hady *et al.*, 2008; Keshtkar *et al.*, 2008). However, present study indicated non-uniformity in germination with different concentrations of $GA_3$ amongst the seed sources as reported by Bhatt *et al.* (2005) for *Swertia angustifolia*. However, exogenous application of $GA_3$ had no effect on the germination of seeds in *Ferula assafoetida* (Otroshy *et al.*, 2009).

Sodium hypochlorite overcomes seed dormancy by either increasing permeability of the seed coat to oxygen through the removal of phenolics (Hurley *et al.*, 1989) or by scarification or modification of the seed coat (Hsiao, 1979; Butola and Badola, 2004a, b, 2007) which might have resulted in early seedling onset and increased percent germination in seeds treated with NaOCl (5 and 10 min) compared to control for all tested sources in the present experiment. The NaOCl (10 min) could significantly stimulated seed germination for only two sources (*forest-slope and green-house*) at $p<0.05$. However, the time period for soaking in NaOCl needs to be standardized from species to species. For instance, seed germination was enhanced by soaking for 5 to 60 sec in *Amaranthus powellii* but declined as the soaking period exceeded 60 sec in annual weed (Tommaso and Nurse, 2004). Similarly, seed germination was increased from 2 to 19% in *Zizania palustris* when soaked in NaOCl for 2 h (Oelke and Albrecht, 1985). A range of soaking time in NaOCl (15-45 min) effectively used in many Himalayan herbs (Butola and Badola, 2004ab, 2006b, 2007). Since, the seeds are susceptible to fungal infection during germination, but, treating with NaOCl further reduces the chances of fungal infection to seeds (Butola and Badola, 2007) thereby resulting in improved germination as in the present study.

In present study, $KNO_3$ 50 and 100 mM proved significantly effective for all sources used, lesser stimulatory comparing to $GA_3$. Whereas, increased concentration of $KNO_3$ (150 mM) was beneficial significantly only in case of shrubberies and net-shade. Contrarily, in *Angelica glauca* (with large sized seed, compared to minute seeds in *S. chirayita*), $KNO_3$ (150 mM) was found effective in stimulating seed germination (Butola and Badola, 2004a), may relate to the seed size as an important factor, need to be considered while standardizing level of concentration. Effectiveness of $KNO_3$ in stimulating seed germination may be possibly due to oxidized form of nitrogen causing a shift in respiratory metabolism to the pentose phosphate pathway (Roberts and Smith, 1977). Nitrogenous compounds in various forms, particularly nitrates (e.g., $KNO_3$) have been used to stimulate germination (Choudhary *et al.*, 1996; McIntyre *et al.*, 1996). They play a critical role in increasing the physiological efficiency (Bhargava and Banerjee, 1994) and influence germination through change in water relationship (Nikolaeva, 1977). The responses of seeds to a particular concentration of $KNO_3$ may vary with species. In Gentianaceae family, in general, physiological dormancy is reported (Nikolaeva, 1977). Contrarily, the researcher's

assessment on 13 wild populations of *S. chirayita* (Gentianaceae) did not show any seed dormancy (Pradhan and Badola, 2008a). However, in the present study, *ex situ* produced seeds were found with high dormancy. The results with improved germination rates in current study under $GA_3$ and $KNO_3$ treatments further correspond well with these generalizations, as both these substances are considered best for breaking physiological dormancy (Nikolaeva, 1977). The present study confirms that the seeds of *S. chirayita* from *ex situ* grown sources, initially using wild seed stock, had physiological dormancy, which was broken by pre-sowing chemical treatments.

Present authors had earlier recorded over 70% (minimum) seed germination in thirteen wild populations of *S. chirayita*, which gradually declined over time and further differed amongst sources under storage conditions (Pradhan and Badola, 2008a). Seed germination potential differences in the same species/genus have been reported (Celiklar *et al*., 2006). Such differences may be due to the environmental variations in the light and temperature (Karlsson and Milberg, 2007; Vandelook *et al*., 2007) and climate present in the native habitats of the species (Celiklar *et al*., 2006), but if proper strategies in respect of seed collection time etc., is maintained, such difference can be minimized to some extent. The present results suggest, there might be various environmental factors, the plants grown in or transplanted from green-house to natural habitats had to encounter during acclimatization and adaptation over two more growing seasons, might have induced greater physiological seed dormancy in second generation, which was later broken by chemical treatments. This can be viewed as a part of defence mechanism of a plant's survival tactics in response to a sudden change in its' eco-climatic condition. More detailed investigations are recommended to assess such eco-physiological changes occur from a sudden snap from wild species adapted environmentally over ages. The study further recommends $GA_3$ (50 to 350 μM) as the best treatment for breaking seed dormancy and greatly improving germination in *ex situ* produced seeds in *S. chirayita*. Good survival in natural conditions following transplantation of plants developed in green-house, using above pre-sowing treatments, has appeared as a technological package for strengthening conservation as well *ex situ* cultivation of targeted endangered taxa, *S. chirayita*, in Himalaya.

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Original contributions

*B. K. Pradhan and H. K. Badola*

# Assessment of seedling emergence and vigour for quality planting material in thirteen populations of *Swertia chirayita* – a high value endangered medicinal herb, using substrate combinations

## Introduction

Indian Himalaya, an immensely rich reservoir of medicinal plant resources, has been targeted to harvest several high value species in meeting out ever-expanding raw material demand of the pharmaceuticals (3). However, with growing herbal market in recent few decades, owing to over harvesting and unsustainable extraction practices several medicinal plant species have faced risk of their survival in natural habitats, and many assessed as threatened (3,6,7,24,35,37,38,39). Since, the raw material supply to herbal industry is a societal requirement, and conservation of genetic material is a vital concern towards its safeguard for long-term survival in nature. *Ex-situ* cultivation by developing suitable and easy to handle propagation and agro-technique packages for the farmers are the only answer to meet-out raw material supply of the pharmaceuticals, ensuring lessening harvesting pressures on natural habitats, thus helping *in-situ* conservation of threatened taxa (2,5,25,30,31). Developing quality planting material is the primary requirement for successful cultivation of wild medicinal plants, especially Himalayan (4, 13,15). The success of productive cropping will largely depend upon the appropriate choice of growing media or substrate combinations and in many Himalayan herbs, these substrates have proved promising to achieve improved seedling emergence, growth and crop productivity (4,14,27,28,29).

*Swertia chirayita* (Roxb. ex Fleming) H. Karst, a critically endangered herb, indigenous to temperate Himalaya, distributed along 1200 m–3000 m asl is considered non toxic and safe in ethnomedicinal use (26). The herb is exploited for bitter bioactive compounds including amarogentin, xanthones and iridoid glycosides (21). The beverage industry has discovered an alternative bitter product from *S. chirayita* (i.e. used in the liquor industries to impart bitter flavour to mouth). *S. chirayita* is valuable Ayurvedic medicinal herb, having high demand in Indian and international markets (6), and possess multiple use value in local medicine (31,32). Seeds are the only means of propagation in *S. chirayita* and poor seed germination and low seed viability in its natural environment has been considered as limitation to commercial cultivation of the species (6,18). The reason for poor natural regeneration in *S. chirayita* may be that the seed matures in dry period (November-December) where the availability of moisture in the natural environment is very low. The seed emerges out in late May prior to start of monsoon shower and the species does not get the required moisture for sufficient germination. The present authors field experiences reveals that the immature fruits of *S. chirayita* provides shelter and the necessary nutrients required for the growth and development of insect larvae which prohibits the fruit from further development and could be another factor resulting in poor natural regeneration in *S. chirayita*.

Micro propagation of *S. chirayita* has been tried by several workers (1,8,18,19,20,22,40,42). There are not many reports on studies relating to germination and viability of *S. chirayita* seeds. Raina et al., (34) reported 91% seed germination after chilling to 3 °C for 15 days as compared to 3.4% germination in control seeds in Himachal population; Chaudhari et al. (18) reported difficulty in germination. However, Pradhan and Badola (30) obtained 100% seed germination, initially, under laboratory conditions, without any treatment in majority of the natural populations and also found out that 5 populations retained above 50% germination after 18 months of storage at 4 °C in *S. chirayita* in Sikkim. However, physiological dormancy was observed in seeds procured from domesticated

**Abstract**

*Swertia chirayita* (Chirayita) is a high value medicinal herb having high global demand for raw material by pharmaceuticals, and prioritized at the top for *ex-situ* cultivation in Himalaya. The variability in seedling emergence is a major problem resulting in the production of non-uniform seedlings, as big constraint to commercial cultivation. The present study aimed to (1) standardize effective substrate combination for obtaining uniform and vigorous seedlings, and (2) identify morphological traits for the assessment of healthy seedlings as quality planting material in *S. chirayita*. The effect of two substrate combination (substrate 1: garden soil, sand, forest humus; substrate 2: garden soil, sand, farm yard manure; 1:1:1) and control (sand and garden soil; 3:1) on seedling emergence, seedling growth, biomass and vigour were tested with the seeds collected from thirteen wild populations in Sikkim. Seedling emergence was significantly higher in substrate 2 ($p<0.05$) over others. Substrate 2 significantly ($p<0.05$) improved all the seedling growth parameters except number of leaves. Of thirteen populations assessed, seven (Sc1, 2, 3, 8, 9, 11, and 13) exhibited higher seedling biomass and SVI using substrate 2. The present study concludes substrate 2 as the most effective substrate combinations resulting in high percent emergence, uniform and vigorous seedlings and high plant survival after field transplantation; besides, collar size appeared to be the strongest morphological trait to identify seedling vigorousness, as an aid to successful *ex-situ* cultivation of high value medicinal crop, *Swertia chirayita*.

**Keywords**

*Ex-situ* cultivation, populations, seedling-emergence, seedling-production, seedling-vigour, substrate combinations, *Swertia chirayita*

plants, and that was broken by suitable chemical treatments (33).

In commercial cultivation of the species, besides having slow growth rate (10), the variability in commencement of seedling emergence has been identified as the major problem resulting in the production of non-uniform seedlings; therefore, the need of the hour was to develop uniform and vigorous seedlings for successful and productive crop cultivation of the species. In view of this, our study aimed to (1) standardize effective substrate combination for obtaining uniform and vigorous seedlings, and (2) identify morphological traits for the assessment of healthy seedlings as quality planting material for cultivation in *S. chirayita*.




**Fig. 1: Availability of *Swertia chirayita* in natural habitat; a and b – plants in vegetative stage; c – plants in reproductive stage.**
**Abb. 1: *Swertia chirayita* am natürlichen Standort; a und b – Pflanzen im vegetativen Stadium; c – Pflanzen im generativen Stadium.**

## Materials and methods

### Experimental design

The matured fruits of *S. chirayita* were collected from thirteen populations (*Tab. 1*) distributed in different parts of Sikkim Himalaya (27°04′46″ and 28°07′48″ N lat. and 88°00′58″ and 88°55′25″E. long.) during the month of November – December 2005. The collection of fruits/seeds was made sustainably in such a way that 15-20 robust plants per site were randomly selected from the middle of each population without disturbing the other plants keeping in view that the seeds are the only means of reproduction in *S. chirayita*; collection from the margins was avoided to prevent the natural spreading of the species and to limit the edge effect, if any.

The collected fruits were brought to the laboratory and seeds were segregated by mashing up fruits which followed the thorough cleaning to remove impurities. The seeds were dried in ambient room temperature for ten days, and stored in refrigerator (4 °C) in properly sealed specimen tubes separately for different populations till nursery experiment initiated. Following good result at the initial test in the laboratory (30); the seeds were tested under nursery conditions in the end of February 2006 using garden soil [pH: 6.43±0.05; moisture (%): 42.00±2.00; nitrogen (%): 0.40±0.11; phosphorus (%): 0.41±0.05], forest humus [pH: 5.91±1.00: moisture (%): 42.67±2.31; nitrogen (%): 0.43±0.01; phosphorus (%): 0.29±0.04], farm yard manure [pH: 8.32±0.06; moisture (%): 46.39±0.17; nitrogen (%): 6.57±0.02; phosphorus (%): 7.29±1.25], and sand [pH: 7.17±0.02; moisture (%): 6.40±0.96; nitrogen (%): 0.25±0.03; phosphorus (%): 0.02±0.02]. At first, two substrate combinations of equal

**Einfluss verschiedener Anzuchtsubstrate auf das Auflaufen und die Vitalität von Sämlingen für die Jungpflanzenproduktion von *Swertia chirayita* – einer wertvollen Arzneipflanze**

**Zusammenfassung**

*Swertia chirayita* (Chirayita) ist eine wertvolle Arzneipflanze, die weltweit in großem Umfang zur Herstellung pflanzlicher Arzneimittel benötigt wird und die besonders hohe Bedeutung für den ex-situ-Anbau im Himalaja hat. Die Ungleichmäßigkeit der Samenkeimung, die zur Entwicklung uneinheitlicher Jungpflanzen führt, stellt eine große Erschwernis für den kommerziellen Anbau dar. Die Untersuchungen wurden mit der Zielstellung durchgeführt, (1) Anzuchtsubstrate für die Gewinnung einheitlicher und vitaler Jungpflanzen zu optimieren und (2) morphologische Merkmale zu finden, die geeignet sind, die Qualität gesunder Jungpflanzen von *S. chirayita* zu charakterisieren. Es wurde der Einfluss von zwei Anzuchtsubstratkombinationen (Substrat 1: Gartenerde, Sand, Waldhumus; Substrat 2: Gartenboden, Sand, Stalldung; 1:1:1) und der Kontrolle (Sand und Gartenerde; 3:1) auf Auflaufen, Wachstum, Biomasse und Wüchsigkeit der Sämlinge von 13 in Sikkim gesammelten wilden Populationen getestet. Das Auflaufen war in Substrat 2 signifikant ($p<0.05$) am höchsten. Substrat 2 steigerte alle Wachstumsmerkmale signifikant ($p<0.05$) mit Ausnahme der Anzahl der Blätter. Von den 13 bewerteten Populationen wiesen sieben (Sc1, 2, 3, 8, 9, 11 und 13) eine höhere Biomasse und einen höheren Sämlingsvitalitätsindex (SVI) bei Verwendung des Substrates 2 auf. Bei den durchgeführten Untersuchungen erwies sich Substrat 2 als besonders geeignete Anzuchterde, da mit ihr eine hohe Keimfähigkeit, einheitliche und vitale Jungpflanzen und eine hohe Überlebensrate nach dem Auspflanzen im Feld erreicht wurden; weiterhin erschien die Rosettengröße als besonders gut geeignetes morphologisches Merkmal zur Beurteilung der Jungpflanzenvitalität als Hilfsmittel für die erfolgreiche ex-situ-Kultivierung der wertvollen Arzneipflanze *Swertia chirayita*.

**Schlagwörter**

Anzuchterden, Auflaufen, Ex-situ-Kultivierung, Jungpflanzenanzucht, Populationen, Sämlingsvitalität, *Swertia chirayita*

Tab. 2: Result of MANOVA characteristics by populations, treatments and their interaction on *S. chirayita*
Tab. 2: Ergebnis der mehrfaktoriellen Varianzanalyse der Prüfmerkmale von *S. chirayita* in Abhängigkeit von den Populationen und Behandlungen und ihre Wechselwirkungen

| Dependent variables (Abhängige Variablen) | Independent variables (Unabhängige Variablen) | | | | | |
|---|---|---|---|---|---|---|
| | Population (Population) (P) | | Treatment (Behandlung) (T) | | P x T | |
| | F-value (F-Wert) | Probability (*) | F-value (F-Wert) | Probability (*) | F-value (F-Wert) | Probability (*) |
| Seedling onset | 3.407 | 0.000 | 38.778 | 0.000 | 3.991 | 0.000 |
| $T_{50}$ | 2.111 | 0.025 | 19.030 | 0.000 | 2.058 | 0.009 |
| Final emergence | 5.086 | 0.000 | 13.000 | 0.000 | 4.363 | 0.000 |
| Emergence (%) | 8.587 | 0.000 | 34.595 | 0.000 | 3.566 | 0.000 |
| Mean emergence time | 3.808 | 0.000 | 40.388 | 0.000 | 4.786 | 0.000 |
| Emergence rate | 3.311 | 0.000 | 33.694 | 0.000 | 4.460 | 0.000 |

* Irrtumswahrscheinlichkeit

Tab. 3: Result of MANOVA characteristics by populations, treatments and their interaction on *S. chirayita*
Tab. 3: Ergebnis der mehrfaktoriellen Varianzanalyse der Prüfmerkmale von *S. chirayita* in Abhängigkeit von den Populationen und Behandlungen und ihre Wechselwirkungen

| Dependent variables (Abhängige Variablen) | Independent variables (Unabhängige Variablen) | | | | | |
|---|---|---|---|---|---|---|
| | Population (Population) (P) | | Treatment (Behandlung) (T) | | P x T | |
| | F-value (F-Wert) | Probability (*) | F-value (F-Wert) | Probability (*) | F-value (F-Wert) | Probability (*) |
| Collar diameter | 10.115 | 0.000 | 119.699 | 0.000 | 6.214 | 0.000 |
| Number of leaves | 7.354 | 0.000 | 384.623 | 0.000 | 8.772 | 0.000 |
| Largest leaf length | 31.524 | 0.000 | 880.279 | 0.000 | 18.160 | 0.000 |
| Largest leaf width | 9.576 | 0.000 | 437.101 | 0.000 | 8.233 | 0.000 |
| Number of roots | 10.922 | 0.000 | 182.709 | 0.000 | 6.502 | 0.000 |
| Total dry biomass | 12.394 | 0.000 | 241.057 | 0.000 | 0.668 | 0.000 |
| Seedling vigour index | 11.053 | 0.000 | 209.046 | 0.000 | 8.592 | 0.000 |

* Irrtumswahrscheinlichkeit

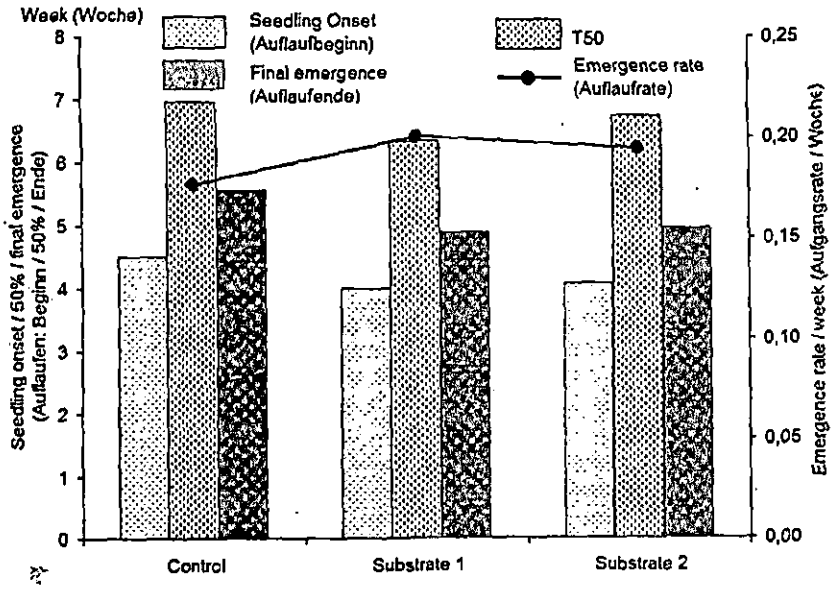


Fig. 3: Effect of different substrate combinations on seedling onset, $T_{50}$, final emergence and emergence rate in *S. chirayita*.
Abb. 3: Einfluss verschiedener Substratkombinationen auf den Beginn des Auflaufens, $T_{50}$, Ende des Auflaufens und auf die Aufgangsrate von *S. chirayita*.

### Seedling survival

For seedling survival test, fifteen seedlings, of more or less uniform size, per substrate (five each of three replicates), including control, were transplanted in 20 x 20 cm distance under a net shade in the Institute's experimental medicinal plant garden [altitude: *ca.* 2000m asl; temperature: 20.1 °C (max.), 6.42 °C (min.); relative humidity: 98.4% (max.), 70.3% (min.)] and monitored by the end of growing season. Populations were not taken into consideration for observing survival. Plant survival for each substrate combinations and control set was recorded.

## Results

Multivariate analysis of variance (MANOVA) revealed significant effect of populations, treatments and their interaction on various seedling emergence parameters and growth parameters in *S. chirayita (Tab. 2* and *Tab. 3)*.

**Effect of substrate combinations on seedling onset, $T_{50}$ and final emergence**

Minimum of four weeks and maximum of five weeks were taken for the onset and completion of 50% seedling emergence ($T_{50}$), and minimum of five weeks and maximum of seven weeks were taken in completing final emergence in all the populations irrespective of substrate combinations, including control *(Fig. 3)*. However, seedling onset, $T_{50}$, and final emergence was significantly faster in substrate 1 ($p<0.05$) than substrate 2 and control.

**Effect of different substrate combinations on seedling emergence, mean emergence time and emergence rate**

Seedling emergence and altitude of populations (seed collection site) showed negative (non-significant) correlation with all the substrate combinations including control (substrate 1, $r$ = -0.304; substrate 2, $r$ = -0.092; control, $r$ = -0.163). Seedling emergence was significantly higher in substrate 2 ($p<0.05$) over substrate 1 and control. Similarly, substrate 1 recorded higher seedling emergence over control ($P<0.05$). The lowest (Sc10; 2841m asl) and the highest (Sc11; 2000m asl) seedling emergence in control and

volume were prepared, substrate 1 (pH: 6.5): Garden soil + Sand + Forest humus (1:1:1) and substrate 2 (pH: 7.08): Garden soil + Sand + Farm yard manure (1:1:1), and substrate combinations were mixed well. These substrate combinations were filled in earthen trays. Trays filled with sand and garden soil in 3:1 ratio (pH: 6.7) were considered as control. The seeds of *S. chirayita* were soaked overnight in double distilled water before sowing. Each set of experiment had three replicates of 30 seeds per replicate; the seeds were sown at 0.5cm depth with equal distance between seeds. The experiment was conducted under an automatic temperature controlled green-house (25 °C ± 5 °C). Mist irrigation was done twice a day (09:30 hrs and 16:30 hrs) till the completion of the seedling emergence which was reduced to once in a day (09:30 hrs) after seedling emergence stopped providing optimal moisture to growing plants. Weeding was done at equal interval.

Seedlings after the completion of emergence (eight weeks) were thinned to mitigate competition, maintaining 8 – 10 cm distances.

After 24 weeks, the effect of different substrate combinations on seedling growth, different morphological traits were evaluated by harvesting five seedlings each of uniform size for all populations. During experiment, the seedlings remained in rosette form; the identified traits included collar diameter, largest leaf length, leaf width and number of roots and biomass. To obtain dry biomass, after recording the fresh weight, seedlings were oven dried at 60°C to constant weight and reweighed and the value obtained was recorded.

**Statistical Analyses**

SPSS 10.0 for Windows (SPSS Inc. 1989) was used for statistical analysis of the data. Mean emergence rate (*e*) was calculated following Labouriau (23) as, $e = 1/\sum(xd)/\sum t$;

where, x = number of newly emerged seedlings in each day d, and t = total number of seedling emerged at the end of the test. Multivariate analysis of Variance (MANOVA) was carried out to determine the effect of the substrate combinations concerning on the seedling emergence and seedling morphological traits. A Bonferroni test ($p<0.05$) was used to determine differences amongst the treatments. Pearson's correlation analyses were performed to analyze the relationship between different morphological traits for each substrate combinations including control. To identify the best substrate combinations, Seedling Vigour Index (SVI) was calculated following Butola and Badola (12) as SVI = (*d*/*e*)*100; where, "*d*" is dry weight/seedling; "*e*" is mean emergence rate and 100 is constant.

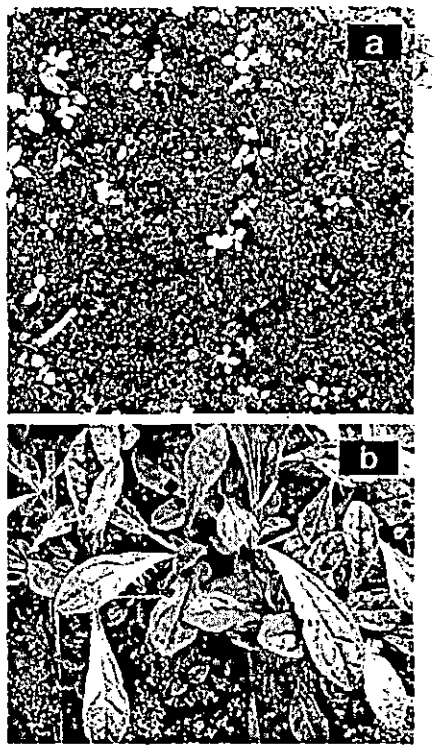


**Fig. 2: a – Seedling emergence in nursery experimentation; b – thinning of seedlings after eight weeks (under substrate 2) in *S. chirayita*.**
Abb. 2: a – Auflaufen der Sämlinge im Anzuchtversuch; b – Ausdünnen der Sämlinge von *S. chirayita* nach acht Wochen (auf Substrat 2).

**Tab. 1: Site characteristics of the populations of *S. chirayita* in Sikkim**
Tab. 1: Beschreibung der Regionen in Sikkim, in denen die Populationen von *S. chirayita* gesammelt wurden

| Site code (Regionscode) | Site Name (Bezeichnung der Region) | Altitude (Höhe) (m) | Aspect (Himmels-richtung) | Slope (Gefälle) (°) | GPS Latitude (Breite) (N) | GPS Longitude (Länge) (E) | Broad habitat (Lebensraum) |
|---|---|---|---|---|---|---|---|
| Sc1 | Luing (ES) | 2126 | NE | 55 | 27°21'47.60" | 88°34'12.17" | Ridge, mixed forest of *Cryptomeria japonica* and *Castanopsis tribuloides* |
| Sc2 | Railgaon (ES) | 1948 | NE | 40 | 27°21'52.71" | 88°33'47.07" | Shrubberies with species of *Cryptomeria japonica* and *Castanopsis tribuloides* |
| Sc3 | U Pangthang (ES) | 2176 | SW | 45 | 27°21'13.54" | 88°33'53.37" | Forest-shrubberies with *Quercus lamellose* and *Castanopsis tribuloides* |
| Sc4 | Guransey dara (ES) | 2107 | SE | 60 | 27°20'51.02" | 88°34'07.92" | Moist mixed Forest with *Cryptomeria japonica* and *Rhododendron* sp. |
| Sc5 | Jaunbari (SS) | 1651 | SW | 50 | 27°11'47.20" | 88°23'33.60" | Moist forest-shrubberies with *Alnus nepalensis* and *Michelia lanuigilosa* |
| Sc6 | U Chamgaon (SS) | 1583 | NW | 55 | 27°11'03.80" | 88°22'26.20" | Forest-shrubberies with *Alnus nepalensis* and *Schima wallichi* |
| Sc7 | Tiffin dara1 (SS) | 1667 | W | 60 | 27°11'14.8" | 88°22'46.01" | Moist grassy slope with *Cryptomeria japonica* and *Alnus nepalensis* |
| Sc8 | U Changrang (ES) | 2055 | S | 65 | 27°21'51.97" | 88°35'20.31" | Forest-shrubberies with *Machilus* sp. and *Castanopsis tribuloides* |
| Sc9 | Phinsyonala (NS) | 1761 | NE | 55 | 27°37'01.6" | 88°37'26.2" | Forest-shrubberies of *Alnus nepalensis* |
| Sc10 | Zema1 (NS) | 2841 | NE | 60 | 27°44'34.7" | 88°32'48.0" | Rocky steep slope with *Selenium* sp. and *Artemesia* sp. |
| Sc11 | L. Pangthang (ES) | 2000 | N | 40 | 27°21'49.52" | 88°33'32.15" | Grassy slope with *Symingtonia populnes* |
| Sc12 | Kalipokhari (ES) | 2450 | N | 50 | 27°21'27.31" | 88°33'24.18" | Open shrubberies with *Osbeckia* sp. |
| Sc13 | Khecheopalri (WS) | 1867 | SW | 55 | 27°21'06.07" | 88°11'21.06" | Moist mixed forest of *Quercus* sp. and *Castanopsis tribuloides* |

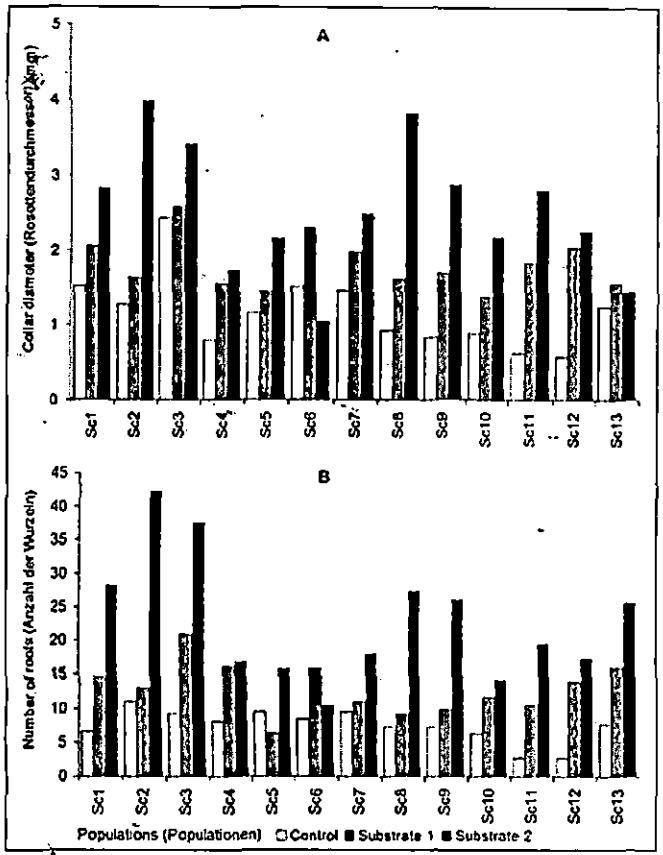


Fig. 6: Effect of different substrate combinations on collar diameter and number of roots in *S. chirayita*
Abb. 6: Einfluss verschiedener Substratkombinationen auf den Rosettendurchmesser und die Wurzelanzahl von *S. chirayita*

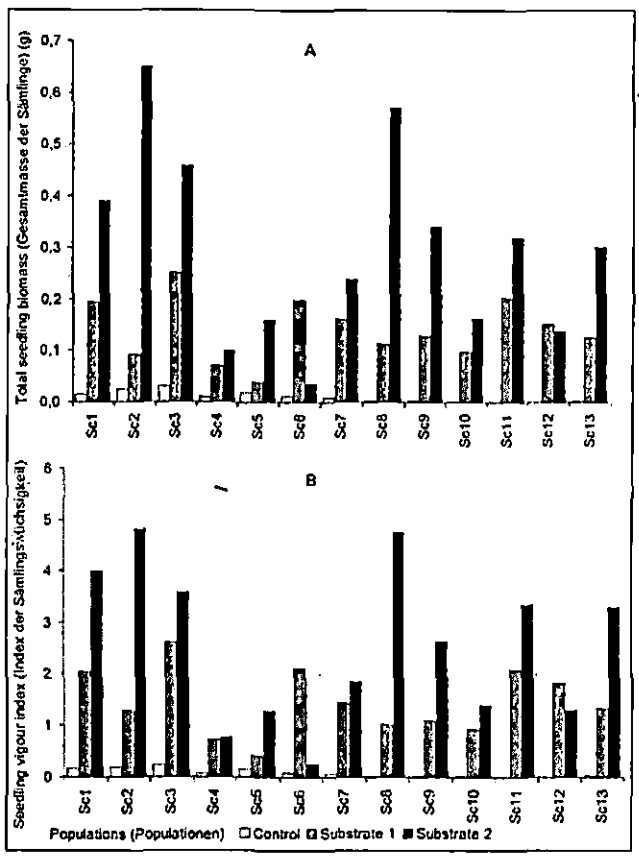


Fig. 7: Effect of different substrate combinations on seedling biomass and seedling vigour in *S. chirayita*
Abb. 7: Einfluss verschiedener Substratkombinationen auf Biomasse und Sämlingswüchsigkeit von *S. chirayita*

obtained with substrate 1 and substrate 2 over control; however the best result achieved with substrate 2 ($p<0.05$) than substrate 1 *(Fig. 7)*. Of 13 populations assessed, seven (Sc1, 2, 3, 8, 9, 11, and 13) exhibited higher total seedling biomass and SVI using substrate 2, of which, Sc2 population followed by Sc8 recorded the highest values. For Sc6, the total seedling biomass and SVI was significantly greater with substrate 1 than substrate 2 ($p<0.05$). All the growth parameters were significantly ($p<0.0001$) correlated with each other *(Tab. 4)*.

**Effect of different substrate combinations on seedling survival**

Seedlings developed using substrate 1 and substrate 2 combinations resulted in 77% and 83% survival, respectively, in nursery beds compared to control (63%). During the experimentation period, no insect infestation was observed in the seedlings.

## Discussion

Difference in germination behaviour between the plant populations (9,17) has been indicated in earlier studies (30). The present study considered that appropriate substrate combinations are essential for the successful establishment of a species. Higher seedling emergence with substrate 1 and substrate 2 combinations compared to control in the present study further strengthens the above assumption *(Fig. 3)*. Of the two substrate combinations, substrate 2 resulted in higher seedling emergence in all the populations than substrate 1, which further significantly enhanced the seedling performance for different growth parameters and resulted in higher percent survival, as well, suggesting substrate 2 as the best substrate combination for *S. chirayita*. The present results are based on a large number of populations and mild use of manure, where all populations exhibited higher performance for both seedling emergence as well as seedling growth, especially greater seedling vigour in majority of populations. That means, the present substrate combination offers wider application for *S. chirayita* in cultivation programme. This is particularly applicable for developing quality planting material, a pre-requisite to successful cultivation of *S. chirayita*, this is also important, as the direct sowing in *S. chirayita* is not suggestive as in the case of *Heracleum candicans* (4) but the transplantation of one season old seedling performed well (33; and other unpublished work). Butola and Badola (14) in their study recorded highest seedling emergence with the mixture of sandy loam and vermicompost and sandy loam and forest humus (mixture in equal proportion, 1:1) in *Angelica glauca* and *Heracleum candicans*, respectively, suggesting that the species responds differently in their nutrient requirements. Compared to very low seed germination achieved in four populations of *S. chirayita* (25.33% to 33.33%) from central and north-western Himalaya in control in growth chamber (11), high seedling emergence was achieved in some of the populations in control (37.33%, Sc11; 33.33%, Sc4 and Sc13) in

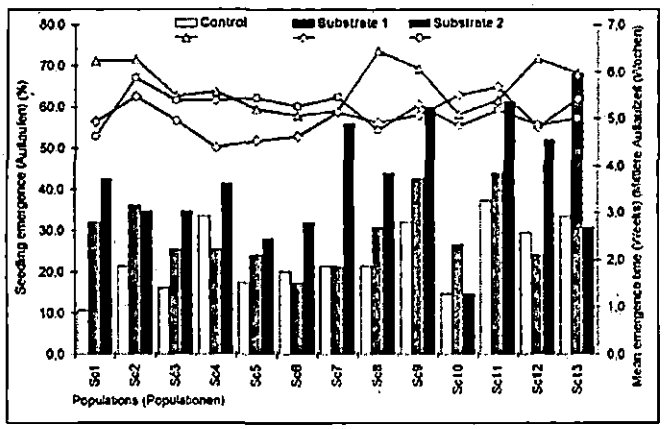


Fig. 4: Effect of different substrate combinations on seedling emergence and mean emergence time in populations of *S. chirayita*
Abb. 4: Einfluss verschiedener Substratkombinationen auf das Auflaufen der Sämlinge und die mittlere Aufgangsdauer der Populationen von *S. chirayita*

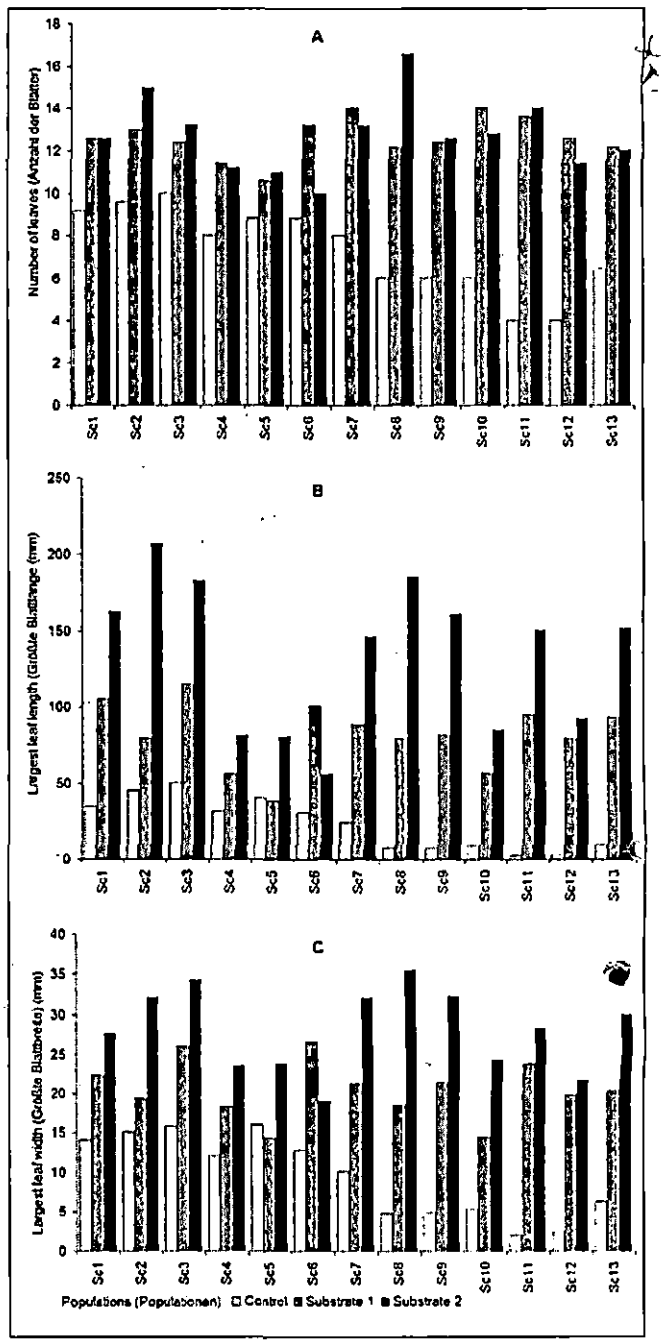


Fig. 5: Effect of different substrate combinations on number of leaves, largest leaf length and leaf width in *S. chirayita*
Abb. 5: Einfluss verschiedener Substratkombination auf die Anzahl Blätter, max. Blattlänge und Blattbreite von *S. chirayita*

Tab. 4: Correlation matrix (Pearson Product-Moment Correlation Coefficient) between different growth parameters in *S. chirayita* after 24 weeks of sowing at *ca.* 2000m asl (n = 195; df = 193)
Tab. 4: Korrelationsmatrix (Pearson Product-Moment Korrelationskoeffizient) zwischen verschiedenen Wachstumsparamtern von *S. chirayita* 24 Wochen nach der Saat in ca. 2000 m ü. NHN (n = 195; df = 193)

| | Collar diameter (mm) | Number of leaves | Largest leaf length (mm) | Largest leaf width (mm) | Number of roots | Total seedling biomass (gm) | SVI |
|---|---|---|---|---|---|---|---|
| Collar diameter (mm) | | | | | | | |
| Number of leaves | 0.668 | | | | | | |
| Largest leaf length (mm) | 0.824 | 0.776 | | | | | |
| Largest leaf width (mm) | 0.773 | 0.798 | 0.908 | | | | |
| Number of roots | 0.741 | 0.610 | 0.832 | 0.737 | | | |
| Total seedling biomass (gm) | 0.864 | 0.674 | 0.916 | 0.804 | 0.843 | | |
| SVI | 0.841 | 0.695 | 0.914 | 0.806 | 0.815 | 0.980 | |

*All seedling growth parameters significant at p< 0.0001

substrate 1 and the lowest (Sc6; 1583m asl) and the highest (Sc13; 1867m asl) seedling emergence in substrate 2 were significant at p<0.05 level *(Fig. 4)*. Mean emergence time was significantly lower in substrate 1 over substrate 2 and control (p<0.05); likewise, it was significantly lower in substrate 2 compared to control (p<0.05). Comparatively, seedling emergence rate was significantly (p<0.05) higher in seeds sown in substrate 1 than substrate 2 and control *(Fig. 3)*.

**Effect of different substrate combinations on seedling growth, biomass and vigour**

Both substrate 1 and substrate 2 combinations were significantly (p<0.05) effective in improving all the seedling growth parameters over control in all the populations. However, on comparing between substrate 1 and substrate 2, the latter substrate combination was more effective (p<0.05) than former, but in the case of number of leaves the effectiveness was non-significant. *Fig. 5* and *Fig. 6* depict the effect of both the substrate combinations including control on various seedling growth parameters in *S. chirayita*. Significant (p<0.05) increase in total seedling biomass and SVI was

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the present study even in nursery condition, suggesting the populations of *S. chirayita* from Sikkim (eastern Himalaya) to be more viable.

The light requirement of the species for germination (author's unpublished work) ensures that the seeds will germinate successfully on or near the soil surface, when other conditions are favourable for seedling emergence. In the present study, the seeds were sown at 0.5cm depth and showed positive result. Similar results have been earlier reported for Himalayan herbs (5,14,16)

Germination of seeds may be inhibited if the amount of water is too low or if it is too much (9). In case of *S. chirayita*, healthy and robust form of the species in open slopes compared to flat lands in natural habitats suggested that the water requirement of the species is very low (present author's personal observation). Therefore, beside appropriate substrate combinations, proper irrigation in terms of volume and timing is also essential to maximize production of high quality plant material and optimum percent survivability. Warren and Bilderback (41) suggests an early morning hour (before 10.00 hrs) as suitable time to irrigate plants to reduce potential of wind blowing the irrigation water from the targeted area and to reduce evaporation of irrigation water. Irrigation in the form of mist has resulted in high percent emergence, as the seeds were protected from being swept away by the force of the water due to its minute size, thereby giving high survivability in *S. chirayita* in the present study.

Identifying appropriate morphological traits helps in assessing plant health and productivity (12). For example, strong correlation of collar diameter to total seedling biomass suggested that these traits are of great use in pre-assessing crop productivity. The plant height could not be considered for identifying seedling vigour in *S. chirayita* as in the case of *Angelica glauca* (12) because, for the first two years the *S. chirayita* plants remains in rosette form and merely exceeds 15 cm height. Instead, collar diameter provides a general idea about the seedling durability and has been considered as one of the best predictors of field survival and healthy growth (36), which is supported by significantly strong and positive correlation between collar diameter and total seedling dry biomass ($r$ = 0.864; $p<0.0001$) and between collar diameter and SVI ($r = 0.841$; $p<0.0001$) in case of *S. chirayita* in present study.

## Conclusion

Our study concludes that substrate 2 (garden soil, sand, farm yard manure in equal proportion) is the most effective substrate combination resulting in high percent emergence, uniform and vigorous seedlings and high field plant survival after transplantation. In addition, the collar size appeared to be the strongest morphological trait by which the seedling vigorousness could be identified, as an aid to successful *ex-situ* cultivation of high value medicinal crop, *Swertia chirayita*.

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ARTICLE

# Chemical Treatments to Improve Seedling Emergence and Vigour using Seeds from Six *ex-situ* Source in *Swertia chirayita*, a Critically Endangered Medicinal Herb in Himalaya

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***Swertia chirayita*** **(Gentianaceae), a critically endangered medicinal herb in Himalaya, is recommended at the top amongst other prioritized species, for its conservation through *ex-situ* cultivation by an international forum of experts. We examined the effects of identified pre-sowing chemical treatments on seedling emergence and vigour using seeds obtained from *ex-situ* raised plants under natural conditions (4 sources) and nursery conditions (2 sources), with an aim to fulfill ever increasing demand of the pharmaceuticals, and to support *in-situ* species recovery programme. Seedling emergence was promoted from 6% (control) to 69% ($GA_3$ 250 µM). The seedling emergence rate was faster in $GA_3$ (250 µM) followed by $NaHClO_3$ (5 min) in all the seed sources. MANOVA indicated significant effect ($p < 0.0001$) of treatments on all seedling emergence variables; seed sources and treatments had significant ($p < 0.0001$) effect on different seedling growth parameters. Collar diameter appeared as the strongest morphological trait, which can be used in identifying vigorous seedlings for plantation.**

*Keywords:* *Ex-situ* conservation, gibberellic acid, medicinal herb, sodium hypochlorite, *Swertia chirayita*.

## Introduction

*In-situ* conservation within protected habitats must remain the primary means to conserve plant species. Plants are very sensitive to the environmental changes around them. The habitat disturbance and change in habitat conditions, ultimately leading to habitat loss, due to undesirable growth of unwanted plant species over important plant species, devoid them of required ecological conditions. This would result in fragmentation of the population thereby restricting its distribution to smaller pockets and finally wiping the species from the entire area. At this crucial point, *ex-situ* mechanism plays an important role in conserving endangered plant species (Badola and Pal, 2002; Pradhan and Badola, 2010b) through their recovery, habitat rehabilitation and restoration. *Ex situ* conservation helps to provide the flexibility in responding to unforeseen environmental changes. The risk of extinction of threatened species in nature can be reduced by reinforcing the *ex-situ* raised individuals (Bowes, 1999). Expensive techniques may not be essential for successful seed germination and developing healthy individuals for some species, but identifying suitable technique, based on species requirements, would minimize the wasting of the resources (Fay, 1994; Benson *et al.*, 2000; Butola and Badola, 2004a, 2007; Pradhan and Badola, 2010b). For raising mass planting material in species conservation programme, especially for Himalayan herbs, the best option would be the production of uniform and vigorous seedlings using identified pre-sowing chemical treatments (Butola and Badola, 2004a; 2006, 2007). Also, high and rapid germination determine good stand establishment which results in higher yields. Therefore, fast and uniform germination is equally important for superior crop production (Afzal *et al.*, 2002).

The genus *Swertia* (Gentianaceae) is represented by over 170 species globally, out of which over 40 species occur in Indian Himalayan region and 13–14 species are recorded in Sikkim Himalaya (India). Amongst them, *Swertia chirayita* is of great importance for its several biologically important compounds

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(Singh, 2008) and its multiple utilities in local medicine (Pradhan and Badola, 2008a,b, 2010a,b). Species bears high pharmaceutical value in national and international markets (Badola and Pal, 2002; Pradhan and Badola, 2010a). Plant possesses anti-inflammatory, hypoglycemic, anti-fungal, anti-bacterial, anti-malarial, anti-oxidant (Joshi and Dhavan, 2005) and anti-pyretic (Bhargava *et al.*, 2009) properties; and can be used as natural larvicide (Balaraju *et al.*, 2009). *S. chirayita* is considered to be a bitter tonic, febrifuge and laxative and is used in fever, burning of body, intestinal worms, skin diseases, etc., in a variety of forms and in combination with other medicines. In ancient Indian literature, *Mādhavachikitsā* text, chapter *Jvarachikitsā*, one of the most important post *Caraka Sushruta Samhita* (*Caraka Sushruta* era: between 5th and 10th century) mentions effectiveness of *S. chirayita* in *Jvara* or fever (Mishra, 2009). As entire plant possesses medicinal properties, *S. chirayita* is uprooted unsustainably in wild at very early stage of development or before seeds are fully matured. Such human pressures have led to its endangerment in nature and has been assessed as critically endangered (Ved *et al.*, 2003; IUCN, 2008). Amongst the prioritized species, *S. chirayita* is further recommended at the top for its conservation through *ex-situ* cultivation (Badola and Pal, 2002). For both strengthening *in-situ* conservation and promoting *ex-situ* cultivation, availability of standardized propagation techniques and quality planting material of targeted species is a prerequisite (Butola and Badola, 2004a, 2006, 2007). To maintain sustainability of genetic stock (seeds), it would be equally important to use seeds from *ex-situ* sources (Butola and Badola, 2007; Pradhan and Badola, 2010b)

The present study was carried out with the aim to fulfill ever-increasing demand of the pharmaceuticals and to strengthen *in-situ* species recovery programme by developing and standardizing suitable technique to improve seedling emergence and raise vigorous and mass scale planting material for *S. chirayita*. The objectives of the present study were: (i) to examine the effect of earlier laboratory tested and identified best pre-sowing chemical treatments on nursery seedling emergence, emergence rate, growth and biomass; (ii) to examine variability amongst seed sources on above parameters in nursery conditions, and (iii) to identify appropriate morphological traits in screening vigorous seedlings in *S. chirayita*.

In the present work, we chose two earlier tested best chemical treatments in improving seed germination potential in a seed germinator, using the same *ex-situ* sources for obtaining seeds, as a continuum (second phase) of earlier work (Pradhan and Badola, 2010b) in nursery condition with addition of assessment on seedling growth and vigour in *S. chirayita*.

## Materials and methods

### *Seed collection*

In December 2007, mature seeds of *S. chirayita* (Roxb. ex Fleming) H. Karst. were harvested from six experimental set ups, viz. four in natural habitats (shrubberies, forest-slope, open-slope and tree-canopy) and two in nursery conditions, on soil amended beds using garden soil and forest humus (open net-shade and temperature controlled greenhouse; 25°C ± 5°C) established by us (Pradhan and Badola, 2010b) at Pangthang-Gangtok (Sikkim), India (*ca.* 2000 m; 27°21′N and 088°34′E). Seeds were collected from all six *ex-situ* sources, targeting over a dozen plants in each source. Seeds were mixed well individually for each source, room dried for 15 days and stored in air tight specimen tubes at 4°C until the experimentation.

### *Experimental design*

During May 2008, seeds from all the six sources were provided with the best chemical treatments, which we had earlier tested and identified in laboratory on germination in *S. chirayita* seeds collected from the same plots (Pradhan and Badola, 2010b), i.e. gibberellic acid ($GA_3$ 250 µM) and sodium hypochlorite ($NaHClO_3$ 5 min soaking time; 5% available chlorine), out of 11 treatments, i.e. $GA_3$ (50, 150, 250, 350 µM); $KNO_3$ (50, 150, 250 mM) and $NaHClO_3$ (5, 10 and 15 min soaking time) including control. In the previous experiment (Pradhan and Badola, 2010b), all four concentrations of gibberellic acid resulted in high percentage of seed germination and $GA_3$ (250 µM) most effectively stimulated seed germination for majority of the sources (open slope, tree canopy, net shade, green house); and amongst three concentrations used for $NaHClO_3$, the 5 min soaking time gave best results; while all the three concentrations of $KNO_3$ were less effective in stimulating seed germination, except for shrubberies, due to which $KNO_3$ was not considered in the current experiment. The seed lots from

each source were divided into three sets containing 150 seeds/set/source. The first set of seeds was soaked in $GA_3$ (250 µM) for 24 h and the second set was soaked in $NaHClO_3$ for 5 min. The third set was soaked in double distilled water (DDW) for 24 h which served as control. The soaked seeds for each set were washed thoroughly with DDW and sown (0.5 cm depth in rows; maintaining 5 cm distance between each seed in three replicates; 50 seeds each per replicate) in nursery trays containing a mixture of garden soil, sand and crushed farm yard manure (pH 7.02) in equal volume. The experiment was performed under the net-house (temperature, max: 25.04°C, min: 13.22°C; relative humidity, max: 91%, min: 65%) located in the Institute campus at an altitude of *ca* 2000 m in a completely randomized design. Prior to seed sowing, the soil mixture was wetted with water in order to avoid washing away of seeds from the trays. Taking into account the importance of soil moisture in seedling emergence, careful watering was done according to the necessity.

Daily observation on seedling emergence was made using a powerful hand lens for 60 days. To avoid competition, thinning was done after 16 weeks of seed sowing, maintaining maximum distances between each seedling (10–12 cm). After 24 weeks of seed sowing, 5 seedlings for each treatment per seed source from experimental trays were randomly harvested and assessed for growth parameters, including the total dry biomass. As the plant remains in the rosette form for consecutive two years (pers obs), the plant height was not taken into consideration. The root length was determined from the root collar to the tip of the primary root. The collar diameter was measured using a digital calliper (Mitutoyo, Japan). The dry biomass was determined by oven-drying seedlings to constant weight at 70°C.

### *Statistical analysis*

Multivariate analysis of variance (MANOVA; $\alpha = 0.05$) in general linear model (GLM) was carried out to determine the effect of seed sources, treatments and their interaction on seedling emergence variables and morphological traits using SPSS 10.0 software (SPSS Inc. 1989). A Bonferroni test ($p < 0.05$) was used to determine the differences amongst mean values for different treatments. Mean days for emergence ($M_d$) was calculated according to the equation of Edmond and Drapala (1958) as: $M_d = \sum nd/\sum N$, where $n$ is the number of newly emerged seedlings in each day $d$, and $N$ is total number of seedlings emerged at the end of the test. Seedling emergence rate was calculated using modified Timson index of germination velocity by Khan and Ungar (1984) as: $T = \sum G/t$, where $G$ is percentage of seeds emerged after two days interval and $t$ is the total emergence period.

To identify the most effective treatment for producing healthy seedlings, seedling vigour index (SVI) was calculated following Butola and Badola (2004a), $SVI = (d/M_d) \times 100$, where $d$ is dry weight/seedling; $M_d$ is seedling mean days of emergence and 100 is constant value. In order to analyse the relationship between different morphological traits and to identify convenient morphological traits to select vigorous seedlings, Pearson's correlation ($r$) analyses were performed by pooling data from all the treatments and the relationship amongst different seedling traits was examined.

## Results

### *Seedling emergence*

Table 1 shows the result of multivariate analysis of variance (MANOVA) showing effect of treatments, seed sources and their interaction on different seedling emergence variables. Different treatments showed significant effect on all the seedling emergence parameters ($p < 0.0001$).

Seedling emergence in all the sources and treatments including control started within 18 to 22 days of seed sowing and maximum number of seedlings emerged within 56 days of sowing. Both $GA_3$ (250 µM) and $NaHClO_3$ (5 min) highly stimulated the seedling emergence in all seed sources over control; however $GA_3$ was more effective than $NaHClO_3$ (Figure 1). Over 59% seedling emergence was recorded with $GA_3$ in all the seed sources and the maximum latitude in stimulation was recorded in seeds sourced from forest slope, where seedling emergence was promoted from 6% (control) to 69% ($GA_3$). Comparatively, 40% seedling emergence was the maximum value recorded in seeds (green house) treated with $NaHClO_3$.

Seedling emergence rate was faster in $GA_3$ followed by $NaHClO_3$ in all the seed sources (Table 2). With

**Table 1.** Results of MANOVA showing effect of different seed sources, treatments and their interaction on seedling emergence variables in *Swertia chirayita*.

| | Independent variable | | | | | |
|---|---|---|---|---|---|---|
| | Source (S) | | Treatment (T) | | S × T | |
| Dependent variable | F | Sig. | F | Sig. | F | Sig. |
| Seedling onset time (days) | 6.902 | 0.000 | 9.581 | 0.000 | 2.633 | 0.016 |
| 50% emergence time (days) | 6.784 | 0.000 | 17.562 | 0.000 | 3.887 | 0.001 |
| Final emergence time (days) | 10.176 | 0.000 | 456.074 | 0.000 | 8.152 | 0.000 |
| Seedling emergence (%) | 2.526 | 0.046 | 689.359 | 0.000 | 2.622 | 0.017 |
| Mean days of emergence (days) | 9.231 | 0.000 | 103.197 | 0.000 | 9.558 | 0.000 |
| Emergence rate | 0.954 | 0.458 | 378.929 | 0.000 | 1.026 | 0.442 |

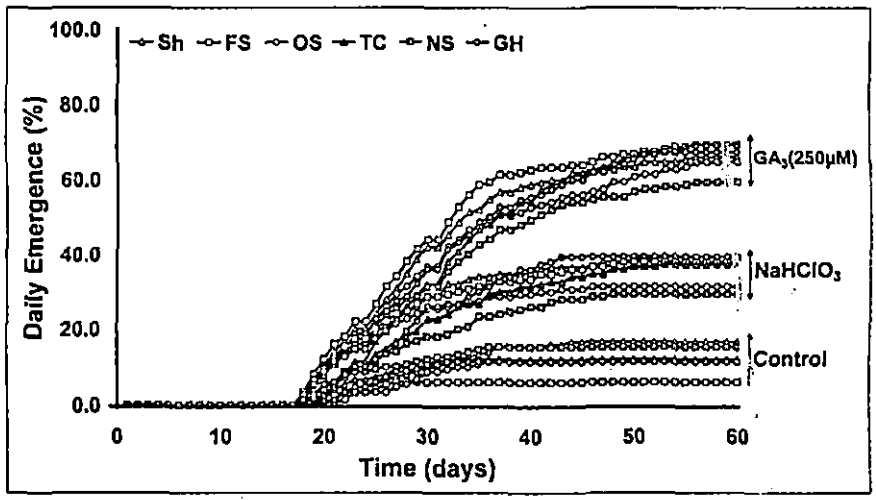


**Figure 1.** Effect of different seed sources and pre-sowing chemical treatments on seedling emergence in *Swertia chirayita*. *Sh:* shrubberies, *FS:* forest slope, *OS:* open slope; *TC:* tree canopy; *NS:* net shade; *GH:* green house.

$GA_3$, amongst six sources, the seedling emergence was faster for green house ($11.53 \pm 0.47$) and slower for net-shade ($9.51 \pm 2.39$), comparatively. With $NaHClO_3$, the seedling emergence was faster for tree canopy ($4.52 \pm 1.35$) and shrubberies ($4.51 \pm 0.93$) compared to other sources.

### *Seedling growth and biomass*

MANOVA revealed significant ($p < 0.0001$) effect of seed sources and treatments on all the seedling growth parameters; however, the effect of their interaction was non-significant for number of leaves and root diameter (Table 3). Amongst the seed sources, forest slope resulted in significantly ($p < 0.05$) higher values for root length, total plant dry biomass and seedling vigour index compared to other sources (Figures 2–4). Significantly ($p < 0.05$) minimum seedling growth was achieved for shrubberies. Significantly ($p < 0.05$) lesser number of leaves was recorded for the seed sourced from open slope compared to other sources. $GA_3$ and $NaHClO_3$ treatment significantly ($p < 0.05$) enhanced all the seedling growth parameters over control; however the effect of $NaHClO_3$ was in-significant for number of leaves. On comparison, $GA_3$ appeared significantly ($p < 0.05$) more effective over $NaHClO_3$ treatment for all the growth parameters including total plant dry biomass and seedling vigour index. All the

**Table 2.** Index of emergence rate in *Swertia chirayita*, using a modified Timson Index of germination velocity.

| | Treatment | | |
|---|---|---|---|
| Source | Control | $GA_3$ (250 µM) | $NaHClO_3$ (5 minutes) |
| Shrubberies | $1.27 \pm 0.35^{a}$ | $10.20 \pm 0.75^{a}$ | $4.51 \pm 0.93^{a}$ |
| Forest slope | $0.20 \pm 0.17^{b}$ | $10.34 \pm 1.38^{a}$ | $4.43 \pm 0.47^{a}$ |
| Open slope | $0.48 \pm 0.13^{bc}$ | $11.33 \pm 1.49^{a}$ | $3.27 \pm 0.96^{a}$ |
| Tree canopy | $0.69 \pm 0.19^{ab}$ | $11.08 \pm 2.53^{a}$ | $4.52 \pm 1.35^{a}$ |
| Net shade | $1.02 \pm 0.13^{ac}$ | $09.51 \pm 2.39^{a}$ | $2.99 \pm 0.69^{a}$ |
| Green house | $0.54 \pm 0.23^{bc}$ | $11.53 \pm 0.47^{a}$ | $4.28 \pm 0.65^{a}$ |

Values in each column with the same superscript are not significantly different at $p > 0.05$, Bonferroni test (±: standard deviation).

**Table 3.** Results of MANOVA showing effect of seed sources, treatments and their interaction on different growth parameters in *Swertia chirayita*.

| | Independent variable | | | | | |
|---|---|---|---|---|---|---|
| | Source (S) | | Treatment (T) | | S × T | |
| Dependent variable | F | Sig. | F | Sig. | F | Sig. |
| Collar diameter | 40.429 | 0.000 | 99.950 | 0.000 | 3.839 | 0.000 |
| Leaf number | 9.842 | 0.000 | 11.358 | 0.000 | 0.626 | 0.787 |
| Largest leaf length | 78.377 | 0.000 | 95.652 | 0.000 | 7.250 | 0.000 |
| Largest leaf width | 24.729 | 0.000 | 26.920 | 0.000 | 4.089 | 0.000 |
| *Root diameter* | 13.780 | 0.000 | 12.200 | 0.000 | 0.662 | 0.755 |
| Root length | 138.446 | 0.000 | 182.174 | 0.000 | 4.929 | 0.000 |
| Seedling dry biomass | 311.877 | 0.000 | 262.684 | 0.000 | 43.899 | 0.000 |
| Seedling Vigour Index | 328.761 | 0.000 | 148.523 | 0.000 | 33.030 | 0.000 |

plant growth parameters showed significant correlation with each other except number of leaves (Table 4).

## Discussion

In a species, variation in germination behaviour between sites (Baskin and Baskin, 1998; Ceraboloni *et al.*, 2004; Pradhan and Badola 2008a) is applicable not only to the natural populations but also to the *ex-situ* produced seeds under different conditions (Pradhan and Badola, 2010b). The present study, with *ex-situ* produced seeds in *S. chirayita*, indicates the germination variability amongst the seed sources in addition to existence of physiological dormancy (Pradhan and Badola, 2010b).

Both $GA_3$ (Mehanna *et al.*, 1985; Tipirdamaz and Gomurgen, 2000; Butola and Badola, 2004ab, 2006; Chandra *et al.*, 2006) and $NaHClO_3$ (Fieldhouse and Sasser 1975; Hsiao 1979; Drew and Brocklehurst 1984; Bewely and Black, 1994; Butola and Badola, 2004ab, 2006, 2007, Pradhan and Badola, 2010b) are known for their stimulatory action on seed germination, breaking seed dormancy and increasing germination rate. In the present study, both $GA_3$ (250 µM) and $NaHClO_3$ (5 min) treatments were stimulatory to seedling emergence and over all seedling growth for all six *ex-situ* seed sources. The commencement of seedling emergence was significantly faster ($p < 0.05$) in $NaHClO_3$ treatment followed by $GA_3$ over control. Such differences in seedling emergence between treated and untreated seeds might be due to alteration of physiological process by the liberated enzymes inside the embryo (Kattimani *et al.*, 1999). Although the mean days of seedling emergence were higher in $GA_3$ than $NaHClO_3$, the faster seedling emergence rate for $GA_3$ indicated it as more effective treatment than $NaHClO_3$ (Table 2).

$GA_3$ proved to be more effective than $NaHClO_3$ in stimulating seedling emergence in *S. chirayita* in all six *ex-situ* seed sources (Pradhan and Badola, 2010b) in the present study, however, higher effectiveness of $NaHClO_3$ over $GA_3$ was observed in case of *Angelica glauca* and *Heracleun candicans* (Butola and Badola, 2004a, 2006). In the present study, greatly stimulated seedling emergence by using higher concentration of

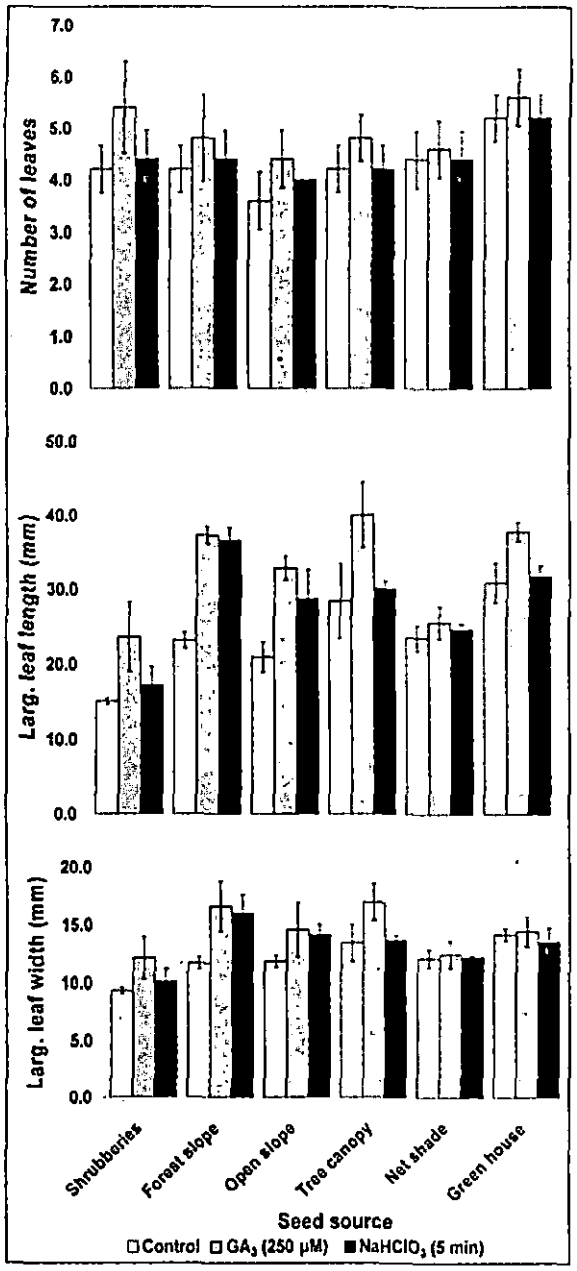


Figure 2. Effect of pre-sowing chemical treatments on number of leaves, largest leaf length and width in *Swertia chirayita* (error bars: standard deviation).

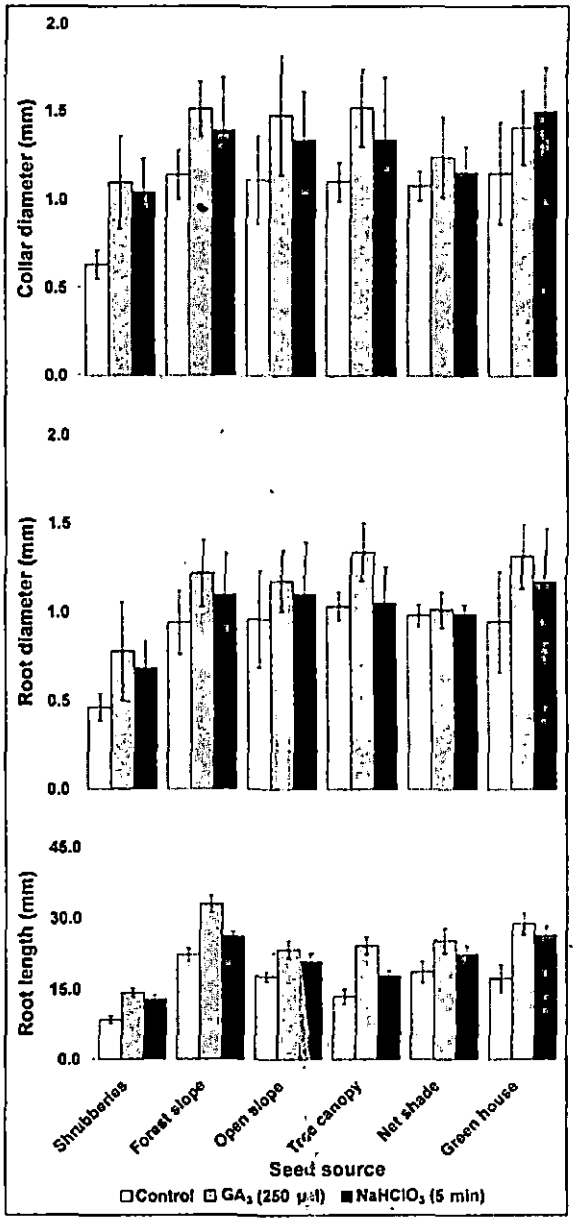


Figure 3. Effect of pre-sowing chemical treatments on collar diameter, root diameter, root length in *Swertia chirayita* (error bars: standard deviation).

Scarification or bleaching of seed coat by $NaHClO_3$ (Vujanovic *et al.*, 2000; Bohm, 2003) or other modification in seed coat (Hsaio, 1979) may not be the cause of stimulation of seedling emergence in *S. chirayita*, as the delicate seeds are enclosed in very thin seed coat which do not need extra physical effort in promoting germination. It may be due to either increase in permeability of seed coat to oxygen through the removal of phenolics (Hurley *et al.*, 1989) or release of oxygen with decomposition of $NaHClO_3$, thus enhancing oxidative respiration, thereby, promoting seed germination (Bewely and Black, 1994; Vujanovic *et al.*, 2000).

Other factors which might be responsible for stimulation of seedling emergence by $NaHClO_3$ are decomposition of germination inhibitors (Mackinnon and Alderton, 2000), overcoming dormancy by lowering of pH thereby promoting oxygen uptake (Bohm, 2003), or promoting an enhancement of α-amylase activity (Kanecko and Morohashi, 2003). Soaking seeds in $NaHClO_3$ for lesser time (5 min) appears relatively useful to achieve successful recruitment of *S. chirayita*. $NaHClO_3$ not only protects the seeds from pathogens in nursery conditions but also facilitates leaching of toxic compounds and improves the uptake of water and oxygen contributing to increase seed germination or seedling emergence.

In the present study, $GA_3$ application resulted to higher seedling dry biomass and seedling vigour index. This may be due to the fact that the action of $GA_3$ applied before sowing could have sustained itself till the plants reached vegetative stage. Further, this could be related to an elaboration of an endo-membrane system and regulated synthesis of proteins required for germination, which in turn could have contributed additionally to the amino acid rescue and protein turnover during active metabolism, later in plant life (Shah, 2007). $NaHClO_3$ is applied as a suitable disinfectant for seed surface sterilization (Bewley and Black, 1994; Kaneko and Morohashi, 2003) and also known to promote seed germination and overcome seed dormancy (Bewely and Black, 1994). However, the effect of $NaHClO_3$ on seedling growth (Fieldhouse and Sasser, 1975; Chun *et al.*, 1997; Butola and Badola, 2004a, 2006, 2007) and its mechanism has rarely been reported.

All the seedling growth parameters were positively correlated with each other but the correlation was non-significant for some of the growth parameters. Both collar diameter and root diameter though showed significant correlation with all the growth parameters except number of leaves; collar diameter is considered the strongest morphological trait for identifying seedling vigorousness in *S. chirayita* because root being present below ground surface is not conveniently visible. Further, the collar diameter is always larger than the root diameter (pers obs) which does not necessitate going further step to identify vigorous seedlings.

## Conclusions

The present study suggests that stimulatory effect of $GA_3$ (250 μM) (preferably) and $NaHClO_3$ (5 min) on seedling emergence and seedling vigour may solve the problem of increasing pressure on the wild population of species for raw material, as a tool to strengthen *ex-situ* cultivation of *S. chirayita*. Higher seedling emergence rate obtained with $GA_3$ (250 μM) may lead to early establishment of the seedlings, which bear the ability to compete and withstand the rough climate. This could be advantageous during the process of species reinforcement in the nature using individuals raised by *ex-situ* means. While reinforcing the species into the natural habitats, collar diameter should be used for identifying the vigourous seedlings which would not only save time but also minimize the wastage of the resources (Fay, 1994; Benson *et al.*, 2000; Butola and Badola, 2004a). Such step will definitely reduce the risk of extinction of endangered species (Bowes, 1999) like *S. chirayita* in Himalaya.

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