Studies on Seed Biology and Mass Multiplication of Two Medicinally Important Plant Species: *Panax pseudoginseng* W. (Araliaceae) and *Paris polyphylla* Smith. (Trilliaceae)

By

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DECLARATION

I, Mr. Sakutemsu L. Jamir, bearing Ph. D. Registration number **474/2012 dated October 14, 2011** hereby declare that, the subject matter of my thesis entitle **'Studies on seed biology and mass multiplication of two medicinally important plant species: *Panax pseudoginseng* W. (Araliaceae) and *Paris polyphylla* Smith. (Trilliaceae)'** is the record of original work done by me, and that the contents of this thesis did not form the basis for award of any degree to me or to anybody else to the best of my knowledge. The thesis has not been submitted by me for any Research Degree in any other University/Institute.

This thesis is being submitted to the Nagaland University for the Degree of **'Doctor of Philosophy' in Botany**.

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Chapter - 1

General Introduction

According to the UN Convention on Biological Diversity (1992), biodiversity is defined as the variability among living organisms from both the terrestrial and marine along with the ecological complexes in which they plays their part. Biodiversity is the results of the evolutionary plasticity of living organisms which has increased geometrically through perhaps 2.5 billion years, proliferating by trial and error, controlled by natural selection, filling almost every one of the habitable ecological niches created in a likewise evolving world environment. The diversity of these natural habitats has expanded over a period of time through natural selection and also through introductions. Biodiversity includes diversity of form right from molecular level through individual, organisms, populations, communities, ecosystems, landscapes and biosphere. The world harbors an estimated over 10 million different types of organisms including plants, micro-organisms and animals, out of which about 1.4 million species are that of plants including algae, fungi, mosses and higher plants (Myers *et. al.,* 2000).

Biodiversity hotspots are geographical regions which are extremely rich in species, have high endemism, and are under constant threat. Recently, the existing 25-hotspots of the world have been updated to 34 hotspots with the list of 9 new hotspots in the great range of the Himalayas and the island nation of Japan. India is a home for 4 of them extending into neighboring countries-the Western Ghats/Sri Lanka, the Himalaya, the North-East and the Nicobar Island (MoEF, 2014) and they figure in hottest hotspots. The determining criteria for a hotspot is that an area must contain at least 0.5% or 1,500 of the world's 300,000 plant species and 27,298 vertebrates (including mammals, birds, reptiles and amphibians) as endemics, and the remaining primary vegetation is less than 30% of its extent area (Sala *et. al*., 2000).

India being one of the 17-mega biodiversity centers having about 10% of the world's biodiversity wealth, which is distributed across 16 agro-climatic zones. India is a tropical country with a tremendous heterogeneity of environments ranging from tropical rain forest of Andaman and Arunachal Pradesh to the hot deserts of Rajasthan and cold desert of Ladakh. It lies at the junction of three biogeographical provinces of Africa, temperate Eurasia and the Orient. Out of 17,000 species of higher plants reported to occur within India, 7500 are known to have medicinal uses. Ayurveda, the oldest medical system in the Indian Subcontinent, has alone reported approximately 2000 medicinal plant species, followed by the Siddha and Unani medical system (Kala and Sajwan, 2007). The practice of ethno-medicine is an important vehicle for understanding indigenous societies and their relationships with nature (Anyinam, 1995). According to the World Health Organization (WHO) as many as 80% of world's population depends today on traditional medicine for their primary health care needs (Azaizeh *et. al*., 2003).

The Indian Himalayan Region (IHR) with 250-300 km across stretches over 2,500 km from Jammu & Kashmir in the west to Arunachal Pradesh in the east spreading between 21˚57' – 37˚ 5' N latitudes and 72˚40' – 97˚25' E longitudes. This great chain of mountains covers the frontiers of Myanmar in the east partially/fully the twelve states of India including Nagaland. Northeast India which is a centre of mega-biodiversity contains more than one-third of the country's total biodiversity. It lies between 22˚9' - 29˚6' N latitude and 87˚7' E longitude. North-East India is the most important floristic region owing to its rich biodiversity and high endemism. The region is one of the 17 hottest hotspots of the world, having at least 7,500 flowering plants. Out of 315 families of angiosperms in India, more than 200 are represented in Northeast India; which accounts for nearly 50% of the total number of plant species in India as a whole. Northeast India lies in the lowland-highland evolution zone as it is the meeting place of the Himalayan mountain and the Peninsular India and because of these geographical transitional zone, it has diverse biomes or ecological communities contributing to the immense richness in its biodiversity (Chakraborty *et. al.*, 2012). For this rich biodiversity, North-East region was named, *'The Cradle of Flowering Plants'* (Takhtajan, 1969). However, though it is known for its diverse and extensive lush forest cover and species composition yet it is one of the major regions facing severe deforestation. The Northeastern Hill region, comprising Nagaland, Manipur, Mizoram, and Tripura, is part of the Indo-Burma hotspot, which has a wide variety of ecosystems, including hilly tracts. The Indo-Burma biodiversity hotspot comprises around 7,000 endemic plant species. Around 8,550 floral species have been recorded from this region, of which 2,500 are from Manipur, 2,250 from Nagaland, 2,200 from Mizoram, and 1,600 from Tripura. Though most of the species are of economic importance, some of the species plays a vital role in rural economy and used as medicines, vegetables, construction materials, as dye etc. Some of the important economically plant species are: *Sapria himalayana, Nepenthes khasiana, Rhododendron* species, *Aloe vera, Dioscorea* species, *Aconitum nagarum., Malaxis acuminate, Panax pseudoginseng, Paris polyphylla, Stobilanthes flaccifolious, Taxus bacata, Berberis* sps., *kiwi, Lassia spinosa, Livistona jenkinsiana, Distemon indicum, Acorus calamus, Tricopus zeylanicus* etc. Due to the varied environmental conditions found in the North east region part of India, diverse species of both plant and animal species can be found. Forest is an important natural resource which is vital in meeting the daily requirements of the people but due to over-exploitation, it has cause imbalances in the ecosystem and loss of its rich biodiversity. This limits the plant recruitment processes and ecosystem productivity, altering the overall ecosystem functioning (Symstad and Tilman, 2001).

Nagaland which lies between 25˚06'–27˚04' N latitude and 93˚20'–95˚15’ E longitudes with an area of 16,579 sq. Km is among the richest bio diverse region in the world and it lies in the Indo-Burma biodiversity hotspots. The geographical location of Nagaland has provided an ideal environment for the growth and nourishment of different medicinal plant species. Unfortunately the plant genetic resources of the region are fast depleting due to indiscriminate felling of forest trees including ground vegetations for ‘Slash and Burn/Shifting Cultivation’ together with ruthless exploitation of plants for trade, poor knowledge of the people on conservation measures and unplanned human activities. In the recent years, extinction has been the destiny of a great number of plant species including several unique and irreplaceable varieties, while many await a similar fate.

According to IUCN, plant species are declining in South and Central America, Central and West Africa and Southeast Asia Malaysia has the most threatened species (681) followed by Indonesia (384) (IUCN, 2000). Globally, the number of threatened plants listed is 5,611, but this is based on an assessment of only 4% of the world's described plants, which suggest that the percentage of threatened plants may be much higher. Recent decades have seen significant changes occurring within several aspects of ethno-medicine as a result of environmental degradation and tremendous changes in modern, social, and economic systems (Anyinam, 1995).These factors in totality resulted in disappearance of ethno medicinal plants at regional as well as global scale (Baillie *et. al*., 2004). Ethno medicines are of particular relevance in developing countries like India (Ali, 1999; Jamir *et. al.*, 1999; Sharma *et. al.*, 2001; Buragohain, 2008; Ignacimuthu *et. al*., 2008), where modern health service is limited. The North Eastern Region (NER) of Indian Himalayan region (IHR), comprising of 8 states harbors more than 180 major tribal communities out of the total 427 tribal communities found in India (Sajem *et. al*., 2008). A large part of the NER of India is comparatively botanically underexplored or even unexplored (Jamir *et. al*., 1999; Sharma *et. al*., 2001). Agriculture is the major livelihood option in India as only cultivators and agricultural laborers comprise more than 58.4% of total workforce in the country. The people of the Himalayan region, especially those in the north-east, over the years had adopted traditional practices of replenishment in the region The '*Jhum*' cycle was once considered to be as long as 25 years, but in the recent past, studies have shown that the cycle has shrunk to as short as 4-5 years which resulted in acceleration in soil erosion. This is a strong indicator of the deteriorating ecological balance of the region and is also a statement on the increasing human pressure on land and growing food needs.

Due to all the above factors plant species of this region have become endangered, threatened or rare plants and many workers have put their efforts in conserving this important plants through investigating single or combined components of the reproductive ecology of such plants, such as their flowering frequency and vegetative reproduction (Morely, 1982), pollination (Macior, 1978; Karron, 1987; Deyrup and Menges, 1997), breeding system (Planisek, 1983; Karron, 1989), seed predation (Gisler and Meinke, 1997), seed germination (Baskin and Quarterman, 1969; Baskin and Baskin, 1979, 1997; Halse, 1988; Jacobs, 1993; Clark *et. al.*, 1997; Florance, 1997), breeding system and Germination (Clampitt, 1987; Menges, 1995), and seed production and predation (Menges *et. al.*, 1986). However, few studies of these plants describe the limits to reproduction at all stages from flower production through dispersal of germinable seed (Crowder, 1978; Massey and Whitson, 1980; Pavlik *et. al.*, 1993).

Plants as a Source of Medicines

Nature has provided a rich store house of herbal remedies to cure all mankind's ill. Throughout the world people have utilized several thousands of plants and plant products as cure for human ailments. In the plant kingdom, almost all plants are medicinal and the application of medicinal plants especially in traditional medicine is well recognized (Chaudhary and Tariq, 2006). In the developing countries, drugs are not only expensive, but also have many side effects during treatment for any disorders that is why in the present era much emphasized on search of medicinally valuable plants are prominent.

India has one oldest, richest and diverse cultural traditions associated with the use of plants and herbs for human, livestock and plant health. Many of the ingredients of Indian cooking which have been handed down from ages contain medicinal properties (Chakraborty and Das, 2010). In India, the '*Ayurvedic System of Medicine*' has been in use for more than 3000 years. '***Charaka & Susruta***' two of the earliest Indian authors had sufficient knowledge of the properties of the Indian medicinal plants. ***'Charaka Samhita'*** **and '*Susruta Samhita*'** which are their medical works are one of the esteemed treasures of literature of indigenous medicine today (Deb *et. al.*, 2009).

Plants have been used in the traditional health care system from the time immemorial, particularly among tribal communities. The World Health Organization (WHO) has listed over 20,000 medicinal plants globally (Laloo *et. al*., 2006); India’s contribution is ~20%. According to WHO estimate, about 80% of the population in the developing countries depends directly on plants for their sole source of medicine (Deb *et. al.*, 2009). In India, about 2000 drugs used are of plant origin (Dikshit, 1999). Though a vast ethno botanical knowledge exists in India from ancient times, very few plants used by locals for medicine are subjected to scientific investigation. The need for conservation of medicinal plants and traditional knowledge, particularly in developing countries like India, taking into account the socio-cultural and economic conditions is urgent (Misra, 1999).

The maximum diversity of medicinal plants in the IHR exists in altitudes of more than 1,800 m and which accounts for ~1400 species. Human habitations with diverse cultures and communities present contribute to their immense knowledge of plant species used in medicine. Thus the practical and uses of ethno-medicinal plants is comparatively higher in the IHR (Samant and Dhar, 1997). Apart from the use of species as medicine, different parts such as roots, tubers, fruits, flowers, seeds and leaves of over 200 species of medicinal plants are consumed either raw, roasted, boiled, fried, cooked or in the form of oil, spice and seasoning materials, jams, pickles, etc. (Dhyani and Khali, 1993; Samant and Dhar, 1997). The nutritional value of most of these species is comparable with cultivated species (Dhyani and Khali, 1993; Samant and Dhar, 1997).

Importance of Floral Biology

There are several important aspects, vital for a clear understanding of floral biology, such as morphology, phenology, the reproductive system, pollination and fertilization. Studies on reproductive ecology are also important for increasing comprehension on features of ecological equilibrium existent in nature, how plants evolved on the planet, and how animals, mainly bees, manage to survive through making use of flowers. Floral biology, through its reproductive system, blends with the ecology of pollination, thereby relating floral diversity with the behavior and morphology of visiting and pollinating animals (Waser, 1983) thus demonstrating their mutual dependence, since most plants rely on these agents for sexual reproduction. Furthermore, floral resources constitute the main food supply for several animal groups (Proctor *et. al.*, 1996). The interaction of plants with pollinating agents is vital for the structural and functional integrity of natural ecosystems, besides the maintenance and increase in the production of food for human consumption. It is believed that three quarters of the plant products thus produced and ninety percent of wild plants, are dependent on animal pollination. Furthermore, the protection of wild species threatened with extinction, the use of plant species in urban environments and the production of fodder for feeding domestic animals are also benefited through understanding floral biology and pollination concepts (Figueiredo, 2000). The system of pollination can serve as an instrument for studying co-evolutionary processes, where the degree of morphological, physiological and behavioral adequacy observed between plant and pollinator can be evaluated (Proctor *et. al.*, 1996). In this context, aspects concerning floral morphology and visiting behavior, as well as those referring to flower physiology and morphology, could serve as parameters for measuring the degree of relationship between plant species and their floral visitors, as well as for discerning which among them effectively contribute to plant reproduction (Faegri and Van der Pijl, 1979; Proctor *et. al*., 1996; Figueiredo and Sazima, 2007).

Importance of Reproductive Biology

Reproductive biology is an important tool in working out the conservation strategy. The reproductive performance of the plants and its efficiency determines its survival and therefore knowledge on reproductive biology is the key factor in the achievement of plant conservation (Kumar *et. al*., 2011). Flowering is a pre-condition for fruiting with seed(s) but many flowers results in poor seed production especially those angiosperm plant species which have small flowers and large fruits. Reproductive bottlenecks includes failure of pollination, pre or post fertilizer barriers leading to no or poor seed set, poor reproductive vigor due to inbreeding depression and very low germination rates imposed constraints on the multiplication and survival of the species. Therefore, any conservation approach has to be based on an in-depth study of plant reproductive biology which provides information on seed germination capacity, survival rate of seedlings and adults, age at flowering, reproductive lifespan and number of flowers and seeds, seed biology. Such studies would prove to be fruitful in planning various programmes specific to different habitats. The knowledge combined with clonal propagation will also help in developing strategies to preserve the genetic potential of rare species crucial for reintroduction.

Plant morphological and biological characteristics may limit its population growth in the wild and therefore macro propagation of those plants are important *ex situ* so as to introduce them back to the wild and ensure its stability in its number of species. Studies on plant reproductive biology, seed biology and macro-propagation and reintroduction to the wild of medicinally important plant species will ensure continued presence of these plants in nature.

Importance of Seed Biology

Recruitment of tree species on the forest floor or in the field is governed by various factors including the seed traits and microhabitat conditions (Kitajima, 2007). Likewise, germination of seeds in nature is strongly influenced by internal as well as external environmental factors (Baskin and Baskin, 1998). The germinating seeds and seedlings are most vulnerable to predation, desiccation, developmental stage of embryos, competition and damage as the seeds and seedlings constitute important resource for the herbivores and pathogen. Seedling predation and disturbance in the form of trampling and microhabitat alternation have direct implications for recruitment and multiplication of species. Consistency in microhabitat condition, right developmental stage of the embryos in the harvested seeds, right desiccation of the seeds and duration of post harvest storage of seeds enhances seedling survival and plantlet growth. One of the important microhabitat factors in the forest floor is light regime which is governed by the tree canopy cover. Tree species vary considerably in terms of light requirement at seedling stage (Pacala *et. al.*, 1994; Davis, 2001). Germinating seeds, depending upon their state such as inherent properties, developmental stage, reserve food materials, moisture content, nature of pericarp, represents their own microenvironments which have rarely been studied. In some species even if one of the factors are not favorable, it may result in initial seed germination but seedlings often fails to establish in the seed bed and subsequently in the field.

Many forests have a high number of species with recalcitrant seeds, or seeds sensitive to desiccation. The intolerance to desiccation leads to difficulties in conserving those seeds (Barbedo and Cicero, 2000). Many programmes that involve the rational and economic use of these species are hampered due to the lack of storage methods of recalcitrant seeds for longer periods. The methods in use preserve such seeds for periods varying from days to months. Thus, inclusion of several species in those programmes is limited. Identification and conservation of recalcitrant seeds as well as studies increasing their tolerance to desiccation were performed and pointed out respectively by several authors (Berjak *et. al.*, 1990; Pammenter and Berjak, 1999; Pammenter *et. al.*, 1991; Neves, 1994; Deb *et. al.*, 2012). In general, there is an agreement about the desiccation intolerance of recalcitrant seeds and their short longevity. Moreover, differences between recalcitrant and orthodox seeds at the end of the process of seed formation are well known. The orthodox seeds undergo a nearly essential process of desiccation, at the end of maturation. This process allows these seeds to change their metabolism from development to germinate (Kermode, 1990). These changes are not observed in recalcitrant seeds due to several factors including hormonal balance, protein and sugar contents and water physical properties, among others (Kermode, 1990; Barbedo and Filho, 1998).

The available methods of seed drying are not suitable for recalcitrant seeds, new approaches on seed germination and storage should be matter of investigation. Although it is not possible to define unequivocally '**critical moisture content**' for viability loss in recalcitrant seeds because they show differential response to dehydration at different drying rates, it is necessary to maintain their water level above critical levels (Pammenter *et. al.*, 1998). Under these conditions, however, seeds may initiate germination and or undergo microorganism infection, which could lead to seed deterioration. High humidity favors the initial steps of seeds germination processes causing damage to seeds if water is not supplied in sufficient. Consequently, storage treatments maintaining high moisture of the seeds are often associated with low temperatures (Cunha *et. al.*, 1995). Nevertheless, many of these seeds have little tolerance to cold conditions. Therefore, studies on these factors will give a better understanding on seed storage, seed germination and seedling establishment of the species which produces recalcitrant seeds.

Inside each seed is a living plant embryo that even in a state of dormancy breathes through the exchange of gases and is consistently undergoing metabolic (aging) process. The natural lifespan of a seed is influenced by several factors including: permeability of the seed coat, dormancy, seed physiology and storage environment. Seeds of many of our native plants and weedy alien species have dormant embryos and hard seed coats, a condition that retards germination and consequently enhance longevity. The presence and degree of seed dormancy and subsequent metabolic rates varies considerably between species and thus influences their lifespan. For most species from temperate and arid climates reducing and maintaining a low seed moisture content, storing seeds at low to moderate temperatures, and taking precautions not to damage seeds during cleaning and handling, slows down the metabolic process and thereby increases their longevity in storage.

Seeds are generally categorized into three types:

1. **Orthodox**: Seeds that can be dried, without damage, to low moisture contents, usually much lower than those they would normally achieve in nature. Their longevity increases with reductions in both moisture content and temperature over a wide range of storage environments.

2. **Recalcitrant**: Seeds that do not survive drying to any large degree, and are thus not amenable to long term storage.

3. **Intermediate**: Seeds that are more tolerant of desiccation than recalcitrant, though that tolerance is much more limited than is the case with orthodox seeds, and they generally lose viability more rapidly at low temperature.

Recalcitrant seeds are not only desiccation-sensitive, but also metabolically active. In contrast, orthodox seeds, owing to their dry state, are metabolically quiescent (Berjak, 2005). One can estimate a species' natural potential for storage tolerance by:

1. ***Seed size***: Large seeds often have high moisture content and are generally recalcitrant in their storage behavior.

2. ***Climate and habitat conditions in which the species grow***: Seeds from plants adapted to tropical or riparian habitats, due to a semi to permanent water source and/or consistently mild and reliable growing conditions, may not require long term seed viability for survival. Conversely, plants from desert, temperate climates, where environmental conditions suitable for germination are often infrequent, are more likely to produce seeds capable of surviving for long periods.

3. ***Seed physiology***: A heavy impervious seed coat even on large seeds, as is often found on desert legumes and lupines, promotes long-term seed viability.

4. ***Life cycle***: Annual and perennials are more dependent on a persistent soil seed bank than woody and long-lived shrub and tree species.

5. ***Ecological association***: Plants that are early succession colonizer species that may occur only after disturbances and species that depend on other plants for their development must maintain viability until a suitable host plant is available.

Macropropagation and Clonal Planting Material

India nurtures enormous plant diversity and as many as 45,000 species of plants have so far been recorded in India of which 5285 species of angiosperms belonging to 140 genera are endemic to the country (Moza and Bhatnagar, 2007). At least 10% of India's recorded wild flora are on the list of threatened species, many on the verged of extinction (Singh, 2004). A number of biologically rich areas like North-Eastern regions have not been fully explore and studied even though it encompasses a broad range of ecological habitats (Kushwaha and Behera, 2002). The plant genetic resources are depleting significantly due to over exploitation of the plants, Jhum cultivation and unplanned human activities. Therefore it is necessary to develop protocols for mass multiplication and conservation of these threatened plant species. Propagation of plants through different macropropagation techniques like cutting, layering, rhizome splitting, seed propagation, suckers etc are some of the widely used techniques for commercially important plants and threatened species (Kuria *et. al*., 2010; Kipkemoi *et. al.*, 2013). Other alternative means are the production of clonal planting materials through plant tissue culture technique. However, tissue culture technique involves higher cost as compared to different conventional technique. Further for a layman it is easy to practice the conventional technique in the rural areas. Moreover, mortality rates of the plant species propagated can be maintained very low as compared to *in vitro* propagation.

As medicinal plants continue to be the key role in the treatment of number of diseases, and they are the only source of medicines in the treatment of people in the developing world in most cases, conservation becomes a very important priority. Moreover the toxicity and health hazard associated with the use of synthetic drugs and antibiotics have renewed the interest in the use of plants and plant based drugs which increase the demand of medicinal plants, but only a small percentage of medicinal plants traded in India are solely cultivated. The remaining huge raw material of medicinal plants is met from their wild population. Over exploitation and indiscriminate collection to meet the market demands of medicinal plants supply in fact threatens the survival of many rare species. Thus, producing clonal planting materials through different techniques like conventional macropropagation and plant tissue culture can solved the problem by meeting the market demands as well as conservation of species in the wild.

Many plant species have been propagated successfully through macropropagation techniques particularly the rare, endangered and threatened plant species and reintroduce into the wild ameliorating their status in nature. Different parts of the plant have been used for macropropagation of different species of plants by many workers for conservation programme. Following are some works done by various workers: *Strychnos henningsii* (stem and root cuttings - Kipkemoi *et. al.*, 2013), *Eucalyptus globules* (stem cuttings- Wilson, 1993), *Triplochiton scleroxylon* (stem cuttings- Leakey, 1983), *Warbugia ugandensis* (stem rooting- Kuria *et. al*., 2010) etc.

Therefore keeping in view of the above points, the present study was undertaken on reproductive, seed biology and mass macro-propagation of these two plant species (***Panax pseudoginseng* W. and *Paris polyphylla* Smith.**) for my Ph.D. programme with the following objectives:

1. The reproductive biology of the two selected species.

2. Morphological and reproductive change in relation to the age of the plant and the effect of climatic factors on flowering, fruiting and seed production.

3. Seed biology, its germination and plant production.

4. Macropropagation of the plants.

5. Reintroduction of the regenerates in the wild.

A brief about the selected species:

A. *Panax pseudoginseng* W. (Araliaceae)

Ginseng has perhaps the longest continuous history of usage of any healing herb. Sometimes referred to as the '***Root of Heaven'***, ginseng is a good example of an ancient herb that Western medicine is just beginning to study and understand. In Latin, the word '***Panax***' means '***Cure all'***, and the family of ginseng plants is one of the most well known herbs. The English word ginseng derives from the Chinese term *rénshēn* literally "man root" (referring to the root's characteristic shape, resembling the body of a person). Ginseng mostly grows in Korea, China, Japan, Siberia, Vietnam, and North America and is considered to be one of the most important plants in herbal medicine, with many health benefits arising from consumption of the root and its extractives. A ginseng seed can remain in the soil for 18-24 months prior to germination before seeing any sign of growth. The flowers from this plant are bisexual (has both male and female organs) and are usually self- pollinating. Wild ginseng is considered to be superior to domestic ginseng by many ginseng consumers and buyers. There are 11-12 recognized species of ginseng, depending on the method of classification (Awang, 2003). The following list contains all known species of ginseng - *Panax bipinnatifidus* Seemann, *Panax bipinnatifidus* Seemann var. *angustifolius* (Burk.) Wen, *Panax ginseng* C. A. Meyer (Korean ginseng), *Panax japonicus* C. A. Meyer, *Panax* *notoginseng* F. H. Chen, C. Y.Wu, et K. M.Feng, *Panax pseudoginseng* Wallich, *Panax quinquefolius* L., *Panax stipuleanatus* H. T. Tsai et K. M. Feng, *Panax trifolius* L., *Panax vietnamensis Ha et Grushvitsky*, *Panax wangianus* Sun, *Panax zingiberensis* C. Y. Wu et K. M. Feng.

Figure 1: a. *Panax pseudoginseng* plant; b. *Panax pseudoginseng* rhizome; c. *Panax pseudoginseng* inflorescence; d. *Panax pseudoginseng* fruits

Panax pseudoginseng W. is a perennial herb which grows to a height of about 1m and a width of 0.7 cm (**Fig. 1 a, b**). It has a slow growth rate. It needs partial to full shade which is best for growing this plant. It usually does best in moist, well drained soils. The plant has got tuberous roots, short and fleshy, stems erect, leaves palmate, long petioted in whorls; leaflets 5-7, margins dendate, hairy on both sides. Inflorescence in terminal simple umbel; flowers small, yellowish-green (**Fig. 1c)**. Berry globose, slightly compressed, red when ripe, mostly 2-seeded (**Fig. 1d)**. Ginseng is used as a stimulant and aromatic bitter, stomachic and demulcent, and is considered alliterative, carminative, tonic, expectorant and antipyretic. It is used as a masticatory. It is reputed to have a sedative effect on the cerebrum and a mildly stimulating action on the vital centers. It is also a gonadotrophic agent containing little toxic substance. Ginseng has a deep influence on metabolism and prevents the development of atherosclerosis. It has the capacity of reducing high blood pressure and raising low blood pressure to the normal level. For this reason, it is administered in cases of hypertension and hypotension. In *Shen Nung Pen Ts'ao Ching*, it is claimed that ginseng is a tonic for the five viscera, quells animal spirits, establishes the soul, allays fear, expels evil effluvia, brightens the eyes, opens up the heart, and benefits the understanding (Bae, 1978). Thus, ginseng was believed to have not only physical effects on the body, but metaphysical effects as well.

The ginseng root has been the focus of many chemical studies in an attempt to understand the nature of its active ingredients. Many plants contain a group of compounds known as *saponins*, but the saponins within ginseng are unique and have been named *ginsenosides*. These compounds resemble steroids and are known as tetracyclic damarane saponins, which have carbohydrate groups attached at several points. Ginseng root also contains a group of compounds known as *polyacetylenes*. One of these, *panaxynol* or *falcarinol*, was able to stimulate neurite growth in cell culture (Wang *et. al*., 2006).

Numerous studies have been done by different workers on *P. quinquefolium* and *Panax ginseng* and they have examined the various ecological relationships (Lewis and Zenger, 1982; Lewis, 1984; Anderson *et. al*., 1984; Stathers and Bailey, 1986; Anderson, 1996; Hackney, 1999; Van der Voort, 2005; Shahi, 2007), reproductive biology (Carpenter and Cottam, 1982; Lewis and Zenger, 1982; Schlessman, 1985, 1987), chemical composition (Shim *et. al*., 1983; Tomoda *et. al*., 1985; Hansen and Bal, 1986; Hikino *et. al*., 1986; Oshima *et. al*., 1987), medicinal properties (Hu, 1976; Dubrick, 1983; Carlson, 1986) and growth, yield and rates of photosynthesis, respiration and transpiration under varied solar radiation and/or temperature conditions (Hu, 1976; Lee *et. al*., 1980; Stoltz, 1982; Strick and Proctor, 1985; Konsler, 1986; Proctor and Tsujita, 1986). Yoo *et. al*. (2001) reported that several Himalayan species of *Panax* were taxonomically problematic due to sympatry of morphologically distinct taxa and the existence of intermediates. *Panax wangianus* (syn. *P*anax *pseudoginseng*) S. C. Sun is a perennial, critically endangered, herb native to sub-tropical wet forests of North-East Himalayan regions especially in Meghalaya (Pushpangadan and Nair, 2005). Its rhizome was used as a blood-regulating medicine a tonic (Wen, 2007). *P. wangianus* was abundantly distributed about a century ago. However, at present their population is decreasing alarmingly because of various human impacts such as urbanization, over exploitation of natural resources, pollution of soil, water and atmosphere due to coalmine activities which contribute to the global climatic change (Pushpangadan and Nair, 2005).

***B. Paris polyphylla* Smith. (Trilliaceae)**

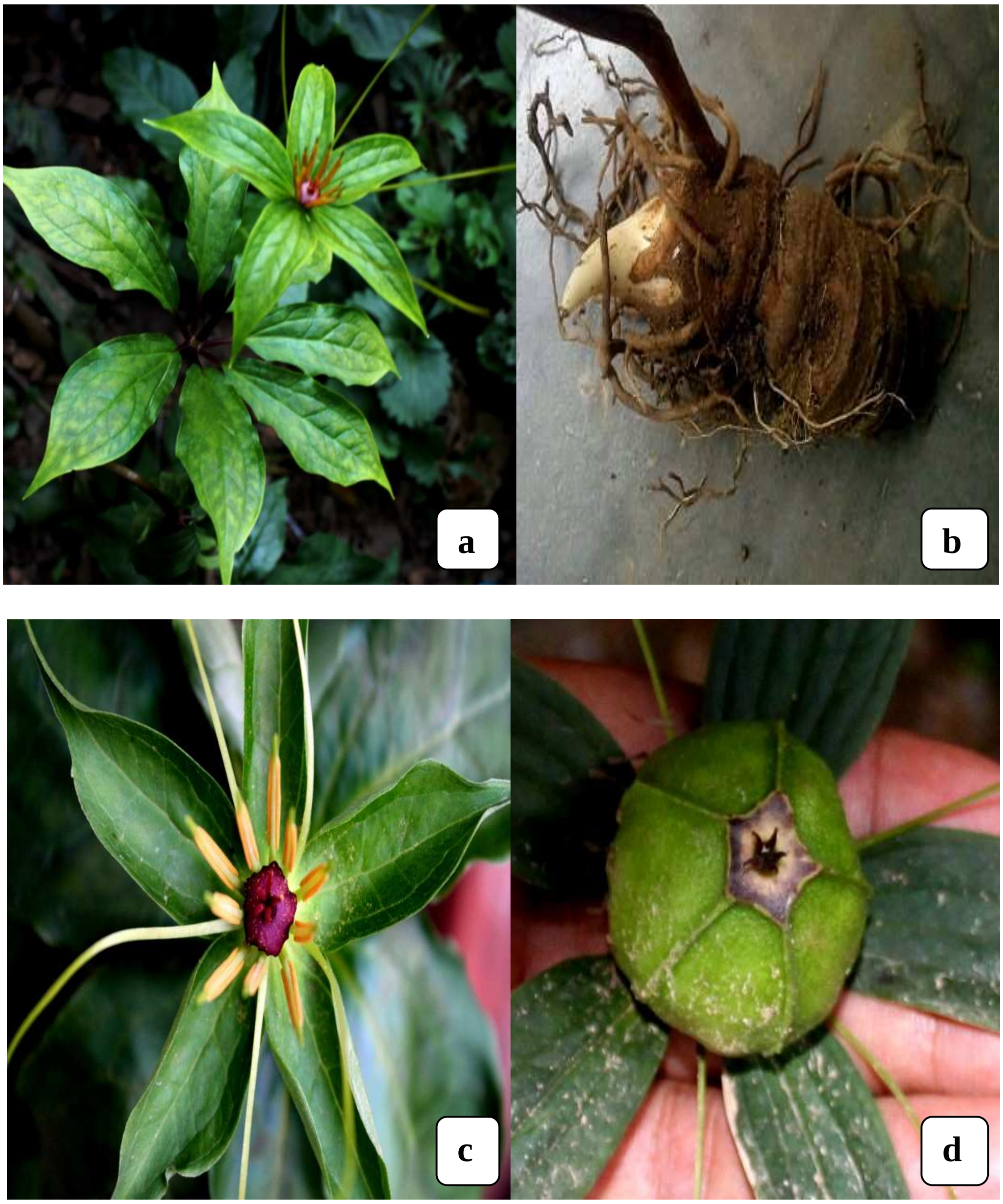

Figure 2: a. *Paris polyphylla* plant; b. *Paris polyphylla* rhizome; c. *Paris polyphylla* fruit

Paris polyphylla Smith is one of the medicinal plants listed as vulnerable under IUCN threat category (Madhu *et al*. 2010). *Paris polyphylla* Smith var. *polyphylla*, Synonym *Daiswa polyphylla* (Smith) belongs to the family Trilliaceae. It is an important perennial medicinal plant growing under the canopy of moist and shady places of the north eastern region and also in parts of Nagaland. Its descriptions are perennial erect herb, 30-100 cm tall; rhizome stout, creeping. Leaves 6-10 in a whorl, short-stalked, elliptic, finely acuminate, base cuneate, glabrous (**Fig. 2 a, b)**. Flowers solitary, terminal, short-stalked; pedicel Sepals (3-)4(-6), lanceolate, acuminate. Petals equaling number of sepals, (½-) 2/3 to longer than sepals, filiform, yellowish or greenish (**Fig. 2c)**. Stamens 10 or more, short. Stigma lobes usually 5, re-curved at tips. Fruit globular; seeds scarlet (**Fig. 2d)**. Usually the flowering season is April to May and fruiting season is July.

Paris polyphylla Smith is an important medicinal plant and its rhizome part is used mainly for medicinal purpose for treatment of different diseases. The plant contains saponin steroids polyphyllin D, dioscin and balanitin7 (Li *et. al.*, 2012). Rhizome of *Paris polyphylla* Smith plant is use as antihelmintic, antispasmodic, digestive stomachic, expectorant and vermafuge (Madhu *et. al.*, 2010; IUCN, 2004; Bhattarai and Ghimire, 2006), scabies, rashes, or itching problems (Jamir *et. al*., 2012), to treat liver cancer (Li *et. al.*, 2012). The roots are also fed to cattle with diarrhea and dysentery (Madhu *et. al.,* 2010). The whole plant can be used as febrifuge, while roots can be used as analgesic, antiphlogistic (removes heat), antipyretic, antitussive and depurative (Yung, 1985, Duke and Ayensu, 1985). The rhizomes are used for injuries from falls, fractures, convulsions and strains (Liang, 2000). It produces vasoconstriction in kidney, vasodilation in spleen and limbs and stimulates the isolated intestine (Dutta, 2007, Baral and Kurmi, 2006). It is used as a primary herb in the treatment of liver, stomach, nose, lung, throat and breast cancer in traditional Chinese medicine (Vassilopoulos, 2009). *Paris polyphylla* Smith var. *polyphylla* plants are facing threat due to unsustainable harvesting either for commercial purpose or local medicinal uses and the population is rapidly decreasing in the wild forest of Nagaland.

Chapter - 2

Floral Phenology and Reproductive and Seed Biology of *Panax pseudoginseng* W.

Flowering is pre-condition for fruiting and seed but many flowers results in poor seed production especially those angiospermic plant species with small flowers and large fruits. The reproductive performance of the plants and its efficiency determines its survival and therefore knowledge on reproductive biology is the key factor in the achievement of plant propagation and conservation. Plant morphological and biological characteristics may limit its population growth in the wild and therefore macropropagation of those plants are important *ex situ*. Reproductive biology is an important tool in working out the conservation strategy. The reproductive performance of the plants and its efficiency determines its survival and therefore knowledge on reproductive biology is the key factor in the achievement of plant conservation (Kumar *et. al.*, 2011). Reproductive bottlenecks includes failure of pollination, pre or post fertilizer barriers leading to no or poor seed set, poor reproductive vigor due to inbreeding depression and very low germination rates imposed constraints on the multiplication and survival of the species. Therefore, any conservation approach has to be based on an in-depth study of plant reproductive biology which provides information on seed germination capacity, survival rate of seedlings and adults, age at flowering, reproductive lifespan and number of flowers and seeds, seed biology. Such studies would be fruitful in planning various programmes specific to different habitats. The knowledge combined with clonal propagation will also help in developing strategies to preserve the genetic potential of rare species crucial for reintroduction.

Seeds are the fundamental component of the plant life cycle, as they store the genetic information necessary for the next generation of plants to disperse, establish, develop and eventually reproduce to maintain the species. The production, dispersal, survival, longevity and germination of a plant are dependent on the environment factors and site locations. The seed quality, pre-treatment methods, germination conditions, pathogenic resistance and the environmental conditions such as temperature, water substrate, and light are the determinants in germination of seeds. Measurement of soil type, *p*H, presence or absence of duff layer and its types, biogeoclimatic zone, elevation, slope and aspect is needed. Canopy cover is the environmental factor which significantly affects the micro climate of a site, influencing the solar radiations, air and soil temperatures, wind speed, humidity and rainfall experienced at the ground **(**Hanley, 1978). Soil nutrient levels are important because they effect seed production, germination and seedling growth. Soil chemical analysis is important for determining site nutrient status on sites. During germination light, temperature and moisture are the most important factors. However, good drainage, soil medium and nutrients and *p*H become important during the seedling establishment. Optimal environmental conditions either artificially or in its natural habitat are advisable so that the seeds pass through the vulnerable stage fast and thus ensure its survival. Litters and duffs are usually found which maintain a cool temperature for the seedlings. Decay wood has high water retention capacity and they are good natural seedbeds for the seeds.

Ginseng has been using as medicine since 2000 years back and its description can be found even in ancient Oriental medical literature (Qi *et. al*., 2011) . Ginseng roots which are more than five years old before harvested is highly valued and prized. *Panax pseudoginseng* W.. which is a native to India can be found in China, South Tibet, North Burma, North-east India and Nepal (Sharma and Sett, 2001). Some plants which are not considered true ginseng are Siberian ginseng (*Eleutherococcus senticosus*), Indian ginseng (*Withania somnifera*), and Brazilian ginseng (*Pfaffia paniculata*) as they are not from the *Panax* species (Baek *et. al*., 2012). Ginseng is widely used as a stimulant, for digestion, increase overall energy, adaptogen, for diabetes, to increase memory, for impotency, for cancer, for aphrodisiac means, for asthmatic attacks, for Alzheimer’s disease and for HIV disease (Choi, 2008.)

Panax pseudoginseng W. is highly threatened in Nagaland due to its over-exploitation by the locals for its local medicinal usage who over-harvest the plants especially the flowering or older plants which are easily located in its natural habitat thus leading to its rapid reduction in its population regeneration and size. Moreover the clearing of forest either for cultivation or developmental activities highly pose a threat to this valuable medicinal plant. The limitation of its population size also lies within the plant itself in its reproductive behavior and its seed habits. Plants shows very slow in its growth and high specific in its habitat conditions for its proper growth. The production of very less seeds as compared to its flower production along with its long month's seed dormancy contributes to its less population. The plant is highly threatened and thus there is an urgent need to conserve this medicinal plant both *ex situ* and *in situ*. Thus, taking into all these consideration, understanding its habitat, its reproductive biology and seed biology becomes an important priority to understand more of this plant and to conserve them.

Materials and Methods

Study Sites

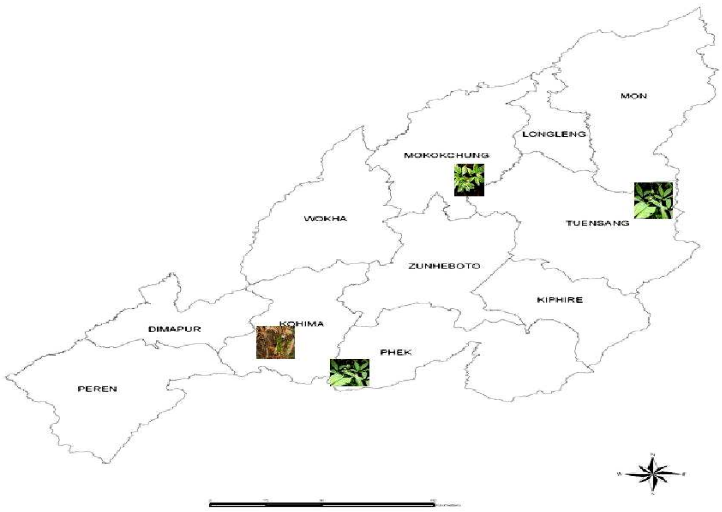

Figure 3: Map of Nagaland showing the four study areas (Natural and artificial).

For the present study two natural habitats was selected. One of the natural habitat was the forest area of Pangsha village near Indo-Burma border at an elevation of 2278 m above sea level (ASL), 26˚14´27.2´´ N and 95°07´05.7´´E under Tuensang district, Nagaland, India and another area was 'Chida' and its surrounding forest area of Khezakenoma village at an elevation of 1874 m ASL, 25˚30´11´´ N and 94°13´37´´E under Phek district, Nagaland **(Fig. 3)**. The habitat and morphological aspects of the plant, its dormancy, reproductive and phenological stage, class description and their reproductive output, mortality rates, percentage of fruit set, plant vigour, fruit setting and potentiality of seed formation etc were studied in the study areas. During the present study the habit and habitat of *Panax pseudoginseng* was studied and also experimental plots was created in both the study areas for experimental purpose. During the study certain microclimatic conditions like canopy cover of the natural habitats, associated plant species and level of anthropogenic disturbances were studied and correlated with reproductive behaviors.

Besides the above two natural habitats, two artificial habitats was created to study the adaptability of the species away from their natural habitat. After collection from the natural habitat, the plants were planted in polybags and transported to the artificial habitats where they were planted in potting mix as described below.

Figure 5: a. *Panax pseudoginseng* in Aradura hill showing stunted growth; b. 2-leaves non-flowering plant; c. *Panax pseudoginseng* plants established in green house in Mokokchung; d. *Panax pseudoginseng* fruits showing non-seed bearing umbel.

Aradura hill and Penli ward: Plants collected from the wild were introduced in the Aradura hill, Kohima district, Nagaland, India in a protected forest area at an elevation of 1384 m ASL, 25˚38´49.5´´ N and 94°06´23.6´´E and Penli ward, Mokokchung district, Nagaland at an elevation of 1259 m ASL, 26˚19´17.1´´ N and 94°31´09.8´´ E (**Fig. 3 and Fig. 5 a, c**). At Aradura hill, the ground soils in the planting area was dug up to a foot deep and the distance between two dug pits was maintained at 1 m. Different soil mixture was prepared by mixing decayed wood, sand and top soil as follows:

1. Manure soil by mixing decayed wood, sand and top black soil at a ratio of 1:1:3.
2. Mixture of cow dung, sand and top black soil at a ratio of 1:1:3.
3. Only to black soil.
4. Mixture of decayed wood, sand and top black soil at a ratio of 1:1:3 with a moss topping.

The mixtures were sundried for one week before putting into the pits or to the pots. The plants were watered only during the dry seasons usually from October to April thrice in a week. Plants were monitored constantly for its growth and development. Change of the soil manure was done every year in potted plants to ensure nutrient availability to the plants.

The experimental beds were provided with different shaded conditions as below:

a. A part of the pits were provided with full shade.

b. Part of the pits was provided full sun light without any shade.

c. Some plants were kept in poly house ca. 75% shade.

Plants grown in different soil mixture, their adaptation outside the natural habitats, effect of temperature and shaded conditions on the plant growth and development were studied.

Reproductive Biology

The reproductive phenology and floral morphology like time of budding, anthesis and stigma receptivity, different stages of anther development, anther dehiscence, different stages of flowering during its inflorescence till its fruit setting, correlation between leaf morphology and flowering, dormancy exhibition and their percentage within the population, reproductive output in the plant as well as comparison of the outputs in different stages were studied. Floral phenology of *Panax pseudoginseng* at Chida and its surrounding forest area (Khezakenoma village) and the forest area of Pangsha village were comparatively studied. Plants were observed from the time of its sprouting of shoot buds from its rhizome till its senescence. Flowers were dissected and studied to know the maturity of the different pollens within the same flower, stigma receptivity, timing of the anthesis were noted and observed. The stigmatic lobes in co-relation to the number of seeds were also studied. In order to estimate flower production, total number of flower per plant was counted manually in the selected plants. Different stages of flowering and the nature of flowering within and among the same species within a growing population with the timing of the flowering was studied in its natural habitat. Correlation between leaf morphology and flowering in different class stages of the plant and understanding the plant vigor in each class stages was also a part of the present study. The associated plant species which were usually found growing alongside the plant were also noted and account for within a five meter parameter range to study their effect on the growth of *Panax pseudoginseng* and their vigor.

Stage class description and reproductive performance**:** Plant height measurements are usually taken during the middle part of April where almost 90% of the plants have already attained their average height. Plants studied in the selected areas in its natural condition were categorized into different stages of plants according to the number of leaves present on them and were tagged as numbers 1, 2, 3, or 4 to indicate the leaves present on them. Relationship between the class stage and reproductive performance *Panax pseudoginseng* plants were studied during the present investigation. Plants were studied both under reserved and non-reserved forest. Besides this the number of plants in each stage class and their percentage of plants that was dormant the following year was taken into account by careful study in its natural condition. Reproductive output of each stage class was studied taken into account the number of pedicels formed per plant in all the class stages of the plant and percentage of pedicels producing fruit.

***Mortality rate*:** Mortality rate of the plants was studied by counting the roots where no plant growth occurs from the rhizomes. The roots that were tagged according to the number of leaves the previous year and which shows no growth the following year are carefully dug to see whether the roots are exhibiting dormancy or has decayed.

Seed Biology

During the present study the quantification of reproductive performance in the form of seed setting, maturation etc. were studies following different parameters as below:

***Correlation of plant morphological vigor, and inflorescence in relation to fruit setting*:** Relationship between morphological plant vigor and reproductive outcome (fruit setting) was also investigated during the study. Besides this, comparison of the number of inflorescence in relation to the percentage of fruit set in *Panax pseudoginseng* was studied. This was done from April-June. Here co-relation of the age of the rhizome and fruit seeding was studied along with the output of fruits in an inflorescence.

Seed setting in relation to different hand pollination treatments:

Three different pollination treatments were performed where 10 healthy three leaves plants were selected for each treatment. These pollination treatments were done in order to find out the effects of seed setting in plants through self- and cross- pollination process. This hand pollination technique was used on the same flower, between two flowers on the same inflorescence and between flowers of different inflorescences on two different plants. A small water colour paintbrush was used. The brush was ensured that it was totally cleaned and dry, so it was washes in running water and air blown dry before its usage. Pollination was done early in the morning at 6:30 -7:30 am when the weather is in dry conditions. The brushes were gently swirled around the selected stamen of the male flower and then the pollens collected on the brush were carefully swirled on the stigma of the selected female flower. They are then wrapped around using mesh clothes and then fasten the wrap around the peduncle using threads. This process was repeated for five-six times using the same selected plants during its flowering period.

Different pollination experiments were done as follows:

a. For self pollination, 5 selected individual flowers from each inflorescence of 10 plants is carefully bagged with fine nylon mesh clothes and the surrounding unselected flowers in the same inflorescence is removed with the help of fine forceps to prevent from cross pollination and overcrowding.

b. For cross pollination of different flowers in the same inflorescence, 10 selected individual flowers from the inflorescence is carefully bagged with fine nylon mesh clothes and the surrounding unselected flowers in the same inflorescence is removed with the help of fine forceps to prevent from cross pollination and overcrowding. Here in each of the 10 inflorescence, 5 selected flowers are emasculated by removing their stamens before anthesis. These emasculated flowers are pollinated randomly from the rest of the 5 selected flowers in the same inflorescence.

c. For cross pollination between two inflorescence of different plants, one plant is selected among them where five flowers per umbel are selected randomly, emasculation of stamens from the five selected flowers were done before anthesis. Both the plants are covered in mesh clothes to prevent pollination from outside.

The flowers are then tagged with their date of emasculation and date of pollination along with the number of flowers emasculated in the case of cross pollination between two flowers.

***Potentiality of seed formation and comparative analysis of seed maturation in the two study areas*:** Random selection of 15 plants in the green house was undertaken for the counting of seeds per berry. Stigmatic lobes during the anther dehiscence were also studied.

***Seed germination*:** *Panax pseudoginseng* W. seeds were collected from the natural habitat/population in the study areas in the month of October when berries were matured. Seeds were collected from both the umbels as well as those fallen from the mother plants. Care was taken to ensure that the seeds collected from the field are not injured or infected. The collected seeds were wrapped in moistened cotton and put in tight polybag and transported to the laboratory within two days. In the laboratory seeds were washed thoroughly in running tap water. The outer cover of the berries were removed manually and washed again in running tap water followed by air dried to the seeds. The seeds were subjected to differential stratification before sowing in the seed beds as described below:

1. The processed seeds were put in autoclaved sand at ~2-3 cm deep in trays. Trays were perforated in order to good water drainage and avoid water logging. The trays were maintained at 25^{o}C for three months and watered thrice a week.

2. These trays were then transferred to 4^{o}C in refrigerator for 1-7 months.

3. The stratified seeds were then sowed in two different seed bed viz. (a) mixture of normal top soil, decayed wood powder and sand at 3:1:1 ratio (seed bed 1), and (b) a mixture of normal top soil, decayed cow dung powder and sand at 3:1:1 ratio (seed bed 2).

The stratified seeds were sowed in both the seed beds at a depth of 4-5 cm below the soil surface and spacing between the seeds and row was maintained at 12-15 cm. The seed bed mixture was sundried before preparing the beds. For each treatment 10 seeds were sowed and repeated thrice. The beds were watered thrice a week and the emergence of radical was used as the marker for seed germination. The seedling developed from the germinated seeds was maintained in the seed bed for one year before transferring to the field/wild.

Results

***Habit and habitat*:** Present study was undertaken in two natural habitats/niches (Forest area of Pangsha village under Tuensang district and Chida and its surrounding forest area of Khezakenoma village under Phek district) and two artificial habitats (**Fig. 3, Table 1**). A total of 5 populations were studied from two niches and one each from the stimulated habitats. During the study it was observed in both the niches that *Panax pseudoginseng* grows luxuriously under canopy cover over 80% shade (**Table 2) (Fig. 4 a.)** and undisturbed areas. It was also recorded that wherever the canopy cover was <80%, did not support healthy growth of plants. In the habitat wherever there was human disturbance, number of individuals were very few or almost absent. Among the two natural habitats studied, Chida and its surrounding forest area supported large numbers of *Panax pseudoginseng*. This is due to the fact that the area was comparatively undisturbed as the area is protected by the village authority.

Figure 4: a. *Panax pseudoginseng* plants in natural habitat; b. Associated species growing in the natural habitat along with *Panax pseudoginseng*.

Table 1: Demographic studies of *Panax pseudoginseng* in Nagaland, India

Sl. No.	**Study location/site**	**Duration of study**	**Number of populations**
1	**Tuensang**	**4 years**	**3**
2	**Phek**	**3 years**	**2**
3	**Kohima**	**3 years**	**1**
4	**Mokokchung***	**4 years**	**1**

* **Under green house condition.**

Note:

1. *Panax pseudoginseng* was studied in *Chang* and *Khiamungan* tribal areas in Tuensang district.
2. In Phek district study was conducted at Chida area and its adjoining forest area.
3. In Kohima study was conducted in artificial habitat in the protected forest of Aradura hill.

Table 2: Site characterization of *Panax pseudoginseng* habitats in Nagaland

Study site	*Panax pseudoginseng* collected and studied	GPS coordinates	Canopy closure* (%)	Disturbances
Tuensang	**Pangsha area**	**2278 m ASL (26˚14´27.2´´N and 95°07´05.7´´E)**	**86**	**High human interference**
Phek	**Chida and its surrounding area**	**1874 m ASL (25˚30´11´´ N and 94°13´37´´E)**	**88**	**Limited human interference**
Kohima	**Aradura hill**	**1384 m ASL (25˚38´49.5´´N and 94°06´23.6´´E)**	**84**	**Protected area**
Mokokchung	**Penli ward**	**1259 m ASL (26˚19´17.1´´N and 94°31´09.8´´)**	**80**	**Green House Condition**

* Most of the *Panax pseudoginseng* was found growing where there is more than 80% shade in the natural habitats.

* There is high human interference in many locations due to its ethno-medicinal uses.

Table 3: Herbaceous common associated plants found in the *Panax pseudoginseng* niches

Sl.no	Name of the herb	Family
1.	***Artemisia nilagirica***	**Asteraceae**
2.	***Dicentra* sps.**	**Fumariaceae**
3.	***Girardina heterophylla***	**Urticaceae**
4.	***Impatiens* sps.**	**Balsaminaceae**
5.	***Pilea umbrosa***	**Urticaceae**
6.	***Aconitium* sps.**	**Ranunculaceae**

Note:
1. The above table gives generalized common plants which are found within 5 m parameter where *Panax pseudoginseng* is found growing in its natural habitats.

During the present study it was observed that there were some common herbaceous plants species found in most of the populations in both the niches within 5 m parameter (**Table 3)**. Some of the plants commonly found associated with the plant and which were found within the vicinity of 5 metres parameter were *Artemisia nilagirica* (Asteraceae), *Dicentra* sps. (Fumariaceae), *Girardina heterophylla (*Urticaceae), *Impatiens* sps. (Balsaminaceae), *Pilea umbrosa* (Urticaceae), *Berberis* sps. (Berberidaceae), *Aconitium* sps. (Ranunculaceae), *Smilax* sps. (Smilacaceae), *Schizostachyum dullooa (*Poaceae), *Schima wallichi* (Theaceae) *Alnus nepalensis* (Betulaceae), *Rhododendron arboreus* (Ericaceae), *Quercus* sps. (Fagaceae) and *Taxus baccata* (Taxaceae). Bamboos, Ferns, Bryophytes, Mushrooms and Lichens were also found in abundance. Oak tree was the most common tree where ginseng seems to grow nearby or at its foot of the trunk (**Fig. 4 b)**. Further it was recorded that wherever these associated species were present, *Panax pseudoginseng* registered healthy growth compare to the areas where these species were absent.

Beside the natural habitats, the plants were also maintained in two artificial habitats-One poly house at Penli ward, Mokokchung and another on wood grown natural forest at Aradura hill, Kohima to study the adaptability of the species away from the natural habitat and correlate with reproductive performance. In general *Panax pseudoginseng* exhibited poor adaptation to artificial habitat. The plants maintained in Aradura hill (Kohima) exhibited stunted growth (**Fig. 5 a**). It was observed that though the plants/rhizome remained alive, the rhizome dormancy was very high and failed to sprout in the subsequent year and only few rhizomes could sprout and produce plants. Most plants were mostly 2-leaf stage and failed to flower (**Fig. 5 b**). However the mortality of the rhizome and roots was very less.

Compare to Kohima, plants maintained in Penli ward, Mokokchung were better adapted though the progressive acclimatization was very poor (**Fig. 5c**). Plants exhibited gradual increase in its height, exhibition of flowering in subsequent years and bearing of young fruits. Though plants started flowering, failed to mature and resulted in pre-mature fruit fall (**Fig. 5d**). During the study it was recorded that if the plants are exposed to sunlight or even increased illumination there was gradual death of the above ground part. Plants in absence of sunlight showed healthy growth and development but most plants were in its vegetative stage only.

Reproductive Biology

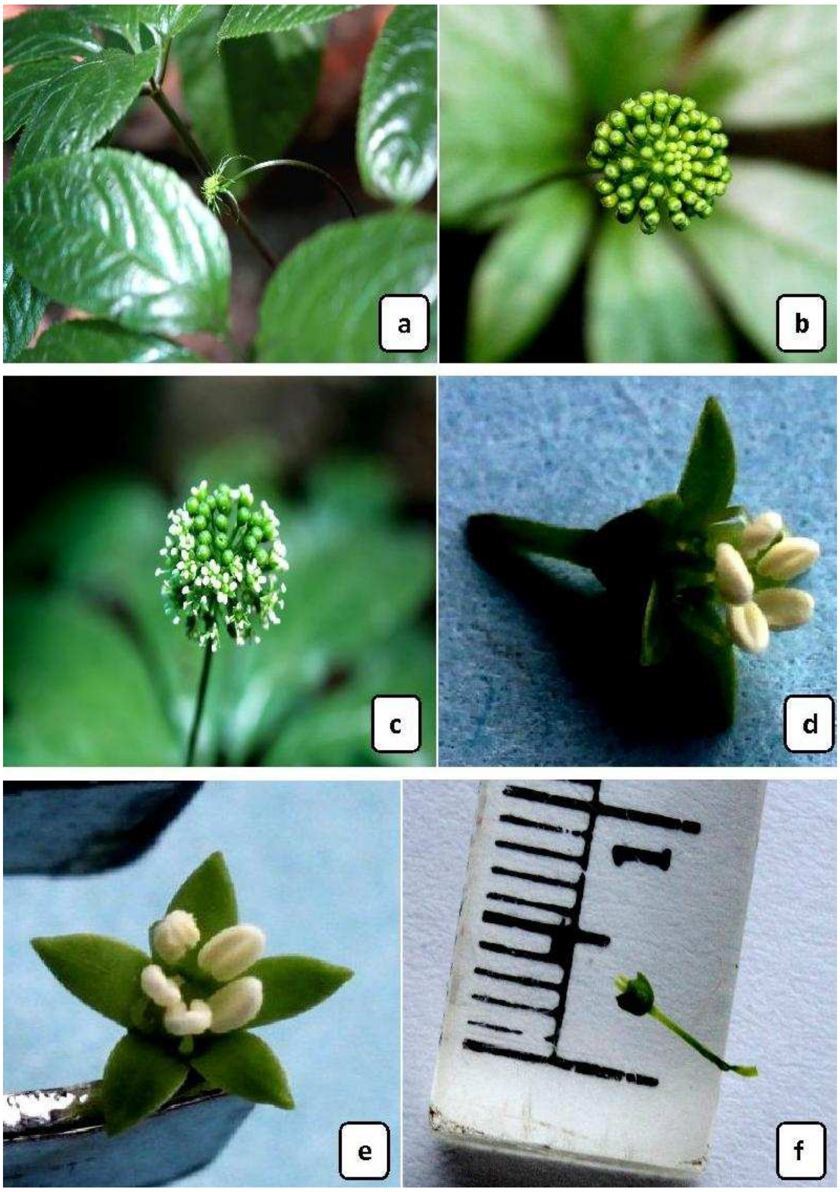

Figure 6: a. *Panax pseudoginseng* inflorescence; b. *Panax pseudoginseng* floral buds; c. Flowers at bloom; d. An enlarged flower showing the different floral parts; e. Anther dehiscence; and f. *Panax pseudoginseng* stigmatic lobe.

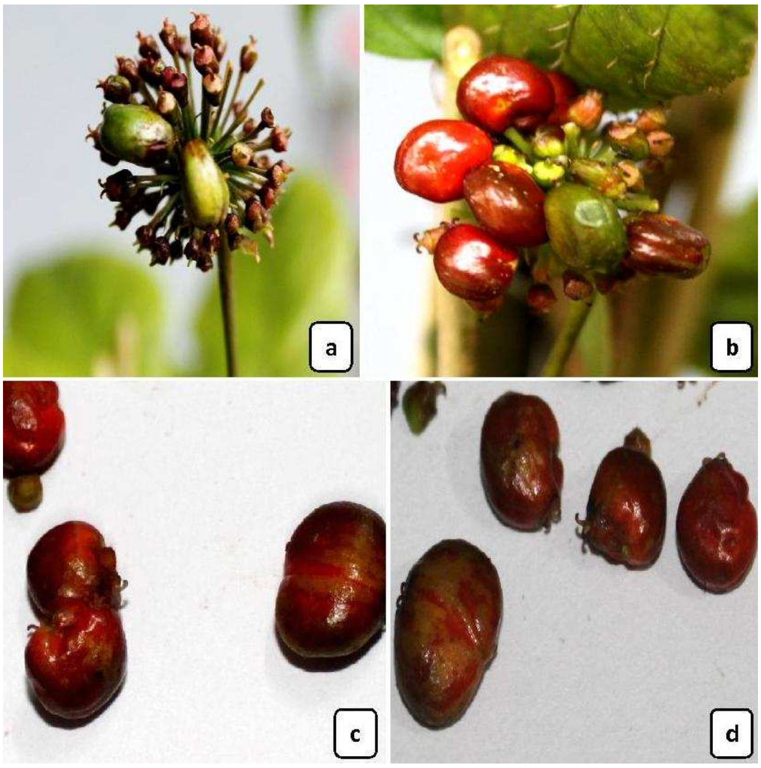

Figure 7: a. ***Panax pseudoginseng*** **umbel inflorescence bearing immature fruits; b. Umbel with matured fruits; c & d. One and two seeded berries.**

Table 4: Reproductive phenology of *Panax pseudoginseng*

Parameters	*Observations*
Height of the plant	**Upto 1 m**
Sprouting of rhizome	**Mid- February**
Full leaf expansion	**2nd week of April**
Bearing of inflorescence	**2-leaves plant onwards**
Type of inflorescence	**Centripetal**
Flower size	**2-3 mm**
Flower colour	**Light green**
Flower type	**Bisexual**
Flowering period	**Late May –Early June**
Flowers per Umbel	**50-65**
Nature of flowering	**Continuous**
Ovary	**Inferior**
Stigma	**Bilobed**
Anther color	**Faint yellowish white**
Number of stamens	**5**
Stamen position	**Between sepals**
Pollination	**Self-pollination**
Time of anthesis	**6:30-7:30 AM**
Time of anther dehiscence	**7:30-8:30 AM**
Temperature for seed production	**18˚C-23˚C**
Transition from flower to immature berries	**12-15 days**
Mature berries	**Last week of June**
Fruit size	**0.4-1.2 cm**
Fruit set %	**4.24**
Fruit dispersal	**September-October**
Dispersal of seeds	**Gravity and birds**
Senescence	**October**

Panax pseudogingseng W. grows to a height of about 1 m in height. Sprouting of the rhizome takes place from mid-February onwards but early and late sprouting can be seen in some plants. Full leaf expansion occurs by second week of April with the bearing of inflorescence from 2nd leaf onwards. Centripetal inflorescence is exhibit where flowering occurs blooming from the base of the inflorescence to the tip of the inflorescence (**Fig. 6 a**). Continue nature of flowering is exhibited. Flowers are light green in color with green petals of 5, calyx lobed with 5 in number, fused carpels with 5 anthers. The flowers are very small and inconspicuous with just 2-3mm in size. The flowers are bisexual. Flowering period starts from late May to early June with 50-65 flowers per umbel. The anthers protrude out and surround the stigmatic lobe. The stigmatic lobe is green when young. Ovary is inferior. The flowers at the outermost edge of the umbel inflorescence open first and then flowering starts in the inner flower buds. Anthers produce large amount of pollens and the pollen from the anther falls on the stigmatic lobes once the petal opens and fertilization begins as it reaches the ovary through the style. Anthers of the same flower dehisce in different timing (**Fig. 6 b-f)**. Insects such as small flies and moths were seen on the flowering plant but they can be either visitors or pollinating agents. The plants are self pollinated. Stigma is usually bilobed. The timing of anthesis is between 6:30- 7:30 A.M, followed by the dehiscence of anther from 7:30-8:30 A.M onwards. Fertilized ovary converts into greenish round berries (**Fig. 7 a)**. The immature green berries forms in its tightly packed umbel and shortly after 1-2 weeks it forms into matured red berries (**Fig. 7b)**. The fruit production increases with age and size of the plant, the berries usually contain 1-3 seeds. The maturity of berries starts from the outer most edge and proceeds towards the centre of the umbel. It was found that two different types of seeds shape forms in *Panax pseudoginseng*. When the berries contain one seed, the seed is roundish in shape and when it has two seeds, the seeds are peanut shaped while the three seeded berries are triangular in shape (**Fig. 7 c, d**). Further it was recorded that only ~4.24% results in setting of fruit and the rest of the fruits were either aborted/seedless. The production of seeds in *Panax pseudoginseng* requires a higher temperature of 18-23˚C which usually occurs during the mid-summer season (**Table 4**). Seeds are usually dispersed by gravity, winds, small animals and birds.

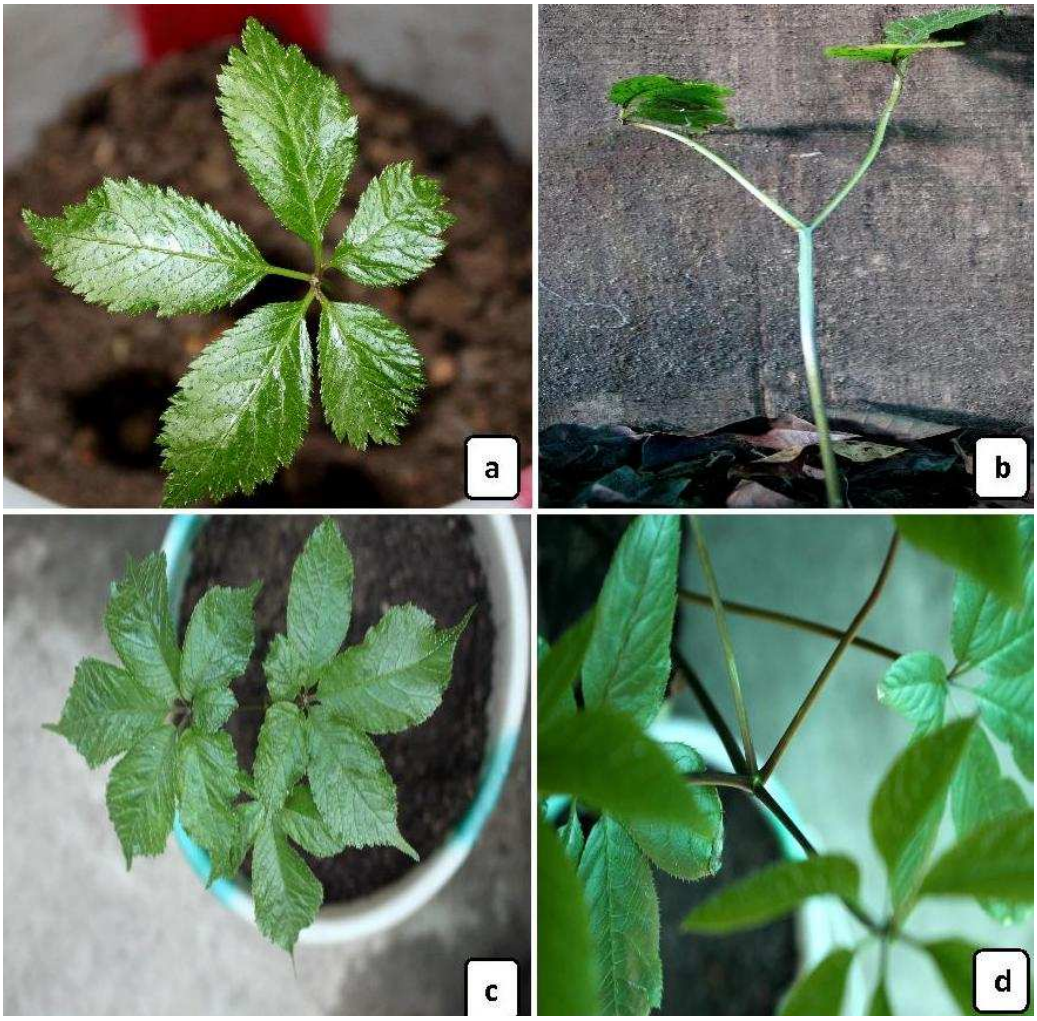

Figure 8. *Panax pseudoginseng* plants of different leaf class stages. a. 1-leaf plant; b. 2-leaves plant; c. 3-leaves plant and d. 4-leaves plant.

Table 5: Stage class description: Number of plants (N) in each stage observed during 2011-2014, in relation to the number of leaflets and height

Stage	N (Number of plants)	Mean number of Leaflets	Height (cm)
Seedling	**192**	**3.4**	**6.3**
1-leaf	**116**	**4.4**	**14.2**
2-leaves	**143**	**9.1**	**16.3**
3-leaves	**177**	**15.8**	**27.8**
4-leaves	**67**	**21.3**	**40.1**

Note:

1. Height measurements are usually taken during the middle part of April where almost 90% of the plants have already attained their maximum height on an average.
2. There is a positive co-relationship with the stage class and the height and the number of leaflets of the plants.

Table 6: Number of plants in each stage class for 4 years interval and their percentage of plants that was dormant the following year under controlled conditions

Year	1-leaf*			2-leaves*			3-leaves*			4-leaves*		
	N	n	%	N	n	%	N	n	%	N	n	%
2011	**13**	**1**	**7.69**	**23**	**2**	**8.69**	**14**	**2**	**14.28**	**3**	**1**	**33.33**
2012	**19**	**1**	**5.26**	**21**	**2**	**9.52**	**18**	**3**	**16.66**	**2**	**0**	**00.00**
2013	**24**	**0**	**0.00**	**34**	**3**	**8.82**	**26**	**3**	**11.53**	**2**	**0**	**00.00**
2014	**31**	**1**	**3.23**	**28**	**2**	**7.14**	**17**	**2**	**11.76**	**3**	**1**	**33.33**
Average %	**4.05**			**8.54**			**13.55**			**16.66**		

*** N: Total plant studied, n: Number of plants remained dormant.**

Note:

- Dormancy in *Panax pseudoginseng* plants are more like in the higher stage plants than in lower stage plants.
- There is a distinct rise in the increase of plant dormancy from 2-leaf onwards.
- However, 4-leaf plants data is too small to be taken into account but the data itself indicates its probability shows it’s more likely to go into the dormancy period than the lower stage plants.
- 2-leaf plants are the most frequently emergent plants once the plant undergoes dormancy.
- Many a times the dormant plants maintain their own class stage; however there is also progress and regress in their class stage after their dormancy.
- Dormancy for even up to 2 years has being noted till now.

Table 7: Reproductive output of each stage class; number of pedicels per plant and percentage of pedicels producing fruit

Stage	Number of plants studies	Pedicels per plant in class	Percentage pedicels forming drupes (%)	Seeds per reproductive plant
2-leaves	26	6.1	16.4	2.1
3-leaves	121	28.3	19.3	8.9
4-leaves	47	46.1	23.7	14.5

Note:

- No inflorescence in 1-leaf plants were observed
- Inflorescence and berry production starts from 2-leaf plants onwards. However their mortality rate is very high.
- Usually the number of seeds present per berry is 1-seeded to 2- seeded. However, 3-seeded berries are also observed usually in 4-leaf plants and onwards.
- Mostly the 2-leaf plants produce 1- seeded berries while in 3-leaf to 4-leaf plants, usually double seeded berries occurs in maximum on an average.
- Reproductive output and their success is more from 3-leaf plants and above.
- There was variation among the different populations of *Panax pseudoginseng* in its time of berry production and ripening.

Table 8: Mortality rates in each stage class

Green House Condition

Year	Seedling N n %	1-Leaf N n %	2-leaves N n %	3-leaves N n %	4-leaves N n %
2011	18 3 16.7	13 3 23.1	23 2 8.7	14 0 00.0	3 0 0
2012	19 4 21.1	19 5 26.3	21 2 9.5	18 2 11.1	2 0 0
2013	15 3 20.0	24 4 16.7	34 0 0.0	26 1 3.8	2 0 0
2014	23 4 17.4	31 4 12.9	28 1 3.8	17 0 00.0	3 0 0
Average	18.8	19.7	5.5	3.7	0

Natural Habitats

Year	Seedling N n %	1-Leaf N n %	2-leaves N n %	3-leaves N n %	4-leaves N n %
2011	41 12 29.3	96 18 18.8	84 06 5.7	103 06 4.7	11 1 9.1
2012	37 09 24.3	83 22 17.7	96 11 7.5	112 04 3.6	13 0 0.0
Average %	26.8	18.3	6.6	4.2	4.6

Note:

- **N-Number of plants**
- **n-Number of plants that died**
- **This table gives an outlook on the mortality of plants in relation to its leaf stages.**

***Stage class description, dormancy and reproductive performance*:** During the present study effort was put into to study the correlation between number of leaves, plant height and reproductive outcome. It was found that there was a difference in number of leaflets among the same class stage. Thus average mean was calculated for both the height and the leaflets present in same class stage (**Table 5**). It was found that mean plant height was directly correlated with number of leaves. Plant height was found to be between 14.2 cm (1 leaf) to 40.1 cm (4 leaves stage) and there was a positive correlation between plant height and the number of leaflets (**Fig.**

8 a-d). The mean number of leaflets ranged between 3.4 to 21.3 from seedling to 4 leaves stage which are corresponding with plant height (6.3–40.1 cm). Seedlings exhibits lesser height as compared to other class stages.

In the present study it was found that rhizome dormancy was associated with the leaf class stage. The number of plants in each stage class for and their percentage of plants that was dormant the following year was taken into account by careful study in its natural condition. It was found that lesser the number of leaves, rhizomes were less dormant in the subsequent year (**Table 6)**. Dormancy in *Panax pseudoginseng* plants are more likely in the higher stage plants than in lower stage plants. There is a distinct rise in the increase of plant dormancy from 2-leaves onwards. However, 4-leaves plants data is too small to be taken into account but the data itself indicates its probability which shows it's more likely to go into the dormancy period than the lower stage plants. 2-leaves plants are the most frequently emergent plants once the plant breaks its dormancy period. Many a times the dormant plants maintain their own class stage; however there is also progress and regress in their class stage after their dormancy. Dormancy exhibition even up to 2 years have being noted.

Besides rhizome dormancy, reproductive output of each stage class were studied taken into account the number of pedicels formed per plant in all the class stages of the plants and percentage of pedicels producing fruit. There was no inflorescence developed in the one-leaf plant. Inflorescence and berry production starts from 2-leaves plants onwards (**Table 7**). In the present study highest number of pedicels formation was recorded from 4-leaves plant (~46) followed by 3 leaves (~28) and two leaves (~6). Percent pedicle forming drupes and formation of seeds per flowering plants also exhibited a similar trend. However their mortality rate was very high. Usually the number of seeds present per berry is 1-seeded to 2- seeded, however 3-seeded berries were also observed usually in 4-leaves plants and onwards. Mostly the 2-leaves plants produced 1- seeded berries while in 3 to 4 leaves plants usually double seeded berries are yielded. Reproductive output and their success was more from 3-leaves plants and above.

While there was a positive correlation between leaf class stages, dormancy of rhizome, seed development but as far as mortality is concerned, it exhibited reverse correlation. Mortality rate of the plants was studied by counting the roots where no growth of plants occurs during the growing season. The roots that were tagged according to the number of leaves the previous year and which shows no vegetative growth the following year are carefully dug to see whether the roots are exhibiting dormancy or has decayed. It was observed that highest mortality was in seedling stage followed by one leaf, two leaves, three leaves and four leaves respectively. This study was conducted in both natural habitat as well as artificial habitats. In both kinds of habitat similar response was observed though the mortality rate was comparatively lower in the artificial habitats (**Table 8**).

Class stage and reproductive performance**:** During the present study effort was put into to study the correlation between leaf class stage and flowering. The study was conducted in the natural habitat as well as in the artificial habitat. In most of the cases it was found that plants remained non-flowering. Further it was found that plant with one or two leaves remained non-flowering. There was no flowering from the plant with one leaf while two leaves plants ~81% plants remained recalcitrant to flowering. Flowering status improved with the increase in leaf number. Among the plants studies, ~90% plants produced flower with the plants with 4 leaves (**Table 9**).

It was found that there was a direct relation between the age of the rhizome and the morphological vigor of the plant such as the stem height, mean number of leaves/leaflets and potentiality of fruit setting (**Table 10)**. The plant height ranged between 7.2 cm (one year old rhizome) to 29.2 (9 year old rhizome). It was observed that plants produced from younger rhizomes were mostly non-fertile and up to two years there was no blooming of the buds. Inflorescence, flowering and fruit setting were recorded from the third year onward. Though the fruit setting was observed from the third year rhizome, number of flowers per inflorescence and *per cent* fruit setting was very low (1.71%) (**Table 10, 11**). As the rhizome aged, bud blooming and fruit setting performance improved and as high as ~10% fruit setting registered from the plants with 9 years old rhizome (**Table 10, 11**).

Table 9: Relationship between the class stage and reproductive performance of *Panax pseudoginseng* plants

Sl. No	Class stage	Number of plants studied	No. of plants flowering/non-flowering	% non-flowering plants
1	1-Leaf	23	0/23	100.00
2	2-Leaves	48	9/39	81.25
3	3-Leaves	16	11/5	31.25
4	4-Leaves	21	19/2	9.50

Note:

- Plants were selected in a given location where they were found under reserved forest.
- Vegetative plants were very high in *Panax pseudoginseng* plants.
- Most of the vegetative plants lie in the class of 1-leaf and 2-leaf plants.
- In nature, majority of the plants were 2-leaf plants and many of them were found to be non-flowering.

Table 10: Relationship between morphological plant vigor and reproductive outcome (fruit setting)

Age of the plant	Stem height (cm)	Mean number of leaves	Mean number of leaflets	Mean percentage of fruit set
1 year	7.2	1.00	3.42	0
2 years	8.3	1.01	4.48	0
3 years	10.1	1.63	5.62	1.71
4 years	13.8	1.96	8.34	2.02
5 years	15.3	2.17	11.01	3.29
6 years	17.6	2.43	11.97	5.11
7 years	20.2	2.72	12.44	7.14
8 years	24.8	2.95	13.84	8.75
9 years	29.2	3.22	15.46	10.22

Note:

- The morphological characters of the plant highly determine the plant vigor. Matured plants exhibit more vigor as compared with younger plants.

Table 11: Comparison of the number of inflorescence in relation to the percentage of fruit set in *Panax pseudoginseng*

Age	Mean number of flowers per inflorescence	Mean percentage of fruit set
1 year	0	0
2 years	0	0
3 years	6	1.71
4 years	8	2.02
5 years	18.33	3.29
6 years	29.6	5.11
7 years	44.30	7.14
8 years	56.5	8.75
9 years	61.46	10.22

Note:

- Higher aged plants exhibits more berry production than lower aged plants.
- There is a wide distinction in the production of inflorescence from 5 years and above.
- The setting of fruit is generally very low as compared to the number of flowers in the inflorescence. This can be due to the length of the pedicel in relation to the size of the fruit produced and the size of the inflorescence.

Seed Biology

Effort was put into to determine the reproductive output in terms of seed formation. Species of *Panax* are known to produce fruits with variable number of seeds. In *Panax pseudoginseng*, three different types of fruits were recorded viz. one seeded, two seeded and three seeded. It was found that number of seeds per fruit was depending on the number of the leaves in the plant. Lower the number of leaves, there was more one seeded fruits and with increase in leaves, number of seeds per fruit increased (**Table 12)**. The presence of one seeded fruits was very prominent in 2 leaves plant but there is sizeable decrease in the presence of one seeded fruits as the number of leaves on the plant increases. Likewise, gradual increase in three seeded fruits occurs as the plant acquires more leaves. Two seeded fruits were found to be dominant in the plant in all the studied areas.

Panax pseudoginseng W. seeds can be produced from the plant through both self-pollination and cross-pollination. Self pollination was found to be better than cross pollination in terms of seed productivity and output. However there were not much of variable differences between the success in seed productivity, seed setting through self-pollination and cross-pollination.

Figure 9: a & b. Seed bed preparation; c & d. Seedling developed from germinated seed; e. Young plants in the pots; f. One year old plantlets of *Panax pseudoginseng* ready for transfer to the field.

Table 12: Relationship between the class stage and potentiality of seed formation in *Panax pseudoginseng*

Number of leaves per plant	1-seeded fruits %	2-seeded fruits %	3-seeded fruits %
2-leaves	**57.1**	**42.9**	**00.0**
3-leaves	**30.8**	**66.7**	**02.6**
4-leaves	**7.9**	**75.0**	**07.1**
5-leaves & above	**7.4**	**69.6**	**13.0**

Note:

- Random selection of 15 plants was undertaken for the counting of seeds in the berries.
- The percentage of 2-seeded berries is more as compared to1-seeded and 3-seeded berries.
- There is however lower 2-seeded berries in 2-leaf plants.
- The leaf number determines the number of the seeds inside the berries.
- Matured plants produce more berries than young plants.

Table 13: Effect of stratification on seed germination of *Panax pseudoginseng*

Warm stratification period at 25˚C (Months)	Cold stratification period at 4˚C (Months)	% germination in seed bed 1* (±SE)	Time for germination (days)	% germination in seed bed 2**	Time for germination (days)
0	0	**10.00 (0.5)**	**425**	**8.20 (0.3)**	**425**
0	1	**0.00**	**-**	**0.00**	**-**
0	2	**0.00**	**-**	**0.00**	**-**
3	3	**38.25 (0.4)**	**12**	**24.20 (0.2)**	**17**
3	**4**	**68.20 (0.7)**	**14**	**52.40 (0.3)**	**18**
3	**5**	**41.30 (0.5)**	**11**	**29.20 (0.2)**	**14**
3	**6**	**26.00 (0.3)**	**17**	**14.10 (0.2)**	**17**
3	**7**	**9.10 (0.2)**	**14**	**3.10 (0.1)**	**8**

±SE: Standard error from mean.
***** Seed bed 1: Mixture of normal soil, decayed wood manure and sand in 3:1:1.
** Seed bed 2: Mixture of normal soil, decayed cow dung manure and sand in 3:1:1.

Panax pseudoginseng seeds were collected from the natural habitat/population in the study areas in the month of October when berries were matured. Seed beds were prepared as described in materials and methods (**Fig. 9 a, b**). The seeds were stratified differentially as described in materials and methods before sowing in the seed beds. The seeds sowed without stratification remained recalcitrant to germination. Seeds stratified at 25^{o}C for three months followed by cold stratification at 4^{o}C for 1 to 7 months proved to be beneficial for seed germination (**Table 13**). Under controlled condition ~10% seed germination recorded after 425 days in seed bed 1 while 8% seed germination registered in seed bed 2 after 450 days of sowing. Warm stratification for 3 months followed by cold stratification beyond 3 months was found to be promotory for seed germination in both the seed beds. Of the two seed bed condition studied in the present study, seed bed prepared by mixing normal soil, decayed wood powder and sand at 3:1:1 ratio found to be better for seed germination. Of the different stratifications tested in the present study, warm stratification followed by cold stratification for 4 months before sowing supported optimum seed germination in both the seed beds. Within 14 days after sowing in the seed bed 1 ~68% seeds germinated by emergence of radical followed by formation of seedling (**Fig. 9 c),** while in seed bed 1 ~52% seeds registered germination after 18 days of sowing. Though emergence was observed within 14 days of sowing, seedling formation was achieved after ~6 week of sowing seedlings with green leaves formed after 3-4 months (**Fig. 9 d**). Seed stratified at 4^{o}C beyond 4 months reduced the germination and seed stratified for 7 months registered only 9% germination. The seedling consists of one leaf with 3-5 leaflets and leaves were light green immediately after sprouting and slowly turned green as they matured. It was found that seedlings maintained in the seed bed 1 were healthy and dark green while seedling maintained in the seed bed 2 exhibited stunted growths. Of the germinated seedlings ~ 27%

seedling died within one month due to stem rooting and yellowing of leaves. The seedlings/young plantlets were maintained for 1 year before transferring to the wild (**Fig. 9 e**). The seedlings were transferred in the wild and monitored for one year.

Discussion

Present study was undertaken in two natural habitats/niches- Forest area of Pangsha village in Tuensang district and Chida and its surrounding forest area of Khezakenoma village in Phek district along with two artificial maintained habitats. During the present study it was observed that growth and population density of *Panax pseudoginseng* greatly differed in different natural habitats. It is well established fact that every individual species has its own requirement for growth whether natural or artificial habitat. In general the genus *Panax* is shade loving plant (Xiao *et. al.*, 1987) and primarily grows in primary forest. The observation of the present study is also in agreement with the reports of other workers (Xiao *et. al.*, 1987; Venugopal and Ahuja, 2013). It was found in all the natural habitats studied that *Panax pseudoginseng* grew luxuriously under canopy cover over 80% shade and altitude above 1850 m ASL in undisturbed areas. At lower altitude and higher illumination, plants did not grow healthy.

During the present study one interesting fact observed was the presence of some common herbaceous plants species in all the natural niches. Wherever these associated species were present, *Panax pseudoginseng* registered healthy growth compare to poor growth of the plants where these associated species were absent. It is assumed that there might be some kind of mutual interrelationship (symbiotic?) that exists between *Panax pseudoginseng* and its associated herbaceous plants. In the recent past a similar finding was reported by Langhu and Deb (2014) with *Aconitum nagarum* where they have reported the influence of associated species on flowering of *A. nagarum*. In the present study some of the common associated species recorded are *Artemisia nilagirica* (Asteraceae), *Dicentra* sps. (Fumariaceae), *Girardina heterophylla (*Urticaceae), *Impatiens* sps. (Balsaminaceae), *Pilea umbrosa* (Urticaceae), *Berberis* sps. (Berberidaceae), *Aconitium* sps. (Ranunculaceae), *Smilax* sps. (Smilacaceae), *Schizostachyum dullooa (*Poaceae), *Schima wallichi* (Theaceae), *Alnus nepalensis* (Betulaceae), *Rhododendron arboreus* (Ericaceae), *Quercus* sps. (Fagaceae) and *Taxus baccata* (Taxaceae). Bamboos, Ferns, Bryophytes, Mushrooms and Lichens are also found in abundance where *Panax pseudoginseng* was usually found.

Besides the natural habitats, two artificial habitats were used for the experimental purpose to study the adaptability of the species to new environment. *Panax pseudoginseng* exhibited poor adaptation to artificial habitat. Many *Panax pseudoginseng* rhizomes either remain dormant and fail to sprout out or the plant fail to flower in the wild. The plant is a long lived perennial plant but the species is being over-exploited along with other species of *Panax* for medicinal purpose in Nagaland and their natural habitats are getting destroyed rapidly. The plant has reduced drastically and entered under threatened category as the species has very poor adaptive capacity. The species can survive over 40-45 years but in Nagaland it is very rare to find rhizome above 30 years. Besides anthropogenic disturbance, another reason for downsizing of their population is the susceptibility to fungal diseases and nematodes. These attack the root and stem of the plant and often small colonies of ants form on the soil where the plant grows that attack the peduncle and rhizome. Empty skins of the roots were found frequently in cultivated older *Panax pseudoginseng* plants.

It was observed that compare to Kohima; plants maintained in Penli ward, Mokokchung were better adapted though the progressive acclimatization was very poor. Plants exhibited gradual increase in its height, exhibition of flowering in subsequent years and bearing of young fruits. This could probably due to the fact that though the altitude in Mokokchung district is lower compare to Kohima experimental plot, yet intensive and constant care can be easily given and done. Moreover unlike the open natural forest like in Aradura hill, the green house monitors the humidity, temperature and sunlight.

In the present study it was found that *Panax pseudoginseng* generally starts flowering only after they are 3-4 years old but many plants failed to flower even after 5-6 years or did not flower for 3-4 years consecutively. Flowering of plants are influenced by various factors like morphophysiological and physical environment surrounding the species. These factors influence a lot on the reproductive age and reproductive potentiality of the species. Favorable sites may quicken the juvenile phase and thus reproduces at a younger age. *Panax pseudoginseng* gives flowers when the plants attains two leaves, however the flowers productions are very less and the fruit production is negligible and many a times all the flowers are aborted before fruit formation. The fruit setting of the plant is very poor and many of the berries fails to enlarge or dehisce off before getting matured, this can be due to the individual seed stalk which is weak and it becomes weaker due to the weight of the berry or largely attributed to overcrowding.

Most of the fruits are dispersed due to gravity dispersal method and therefore small plantlets are found near the parent plant within a range of 1-2 m. However some seeds are found at more distance due to the slope where the dry twigs and leaves present may aid in its movement. A special case of ingestive dispersal is ‘mimicry’ in which the seeds are bright colored to attract birds, but the fleshy nutritious substance normally associated with ingestive dispersal is absent. Such Diasporas are believed to ‘mimic’ nutritious berries or other fleshy fruits without offering any reward to the dispersal agent (McKey, 1975;Van der Pijl, 1982).

Along with gravity dispersal, birds and jungle rats also act as the plant dispersal agents and reports of fruits eaten by the bird *Plytes dragopan* in *Panax ginseng* plant has also been reported (Changkija, 1999). One of the documentation from locals is that the germination of seeds from the waste of the birds is very high, which needs to be further studied. Although fruit maturation is variable across the species' range within the five districts of Nagaland , but it usually begins in mid to late summer with fruits turning red at maturity in late summer to early fall. The fruit sometimes are found even during the end of October month. The plant needs sunlight illumination nearly 10-15% of total sunlight receive in a day for its production of seeds and it was found that most plants which were experimentally kept in total shade condition produces flowering but no seeds and the plant withers off. Reproduction of *Panax pseudoginseng* is by seeds itself because rhizome fragmentation rarely occurs in natural condition.

The time for emergence of radicals from the germinated seeds, germination time, germination rate, seedling morphology, post germination establishment of seedlings is influenced by various factors and appears to be species specific (Langhu and Deb, 2014). Seedling survival on the seed bed and or in the natural habitat is governed by the availability of suitable substrata, light, nutrients, soil moisture etc. (Kitajima, 2007; Langhu and Deb, 2014). Plant species differ in their habit preference, temperature requirement, and post harvest storage, specific pre-treatment of seeds for germination, seedling emergence and survival. A number of species exhibit positive as well as negative correlation between canopy cover, humidity, temperature etc. (Kwit and Platt, 2003; Pages *et. al*., 2003; Deb *et. al*., 2012; Langhu and Deb, 2014). There are several reports available enhancement of seed germination by pre-germination treatment of seeds with chemicals, low temperature and systematic stratification (Pandey *et. al.*, 2000; Srivastava *et. al.*, 2011; Sharma and Gaur, 2012; Deb *et. al.*, 2012; Langhu and Deb, 2014).

Though the practice of seed propagation, seed storage are not new, but systematic study of seed biology like storage, stratification for germination etc. have been a development of the 20th century. Presently there are over 1500 seed gene banks throughout the world. Viability of a seed lot declines over time, though the seed may germinate, the resulting seedling may have reduced vigor and fail to establish in the field (Walters, 2004). So, for some plant species, using relatively fresh seeds gives better germination over stored seeds.

The seed setting in the plants are affected during the pollination process from the time of pollen reaching to the stigma to fertilization process when the pollen tube grows to the stylar region. Moreover the formation of seed from its development to its maturation is all accounted to for quality of seed setting (Mooney and McGraw, 2007). Environmental factors and changes will play a major role during the development of seeds. In *Panax pseudoginseng*, self-pollination is better with small variable difference than cross-pollination. This is in accordance with (Mooney and McGraw, 2007) where seed production was more in self- than cross-pollinated flowers. This may also indicate why the plants are usually self-pollinated in nature.

Panax pseudoginseng seeds were collected from the natural habitat/population in the study areas in the month of October when berries were matured. The seeds were stratified in two phases for ~7 months before sowing in the seed beds. While non-stratified seeds registered germination after ~14 months of sowing. These results indicates that *Panax pseudoginseng* seeds are morpho-physiological dormant at the time of harvest. Morpho-physiological dormancy is common in many plant species but not as wide as in the genus *Panax* (Li, 2002; Venugopal and

Ahuja, 2013). Dormancy could be due to several factors like embryos not fully developed in the freshly harvested ginseng seeds (Baskin *et. al*., 1995,; Li, 2002). Besides *Panax,* seed dormancy is also reported in species like *Ginkgo biloba* (Tommasi *et. al*., 2006). In the present study it was necessary to stratify the seeds in two phases for successful germination. Seeds stratified at 25^{o}C for three months followed by cold stratification at 4^{o}C for 1 to 7 months proved to be beneficial for seed germination. Under controlled condition ~10% seed germination recorded after 425 days in seed bed 1 while 8% seed germination registered in seed bed 2 after 450 days of sowing. This result is in agreement with the reports with the other species of *Panax* (Xiao *et. al*., 1887; Li, 2002; Venugopal and Ahuja, 2013). According to Xiao *et. al.* (1987) it was necessary to cold stratify the seeds for 18-22 months for successful germination of American ginseng. Warm stratification for 3 months followed by cold stratification beyond 3 months was found to be promotive for seed germination in both the seed beds. Of the two seed bed condition studied in the present study, seed bed prepared by mixing normal soil, decayed wood powder and sand at 3:1:1 ratio found to be better for seed germination. The finding of this work indicates that cow dung is not an ideal substratum for *Panax pseudoginseng* seed germination.

Summary and Conclusion

Present study was undertaken to study the distribution of *Panax pseudoginseng* in two natural habitats and the factors influence their establishment in the natural habitat and their adaptability in synthetic habitat. Besides this, effort was made to study the correlation between the plant phenology, rhizome age on flowering and seed setting. It was found that *Panax pseudoginseng* grow healthy in the undisturbed forest where the canopy cover is more than 80% and if the canopy cover decreases <80%, plants results in poor establishment. In the artificial habitats, plants registered poor survival and failed to flower effectively. It was observed that plants bearing less than three leaves did not support healthy flowering and older rhizomes exhibits better viable fruits setting. Further it was recorded that plants with 2-3 leaves supported very less three seeded fruits while in plants with 4-5 leaves, increase number of 3 seeded fruits occurs thereby increased the reproductive outcome. The plant also shows self-pollination better than cross-pollination in terms of seed setting. The collected seeds were stratified following two different techniques. The un-stratified seeds exhibited only 10% germination after almost 14 months of sowing. In contrary when the seeds stratified at $25^{o}C$ for 3 months followed by cold stratification ($4^{o}C$) before sowing to the seed bed registered ~68% germination within 2 weeks of sowing. The seedling resulted from the germinated seeds were transfer to the field after 1 year in the natural habitat.

Panax pseudoginseng is an economically important medicinal plant and the species is extremely threatened. The findings of the present study provide us a clear picture about the natural habitat and their primary requirements for survival, flowering, fruiting, seed setting. Further the findings pinpoints why the species cannot be cultivated easily away from the natural habitat. The findings from the seed biology study will help the commercial growers for propagation of the plant through its seed. The local authority, government agency should join hands to develop *Panax* park/garden in the natural habitats to propagate and conserve this economically important species.

Chapter - 3

Floral Phenology, Reproductive Biology of *Paris polyphylla* Smith.

The reproductive biology, phenology, generation capacity, its distribution power and the level of threats that occurs both naturally and by human activities determine the population size of any medicinally important plant species. The most vulnerable species are usually popular, slow growing or slow to reproduce, or species with specific habitat requirements and a limited distribution (Cunningham, 1993). Phenology is derived from the Greek word '*phainein*' meaning to 'show or appear'. The phenology of plant can be studied in its different stages such as leafing, flowering, fruiting, seed dispersal and germination (Fenner, 1998). Phenological studies of medicinal plants are very important to know the right season of its collection, its effective treatment and its propagation in the right environment (Abera *et. al.,* 2008). Study of the plant reproductive biology is very important for understanding why the plant is endangered, rare or threatened (Schemske *et. al.,* 1994) so that knowledge obtain can be useful for making out any particular plant species for its conservation.

The word 'Paris' is derived from Latin word '*pars'* meaning equal often referring to the symmetry of the plant for its equal number of floral parts and leaves and the word '*Polyphylla*' refers to the many (poly) and leaves (phylla). *Paris* L. (Trilliaceae) genus consists of about 24 species which is distributed from Europe to Asia (Zhang *et. al.,* 2011). Trilliaceace is divided into two genera*, Trillium* L and *Paris* L. *Trillium* is trimerous while *Paris* is 4- to 11- merous (Yun-Heng *et. al.*, 2006). *Paris polyphylla* is found in China, India, Vietnam and Germany (Li *et. al.*, 2012). China is the centre of species diversity and endemism for the genus *Paris* with 22 species found out of which 12 are endemic (Shu-dong *et. al.*, 2008). *Paris polyphylla* has different varieties and there are 10 varieties identified so far (Shu *et. al*., 2000). *Paris polyhylla* is categorized as sparse (Yonzone *et. al*., 2012) in India but it is listed under vulnerable category in Nepal (Madhu *et. al.*, 2010). *Paris polyphylla* Smith is an important medicinal plant containing saponin steroids polyphyllin D, dioscin and balanitin7 (Li *et. al.*, 2012). The rhizome of *Paris polyphylla* Smith is widely used in Chinese tradition medicine under the name 'Chonglou' (Zhang *et. al.,* 2011). Rhizome of the plant is use as antihelmintic, antispasmodic, digestive stomachic, expectorant and vermafuge (Madhu *et. al.,* 2010), scabies, rashes, or itching problems (Jamir *et. al*., 2012), to treat liver cancer (Li *et. al.*, 2012). The roots are also fed to cattle with diarrhea and dysentery (Madhu *et. al.*, 2010).

In Nagaland, forest are lost or fragmented due to intensive ‘Jhum’ cultivation, incidences of forest fires, logging and developmental activities along with large scale landslides which effects and threatens the biodiversity of a forest. Plants which are at constant threat need immediate conservation. These challenges can be overcome with a definite and appropriate conservation strategy. Species conservation can take place both *ex situ* and *in situ*.

Paris polyphylla Smith. is under constant threat because of rampant harvesting for local medicine as well as illegal trade to the neighboring states and countries. The plant which was quite abundant in the last decade has decreased drastically and therefore conservation of this plant is highly important in the wild. Educating the locals on the importance of this plant and techniques for its conservation in the state can be an effective measure. The plant with its certain morphological limitation factors like habitat specific, slow growth and time taken to become reproductively mature along with reproductive limitations such as non-reproductive plants and long term seed dormancy adds to the rapid reduction in its species. Thus, there is an urgent need to conserve this medicinal plant both *ex situ* and *in situ*. Understanding its habitat, its reproductive biology and seed biology therefore becomes an important priority to understand more of this plant and to conserve them through any appropriate tool.

Materials and Methods

Study Sites

Present study was conducted in both natural as well as artificial habitat. In the present study, four natural habitats were selected (**Fig. 10**). The natural habitats were selected in different parts of the state so that an average representative data could be generated. A brief about the study areas are given below:

1. Forest area of Pangsha village near Indo-Burma border at an elevation of 2278 m above sea level (ASL), 26˚14´27.2´´ N Latitude and 95°07´05.7´´E Longitude under Tuensang district, Nagaland, India.
2. ‘Chida’ and its surrounding forest area (Khezakenoma village under Phek district, Nagaland) at an elevation of 1874 m ASL, 25˚30´11´´ N Latitude and 94°13´37´´E Longitude.
3. Aradura Hill at an elevation of 1396 m above sea level (ASL), 25˚38´49.5´´ N Latitude and 94°06´25.8´´E Longitude under Kohima district, Nagaland, India.
4. Longkum village forest area at an elevation of 1404.51 m above sea level (ASL), 29˚15´98.8´´ N Latitude and 94°24´03.5´´E Longitude under Mokokchung district, Nagaland, India.
5. ‘Chida’ and its surrounding forest area (Khezakenoma village under Phek district, Nagaland) at an elevation of 1874 m ASL, 25˚30´11´´ N Latitude and 94°13´37´´E Longitude.
6. Aradura Hill at an elevation of 1396 m above sea level (ASL), 25˚38´49.5´´ N Latitude and 94°06´25.8´´E Longitude under Kohima district, Nagaland, India.
7. Longkum village forest area at an elevation of 1404.51 m above sea level (ASL), 29˚15´98.8´´ N Latitude and 94°24´03.5´´E Longitude under Mokokchung district, Nagaland, India.

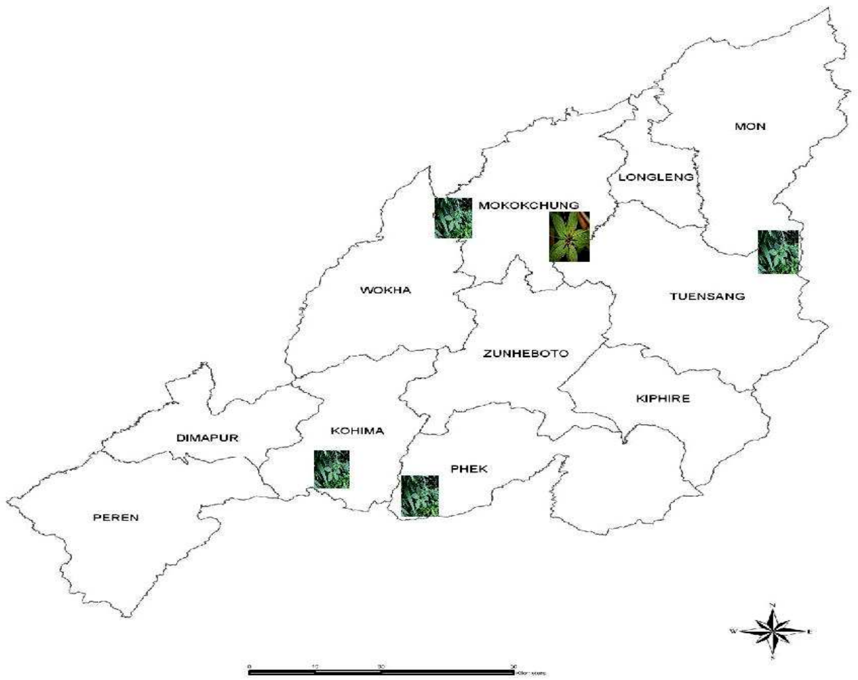

Figure 10: Map of Nagaland showing the natural habitats of *Paris polyphylla* Smith. studied.

During the study, characteristics ecology of the habitats, morphological aspects of the plant along with its phenology and reproductive biology were studied. Experimental plots were created in all the study area for experimental purpose. Certain microclimatic conditions like canopy cover of the natural habitats, associated plant species and level of anthropogenic disturbances were studied.

Reproductive successes of the plant from the time of flower pollination to seed formation both in natural and in artificial condition were studied. The adaptability of the species away from their natural habitat was studied. After collection from the natural habitats, the plants were planted in poly bags and transported to the artificial habitat and transferred to potting mix as described below.

Penli ward: Plants collected from the wild was put under experimental study in Penli ward, Mokokchung district, Nagaland in a poly house at an elevation of 1259 m ASL. Different soil mixture was prepared by mixing decayed wood, sand and top soil as follows:

1. Manure soil by mixing decayed wood, sand and top black soil at a ratio of 1:1:3.
2. Mixture of cow dung, sand and top black soil at a ratio of 1:1:3.
3. Only to black soil.

Plants were monitored constantly for its growth and development. Changes in the soil were done every year to ensure nutrient availability to the plants.

The experimental beds were provided with different shaded conditions as below:

a. Zero shade.

b. Plants kept in association with domestic garden plants and climbers which provides partial shade.

c. Plants kept in poly house ca. 75% shade.

Plants grown in different soil mixture, their adaptation outside the natural habitats, effect of temperature and shaded conditions on the plant growth and development were studied.

***Rhizome characteristics*:** The rhizomes of the plant collected from different areas were studied. The number of nodes formed due to bud scars present on it was initially counted to determine the age of the plant but was not use in the later part when multiple shoot buds were found out in many rhizomes. Rhizome relation to the production of buds was studied.

***Flowering and non-flowering plant*:** The degree of plant survival is related to its reproductive output and success. Therefore, considering this, the plants were carefully studied and tagged to see their inflorescence development and non-development in the studied plant population. The method applied was simple and traditional. Plants in a given population were counted and tagged as reproductive (flowering) and non-reproductive (non-flowering) during the month of April.

***Nature of flowering and plant pollinators*:** Flowering plants were studied both in the wild and in the artificial condition to identify the nature of its flowering along with its pollinating agents. The phenological changes occurring during the flowering stage were carefully studied. Floral parts characteristics and the changes it undergoes were duly noted.

***Post fertilization development in Paris polyphylla* Smith var. *polyphylla*:** The process that undergoes after the plants are fertilized was studied taking into consideration the morphological changes of all the parts of the inflorescence. Ovary development and enlargement during fruit formation was observed.

***Fruiting and seed setting*:** Fruiting and seed formation in *Paris polyphylla* Smith var. *polyphylla* was studied in tagged plants in different locations at both natural and artificial habitats.

***Seed setting in relation to different hand pollination treatments*:** Two different pollination treatments were performed where 10 healthy plants were selected for each treatment. These pollination treatments were done in order to find out the effects of seed setting in plants through self- and cross- pollination process. This hand pollination technique was used on the same flower and between two flowers of different inflorescences on two different plants. A small water colour paintbrush to brush off the pollens from the plant, a small container to collect the pollens and dry cotton swab to put these pollens on the stigma was used. The brush was ensured that it was totally cleaned and dry, so it was washes in running water and air blown dry before its usage. Pollination was done early in the morning at 6:30 -7:30 am when the weather is in dry conditions using the cotton swab. The cotton swab were gently swirled around the selected the stigma of the selected female flower. They are then wrapped around using mesh clothes and then fasten the wrap around the peduncle using threads. This process was repeated for three-four times using the same selected plants.

Different pollination experiments were done as follows:

a. For self pollination, 10 selected individual flowering plants are carefully bagged with fine nylon mesh clothes.

b. For cross pollination between different flowers. 5 selected individual flowers from the inflorescence are carefully bagged with fine nylon mesh clothes and the other 5 selected flowers are emasculated by removing their stamens before anthesis and are bagged to prevent from outside pollinators. These emasculated flowers are pollinated randomly from the rest of the 5 selected flowers in the same inflorescence.

   The flowers are then tagged with their date of emasculation and date of pollination.

***Paris polyphylla* Smith. *in relation to climatic conditions and its susceptibility to predators*:** *Paris polyphylla* Smith var. *polyphylla* in relation to climatic factors and susceptibility to predators were studied in both habitats (natural as well as artificial) so as to identify the plants susceptibility as it is an important factor in its reproductive success.

Results

***Habit and habitat*:** *Paris polyphylla* Smith var. *polyphylla* is an important medicinal perennial plant. During the present investigation, study was conducted in 4 natural habitats (**Fig. 11 a-d)** with a total of 8 populations for 4 years (**Table 14**). Locations of *Paris polyphylla* Smith var. *polyphylla* populations in Nagaland were recorded at an altitude of 1300 m ASL and above but healthy populations were found above 1400 m ASL (**Table 15)**. At lower altitude (<1300 m ASL) no population was reported. It was observed that the species exhibits healthy growth in undisturbed areas with less human interference. *Paris polyphylla* Smith var. *polyphylla* is a shade loving plant and grows under the canopy of moist temperate forest with canopy closure of more than 80% at an altitude of 1300-2500 m ASL in Sub-tropical broad leaved hill forest to temperate broad leaf forest. The plant requires moist and well-drained soil. It grows in slope areas usually north facing slopes with soils that are usually covered with dry and decay organic matters. The plant is an erect herb, 30-100 cm in height (**Fig. 12 a**). The species grows in acidic, well-drained humus rich soil. The soil sample collected from the study area where the plants were found indicates high carbon content, moderate potassium but the soil was poor in its phosphorous content (**Table 16)**. The level of disturbances in all the four study areas ranges from low to high disturbances. High level of disturbances was found in the unprotected forest area of Pangsha village. *Paris polyphylla* Smith var. *polyphylla* found in and around the area of Chida (Khezakenoma) area were found growing the best in slope areas with well-drainage. Some plants were found growing for even more than 1 m in good soil.

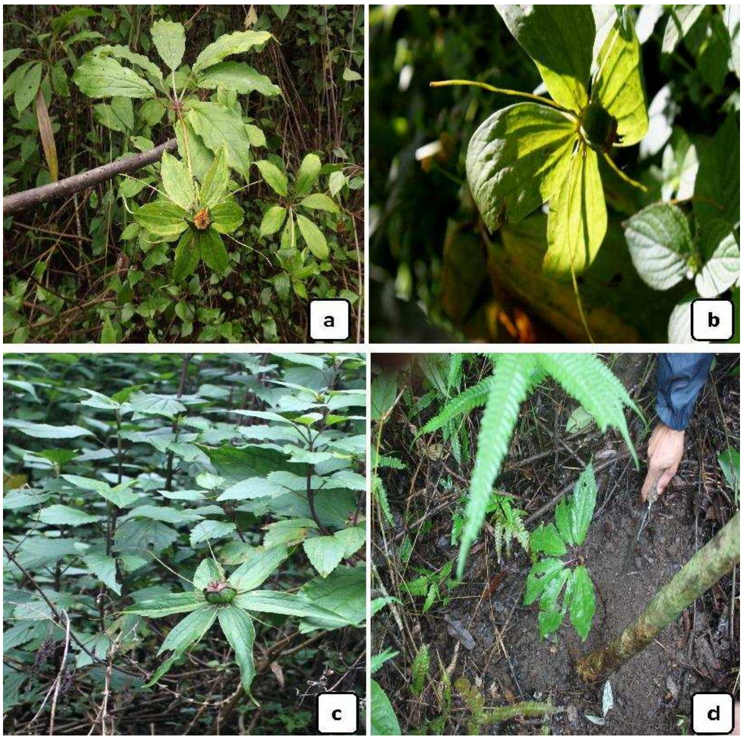

Figure 11: *Paris polyphylla* in all the natural habitats studied. a. Chida area under Khezakenoma village (Phek district); b. Pangsha area (Tuensang district); c. Aradura Hill (Kohima) and d. Longkum village forest area (Mokokchung).

Figure 12. a. *Paris polyphylla* habitat; b. Associated species in area where *Paris polyphylla* Smith var. *polyphylla* grows.

Table 14: Demographic studies of *Paris polyphylla* in Nagaland

Sl. No.	Study location/site	Length of study	Number of populations
1	Tuensang	4 years	3
2	Phek	4 years	2
3	Kohima	4 years	1
4	Mokokchung	4 years	2

Table 15: Niche characterization of *Paris polyphylla* in Nagaland

Site name	Locality	Elevation	Canopy Closure*	Disturbances
Tuensang	Pangsha area	2278 m ASL (26˚14´27.2´´N and 95°07´05.7´´E)	86%	High human interference
Phek	Chida and its surrounding area.	1874 m ASL (25˚30´11´´ N and 94°13´37´´E)	88%	Limited human interference
Kohima	Aradura hill	1384 m ASL (25˚38´49.5´´N and 94°06´23.6´´E)	84%	Protected area
Mokokchung	Longkum village	1404.51 m ASL (29º15´988´´ N and 094°24´035´´E)	87%	Limited human interference

Table 16: Report of forest soil (top soil) analysis from different niches of *Paris polyphylla*

Area from where soil collected	Soil *p*H	Organic carbon (%)	Available Phosphorus (Kg/ha)	Available Potassium (Kg/ha)
Pangsha area	6.80	2.24 (H)	8.00 (L)	138.80 (M)
Chida and its surrounding area.	6.54	2.35 (H)	7.85 (L)	140.00 (M)
Aradura hill	6.55	2.15 (H)	8.10 (L)	137.50 (M)
Longkum village	6.40	2.42 (H)	8.00 (L)	138.50 (M)

Reference: N: Normal; H: High; M: Medium; L: Low

Table 17: Associated herbaceous plants found near *Paris polyphylla*

Sl. No.	Plant species	Family
1.	*Aconitum* sps.	Ranunculaceae
2.	*Ageratum conyzoides*	Asteraceae
3.	*Berberis* sps.	Berberidaceae
4.	*Bidens pilosa*	Asteraceae
5.	*Curculigo capitulata*	Hypoxidaceae
6.	*Drymeria cordata*	Schrophulariaceae
7.	*Eupatorium adenophorum*	Asteraceae
8.	*Fagopyrum esculentum*	Polygonaceae
9.	*Hottuynia cordata*	Saurauiaceae
10.	*Impatiens* sps.	Balsaminaceae
11.	*Lycopodium* sps.	Lycopodiaceae
12.	*Spilanthes acmella*	Asteraceae

Note:
The above table gives generalized common plants which are found within 5 m parameter where *Paris polyphylla* Smith var. *polyphylla* is found in its natural habitat.

It was found that the growth of *Paris polyphylla* Smith. was influenced by other plant species growing in adjoining vicinity in the natural habitat and the presence of some common plants species within 5 m parameter were observed and taken into account (**Table 17, Fig. 12 b**). Further it was recorded that wherever these associated species were present, *Paris polyphylla* Smith var. *polyphylla* registered healthy growth compare to the areas where these species were absent. Some of the common associated species identified are:

Trees - *Schima wallichi* (Theaceae), *Alnus nepalensis* (Betulaceae), *Bauhinia purpurea* (Caesalpiniaceae), *Albizia procera* (Mimosaceae), *Pinus roxburghii* (Pinaceae), *Rhododendron arboreus* (Ericaceae), *Quercus* sps. (Fagaceae), *Taxus baccata* (Taxaceae) and Bamboos.

Herbs - *Berberis* sps. (Berberidaceae), *Aconitium* sps. (Ranunculaceae), *Impatiens* sps. (Balsaminaceae), *Ageratum conyzoides* (Asteraceae), *Bidens pilosa* (Asteraceae), *Curculigo capitulata* (Hypoxidaceae), *Drymeria cordata* (Caryophyllaceae), *Eupatorium adenophorum* (Asteraceae), *Fagopyrum esculentum* (Polygonaceae), *Hottuynia cordata* (Saururaceae), *Spilanthes acmella* (Asteraceae), *Lycopodium* sps. (Lycopodiaceae), Ferns and Bryophytes.

Climbers - *Dioscorea* sps. (Dioscoreaceae) and *Smilax* sps. (Smilacaceae).

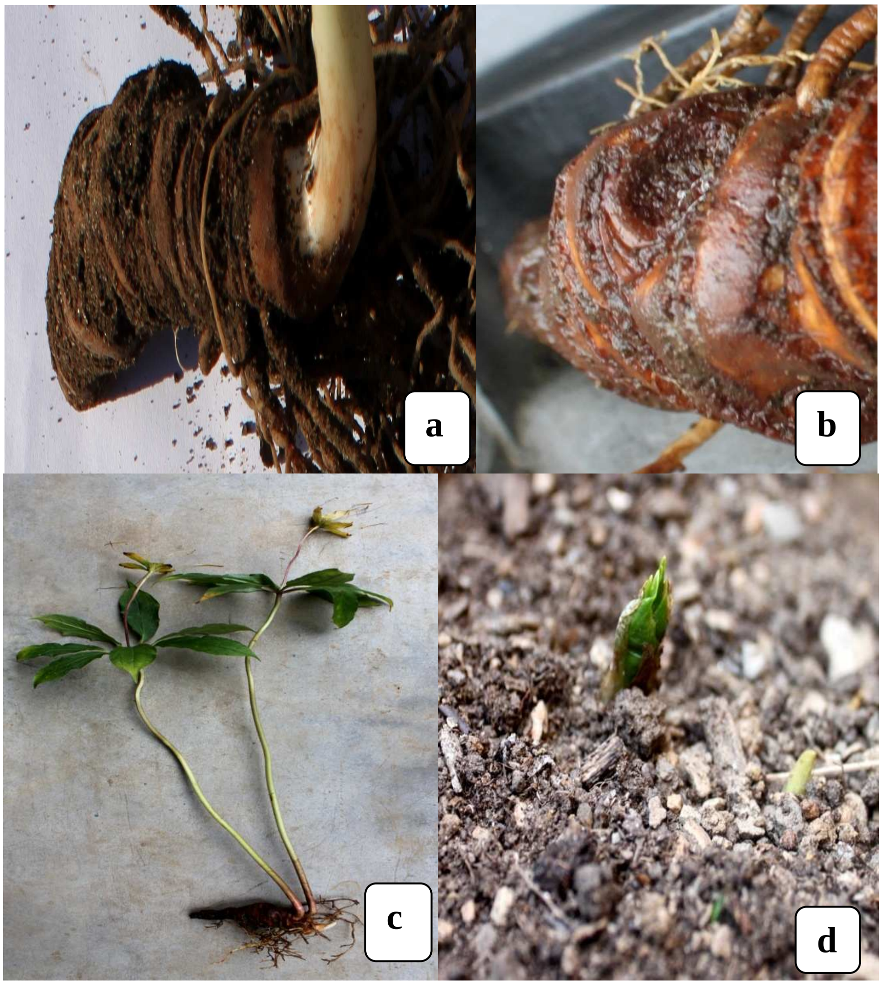

Figure 13. a. Horizontal rhizome of *Paris polyphylla*; b. Rhizome showing transverse rings; c. Formation of buds before the senescence of the above ground plant body; and d. Sprouted buds formed plants.

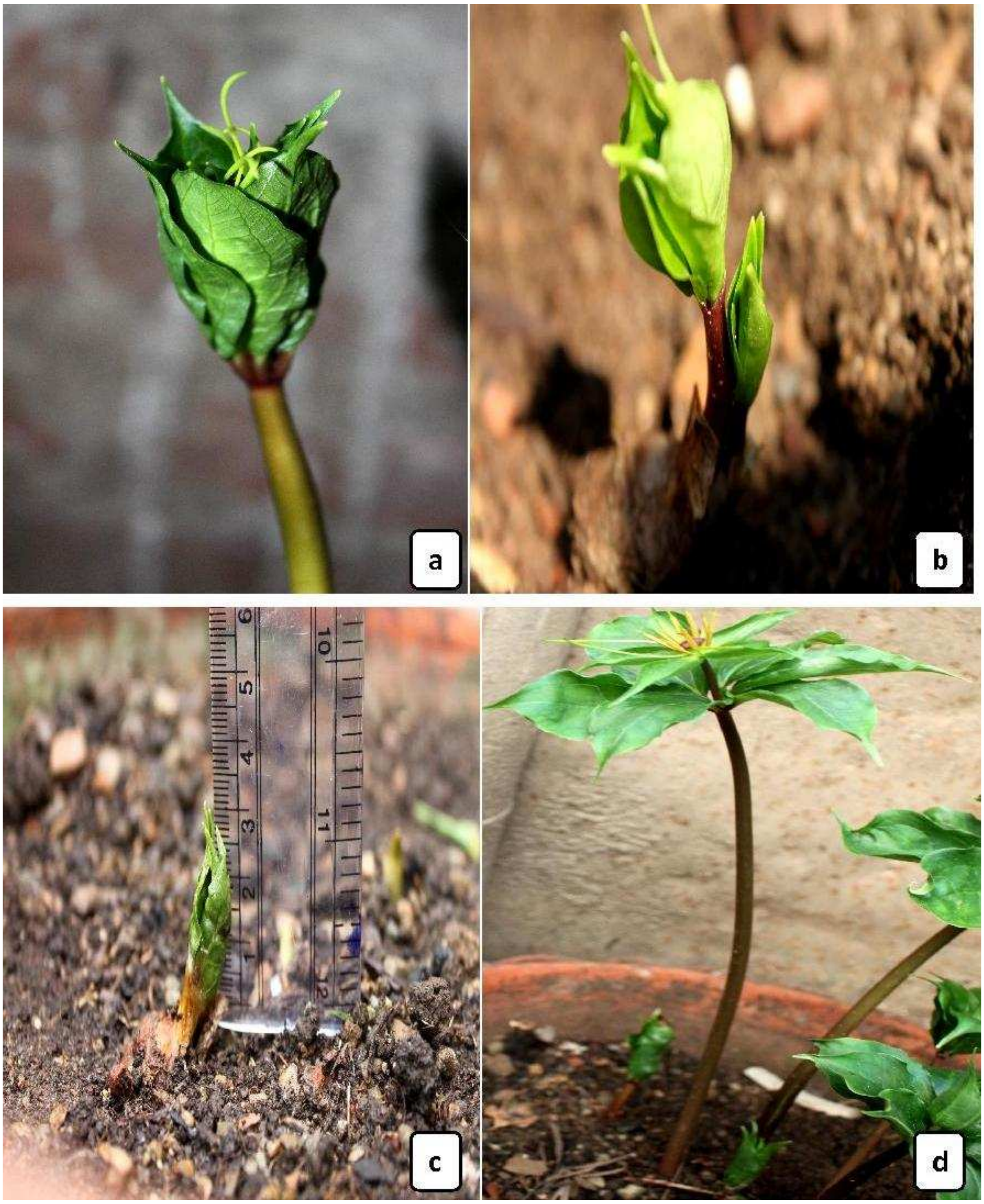

Figure 14: a. Plants with young leaves and inflorescence forms as closed whorl; b. Formation of compound plants from rhizome; c. Formation of single plant from rhizome; d. Formation of new shoots in mid-season when plants are at bloom.

***Rhizome characteristics*:** It was found that in most of the cases the rhizomes were horizontal and creeping (**Fig. 13 a**). *Paris polyphylla* has got transverse nodes on its rhizome formed from the bud scars (**Fig. 13 b**). A small bud outgrowth appears on its rhizome just before/after the above ground part of the plant dies during late autumn (**Fig. 13 c)**. The bud remains dormant for 4-5 months during the winter season until the next sprouting season. *Paris polyphylla* starts sprouting from February onwards till the month of March. The dormant bud from the previous season gives rise to new above ground stems, leaves and inflorescence (**Fig. 13 d**). Usually a single bud sprouts from an individual rhizome but 2-3 buds formation also occurs in some rhizomes. The young leaves and the inflorescence forms a closed whorl as they sprouts out (**Fig. 14 a**). The plants grow fast once the dormancy of its bud is broken. The counting of the bud scars or nodes on the rhizome for determining its age cannot be done as the number of bud shows variations from one to three in different plants. The number of bud in any particular rhizome does not remain constant. If a double bud occurs in a particular rhizome, then it will give rise to compound plants but that particular rhizome can also give out a single bud in the subsequent years (**Fig. 14 b, c**). Further it was found that in some cases a rhizome which already has a matured flowering plant gives rise to new buds in the middle of the year usually during the month of April and May (**Fig. 14 d**). However, the young plants are mostly non-reproductive (absence of floral parts) and shorter than the matured flowering plant.

Table 18: Percentage of non-reproductive *Paris polyphylla* plants

Location	Total number of plants observed	Non-reproductive plants	% non-reproductive plants
Tuensang	332	114	65.67
Phek	252	103	59.13
Kohima	127	44	65.36
Mokokchung	214	92	57.01
Total	925	353	61.84

Note:

- Plants were studied in different locations within the whole district.
- Non-flowered plants were very high in *Paris polyphylla* Smith var. *polyphylla* plants and it accounted for more than 60% in total plant populations.

Figure 15: a. A clump showing flowering plants; b. A clump showing non-flowering plants.

Flowering and non-flowering plant**:** In the present study, an observation with reference to the flowering ability of the plants was made. It was found out that majority of the plants either in natural habitats or plants grown under controlled conditions were found to be non-reproductive or in other words absence of inflorescence. Over 60% of the studied plants were found to the non-reproductive (**Table 18)**. Non-reproductive plants are easily identified at the initial growth of the plants as they will lack the inflorescence stalk and will be shorter in height (**Fig. 15 a, b**). The non-reproductive plants are independent in relation to the age of its rhizome in all matured plants. Matured plants which are non-reproductive may remain the same in the next season or may become reproductive which is vice-versa for reproductive plants.

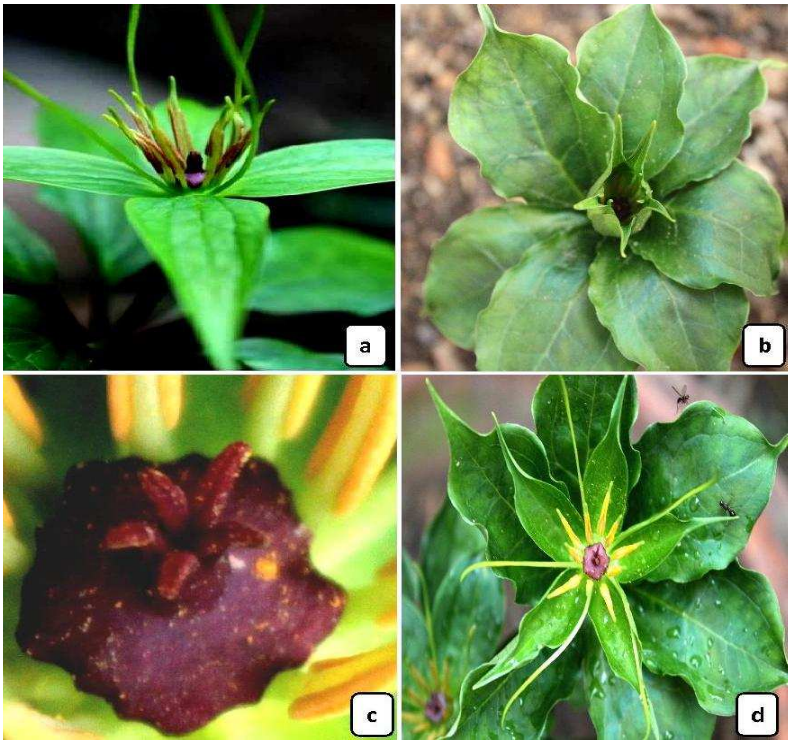

Figure 16: a. *Paris polyphylla* in full bloom; b. Early stage of bloom with closed whorl; c. Purple stigma; d. Flowers showing leafy green outer tepals and yellowish green, filiform inner tepals.

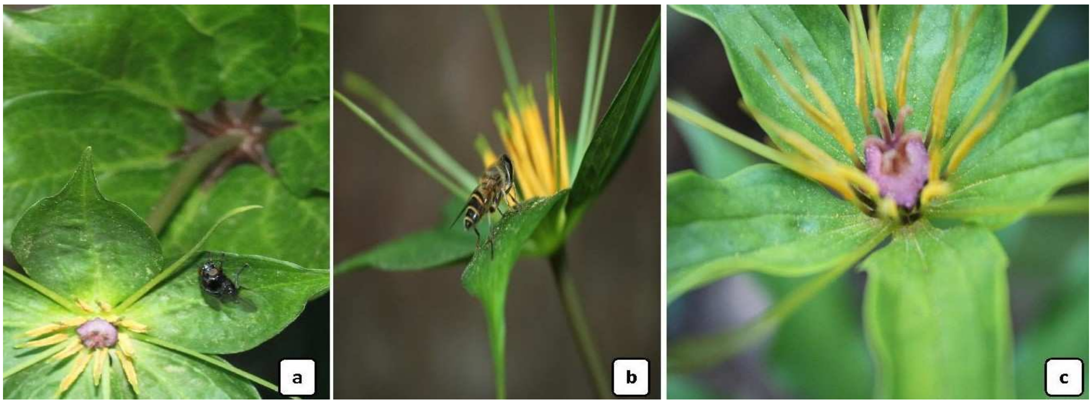

Figure 17: Possible pollinators of *Paris polyphylla*. a. Flies; b. Bee; and c. Pollens dehisced on tepals.

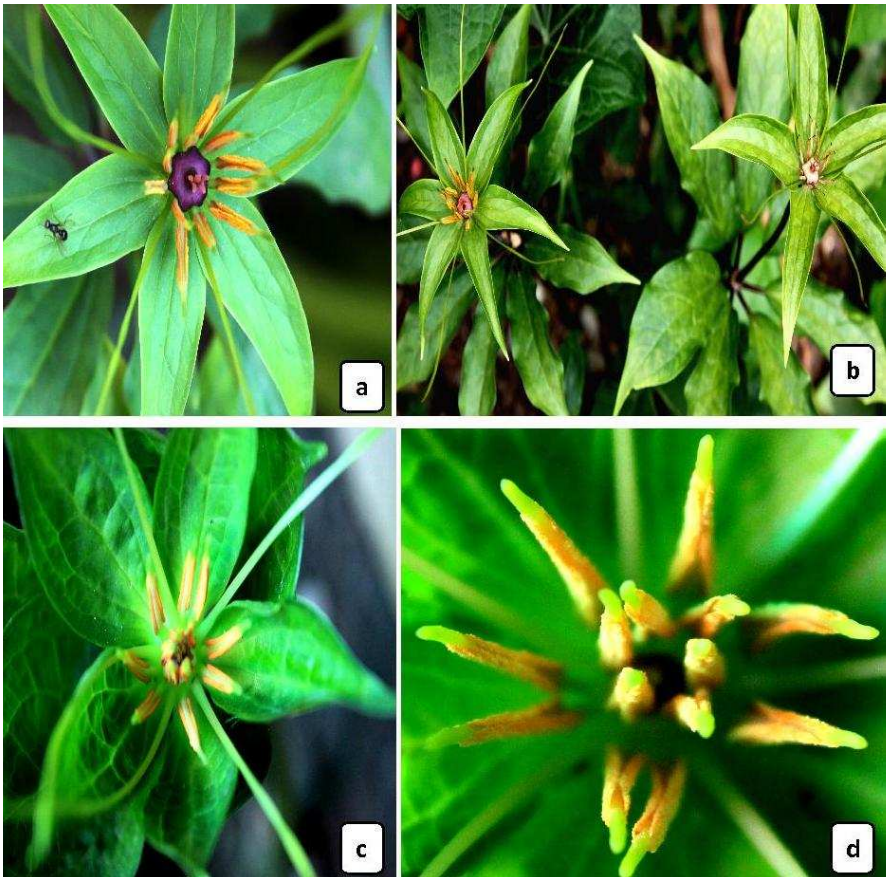

Figure 18: a. *Paris polyphylla* ovary before pollination; b. Ovary during and after pollination; c. *Paris polyphylla* Smith var. *polyphylla* anthers arranged in two whorls; and d. Typical arrangement of inner and outer whorls of *Paris polyphylla*

***Nature of flowering and plant pollinators*:** In *Paris polyphylla* flowering begins in March-April where the flowers bloom at the terminal and they are solitary (**Fig. 16 a**). At the initial growth, the inflorescence forms a closed whorl covered by the sepals enclosing the tepals, the anthers and the stigma (**Fig 16 b**). The ovary is situated in the middle with usually 5 stigmatic lobes. The stigmatic lobes are wet type in nature. Usually the stigma appears deep purple in color during the pollination period (**Fig. 16 c**). In *Paris polyphylla* Smith var. *polyphylla,* the male function first than the female, therefore the flowers are protrandous. Flowers are perfect flowers as they contain both the male and female gametes within the same flower. Outer tepals are leafy and green, while the inner tepals are yellowish green and filiform (**Fig. 16 d)**. Usually the numbers of outer and inner tepals are same. The number of anthers is usually more than double that of tepals. Stigma re-curved at the tips. Plants generally remain at bloom for a period of 2-3 weeks. The timing of anthesis is between 6:30-7:30 AM and the anther dehisces by 7:30-8:30 AM (**Table 19**). The flowers are capable of self-pollination. However pollination can occur through small insects as the plants are visited by different insects such as different types of flies and bees especially during the middle of daytime from 11:00 A:M till 3:00 pm (**Fig. 17 a, b**). Wild bees may act as pollinators. The buzzing of their wings might facilitate the pollen dispersal to the stigma within the flower itself or even to the surrounding flowers as the pollens are very light (**Fig. 17 a, b**). Since the pollen grains are very light, wind may also act as a pollinating agent. The plants are self compatible and they produce large amount of pollens. Huge amount of pollens are wasted which is indicated by the falling of pollens all over the outer tepals (**Fig. 17 d)**. The pollen from the anther falls on the stigmatic lobes once the petal opens and fertilization begins as it reaches the ovary through the style. Stigmatic lobes and the ovary capsule are usually bright purple but turns dull purple once the pollination takes place (**Fig. 18 a, b**).

***Unique character of the anther*:** The anthers of *Paris polyphylla* occur in two whorls (**Fig. 18 c**). Closing and opening of the anthers takes place. Usually the inner whorl has two lesser anther filaments compare to outer whorl (**Fig. 18 d**). The anther filaments of the flower are arranged in a pattern as such that an inner whorl is followed by an outer whorl in a consecutive manner. Two pairs of outer anther filament will lack in its inner whorl filament and this two pairs will always be on adjoining side. This anther display is same in many flowers of *Paris polyphylla.*

Table 19: Reproductive phenology of *Paris polyphylla*

Parameters	Observations
Height of the plant	Up to 1 m
Rhizome type	Horizontal and creeping
Sprouting of plant from rhizome	February-March
Dormancy of rhizome	4-5 Months
Leaves	Lanceolate or sometimes oblong
Outer tepals	Leafy green
Inner tepals	Yellowish green and filiform
Flowering	March-April
Flowers	Solitary and terminal
Time of anthesis	6:30-7:30 AM
Time of anther dehiscence	7:30-8:30 AM
Stigma	Wet type
Stigma lobes	5
Pollination	Usually self-pollinated
Ovary capsule before and during pollination	Bright and deep purple
Ovary capsule after pollination	Dull purple
Fruiting	May
Temperature for seed production	18˚C-23˚C
Seed	Bright orange color
Seed maturation	September-October
Dispersal of seeds	Gravity
Senescence	October-November

Figure 19: a. Recalcitrant rhizome failed to form shoot buds during growing season; b. Sprouting of shoot buds from rhizome and formed plantlets.

Flowering in *Paris polyphylla* is dependent on the rhizome. A recalcitrant rhizome (**Fig. 19 a**) never flowers as they fails even to give out their shoot buds and only rhizome that gives rise to shoot buds (**Fig. 19 b**) will have a probable chance of giving rise to flowering in plants.

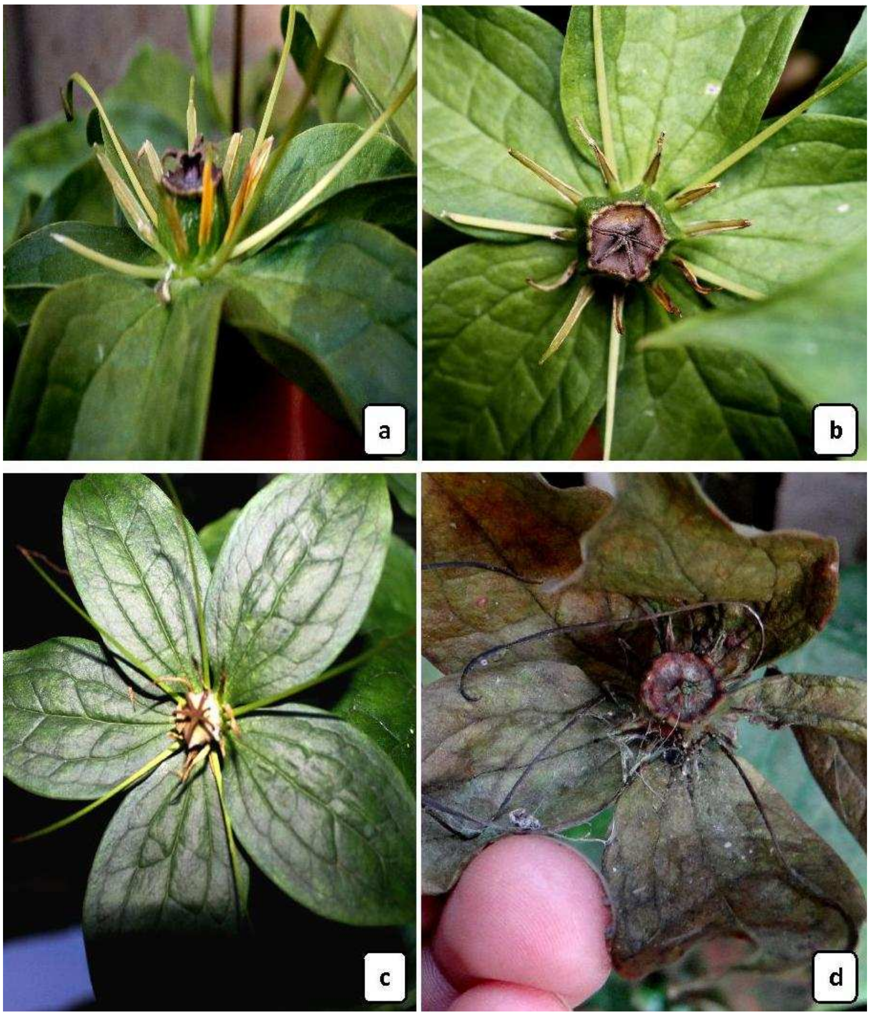

Figure 20: a. Disintegration of floral parts of *Paris polyphylla* after pollination and formation of young fruit; b. Developing ovary (globular capsule type); c. The developed ovary dies off without forming seeds; and d. Post-fertilized ovary pre-mature death along with plant floral parts.

***Post fertilization development in Paris polyphylla*:** After pollination, the inner tepals curls slowly and the ovary color also changes from bright purple to dull purple which finally fades away (**Fig. 20 a**). After fertilization, the ovary enlarges forming a globular capsule. The anther and its filaments and the inner tepals slowly disintegrate off from its tip end towards the bottom (**Fig. 20 b**). It was observed that a significant *per cent* of *Paris polyphylla* though successful in yielding inflorescence and flowers (considered as fertile plants) failed to produce seeds. The ovary after its post-fertilization begins to form a rounded capsule to form fruit which enlarges to a certain level and dies off without developing seeds (**Fig. 20 c)**. Sometimes the ovary does not enlarge itself but dies off along with the plant floral parts (**Fig. 20 d**). Besides these, some plants often failed to undergo ovary enlargement after fertilization and dies off along with the above ground parts.

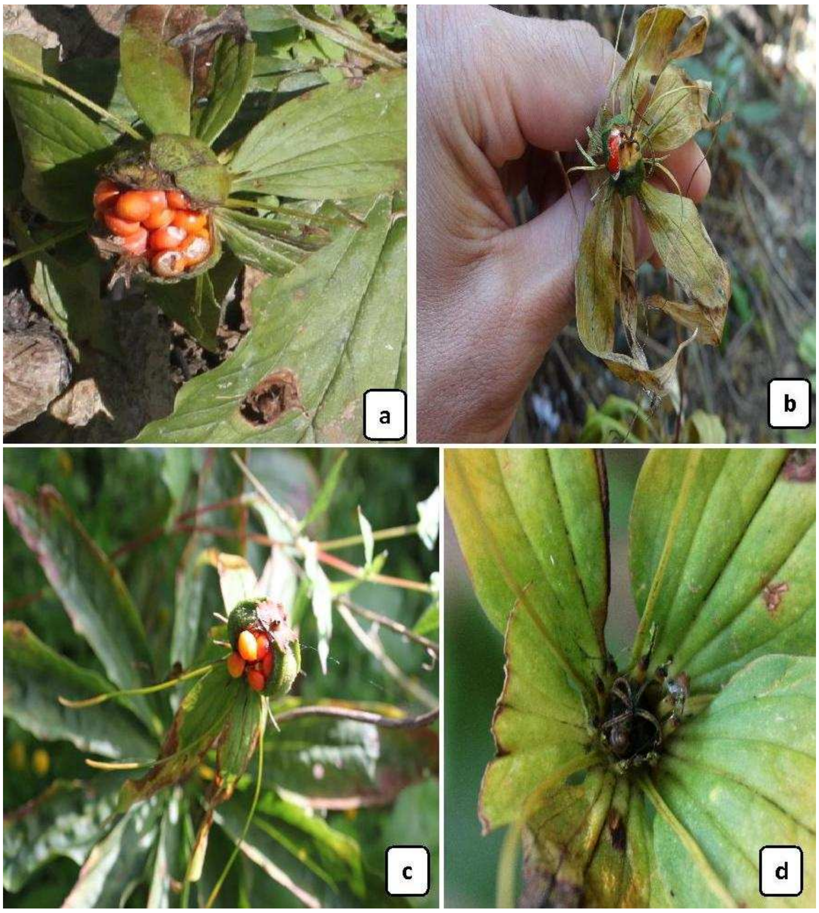

Figure 21: a. Mature fruit of *Paris polyphylla* with bright orange seeds; b. Fruits bearing fewer seeds when maintained at higher light intensity; c. Mature fruit slit opening exposing the seeds; and d. Predators affecting *Paris polyphylla* Smith var. *polyphylla* fruit.

Fruiting and seed setting**:** *Paris polyphylla* seed formation is very crucial and climatic factors plays an important role. The fruit of *Paris polyphylla* which is a globular capsule bears the seeds inside them. The seeds are bright orange in color when the fruit matures (**Fig. 21 a**). It was observed that the seed production is affected by the sunlight and heat. During the development phase, if the plants are exposed to direct sunlight, the seed production is very low. If the plants are maintained under <80% shade compare to normal day light, seed production is affected. Shade cover of less than 50% either produce fruits that are seedless or with very few seeds (**Fig. 21 b**). It can be at times as few as 1-2 seeded fruits. Generally seeds are produce in large quantity in well-suited plant habitats. The outer covering of the capsule slits open exposing the seeds (**Fig. 21 c**). The occurrence of a slit on the side of the globular capsule fruit indicates the maturation of the fruit. In general the seeds are dispersed by gravity and the seeds usually fall near its mother plant. It was found that seeds dispersed in the first year remained more or less same on the soil the following year without germinating indicating that seeds exhibits dormancy.

Seed setting in relation to different hand pollination treatments**:** *Paris polyphylla* seeds can be produced from the plant through both self-pollination and cross-pollination. However in terms of seed productivity and output, self- and cross pollination does not show any variable difference.

Paris polyphylla in relation to climatic conditions and its susceptibility to predators**:** During the present study, it was found that the plants were highly competitive with other herbaceous plants but at the same time they were found to be susceptible to the attack by various predators. In most of the habitats it was observed that snails and slugs were the predators of the fruits and the presence of them causes the death of the enlarging ovary (**Fig. 21 d)**. Besides the biological agents, the plants are also highly sensitive to the heat and sun light. Prolonged exposure to sunlight causes the plant to droop down and later dies off. Moisture content in the soil was also found to be very crucial for the growth of the plant but too much water in the soil causes the rotting of the stem base of the plant.

Discussion

Paris polyphylla is an important perennial medicinal plant in Nagaland where the rhizome of the plant is widely used in local medicines by the local healers. The plant species is widely distributed in the high altitude areas across the state but its population is decreasing at an alarming rate. Like *Panax pseudoginseng,* the main dispersal agent is gravity and the young plantlets are found in and around the mother plant. This is why *Paris polyphylla* is often found in patches in its natural habitat. The local medicinal healers and illegal harvesters can easily collect large amount of rhizomes from the natural populations within a small area. This is one of the key factors for rapid decline of the population of *Paris polyphylla* in Nagaland. Thus conservation of the species is very important and the first step is the location of the plant. In order to locate the species whose population is very thin, knowledge about its associated species could be an important key to locate the species of interest whose population otherwise is very difficult to locate. There are very reports available on this approach of population inventory of RET species. Madhu *et. al*., (2010) used *Artisaema* species for determining the presence or absence of *Paris polyphylla* in Nepal while Langhu and Deb (2014) successfully used the associated species for locating *Aconitum nagarum* in Nagaland from various habitats. In the present study this approach was found to very helpful for locating the *Paris polyphylla* population. Besides this, the information on associated species could be an important tool for characterization of the niches for the species, rehabilitation of the threatened species from conservation view point. There might be a possibility that wherever the associated species are growing, the key species is absent. This could be due to sweeping species from that area due to various anthropogenic activities, but could be a place where the key species may be introduced for propagation and or conservation.

During the present study it was found that there was great amount of variations/polymorphism in phenology of the plants. In the present study, morphological variations was observed in some of the *Paris polyphylla* in different years during their growing seasons even from a single rhizome. From literatures available, it reflects that the polymorphism in the genus *Paris* is universal. According to Zhang *et. al.* (2011) *P. polyphylla* var. *yunnanensis* also exhibits morphological variations and phenotypic diversity which correlates with the morphological diversity of *Paris polyphylla* found in Nagaland. It was also found that anthers are arranged in two whorls and large numbers of pollens are wasted. Presence of large number of anthers and wastage of large number of pollens indicates that the species is not efficient in energy economy. In *Paris polyphylla* var. *yunnanensis* also reported the anthers are arranged in two whorls (Dingkang *et. al.*, 2009) where they also studied the closing and opening of anthers. According to them conservation of pollens in the flowers and enhanced the male fitness by its closing during night and during rains which was not observed in the present study. However, the occurrence of large amount of pollen waste during pollination might give heavy loss and expenditure of the plant energy, which shows plants poor effective mechanism in pollen dispersal to the stigma of the flower.

Seed production is very important for sexually reproduced plants for new generations. In *Paris polyphylla* seed produced during warm weather usually mid-summer but if the plants are exposed to direct sunlight, aborts the seed or seed production is affected significantly. As the plant are shade loving, drooping of the plants usually occurs if they are exposed to direct sunlight even for 1-2 hours indicates that for good reproductive output the commercial growers should ensure proper care especially during flowering and seed setting. The lack of differences in seed production suggest that both self- and cross pollination occurs in the plant and are of equal importance. Important pollinators such as wind, bees, and flies play a vital role in the self- and cross-pollination of *Paris polyphylla* plants.

During the present study, it was found that seeds of *Paris polyphylla* germinate in the natural habitats during February-March after almost 18-20 months of maturation and shading. But according to Madhu *et. al.* (2010) *Paris polyphylla* Smith seeds germinate in April in Nepal. This could be due to variation among the different populations of different areas and or geo-climatic condition of that area. Besides this, there seems to be variations in the phenological calendar difference of *Paris polyphylla* Smith of Nagaland and mid-hills of Nepal.

Summary and Conclusion

During the present investigation efforts were put into to study the distribution of *Paris polyphylla* in natural habitats and the factors influence their establishment in the natural habitat and their adaptability in synthetic habitat. Besides this, effort was made to study the floral morphology, effect of environmental conditions on flowering, pollination, seed setting, reproductive output etc. It was found that *Paris polyphylla* grow healthy in the undisturbed forest where the canopy cover is more than 80% and if the canopy cover decreases <50%, plants fails to establish and flower. Healthy population growth of *Paris polyphylla* studied so far in its natural habitat at its lowest elevation was on Aradura Hill at an elevation of 1396 m above sea level (ASL), therefore an artificial experimental environment in the form of poly green house was set up to see its adaptability to lower elevation below 1300 m above ASL at Penli Ward, Mokokchung at an elevation of 1259 m ASL. However, in this artificial habitat, plants registered poor survival and failed to flower effectively. However at experimental plot areas near where the plant naturally grows, the plant shows much better growth. *Paris polyphylla* has unique morphological feature of anther and produced large volume of pollens.

Paris polyphylla is an economically important medicinal plant and the species is extremely threatened. The findings of the present study provide us a clear picture about the natural habitat and their primary requirements for survival, flowering, fruiting, seed setting. Further the findings pinpoints why the species cannot be cultivated easily away from the natural habitat. *Paris polyphylla* does not show any variable difference in seed setting through self- and cross- pollination. The findings from the seed biology study will help the commercial growers for seed propagation of the seed. The local authority, government agency should join hands to develop *Paris* park/garden in the natural habitats to propagate and conserve this economically important species.

Chapter - 4

Propagation of *Panax pseudoginseng* W. and *Paris polyphylla* Smith.

Forest plant species usually regenerates through their seeds or by vegetative means for their continued survival. Many of the plants are facing threat and their population in the wild is being reduced drastically due to unsustainable harvesting for local medicinal use, removal of natural habitats for 'Jhum cultivation', unplanned developmental activities and other anthropogenic activities. The percentage of plants used for medicinal purpose in relation to the totally availability of medicinal plants is found to be highest in India with 20% of the total species being used as medicinal plants (Schippmann *et. al.*, 2002). However, most of these medicinal plants are being collected from the wild and only 20 species of medicinal plant species used in the Indian herbal industry is being cultivated out of more than 400 species (Schippmann *et. al.,* 2002). This factor imposes a great threat to the existing wild plant species unless appropriate conservation steps are taken up. In order to conserve the economically important and medicinally important plants, efficient mass propagation for the conservation of these species is required. Mass propagation is important for mass production of both wild species under threat and also for domesticated crops. For instance, ginger cultivation in the Northeastern region of India usually follows the traditional method which is ecologically friendly, low cost and which utilizes the local resources, knowledge and labor (Rahman *et. al.,* 2009). Species such as *Garcinia afzelii*, *Panax quinquefolius*, *Saussurea costus, Warburgia salutaris* etc. are threatened species but are now cultivated (Schippmann *et. al.,* 2002) and thereby reduces the risk of population being threatened further. The simplest and most economical method of propagation is through seeds but some species failing to establish through seeds in its natural condition like *Aconitum atrox* (Banday *et. al.,* 2014). Root division and rhizome splitting can be done for plants having long period of dormancy in seeds. Important plants having commercial and medicinal values are being cultivated through mass propagation by using their vegetative rhizome parts such as Ginger (Rahman *et. al.,* 2009), *Jurinea dolomiaea* through rhizome cuttings (Banday *et. al.,* 2014), tuberous roots of *Aconitum atrox* (Banday *et. al.,* 2014), splitting of roots of *Nardostachys jatamansi* (Banday *et. al.,* 2014), stem cuttings of *Strychnos henningsii* (Kipkemoi *et. al.,* 2013), stem cuttings of *Casuarina cunninghamiana* (Karoshi *et. a.l.,* 2000), stem cuttings of *Gongronema latifolia* (Abbo and Obi, 2006), stem cuttings of *Shorea guiso* (Patricio *et. al.,* 2006) and through sucker plantlets in *Musa* species (Baiyeri, 2005). The total percentage of species threatened in the Araliaceae family is 16 and one of the over-harvested species in

Araliaceae family is the *Panax* species (Schippmann *et. al.*, 2002). Ginseng is being cultivated in many parts of the world (Schluter and Punja, 2000). Both *Panax pseudoginseng* Wall. and *Paris polyphylla* Smith var. *polyphylla* can be cultivated deliberately under a forest canopy provided that the environmental conditions are suitable for its growth and reproduction. Both the plants are highly medicinal, herbaceous and long lived. In Nagaland, there is hardly any distinction between these two plants and the locals refer to both the plants as ginseng plant. Both this plant grows at its best in its natural condition but since their population is dwindling, it is important to propagate them to ensure its population survival. One of the possible methods for propagation could be through cutting method. True-to-type copies and preventing extinction of the plant species in the local population (Kipkemoi *et. al.*, 2013) can be achieved through macro propagation. The problem related to threatened species in the wild can be overcome by introduction of any rare or threatened medicinal plants into the wild through mass propagation in the nurseries or through *in vitro* propagation. The time taken for bud emergence in *Paris polyphylla* Smith takes ~6 months under *in vitro* culture (Verma *et. al.*, 2012), therefore rhizome splitting can be an advantage of macro propagation than *in vitro* culture. Propagation through rhizome cutting/splitting is convenient, cost-effective and ensures large scale cultivation and for its long term process of conservation systematic cultivation is important (Banday *et. al.*, 2014). Cultivation of medicinal plants is the only means of meeting the ever increasing current and future demands of the people and the growing industry (Schippmann *et. al.*, 2002). Present study was aimed to produce clonal planting materials of two economically important and threatened species through different macropropagation techniques.

Materials and Methods

Plant materials

Panax pseudoginseng* W.*: Rhizomes of *Panax pseudoginseng* were collected from the natural forest habitat area of Pangsha village near Indo-Burma border at an elevation of 2278 m ASL under Tuensang district, Nagaland, India and another area from 'Chida' and its surrounding forest area of Khezakenoma village under Phek district, Nagaland, India at an elevation of 1874 m ASL. Digging of ginseng roots is very easy as they are usually found in loose soil of primary forest but it should be done very carefully. Accordingly the present exercise was executed very slowly in order to avoid damages to the rhizome and that nick of any roots is avoided. After collection, a part of plants collection of both the species were kept in the polyhouse set up at Chida area and the remaining part of the plants were planted in poly-bags and transported to Penli ward, Mokokchung district, Nagaland at an elevation of 1259 m ASL. Rhizome and root cuttings/splittings were performed at both the selected areas.

***Paris polyphylla* Smith. :** The plant materials were collected from four natural habitats –forest of Pangsha village, 'Chida' and its surrounding forest area of Kezakenoma village, Aradura hill forest area, Kohima and forest of Longkum village After collection, part of the plants were planted in polybags and transported to Penli ward, Mokokchung district, Nagaland and part of the plants were kept at Chida area where rhizome cuttings/ splitting and group rhizome fragmentation was performed.

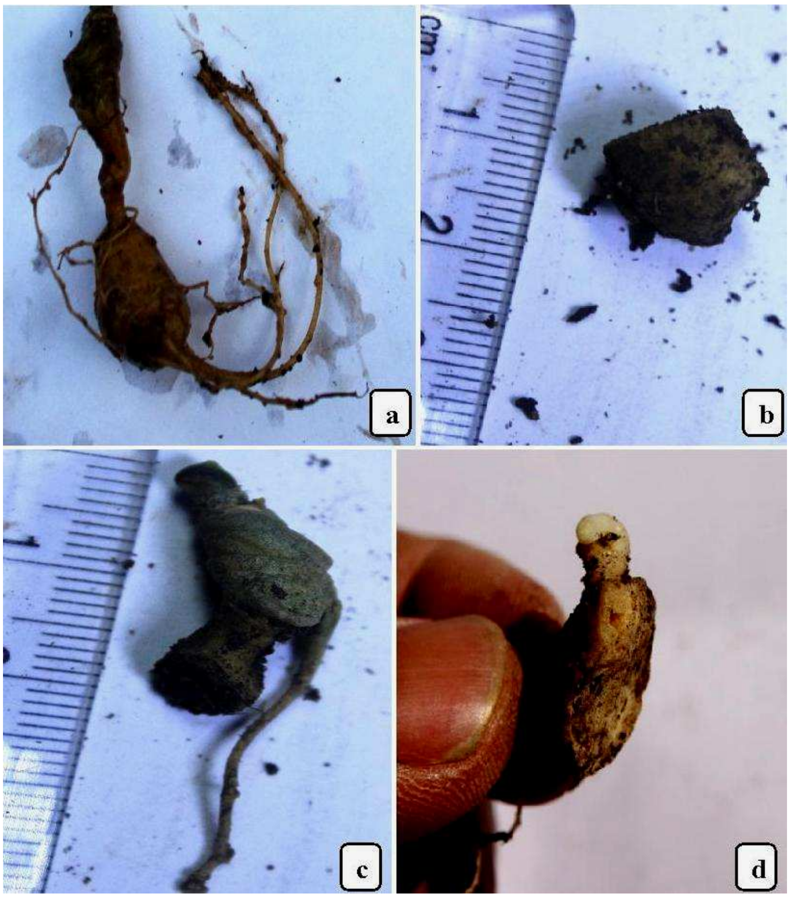

Figure 22: a. *Panax pseudoginseng* rhizome with scars and rootlets; b. Horizontal cut of root; c. *Panax pseudoginseng r*hizome segment; and d. Inclined cut of root.

***Rhizome cuttings in Panax pseudoginseng*: ***Panax Pseudoginseng* has a small neck–like rhizome with bud scars/ nodes connected with a fleshy root that bears individual small rootlets (**Fig. 22 a)**. The age of the plant were counted from the number of bud scars on the rhizome. Each individual rhizome was cut according to the ages as counted from the bud scars of the rhizome by ensuring that at least minimum two bud scars are present at the resultant cut rhizome. The rhizome cuttings was done in the month of November 2011, healthy rhizomes were selected randomly. The age of the rhizomes were between 5 years old to 11 years old as counted from the bud scars on their rhizome. The rhizomes were cut into number of pieces- 2 bud scars, 3 bud scars and 5 bud scars which were used for the present study (**Fig. 22 c**). The segments were mixed and used randomly in the present study.

***Root cuttings in Panax pseudoginseng*:** The root cuttings were done in the month of November 2011. A single root is cut into two equal parts. The roots were cut in two types – horizontal root cuttings and inclined root cuttings (**Fig. 22 b & d**) and maintained in the bed. They were monitored for three years from 2012-2014. Horizontal root cutting was done by cutting into two equal halves in the middle-lower cut root and upper cut root. All the roots were first inspected to ensure that they are healthy. Simple traditional method of cutting was used during the experimental process.

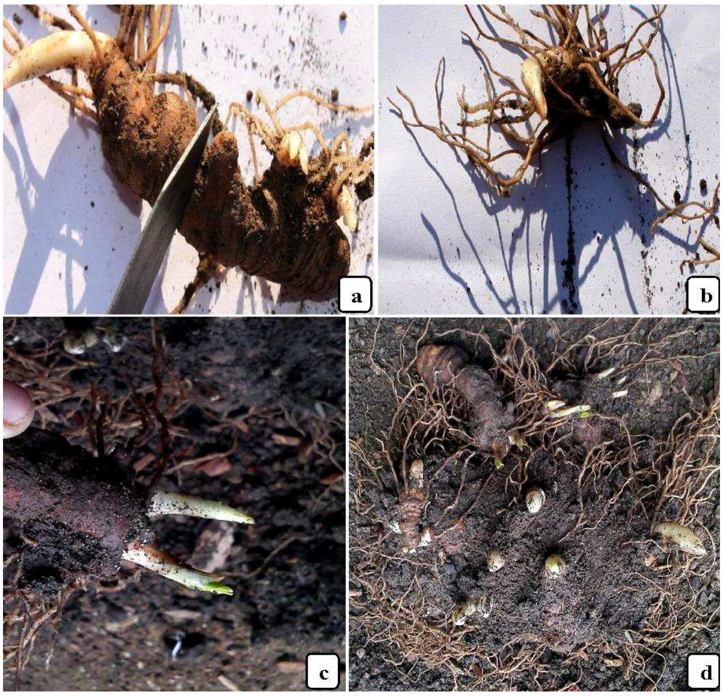

Figure 23: a. *Paris polyphylla* rhizome with multiple shoot buds; b. Rhizome fragment with single shoot bud; c. Rhizome fragment with multiple shoot buds; and d. Compound rhizome of *Paris polyphylla*.

Rhizome cuttings in Paris polyphylla**:** A single rhizome may give rise to multiple off-shoots as the plant ages; *Paris polyphylla* often gives rise to multiple shoot buds (**Fig. 23 a)**. The shoot buds sometimes bifurcates in different directions at one end point. This type of rhizome can give rise to two different directional shoot buds. This form of rhizomes can be propagated through cuttings where rhizome with multiple buds are separated and planted. Rhizome pieces without buds are also cut from large rhizomes. Basing on the nature of the growing tips (single or double buds) rhizome pieces were cut accordingly and used for the present study (**Fig. 23 b & c)**.

***Group Rhizome fragmentation in Paris polyphylla* Smith.:** *Paris polyphylla* rhizome sometimes occurs in masses. This may be due to large number of seeds falling in a particular site from their mother plant and where most seeds are healthy and viable. The roots of *Paris polyphylla* are long and wavy and so when a number of rhizomes are together they become entwined to each other forming a large mass of rhizomes (**Fig. 23 d)**. This might hamper the healthy growth of the individual plants. Therefore the rhizomes need to be individually separated and at the same time, rhizomes that show compound buds on the clumps are selected and used in cutting propagation. Rhizome splitting was simply done by breaking off the clump of rhizomes into each individual rhizome and sowed in the prepared bed in both the two polyhouses. The selected clump rhizomes were obtained from those plants that were flowering in group in the previous season.

Preparation of bed for planting

For the present study, two different soil mixtures were prepared:

4. Mixture of decayed wood, sand and top black soil at a ratio of 1:1:3.
5. Mixture of well dried rotted cow dung, sand and top black soil at a ratio of 1:1:3.

All the soil components were individually sun dried. Before mixing, any sticks, stones, seeds of other plants, or insects were removed from the soil. The soil was watered to see that water drains well out from them. Poly-bags and wooden boxes were used for planting. The experiment was performed during the month of November after the above ground body dies off. The experiment was done in both the poly-house with good ventilation and drainage. Artificial shade was provided in such a way that it fulfills the shade requirements.

***Planting of rhizomes segments, roots in the beds*:** Site selection is very important and it should be the first priority for its mass propagation. In the selected sites, the soil is first dug up to 15 cm to remove the existing top soil. The soils mixture to be put on the dug soil is made loose and good in organic content by adding mixture of cow dung, sand and black soil in 1:1:3. Prepared soil mixture is then put on the dug soil. The plant parts meant for propagation of both the species were sowed in their distinct beds made on a mounded a bit high than the normal ground level so that water is well drained during the rainy season. If any depressions are found on the soil, it is quickly covered with more organic soils. The plant is grown facing the northern slopes as they are usually cooler and have more moisture than south or west facing slopes. Before planting, the soil beds were watered to moisten the soil. The cut rhizomes were planted just below the soil and those rhizomes with buds on it are planted by exposing the bud a little less than a 0.5 cm outside the soil. The experimental beds are watered three times a week as the propagation process is done during the dry season of the year. Continuous monitoring of the soil bed was done and weeds were removed from the soil.

Results

***Regeneration potential of rhizome cuttings of Panax pseudoginseng*:** In the present study with the rhizome cuttings, over 55% of the rhizomes remained recalcitrant to morphogenetic response.

The lowest morphogenetic response was registered in 2012 where ~19.44% rhizome segments potentiated morphogenetic response and formed plantlets while in 2014, the response increased to 51.72%. There is a gradual increase in positive plant formation from the cut rhizomes during the three years of study (**Table 20)**. New shoot buds developed from the responding segments which were mostly one leaf plants (**Fig. 24 a**) but in the third year, two leaves plants were also noted in the seed bed along with one leaf plants (**Fig. 24 b**).

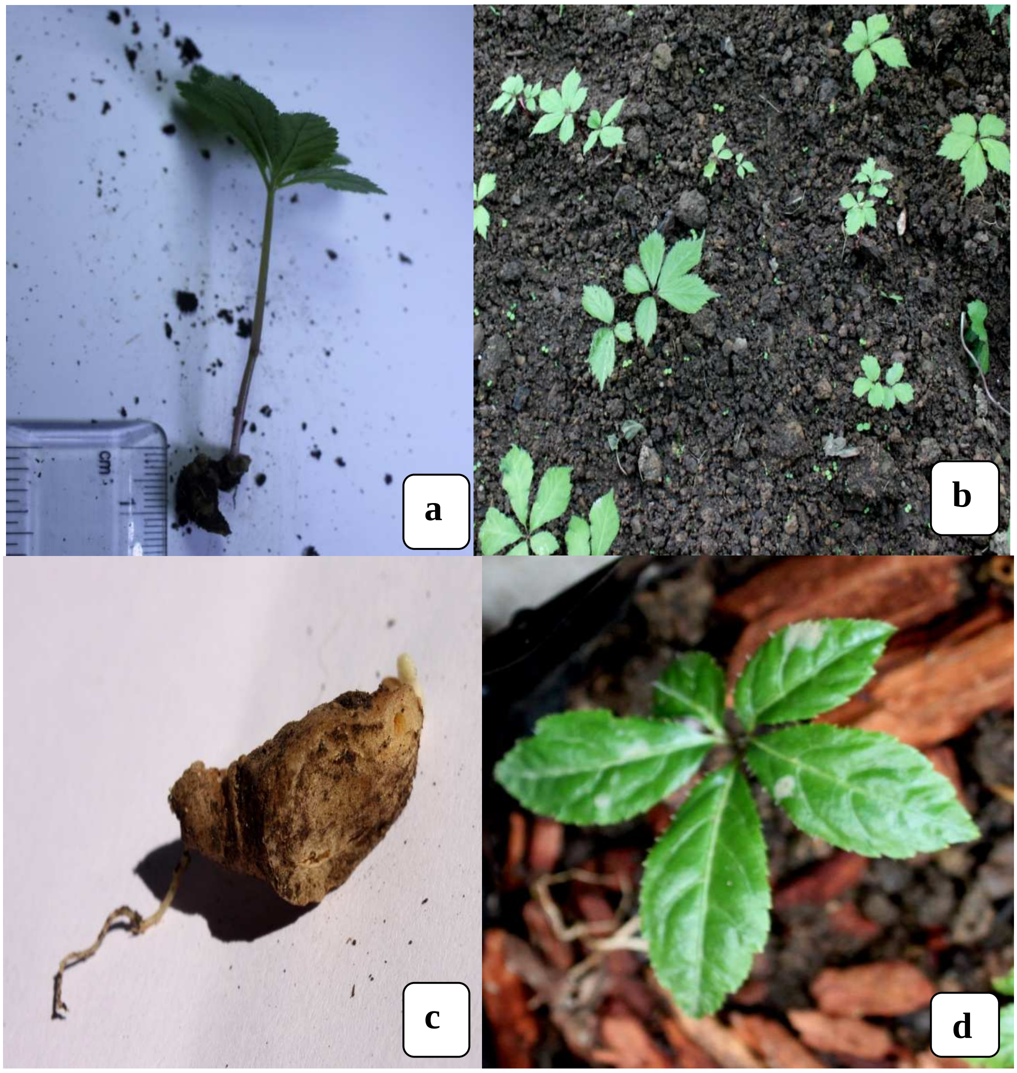

Figure 24: a. Sprouting of shoot bud from rhizome fragment of *Panax pseudoginseng*; b. Regenerated plants in the bed ready for transfer in the wild; c. One rhizome segment with shoot bud and rootlet; d. Development of 1-leaf plant from inclined root cutting

Table 20: Propagation of *Panax pseudoginseng* through rhizome cuttings

Year	Total number of cut rhizomes	Number of plants sprouted	Dormancy exhibited by the cut rhizomes	Number of cut rhizomes that died	% rhizome segments formed plants
2012	72	14	52	6	**19.44**
2013	66	21	37	8	**31.81**
2014	58	30	25	3	**51.72**
Mean	**65.33**	**21.66**	**38**	**5.66**	**34.32**

Note:

- All the rhizomes used for propagation were healthy rhizomes which were flowering the previous season.

Table 21: Propagation of *Panax pseudoginseng* through root cuttings

Type of cuttings	Total number of cut roots tested	Year	Number of rhizomes survived	Number of plants sprouted	Dormancy exhibited by the cut roots	Number of roots that died	% of plant set
Horizontal root cuttings	30	2012	30	0	19	11	0
		2013	19	0	12	7	0
		2014	12	0	8	4	0
Mean	**-**	**-**	**20.33**	**0**	**13**	**7.33**	**0**
Inclined root cuttings	30	2012	30	0	23	7	0
		2013	23	1	18	4	4.34
		2014	19	2	11	6	10.52
Mean	**-**	**-**	**24**	**1**	**17.33**	**5.66**	**4.16**

Note:.

- Horizontal cutting of both the lower horizon and the upper horizon root did not sprout but some of them show young rootlet formation on them.
- Inclined root cutting showed sprouting though the plants was only 1-leaf plant.
- Most of the plants sprouted shows stunted in its growth and all of them were non-reproductive plants.

Root cuttings in Panax pseudoginseng**:** In general the roots that were cut horizontally remained dormant morphogenetically. In the present study, conducted for three years there was no shoot bud formation but some segments showed formation of rootlets (**Table 21**). Inclined root cutting shows both rootlet formation and buds (**Fig. 24 c**). Only 4.16% plant was set in the inclined root cuttings but horizontal root cuttings showed no plant set during the three years of observation. While, compare to horizontal root cuts, inclined root cuts exhibited better response but the morphogenetic response is very poor in both the cuttings. It was observed that the plantlets developed were 1 leaf in inclined root cutting (**Fig. 24 d**).

Rhizome cuttings and group rhizome fragmentation in Paris polyphylla **Smith.:** In the study with rhizome cuttings, it was observed that ~50% rhizomes remained recalcitrant while the remaining segments registered morphogenetic response and formed shoot buds (**Fig. 25 a & Table 22**). The shoot buds was converted into plants in the planted pots within 4-5 months (**Fig. 25 b)** but majority of them were non-flowering in nature. On the regenerated plants of ~49%, only 7 % were reproductive plants that bear the inflorescence. During the present study it was found that the rhizome length and the number of buds are very important during the fragmentation process. If multiple buds are present in a single off-shoot, the length of the rhizome must be longer. However sometimes the off shoot rhizome is found to be very short even when multiple shoot buds are present. In this case the rhizome is taken as this short off-shoot. Appropriate pieces should be cut to achieve the optimum morphogenetic response and the existence of a bud in the rhizome is an added advantage during cuttings as most dormant rhizomes are from those cut parts where the bud was absent.

Figure 25: a. Sprouting of shoot bud from the rhizome segment of *Paris polyphylla*; b. Plantlets developed from the sprouted shoot buds; and c. Regenerated plants in the bed.

Table 22: Propagation of *Paris polyphylla* through rhizome cuttings

Study year	No. of segments sowed in the field	No. of rhizome segments sprouted and formed reproductive plants	No. of rhizome segments sprouted and formed non-reproductive plants	No. of rhizome segments remained dormant	% rhizome segments sprouted shoot buds and plants
2011-2012	100	09	40	51	49
2012-2013	100	04	39	57	43
2013-2014	100	08	48	44	56
Mean	**100**	**7**	**42.33**	**50.66**	**49.33**

Note:

- *Paris polyphylla* propagation was done every year during the month of November.
- All the rhizomes used for propagation were healthy rhizomes which were flowering the previous season.
- Rhizomes were cut into pieces having atleast two to three node/bud scars and sowed in the field.

Table 23: Propagation of *Paris polyphylla* through group rhizome fragmentation

Year of study	No. of rhizome pieces tested	No. of rhizome sprouted	No. of dormant rhizome	Rhizome died	% rhizome sprouted
2011-2012	43	32	10	01	74.41
2012-2013	62	46	15	01	74.19
2013-2014	51	39	10	02	76.47
Mean	**52**	**39**	**11.66**	**1.33**	**75**

Note:

- Compound rhizome clumps were collected from the wild and individual rhizomes were fragmented/ separated from the clumps and sowed in the field and in the poly-bags.

For regeneration of plants from the group rhizome, it was necessary to split the individual rhizome from the clump to optimize the multiplication. Of the total separated rhizomes planted, ~75% rhizomes could sprout successfully and formed plants (**Table 23**) while the remaining rhizomes failed to register morphogenetic response. Mortality rate and dormancy exhibition by the plant was very low. The regenerated plants were maintained in the bed for 6 months before transferring to the field (**Fig. 25 c**).

Discussion

During the present study attempts were made to propagate and produce clonal planting materials of *Panax pseudoginseng* and *Paris polyphylla* from rhizomes of both the species through cuttings and fragmentation. The propagation of *Panax pseudoginseng* through rhizome cuttings was found to be applicable for macro propagation of the plant species. However, dormancy of the cut rhizomes was very high. Out of cut rhizomes, ~34% were morphogenetic. It was observed that for successful morphogenetic response rhizome segments should be more than 3 bud scars. Fragments with fewer bud scars supported poorer morphogenesis. This is probably due to smaller size of the segments and lesser reserve food which failed to support morphogenesis. The cut rhizomes mostly sprouted as 1-leaf plants in all the three years of study along with few 2-leaves plants. In the second year a plant with 5 bud scars cut rhizome give rise to 3-leaves plant which was stunted in growth and was less than 10 cm in height.

The soil mixture preparation did not have much of a profound effect or differences in relation to the plant sprouting, dormancy and death. The propagation of *Panax pseudoginseng* through root cuttings is not appropriate for mass propagation, even though inclined root cuttings can give some positive results on the plant production. The propagated underground parts for *Panax pseudoginseng* are its rhizomes. The roots would be best planted as a whole. Roots having one or two bud scars can still survive very well and formation of buds can take place from the root. Splitting of rhizomes is rare in ginseng as the plant occurs as distinct individuals (Van der Voort *et. al.*, 2003) in its natural condition.

Experiments on growth and multiplication of rhizomes of *Panax sikkimensis* and *Panax bipinnatifida* was shown using different hormones (Rao *et. al*., 1998) however, the protocols developed in the present study is based on traditional method without any usage of inorganic nutrients or different hormone applications so that it will help the locals/farmers in practical application by its easier method, cost effective and convenient in propagating these economically important threatened species and workout the conservation strategies of these species in the natural habitats.

Paris polyphylla propagates efficiently from the underground cut rhizomes and it was more efficient than propagation through the seeds. Rhizome cuttings and fragmentation show better result on the soil bed. The fragmentation of rhizomes enables the individual rhizome to grow healthier. This might be due to free spacing as compared in potted polybags. The plant grows well when more appropriate spacing is given to them. Some of the cut rhizomes shows flowering but majority of them were in the non-flowering stage. *Paris polyphylla* grows well in slope areas. Naga Hills forest with 1300 m and above is an ideal place for its propagation. However, planting should not be done in too slope areas as it drains off the top soil during heavy rains.

Appropriate site habitat for cultivation of the plants is very important to ensure its establishment, growth, reproduction and being disease free. Both the plant takes long time to mature and bear fruits which is their only natural way of propagation. Sustainable harvest from wild populations can be a prior conservation option that requires a sound management system and sound scientific information (Schippmann *et. al.*, 2002) and therefore the locals should adopt sustainable means of harvest of these wild medicinal species. Domestication and propagation of these plants for their uses can be helpful in protection of the wild population. Further, selective harvest should be done by harvesting only those plants that do not produce inflorescence and collection should take place only during the fall after the plants have dispersed their seeds. Sustainable regeneration of the plant species of both the plant in the wild is required so proper conservation measures, legislation laws and acts needs to be impose especially for protecting this medicinal plant species.

Summary and Conclusion

In the present study an attempt was made to propagate and produce clonal planting materials of *Panax pseudoginseng* and *Paris polyphylla*, two threatened medicinal plants of North East India. The seed propagation of *Panax pseudoginseng* is not a very efficient means for producing planting materials as the seeds exhibits physiological dormancy and require over one year for germination. In *Panax pseudoginseng* clonal planting materials could be successfully produced from the rhizome fragments of 3-5 bud scars while in *Paris polyphylla,* fragmentation and cutting of rhizomes could be successfully used for producing clonal planting materials. Both the plants species produced through macropropagation have been introduced into their natural habitats in the studied area locations and also to other new locations of high altitudes. The protocols developed in the present study will help the locals to propagate these economically important threatened species and workout the conservation strategies of these species in the natural habitats.

Chapter - 5

Summary

Nagaland which lies between 25˚06'–27˚04' N latitude and 93˚20'–95˚15’ E longitudes with an area of 16,579 sq. Km is among the richest bio diverse region in the world and it lies in the Indo-Burma biodiversity hotspots. The geographical location of Nagaland has provided an ideal environment for the growth and nourishment of different medicinal plant species but in the recent years, extinction has been the destiny of a great number of plant species including several unique and irreplaceable varieties, while many await a similar fate. Nature has provided a rich store house of herbal remedies to cure all mankind’s ill. Plants have been used in the traditional health care system from the time immemorial, particularly among tribal communities. As medicinal plants continue to be the key role in the treatment of number of diseases, and they are the only source of medicines in the treatment of people in the developing world in most cases, conservation becomes a very important priority.

The thesis 'Studies on seed biology and mass multiplication of two medicinally important plant species: *Panax pseudoginseng* W. (Araliaceae) and *Paris polyphylla* Smith. (Trilliaceae)' presents the findings of the present investigation on niche characterization, floral phenology, reproductive biology, seed biology and propagation of two threatened medicinally important species of North East India viz. *Panax pseudoginseng* and *Paris polyphylla* Smith. Present study was conducted in different districts of Nagaland in their natural habitats. Artificial habitats were established to check the adaptability and establishment of the species to new environments. During the present study, it was found that distribution of both the species are restricted to very few patches in Nagaland. Further it was found that both the species grow well at higher altitude and under >80% canopy cover. The species wise findings are given below:

***Panax pseudoginseng* W.:** Present investigation was conducted in two natural habitats to study the distribution of *Panax pseudoginseng* and the factors that influence their establishment in the natural habitat and their adaptability in synthetic habitat. Efforts were also made to study the correlation between the plant phenology, rhizome age on flowering and seed setting. It was found that *Panax pseudoginseng* grow healthy in the undisturbed forest where the canopy cover is more than 80% and if the canopy cover decreases <80%, plants fails to establish proper growth and development. In the artificial habitats plants registered poor survival and failed to flower effectively. Normally, sprouting of the rhizome takes place from mid-February onwards. Centripetal inflorescence is exhibit where flowering occurs blooming from the base of the inflorescence to the tip of the inflorescence. Flowering period starts from late May to early June with 50-65 flowers per umbel. The plants are self pollinated. The timing of anthesis is between

6:30- 7:30 A.M, followed by the dehiscence of anther from 7:30-8:30 A.M onwards. Rhizome dormancy was associated with the leaf class stage and there is a distinct rise in the increase of plant dormancy from 2-leaves onwards. Mortality rate of the plants was studied by counting the roots where no growth of plants occurs during the growing season. It was observed that highest mortality was in seedling stage followed by one leaf, two leaves, three leaves and four leaves respectively. Flowering status improved with the increase in leaf number. Among the plants studies, ~90% plants produced flower with the plants with 4 leaves. It was observed that plants bearing less than three leaves did not support flowering and plants produced from the rhizome more than 5-6 years old supported viable fruits setting. Further it was recorded that plants with 2-3 leaves supported mostly one-seeded and two-seeded fruits while from plants with 4-5 leaves, one-seeded fruit starts reducing and three-seeded fruit starts increasing. Higher leaves plants increase the reproductive outcome. The plant showed self-pollination better than cross-pollination in terms of seed setting. The collected seeds were stratified following two different techniques. The un-stratified seeds exhibited only 10% germination after almost 14 months of sowing. In contrary when the seeds stratified at 25^{o}C for 3 months followed by cold stratification (4^{o}C) before sowing to the seed bed registered ~68% germination within 2 weeks of sowing. The seedling resulted from the germinated seeds were transfer from the field to their natural habitat after 1 year.

Panax pseudoginseng is an economically important medicinal plant and the species is extremely threatened. The findings of the present study provide us a clear picture about the natural habitat and their primary requirements for survival, flowering, fruiting and seed setting. Further the findings pinpoints why the species cannot be cultivated easily away from the natural habitat.

***Paris polyphylla* Smith.**: During the present investigation efforts were put into to study the distribution of *Paris polyphylla* Smith var. *polyphylla* in natural habitats and the factors influence their establishment in the natural habitat and their adaptability in synthetic habitat. Besides this, effort was made to study the floral morphology, effect of environmental conditions on flowering, pollination, seed setting, reproductive output etc. It was found that *Paris polyphylla* grow healthy in the undisturbed forest where the canopy cover is more than 80% and if the canopy cover decreases <50%, plants fails to establish and flower. In the artificial habitat plants registered moderate survival but there was poor and effective establishment in seed setting. *Paris polyphylla* Smith var. *polyphylla* is influenced by other plant species in its natural habitat and wherever these associated species were present, *Paris polyphylla* registered healthy growth. A small bud outgrowth appears on its rhizome just before/after the above ground part of the plant dies during late autumn. The bud remains dormant for 4-5 months. The number of bud in any particular rhizome does not remain constant. *Paris polyphylla* flowering begins in March-April. Flowers are perfect flowers as they contain both the male and female gametes within the same flower. Outer tepals are leafy and green, while the inner tepals are yellowish green and filiform. Both self- and cross- pollination occurs in the plant. Pollination can occur through small insects as the plants are visited by different insects such as different types of flies and bees. Huge amount of pollens are wasted. After fertilization, the ovary enlarges forming a globular capsule. The anther and its filaments and the inner tepals slowly disintegrate off from its tip end towards the bottom. The outer covering of the capsule slits open exposing the seeds. Seeds are dispersed by gravity and the seeds usually fall near its mother plant. The unique feature of anther; anthers are arranged in two whorls in a definite pattern and the production of large volume of pollen by an inflorescence. When tested for reproductive output of the plants, around 60% were non-flowering/ non-reproductive which add to the limitation of the plants in the natural population.

Paris polyphylla is an economically important medicinal plant and the species is extremely threatened. The findings of the present study provide us a clear picture about the natural habitat and their primary requirements for survival, flowering, fruiting and seed setting. Further the findings pinpoints why the species cannot be cultivated easily away from the natural habitat.

Macropropagation of *Panax pseudoginseng* Wall. and *Paris polyphylla*: In the present study attempt was made to propagate and produce clonal planting materials of *Panax pseudoginseng* and *Paris polyphylla*, two threatened medicinal plants of North East India. The seed propagation of *Panax pseudoginseng* is not very efficient means for producing planting materials as the seeds exhibits physiological dormancy and require over one year for germination. In *Panax pseudoginseng*, clonal planting materials could be successfully produced from the rhizome fragments of 3-5 bud scars. The plants produced were transferred to the wild after three years of its observation and maintenance in the prepared bed. While in *Paris polyphylla*, fragmentation of rhizome and splitting of group rhizomes could be successfully used for producing clonal planting materials. Selection of rhizomes and cutting part of the plant is highly important in both the plant species during propagation.

The findings from the niche characterization of both the species will help in conservation and rehabilitation of these two threatened medicinal plant species. Studies on floral phenology, reproductive biology will help the growers to understand the reproductive bottle necks of these species and why these species fails to produce sufficient seeds and the problems associated with the seed propagation of the species. Further, outcome of the macropropagation experiments provides information how the rhizomes could be used for successful production of clonal planting materials which are easy, inexpensive and could be followed by even common farmers/growers.

References

Abera, B.; Negash, L. and Kumlehn, J. 2008. Reproductive biology in the medicinal plant *Plumbago Zeylanica* L. *Afr J Biotech*, 7(19): 3447-3454.

Agbo, C. U. and Obi, I. U. 2006. Macro-propagation technique for different physiological ages of *Gongronema latifolia* Benth cuttings. *Afr J Biotech*, 5(13): 1254-1258.

Ali, Z. A. 1999. Folk veterinary medicine in Moradabad District (Uttar Pradesh), India. *Fitoterapia*, 70: 340-347.

Anderson, R. C.; Fralish, F. S.; Armstrong, J. and Benjamin P. 1984. Biology of ginseng *(Panax quinquefolium)* in Illinois. Department of Conservation, Division of forest resource and natural heritage, Springfield. Illinois.

Anderson, M. P. 1996. American ginseng *(Panax quinquefolium* L.) in southwestern Wisconsin: patterns of demography and habitat association. MS thesis, University of Wisconsin-Madison.

Anyinam, C. 1995. Ecology and ethnomedicine: Exploring links between current environmental crisis and indigenous medical practices. *Soc Sci Med*, 40(3): 321-329.

Awang, D. V. C. 2003. What in the name of *Panax* are those other. *Herbal Gram J Ame Bot Conc,* 57: 35-40.

Azaizeh, H.; Fulder, S.; Khalil, K. and Said, O. 2003. Ethnomedicinal knowledge of local Arab practitioners in the Middle East Region. *Fitoterapia,* 74: 98–08.

Bae, H. W. 1978. Korean Ginseng. Korea: Korean Ginseng Research Institute.

Baek, S. H.; Bae, O. N. and Park, J. H. 2012. Recent Methodology in Ginseng Analysis. *Ginseng Res*, 36(2): 119-134.

Baillie, J. E. M.; Hilton, T. C. and Stuart, S. N. 2004. How Many Threatened Plants are There? A Global Species Assessment. The IUCN Species Survival Commission. The IUCN Species Programme Rue Mauverney. 28 CH-1196. *Gland*, Switzerland. 217.

Baiyeri, K. P. 2005. Response of *Musa* species to Macro-propagation. II: The effects of genotype, initiation and weaning media on sucker growth and quality in the nursery. *Afr J Biotech*, 4(3): 229-234.

Banday, A.; Nawchoo, I. A.; Kaloo , Z. A.; Shabir, P. A. and Rather, A. A. 2014. Efficient propagation of an endangered medicinal plant *Jurinea dolomiaea* Boiss in the North Western Himalaya using rhizome cuttings under *ex situ* conditions. *J Pl Breeding Crop Sci*, 6(9): 114-118.

Baral, S. R. and Kurmi, P. P. 2006. A Compendium of Medicinal Plants in Nepal. IUCN, The World Conservation Union, Mass Printing Press, Kathmandu, Nepal.

Barbedo, C. J. and Filho, J. M. 1998. Desication tolerance on seeds. *Acta Botanica Brasilica*, 12: 145-164.

Barbedo, C. J. and Cicero, S. M. 2000. Effects of initial quality, low temperature and ABA on the storage of seeds of *Inga uruguensis*, a tropical species with recalcitrant seeds. *Seed Sci Technol*, 28: 793-808.

Baskin, C. C. and Baskin, J. M. 1988. Germination ecophysiology of herbaceous plant species in a temperature region. *Ame J Bot*, 75: 286-305.

Baskin, C. C. and Baskin J. M. 1998. Seed Ecology, biogeography and evolution of dormancy germination. *Pl Ecol*, 152(2): 204-205.

Baskin, C. C and Quarterman, E. 1969. Germination requirements of seeds of *Astragalus tennesseensis*. *Bull Torrey Bot Club*, 96:315-321.

Baskin, C. C.; Meyer, S. E. and Baskin, J. M. 1995. Two types of morphophysiological dormancy in seeds of two genera (*Osmorhiza* and *Erythronium*) with an Arcto-Tertiary distribution pattern. *Am J Bot*, 82(3):293-298.

Baskin, J. M. and Baskin, C. C. 1979. The ecological lifecycle of the cedar glade endemic *Lobelia gattingeri*. *Bull Torrey Bot Club*, 106: 176–181.

Baskin, J. M. and Baskin, C. C. 1997. Methods of breaking seed dormancy in the endangered species *Iliamna corei* (Sherff) Sherff (Malvaceae), with special attention to heating. *Nat Afr J*, 17: 313–323.

Berjak, P. 2005. Protector of seeds: seminal reflections from Southern Africa. *Sci*, 307: 47-49.

Berjak, P.; Farrant, J. M.; Mycock, D. J. and Pammenter, N. W. 1990. Recalcitrant (homeohydrous seeds): the enigma of their desiccation-sensitivity. *Seed Sci Technol*, 18: 297-310.

Bhattarai, K. R. and Ghimire, M. D. 2006. Cultivation and Sustainable Harvesting of Commercially Important Medicinal and Aromatic Plants of Nepal. *Her Res Dev For*, Nepal. Pp. 369-372.

Buragohain, J. 2008. Folk medicinal plants used in gynecological disorders in Tinsukia district, Assam, India. *Fitoterapia*, 79: 388–392.

Carlson, A. W. 1986. Ginseng: America's botanical drug connection to the orient. *Econ Bot*, 40(2): 233-249.

Carpenter, S. G. and Cottam, G. 1982. Growth and reproduction of American ginseng (*Panax quinquefolius*) in Wisconsin, USA. *Can J Bot,* 60: 2692–2696.

Chakraborty, U. and Das, H. 2010. Antidiabetic and antioxidant activities of *Cinnamomum tamala* leaf extracts in Stz –treated diabetic rats. *Global J Biotech Biochem*, 5(1): 12-18.

Chakraborty, R.; Deb, B.; Devanna, N. and Sena, S. 2012. North-East India an Ethnic Storehouse of Unexplored Medicinal Plants *J Nat Prod Plant Resource*, 2(1):143-152.

Changkija, S. 1999. Folk Medicinal Plants of the Nagas in India. *Asian Folklore Studies,* 58: 205-230.

Chaudhary, N. M. A. and Tariq, P. 2006. Anti-microbial activities of *Cinnamomum cassia* against diverse microbial flora with its nutitional and medicinal impacts. *Pak J Bot*, 38(1): 169-174.

Chetri, M. S.; Timilsina, Y. P.; Tripathee, H. P. and Devkota K. P. 2012. Socio-ecological Status and Antibacterial Activity of *Paris polyphylla* from Panchase Area of Kaski District. *Nepal J Sci Technol*, 13(2): 167-174.

Choi, K. T. 2008. Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax ginseng* C A Meyer. *Acta Pharmacol Sin,* 29(9): 1109–1118.

Clampitt, C. A. 1987. Reproductive biology of *Aster curtus* (Asteraceae), a Pacific Northwest endemic. *Ame J Bot*, 76: 941–946.

Clark, D. L.; Ingersoll, C. A. Finley, K. K. 1997. Regeneration of *Erigeron decumbens* var. *decumbens* (Asteraceae), the Willamette Daisy. *In*: Kaye T.N., Liston A., Love R.M., Luoma D., Meinke R.J., and Wilson M.V., (Eds.), *Conservation and management of native plants and fungi*. National Publication Society of Oregon, Corvallis. Pp. 41–47.

Crowder, C. A. 1978. The ecology and reproduction of *Sophora leachiana* Peck (Fabaceae). M. Sc. thesis, Oregon State University, Corvallis.

Cunha, R.; Eira, M. T. S. and Rita, I. 1995. Germination and desiccation studies on wild nutmeg seed (*Virola surinamensis*). *Seed Sci Technol*, 23: 43-49.

Cunningham, A. B. 1993. African medicinal plants: setting priorities at the interface between conservation and primary health care. *People and Plants working paper 1*. Paris. UNESCO. Pp. 1-50.

Davies, S. J. 2001. Tree mortality and growth in 11 sympatric *Macaranga* species in Borneo. *Ecology*, 82: 920-932.

Deb, C. R.; Deb, M. S.; Jamir, N. S. and Imchen, T. 2009. Orchids in indigenous system of medicine in Nagaland, India. *Pleione*, 3(2): 209-211.

Deb, M. S.; Jamir, N. S. and Deb, C. R. 2012. Studies on seed biology of *Cinnamomum tamala* Nees (Lauraceae): a valuable multipurpose tree. *Intl J Ayurved Med*, 2(5): 817-827.

Deyrup, M. and Menges, E. S. 1997. Pollination ecology of the rare scrub mint *Dicerandra frutescens* (Lamiaceae). *Florida Scienist*, 60: 143–157.

Dhyani, P. P. 2000. Common plant species have potential for economic upliftment of rural populace – Bantulsi a case in point. *Hima-Paryavaran*, 12 (1): 11-13.

Dhyani, P. P. and Khali, M. P. 1993. Fruit yield and economics of jelly and jam production from fruits of some promising *Ficus* (Fig) tree crops. *Ecol Food Nutrition*, 30: 169-178.

Dhyani, P. P. and Dhar, U. 1994. Box Myrtle (*Myrica esculenta*, Kaiphal): A promising underexploited tree crop of the Himalaya. Himavikas Occasional Publication No. 3, GBPIHED, Almora, U.P.

Dikshit, V. K. 1999. Export of medicinal plants from India: need for resource management. *In*: Kharbuli, B.; Syiem, D. and Kayang, H. (Eds.), *Biodiversity – North East India perspectives: people's participation in biodiversity conservation*. NEBRC, North Eastern Hill University, Shillong, India. Pp 85-88.

Dingkang, W.; Guifang, S.; Lifang, W.; Shuhua, Z. and Xiaojiang C. 2009. A novel mechanism controls anther opening and closing in *Paris polyphylla* var. *yunnanensis*. *Chinese Sci Bull*, 54(2): 244-248.

Dubrick, M. A. 1983. Dietary supplements and health aids: a critical evaluation. *J Nutrition Edu*, 15: 123–129.

Duke, J. A. and Ayensu, E. S. 1985. Medicinal Plants of China. Reference Publications. ISBN 0-917256-20-1.

Dutta, I. C. 2007. Non Timber Forest Products of Nepal: Identification, Classification, Ethnic Uses and Cultivation. *Hillside Press*. Kathmandu.

Faegri, K. and Van der Pijl, L. 1979. The principles of pollination ecology. 3 ed. Oxford, Pergamon Press.

FAO. 2002. Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries. Satellite event on the occasion of the Ninth Regular Session of the Commission on Genetic Resources for Food and Agriculture. Rome, 12-13 October. Inter-Departmental Working Group on Biological Diversity for Food and Agriculture. Rome. Pp 1-21.

Fenner, M. 1998. The phenology of growth and reproduction in plants. *Perspectives Pl Ecol, Evolution Systematics*, 1(1):78-91.

Figueiredo, R. A. 2000. Biologia floral de plantas cultivadas. Aspectos teóricos de um tema praticamente desconhecido no Brasil. *Argumento*, 2(3): 08-27.

Figueiredo, R. A. and Sazima, M. 2007. Phenology and pollination biology of eight *Peperomia* species (Piperaceae) in semideciduous forests in southeastern Brazil. *Pl Biol*, 9(1): 136-141.

Florance, E. R. 1997. Structure, dormancy, and germination of seeds from *Frasera albicaulis* and *F. umpquaensis* (Gentianaceae). *In*: Kaye T.N., Liston A., Love R. M., Luoma D., Meinke R.J., and Wilson M.V., (Eds,), *Conservation and management of native plants and fungi*, National Publication Society of Oregon, Corvallis. Pp 62–65.

Gisler, S. D. and Meinke, R. J. 1997. Reproductive attrition by predispersal seed predation in *Sidalcea nelsoniana* (Malvaceae): implication for the recovery of a threatened species. *In:* Kaye T.N., Liston A., Love R.M., Luoma D., Meinke R.J., and Wilson M.V., (Eds,), *Conservation and management of native plants and fungi*.National Publication Society of Oregon, Corvallis. Pp 56–61.

Hackney, E. E. 1999. The effects of small population size, breeding system, and gene flow on fruit and seed production in American ginseng (*Panax quinquefolius L.*, Araliaceae). M. Sc. Thesis, West Virginia University.

Halse, R. R. 1988. Seed germination in *Sidalcea nelsoniana* (Malvaceae). *Phytologia*, 64: 179–184.

Hanley, T. A. 1978. A comparison of the line-interception and quadrat estimation methods of determining shrub canopy coverage. *J. Range Manage*, 31: 60-62.

Hansen, L. and Boll, P. M. 1986. Polyacetylenes in Araliaceae: their chemistry, biosynthesis and biological significance. *Phytochem*, 25: 285–293.

Hikino, H. M.; Takahash, M.; Otake, K. and Konno, C. 1986. Isolation and hypoglycaemic activity of eleutherans A, B, C, D, E, F and G: glycans of *Eleuthercoccus serticosus* roots. *J Natural Products*, 49: 293–297.

Hu, S. Y. 1976. The genus *Panax* (ginseng) in Chinese medicine. *Eco Bot*, 30: 11-28.

Ignacimuthu, S.; Ayyanar, M. and Sankarasivaraman, M. 2008. Ethnobotanical study of medicinal plants used by Paliyar tribals in Theni district of Tamil Nadu, India. *Fitoterapia*, 79(7): 562-568.

IUCN. 2000. IUCN Red list of threatened Species, Switzerland: The World Conservation Union.

IUCN. 2004. IUCN Red list of threatened Species. A Global Species Assessment. Jonathan E.M Baillie, Craig Hilton Taylor and Simon N. Stuart (Eds.). Pp. 1-217.

Jacobs, J. 1993. New hope for the Peter’s Mountain mallow. *Endangered Sps Tech Bull,* 18: 13–14.

Jamir, T. T.; Sharma, H. K. and Dolui, A. K. 1999. Folklore medicinal plants of Nagaland, India. *Fitoterapia*, 70: 395-401.

Jamir, N. S.; Lanusunep and Pongener, N. 2012. Medico-herbal Medicine Practiced by the *Naga* tribes in the state of Nagaland (India). *Indian J Fundamental Appl Life Sci,* 2 (2): 328-333.

Kala, C. P. and Sajwan, B. S. 2007. Revitalizing Indian systems of herbal medicine by the National Medicinal Plants Board through institutional networking and capacity building. *Curr Sci*, 93: 6-25.

Karoshi, V. R.; Hegde, G. V. and Hiremath, S. M. 2000. Macropropagation of *Casuarina cunninghamiana* Miq via mistless polytunnel (hydropit). *J Tropical For*, 16: 79-81.

Karron, J. D. 1987. The pollination ecology of co-occurring geographically restricted and widespread species of *Astragalus* (Fabaceae). *Biol Cons*, 39: 179–193.

Karron, J. D. 1989. Breeding systems and levels of inbreeding depression in geographically restricted and widespread species of *Astragalus* (Fabaceae). *Amer J Bot*, 76: 331–340.

Kermode, A. R. 1990. Regulatory mechanisms involved in the transition from seed development to germination. *Critical Rev Pl Sci*, 9: 155-195.

Kipkemoi, N. K.; Kariuki, N. P.; Wambui, N. W.; Justus, O. and Jane, K. 2013. Macropropagation of an endangered medicinal plant, *Strychnos henningsii* (gilg), (loganiaceae) for sustainable conservation. *Intl J Med Pl Res*, 2(7): 247-253.

Kitajima, K. 2007. Seed and seedling ecology. In: Pugnaire, F. I. and Valladares, F.(Eds.) Functional Plant Ecology, 2nd Edn. CRC Press, Tylor & Francies Group. Pp. 549-580.

Konsler, T. R. 1986. Effect of stratification temperature and time on rest fulfillment and growth in American ginseng. *J Ame Soc Horti Sci*, 111: 651–654.

Kumar, P.; Chauhan, S. and Rana, A. 2011. Phenology and reproductive biology of *Abutilon indicum*(L.) Sweet (Malvaceae). *Intl J Pl Repro Biol*, 3(1): 55-62.

Kuria, M. W.; Ngumi, V. W.; Njenga, P. K. and Odee, D. W. 2010. *Ex vitro* rooting of *Warbugia ugandensis* (Sprague) ssp. Ugandensis Sprague. *East Afri J Botany*, 2(1): 243–248.

Kushwaha, S. P. S. and Behera, M. D. 2002. Biodiversity characterization in Subansiri District, Arunachal Pradesh using remote sensing and GIS. *In*: Das, A. P. (Ed.), *Perspectives of plant biodiversity*. BSMPS, Dehra Dun, India. Pp. 529-536.

Kwit, C. and Platt, W. J. 2003. Disturbance history influences regeneration of non-pioneer understory trees. *Ecology*, 84: 2575-2581.

Laloo, R. C.; Kharlukhi, L.; Jeeva, S. and Mishra, B. P. 2006. Status of medicinal plants in the disturbed and the undisturbed sacred forest of Meghalaya, North East India: population structure and regeneration efficacy of some important species. *Curr Sci*, 90: 225-232.

Langhu, T. and Deb, C. R. 2014. Studies on the reproductive biology and seed biology of *Aconitum nagarum* Stapf: a threatened medicinal plant of North East India. *J Res Biol*, 4(7): 1465-1474.

Leakey, R. R. B. 1983. Stock plant factors affecting root initiation in cuttings of *Triplochiton scleroxylon* K. Schum., an indigenous hardwood of West Africa. *Afr J Hort. Sci*, 58(2): 227-290.

Lee, J. C.; Cheon, S. K.; Kim, Y.T. and Jo, J. S. 1980. Studies on the effect of shading materials on the temperature, light intensity, photosynthesis and the root growth of the Korean ginseng (*Panax ginseng* C. A. Meyer). *Korean J Crop Sci*, 25: 91-98.

Lewis, W. H. 1984. Population structure and environmental corollaries of *Panax quinquefolium* (Araliaceae) in Delaware country, New York. *Rhodora*, 86: 431–438.

Lewis, W. H. and Zenger, V. E. 1982. Population dynamics of the American ginseng *Panax quinquefolium* (Araliaceae). *Ame J Bot*, 69: 1483–1490.

Li, T. S. 2002. Stratification of American Ginseng Seeds-Problems and Solutions. *Nat Pl J*, 3(2):109-111.

Li, F.; Jiao, P.; Yao, S.; Sang, H.; Qin, S.; Zhang, W.; Zhang,Y. and Gao, L. 2012. *Paris polyphylla* Smith Extract Induces Apoptosis and Activates Cancer Suppressor Gene Connexin 26 Expression. *Asian Pacific J Cancer Prevention*, 13: 205-209.

Liang, S. Y. 2000. Flora of China. *Science Press*, Beijing and Missouri Botanic Garden, St. Louis. 24: 90-91.

Macior, L. W. 1978. The pollination ecology and adaptation of *Pedicularis furbishiae* (Wats S.). *Bull Torrey Bot Club*, 105: 268–277.

Madhu, K. C.; Phoboo, S. and Jha, P. K. 2010. Ecological study of *Paris polyphylla* Sm. *Ecoprint*, 17: 87-93.

Massey, J. R. and Whitson, P. D. 1980. Species biology, the key to plant preservation. *Rhodora*, 82: 97–103.

McKey, D. 1975. The ecology of coevolved seed dispersal systems. *In*: Gilbert, L. E. and Raven, P. H. (Eds.). *Coevolution of animals and plants*. University of Texas Press, Austin. Pp. 159-191.

Menges E. S., 1995. Factors limiting fecundity and germination in small populations of *Silene regia* (Caryophyllaceae), a rare hummingbird- pollinated prairie forb. *Ame Midland Naturalist*, 133: 242–255.

Menges, E. S.; Waller, D. M. and Gawker, S. C. 1986. Seed set and seed predation in *Pedicularis furbishiae,* a rare endemic of the St. John River, Maine. *Ame J Bot*, 73: 1168–1177.

Meyers, N.; Mittermeier, R. A.; Mittermeier, C. G.; Da Fonseca, G. A. B. and Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature,* 403: 853-858.

Misra, M. K. 1999. Need for conservation of indigenous medicinal knowledge and the herbs. *J Hum Ecol*, 10: 403-406.

MoEF. 2014. India's Fifth National Report to the Convention on biological diversity. *Ministry of Environment and Forests*, Government of India. Pp. 142.

Mooney, E. H. and McGraw, J. B. 2007. Effects of self-pollination and outcrossing with cultivated plants in small natural populations of American Ginseng, *Panax quinquefolius* (Araliaceace), *Ame J Bot*, 94(10): 1677-1687.

Morely, T. 1982. Flowering frequency and vegetative reproduction in *Erythronium albidum* and *E. propullans,* and related observations. *Bull Torrey Bot Club,* 109: 169–176.

Moza, M. K. and Bhatnagar, A. K. 2007. Plant reproductive biology studies crucial for conservation. *Curr Sci*, 92(9): 1207.

Nayar, M. P. and Sastry, A. R. K. 1990. Red data Book of Indian Plants. *Bot Sur India*, *Calcutta*, Vol III, Pp. 28-29.

Neves, C. S. V. J. 1994. Recalcitrant seeds: A review. *Pesquisa Agropecudria Brasileira,* 29: 1459-1467.

Oshima, Y.; Sat, K. and Hikino, H. 1987. Isolation and hypoglycaemic activity of natural products. *J Nat Prod,* 50(2): 188–190.

Pacala, S. W.; Canham, C. D.; Silander, J. A. and Kobe, R. K. 1994. Sapling growth as a function of resources in a north temperate forest. *Canadian J For Res*, 24: 2172-2183.

Pages, J. P.; Pache, D.; Joud, N.; Magnan, N. and Michalet, R. 2003. Direct and indirect effects of shade on four tree seedlings in the French Alps. *Ecology*, 84: 2741-2750.

Pammenter, N. W. and Berjak, P. 1999. A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. *Seed Sci Res*, 9: 13-37.

Pammenter, N. W.; Vertucci, C. W. and Berjak, P. 1991. Homeohydrous (Recalcitrant) seeds: dehydration, the state of water and viability characteristics in *Landolphia kirkii*. *Pl Physiol*, 96: 1093-1098.

Pammenter, N. W.; Graggains, V.; Kioko, J. I.; Wesley-Smith, J.; Berjak, P. and Finch-Savage, W. E. 1998. Effects of different drying rates on viability retention of recalcitrant seeds of *Ekebergia capensis*. *Seed Sci Res*, 8: 463-471.

Pandey, H.; Nandi, S. K.; Nadeem, M.; Palni, L. M. S. 2000. Chemical stimulation of seed germination in *Aconitum heterophyllum* wall and *A.balfuorii* Stapf.: important Himalayan species of medicinal value. *Seed Sci. Technol*., 28: 39-48.

Patricio, H. P.; Castaneto, Y. T.; Vallesteros, A. P. and Castaneto, E. T. 2006. Macropropagation of *Shorea guiso* using stem cuttings. *J Tropical For Sci*, 18(3): 198-201.

Pavlik, B. M.; Ferguson, N. and Nelson, M. 1993. Assessing limitation on the growth of endangered plant populations. II. Seed production and seed bank dynamics of *Erysimum capitatum* ssp. *Angustatum* and *Oenothera deltoides* ssp. *howellii*. *Biol Cons,* 65: 267–278.

Planisek, S. L.1983. The breeding system, fecundity, and dispersal of *Iris lacustris*. *Michigan Bot,* 22: 93–102.

Proctor, J. T. A. and Tsujita, M. J. 1986. Air and root-zone temperature effects on the growth and yield of American ginseng. *J Horti Sci,* 61: 129–134.

Proctor, M.; Yeo, P. and Lack, A. 1996. The natural history of pollination. Harper Collins Publishers, London.

Pushpangadan, P. and Nair, K. N. 2005. Medicinal plant wealth of Meghalaya: its conservation, sustainable use and IPR issues. *In*: Tondon P., Sharma M., and Swarup R., (Eds.), Biodiversity status and prospects. Pp. 16–23.

Qi, L. W.; Wang, C. Z. and Yuan, C. S. 2011. Isolation and analysis of ginseng: advances and challenges. *Nat Prod Rep,* 28:467-495.

Rahman, H.; Karuppaiyan, R.; Kishore, K. and Denzongpa, R. 2009. Traditional practices of ginger cultivation in Northeast India. *Indian J Trad Knowl*, 8(1): 23-28.

Rao, A. N.; Haridasan, K. and Bisht, N. S. 1998. Vegetative propagation of *Panax* species in Arunachal Pradesh. *Indian Forester*. 124(9): 702-704.

Sajem, A. L.; Rout J. and Nath, M. 2008. Traditional Tribal knowledge and Status of some Rare and Endemic Medicinal Plants of North Cachar Hills District of Assam, Northeast India. *Ethnobot Leaflets*, 12: 261-275.

Sala, O. E.; Chapin, I. F. S.; Armesto, J. J.; Berlow, E.; Bloomfield, J.; Dirzo, R.; Huber Sanwald, E.; Huenneke, L. F.; Jackson, R.B.; Kinzig, A.; Leemans, R.; Lodge, D. H.; Mooney, H. A.; Oesterheld, M.; Poff, N. L.; Sykes, M. T.; Walker, B. H.; Walker, M. and Wall, D. H. 2000. Global biodiversity scenarios for the year 2100. *Sci*, 287: 1770–1774.

Samant, S. S. and Dhar, U. 1997. Diversity, endemism and economic potential of wild edible plants of Indian Himalaya. *Intl J Sustainable Dev World Ecol*, 4: 179-191.

Schemske, D. W.; Husband, B. C.; Ruckelshaus, M. H.; Goodwillie, C.; Parker, L. M. and Bishop, J. G. 1994. Evaluating approaches to the conservation of rare and endangered plants. *Ecol,* 75: 584–606.

Schippmann, U.; Leaman, D. J. and Cunningham, A. B. 2002. Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity: Global Trends and Issues. *In:* Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries. Food and Agriculture Organization (FAO).

Schlessman, M. A. 1985. Floral biology of American ginseng (*Panax quinquefolium*). *Bull Torrey Bot Club*, 112: 129–133

Schlessman, M. A. 1987. Gender modification in North American ginsengs: dichotomous choice versus adjustment. *Biosci*, 37: 469–475.

Schluter C.; and Punja P. Z. K., 2000. Floral Biology and Seed Production in Cultivated North American Ginseng (*Panax quinquefolius*). *J Ame Soc Horti Sci,* 125(5): 567-575.

Shahi, D. P. 2007. Effects of density on reproduction and demographic structures of American ginseng (*Panax quinquefolius* L.) population in Ohio. M. Sc. thesis. Graduate college of Bowling Green State University.

Sharma, E. and Gaur, A. k. 2012. *Aconitum balfourii* stapf: A rare medicinal herb from Himalayan Alpine. *J Medicinal Pl Res,* 6(22):3810-3817.

Sharma, P. and Sett, R. 2001. Micropropagation of Indian Ginseng (*Panax pseudoginseng* Wall: A Proposition to Save an Endangered Plant. *J Hum Ecol,* 12(3): 201-205.

Sharma, H. K.; Chhangte, L. and Dolui, A. K. 2001. Traditional medicinal plants in Mizoram, India. *Fitoterapia,* 72: 146-161.

Shim, S. C.; Chang, S. K.; Hur, C. W. and Kim, C. K. 1983. Polyacetylenes from *Panax ginseng* roots. *Phytochem,* 22: 1817–1818.

Shu, C. L.; Songyun, L. and Soukup, V. G. 2000. *Paris* Linnaeus, Sp. Pl. 1: 367.1753. *Flora China*. 24: 88-95.

Shu-dong, Z.; Hong, W. and De-zhu, L. 2008. A New Species of *Paris* (Melanthiaceae) from Northeastern Yunnan, China. *Novon,* 18: 550-554.

Singh, P. K. 2004. Biological diversity and conservation strategy of some important medicinal plants of Manipur. Reading material for UGC/XI Refresher course Lecture 12.

Srivastava, N.; Sharma, V.; Dobriyal, A. and Kamal, B. 2011. . Influence of pre-sowing treatments on *in vitro* seed germination of Ativisha (*Aconitum heterophyllum* Wall) of Uttarakhand. *Biotechnol.* DOI 10.3923/biotech.

Stathers, R. J. and Bailey, W. G. 1986. Energy receipt and partitioning in ginseng shade canopy and mulch environment. *Agricul For Meteorology*, 37: 1–14.

Stoltz, L. P. 1982. Leaf symptoms, yield, and composition of mineral-deficient American ginseng. *HortSci*, 17: 740–741.

Strick, B. C. and Proctor, J. T. A. 1985. Dormancy and growth of American ginseng as influenced by temperature. *J Ameri Soc Horti Sci*, 110: 319–321.

Symstad, A. and Tilman, D. 2001. Diversity loss, recruitment limitation and ecosystem functioning: lessons learned from a removal experiment. *Oikos*, 92: 424-435.

Takhtajan, A. 1969. Flowering plants: Origin and dispersal. Oliver and Boyd, Edinburgh, U.K.

Tomoda, M.; Shimada, K.; Konno, C. and Hikino, H. 1985. Structure of panaxan B, a hypoglycaemic glycan of Panax ginseng roots. *Phytochem*, 24: 2431–2433.

Tormmasi, F.; Paciolla, C.; De Pinto, M. C. and De Gara, L. 2006. Effects of storage temperature on viability, germination and antioxidant metabolism in *Ginkgo biloba* L. seeds. *Pl Physiol Biochem*, 44:359-368.

Van der Pijl L. 1982. Principles of Dispersal in Higher Plants. Springer-Verlag, New York, USA.

Van der Voort, M. E. 2005. An ecological study of *Panax quinquefolius* in central Appalachia: seedling growth, harvest impacts and geographic variation in demography. Ph. D. Thesis, West Virginia University.

Van der Voort, M. E.; Bailey, B.; Samuel, D. E. and McGraw, J. B. 2003. Recovery of populations of Goldenseal (Hydrastis Canadensis L.) and American ginseng (*Panax quinquefolius* L.) following harvest. *Ame Midl Nat*, 149(2): 282-292.

Vassilopoulos, Y. 2009. *Paris Polyphylla*. Medicine Section. *Newsfinder*. A literary favor to world culture.

Venugopal, N. and Ahuja, P. 2013. Seed germination of *Panax wangianus* S.C. Sun (Araliaceae): a critically endangered medicinal plant of Meghalaya, Northeast India. *Intl J Pl Repro Biol*, 5(2):156-160.

Verma, P.; Mathur, A. K.; Jain, S. P. and Mathur, A. 2012. *In Vitro* Conservation of Twenty-Three Overexploited Medicinal Plants Belonging to the Indian Sub Continent. *Scientific World J*, 2: 1-10.

Wang, Z. J.; Nie, B. M.;. Chen, H. Z. and Lu. Y. 2006. Panaxynol induces neurite outgrowth in PC12D cells via cAMP- and MAP kinase-dependent mechanisms. *Chem Biol Interact*, 159 (1): 58-64.

Waser, N. M. 1983. Competition for pollination and floral character differences among sympatric plant species: A review of evidence. *In*: Jones, C. E. and Little, R. J. (Eds.), *Handbook of Experimental Pollination Biology*, Van Nostrand Reinhold, N.Y., Pp. 277-293.

Wen, J. 2007. Mt. Sanqingshan, a botanical treasure. *The Plant Press*, Smithsonian. *National Museum Natural History*, 10(4): 1-12.

Wilson, P. J. 1993. Propagation characteristics of Eucalyptus globules *Labill. ssp*. Globules stem cuttings in relation to their original position in the parent shoot. *J Hortic Sci*, 68: 75-724.

Xiao, P. G.; Zim, Z. Y.; Zhang F.Q.; Zim, W. H.; Chen J. I.; Zhang G. D. and Liu, G. T. 1987. Ginseng research and cultivation. Bejing, China: Agricultural Publishing House.

Yonzone, R.; Bhujel, R. B. and Samuel Rai, S. 2012. Genetic resources, current ecological status and altitude wise distribution of medicinal plants diversity of Darjeeling Himalaya of West Bengal, India. *Asian Pacific J Tropical Biomed*, 12: 439-445.

Yoo, K. O.; Malla, K. J. and Wen, J. 2001. Chloroplast DNA variation of *Panax* (Araliaceae) in Nepal and its taxonomic implications. *Brittonia*, 53: 447–453.

Yun-Heng, J.; Heng, L. and Zhe-kun, Z. 2006. *Paris caobangensis* Y. H. Ji, H. Li & Z. K. Zhou (Trilliaceae), A new species from northern Vietnam. *Acta Phytotaxonomica Sinica*, 44(6): 700-703.

Yung, H. C. 1985. Handbook of Chinese Herbs and Formulas. Institute of Chinese Medicine. Los Angeles.

Zhang, J-Y.; Zhang, J.; Yang, W-Z.; Wang, Y-Z.; Yu, H. and Jin, H. 2011. Morphological diversity of wild medicinal *Paris* L. from China and Vietnam. *Afr J Biotec* 10(73): 16421-16428.

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