NICHE CHARACTERIZATION AND STUDY OF GENETIC VARIABILITY IN PANAX SPECIES COMPLEX OF NORTH-EAST INDIA

BY

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THESIS SUBMITTED

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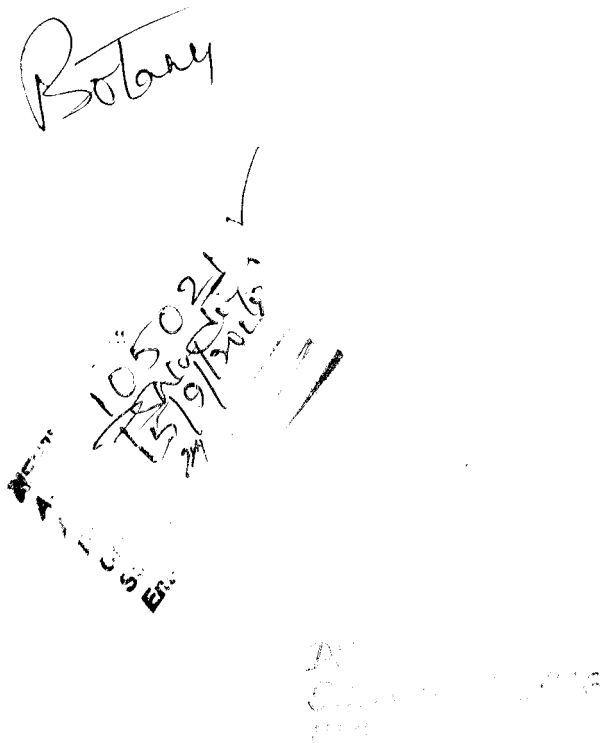
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DECLARATION

I, Lucy Badaplin Nongbri, hereby declare that the subject matter of this thesis entitled ***"Niche characterization and study of genetic variability in Panax species complex of North-east India"*** *is the record of work done by me. The contents of the thesis did not form basis of award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any University/Institute.*

This is being submitted to the North-Eastern Hill University for the award of the degree of Doctor of Philosophy in Botany.

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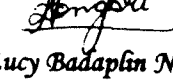

(Lucy Badaplin Nongbri)

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CHAPTER 1
INTRODUCTION

Being the producers, plants are the most important component of an ecosystem. They also support a diversity of other organisms. At least 3,98,650 plant species have so far been scientifically described and named. Plants provide food and other materials essential for humans, and are involved in many ecological processes. The plant cover of the land can significantly influence climate, water supplies and the stability of the soil. During the past 500 years the relationship between human and the planet earth, including plants and animals has undergone several changes. For instance, during the early ages, the vast majority of human population was dependent on wild plants only. Subsequently, the dependency shifted more towards domesticated and cultivated plants those grow in vicinity of their habitations. Today, people worldwide are part of an extensive market economy driven by high consumerism. As a result, exploitation of the earth's resources is continuously increasing. Coupled with the increasing needs, dramatic rise in the size of the human population is putting tremendous pressure on the natural resources that were once well-preserved by our ancestors. The problems of conservation have emerged largely due to these two reasons i.e. increase in the population and excessive pressure on the natural resources. Human populations have long caused local extinction of species and have been responsible for local environmental degradation such as deforestation and soil erosion.

Medicinal plants are important natural resources and are used widely in both developed and developing countries for health care. About 80% of the population in the developing countries depends directly on plants for health care (Pareek, 1996; Mukhopadhyay, 1996). Plants have been used in the traditional healthcare system, particularly among the tribal communities world-wide since time immemorial. This interrelationship has evolved over

generations of experience and practice. About half of the world's medicinal compounds are still obtained or derived from plants (Hamann, 1991). There are more than 25,000 species of medicinal plants described world-wide (Heywood, 1991), several of which represent the great repository of traditional knowledge in the various cultures of people using medicinal plants. The demand for herbal medicine on a global scale is growing rapidly (Srivastava, 2000). A major aspect of the conservation of medicinal plants is enhancing their social and economic value (Farnsworth, 1988). With increased, often unregulated extraction of medicinal plants, the conservation needs of these plant resources are increasingly felt (Akerele *et al.*, 1991).

The utilization of herbs as a source of food, medicine, fragrance, flavour and dyes in India is well-known and is an age-old practice. Out of the 17,500 flowering plant species in India, around 6,500 are used in traditional medicinal system (Ved *et al.*, 2003). The market for Ayurvedic medicine is estimated to be expanding at 20% per annum in India (Subrat, 2002). The emerging field of herbal products industry holds a great potential to the economic development of the Indian region. It is estimated that, 95% of the medicinal plants used in Indian herbal industry today are collected from wild. Majority of these come from the sub-alpine and alpine ecoregions of the Himalayas. With the increase in population, rapid expansion of area under food and commercial crops, deforestation, extension of urban area, and establishment of industries in rural areas, there is considerable depletion of medicinal plant genetic resources. Many of the high value medicinal plant species in fact are in the process of extinction (Vijayalatha, 2004; Singh, 2005).

While reduction of pressure on plant resources through adopting sustainable harvest methods and habitat conservation, remain the preferred conservation action for the plant resources of India, species-specific conservation approach through identification,

prioritization and undertaking recovery actions for threatened species did constitute an important component of conservation action in India.

The Botanical Survey of India established in the year 1890 with the basic objective of exploring the plant resources of the country and identifying the plant species with economic virtues, initiated work on identifying rare and endangered species in early 1980's. Under this initiative, Nayar and Sastry (1987, 1988, 1990) documented at least 621 plant species from different parts of the country. About 1,236 (7%) of the 17,500 plants recorded for the Indian subcontinent are threatened globally (Walter and Gillett, 1998). The science of classification of threatened species in the meanwhile has undergone sea change and much more objectively defined criteria than before are being used to define the categories of threatened species (IUCN, 2001).

While efforts to conserve the species through habitat and species-specific conservation actions are being accelerated, adequate policy measures to ensure species protection both at national and global levels have been undertaken. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is such an international agreement between the governments to ensure that international trade in specimens of wild animals and plants does not threaten their survival. It has been among the largest conservation agreements in existence, with 180 nations as parties to the agreement. India is one of the signatories to the CITES. The Red List prepared by the International Union for the Conservation of Nature and Natural Resources (IUCN) is the world's most comprehensive information source on the global conservation status of animal, fungi and plant species and their links to livelihoods. It provides information on population size and trends, geographic range and habitat needs of species. The IUCN Red List of Threatened Species provides taxonomic, conservation status and distribution information on plants, fungi and animals that have been globally evaluated using the IUCN Red List Categories and Criteria which is

designed to determine the relative risk of extinction. Since its existence, IUCN has attempted to list species that are threatened with extinction globally. The criteria used to define the categories of threatened species changes with new information from 2001 up to 2014 at present and the lists of threatened species are accordingly upgraded.

The North-eastern states of India constitute a characteristic narrow passageway that connects the Indian subcontinent to East Asia and Southeast Asia and comprises eight states viz., Arunachal Pradesh, Assam, Mizoram, Manipur, Meghalaya, Nagaland, Tripura and Sikkim. The climatic, edaphic and altitudinal variations in North-eastern India have resulted in a great range of ecological habitats. North-east India is regarded as the geographical 'gateway' for much of India's flora and fauna, therefore is one of the richest areas of India in biological values (Pareek, 1996; Dash and Sharma, 2001). The north eastern region has been in spotlight for its high biodiversity and traditional knowledge, and has been a priority for leading conservation agencies of the world. The region is affluent in medicinal plants and many other threatened taxa. Since the region has high endemism in higher plants, vertebrates, avian diversity and 70% of its original habitats/geographical area has been destroyed, the region today is one of the 34 biodiversity hotspots in the world.

The region is also the abode of approximately 225 of India's 450 tribes, the culture and customs of which have an important role in biodiversity conservation and management. The immense biodiversity of the north-eastern region has made it a priority area for investment by the leading conservation agencies of the world. WWF has identified the entire eastern Himalaya as a priority Global 2000 ecoregion. The region is part of world's two "biodiversity hotspots" (out of the total 34 hotspots globally), viz., Himalaya, and Indo-Burma (Mittermeier *et al.*, 2011). The richness of the region's avifauna largely reflects the diversity of habitats associated with a wide altitudinal range. Northeast India supports one of the highest bird diversities in the Orient, with about 850 bird species. The region's

lowland and montane moist to wet tropical evergreen forests are considered to be the northernmost limit of true tropical rainforests in the world (Proctor *et al.*, 1998). The north-eastern India alone harbours about 8,000 plant species of which about 2,500 have various medicinal properties. Within the region, each constituent state is also extremely rich in biodiversity. For instance, Meghalaya is home to 3,126 plant species (Khan *et al.*, 1997) of which 834 are pharmaceutically or locally used in health care (Barik *et al.*, 2007).

There is an increasing focus on medicinal plants and traditional healers in addressing the health care problems of the world. Herbal products are gaining global popularity owing to their negligible side effects. The traditional herbal medicines, supplements and cosmetics are increasingly preferred over synthetic compounds as they are cost-effective, easily available, and compatible with culture and tradition. The demand for herbal product in fact is increasing day by day and the international trade in medicinal plants is growing in very fast rate, leading to the depletion of mother populations in the countries of origin (Bhutani, 2008; Mao *et al.*, 2009; De *et al.*, 2010).

About 90% of the medicinal plants of India are found in forest habitats, while only 10% of the medicinal plants are distributed in other landscape elements like open grasslands, agricultural pastures, wasteland, and in and around fresh water bodies. North-east India represents several unique ecosystems rich in medicinal plants associated with folk medicine, Ayurveda, Siddha, Amchi, Unani and Homeopathy system of medicines. Many of these species of North-east origin have revolutionized the allopathic systems of medicine. *Taxus wallichiana* is such an example that revolutionized the treatment of cancer. As such, the medicinal plants occupy an important position in the socio-cultural, spiritual and health care domain of people's life in the region. Traditional medical practice in fact is an integral part of the culture of people of North-east India.

Araliaceae (the ginseng family) includes about 50 genera and approximately 1500 species (Wen *et al.*, 2001a) and is distributed mainly in the tropics and subtropics especially in southeastern and southern Asia and the Pacific islands, with some genera occurring in the temperate zone (e.g. *Aralia* L., *Hedera* L., *Oplopanax* (Torr. & Gray) Miq. and *Panax* L.). The family includes a number of medicinally important taxa, such as *Panax* (ginseng) and *Eletheurococcus* Maxim. (Siberian ginseng). Ginsengs are among the best known herbal medicines widely used in China (Anderson *et al.*, 2003). Ginseng root has been used for thousands of years in the traditional medical system in oriental countries (Ang-Lee *et al.*, 2001; Attele *et al.*, 1999; Wang and Yuan, 2008). It occupies a prominent position on the list of the best-selling medicinal plants in the world (Yun, 2001). The Chinese have been using ginseng for over 2000 years as a tonic, a stimulant and a fatigue-resistance medicine.

Nearly all of the world's 80,000 tons of ginseng trade is dependent on the production by four countries, viz., South Korea, China, Canada and the United States (Evans, 1985). The product is being marketed in over 35 countries. Sales exceeded $2.1 billion in 2010, of which half came from South Korea (Baeg and Seung, 2013). Medicinal plants which are over-extracted from north-eastern India include *Cordyceps sinensis* (Berk.) Sacc., *Dendrobium denudans* D.Don, *D. eriaflorum* Griff., *D. transparens* Wall., *D. devonianum* Paxt., *Lemanea australis* Alkins, *Lycopodium pseudoclavatum* Ching, *Panax assamicus* Banerjee, *Paris polyphylla* Sm., *Rubia manjith* Roxb. ex Fleming and *Taxus wallichiana* Zucc. (Mao *et al.*, 2009).

All the species of *Panax* are important medicinal herbs and are widely used as tonic that supports the function of the adrenal glands, particularly in production of corticosteroids and male sex hormones. It also helps in improving blood flow through the coronary arteries, thus finding use in treatment of arteriosclerosis, high blood pressure and angina. The roots are reported to be analgesic, anti-inflammatory, antiphlogistic, antiseptic, astringent,

cardiotonic, discutient, diuretic, haemostatic, hypoglysaemic, styptic, tonic and vulnerary. They are used in the treatment of contused wounds, soft tissue injuries and different kinds of bleeding, both internal and external, such as haematuria, nose bleeds, haematemesis, and uterine bleeding. They are also used in the treatment of coronary heart disease and angina pectoris. Saponins in the alcohol-soluble pseudoginseng component decreased bleeding time in rat by 52%. Thus the therapeutic use of the species is well-established both in modern and traditional system of medicine. The principal reference components, to which pharmocological effects have been attributed, are in its ginsenosides (steroidal glycosides). Because of its medicinal value and high price in the market, the species have been over-exploited from all its natural habitats pushing them to the verge of extinction.

Estimation of size of natural populations and locating all the populations in nature, particularly those of threatened species are the greatest challenges for the conservationists. Even to develop a sustainable business model involving the rare but important medicinal plants such as *Panax*, these data on species distribution are pre-requisites. Ecological Niche Modelling (ENM) has been proved to be an effective tool in overcoming these challenges. In fact the concept of ecological niche has remained as one of the core ideas in ecological research for almost a century allowing species coexistence because of differentiation in resource use and habitat distribution (Grinnell, 1917; Elton, 1927; Gause, 1934; Hutchinson, 1957; MacArthur and Levins, 1964). The concept of an organism's niche is fundamental to ecological theory contributing two core ideas: (1) that environmental factors affect an organism's performance and thereby limit its geographical distribution, and (2) that organisms are adapted genetically to a limited range of environmental conditions. Research into the habitat requirements of species plays a fundamental role in planning for their future conservation, particularly if their persistence is threatened by external pressures such as disturbance and climatic change.

For effective conservation and management, it is important to know the geographic distribution and potential habitat for a species (Margules and Pressey, 2000). But species distribution data are usually not available and acquiring such data is usually costly and labour intensive (Margules and Austin, 1991; Scott *et al.*, 2002). Many geographic applications have been developed recently that propose new possibilities for understanding biological diversity (Scott *et al.*, 1996). Geographic information systems (GIS) make it possible to construct maps of species richness and endemism, to prioritize areas for conservation based on principles such as complementarity, and to assess the completeness of existing protected areas networks (Peterson *et al.*, 2000). It also offered a powerful tool to predict species distribution in the context of spatially and temporally variable habitats (Guisan and Thuiler, 2005; Hijmans and Graham, 2006).

Several modelling techniques for predicting species distributions have attracted increasing attention (Guisan and Zimmermann, 2000; Austin, 2002). In particular, correlative species distribution modeling (SDM) techniques have utilized a niche-based approach that distinguish regions containing suitable environmental conditions based on habitat characteristics at locations of known species occurrences. Suitable areas are extrapolated onto other geographic regions or into the future using forecasted environmental conditions to predict the distribution of a species (Elith *et al.*, 2010; Dombrowski *et al.*, 2011). This method has proven useful for conservation planning by predicting the occurrence of rare species (Guisan *et al.*, 2006), responses of species to global climate change (Wenger *et al.*, 2011), and the impacts of invasive species (Kulhanek *et al.*, 2011). There are various applications available for distribution modelling techniques, which includes providing insight into limiting factors, exploring populations of rare and endangered species (Elith and Burgman, 2002; Engler *et al.*, 2004), identifying suitable habitats for introductions or translocations, and management of species diversity (Austin and Meyers, 1996), and

predicting ranges under past and future climates (Hilbert *et al.*, 2004). Such models perform more in addition to filling gaps in distribution maps. By delineating favourable habitats, distribution models can help target field surveys (Engler *et al.*, 2004), aid in the design of reserves (Li *et al.*, 1999), inform wildlife management outside protected areas (Milsom *et al.*, 2000) and guide mediatory actions in human–wildlife conflicts (Sitati *et al.*, 2003).

Plant species exist in a series of more or less isolated populations and among them some level of seed or pollen migration takes place. In any population, the members always share a number of features. Unless precluded by virtually complete uniparental reproduction such as autogamy or apomixis, they are likely to share a common ancestor. They intrude each other competitively, they support the same local population of symbionts, herbivores and parasites, and through mating they share a common evolutionary future. Hence the population is the basic unit of conservation at the level of the individual species which led biologist to expand the concept of population biology from local level research and experimentation to a regional outlook. Population ecology basically deals with how the populations of plants, animals and other organisms change in time and space and their interaction with the environment and therefore provides information of the population distribution or size and to evaluate whether a population will increase or decrease with time.

With the advancement in technology, knowledge on population ecology has improved extensively. Metapopulation and extinction risk analyses are being carried out using powerful tools such as metapopulation modelling incorporated with GIS and today population ecology has evolved as a powerful quantitative discipline with popularization and importance in metapopulation studies of both plant and animal species. Studies in metapopulation dynamics and the application of metapopulation models to species-specific problems in conservation have contributed significantly to the discipline of conservation

biology (Brito and Fernandez, 2002; Yuttham *et al.*, 2003) and has prompted many field studies for collecting key data on demography and movement of several plant and animal populations, especially those facing the risk of continuous decline (Holyoak and Ray, 1999).

Concrete assessment of the threat status of declining plant species based on their population size and distribution is a prerequisite for any conservation action. The parameters such as metapopulation dynamics, rate of decline, environmental stochasticity and external perturbations are important determinants of species perpetuation and need to be quantified in respect of a species for correct threat categorization. Metapopulation studies therefore include a wide range of habitat inventory so that the regional cover of the species is known. Therefore, the first step towards this endeavor is scientific baseline information on the habitat and distribution of these species prior to any in-depth research on their populations. Modelling the habitat and potential distribution of species is therefore an important component of conservation biology. The outputs of which would help detailed population sampling and study. For this purpose, an inventory for acquiring maximum presence localities is a pre-requisite.

Studies using metapopulation approach, first introduced by Levins (1969), generally subdivide the general population into a series of local populations with a balance between extinctions and re-colonization of local populations that facilitates long-term persistence of the metapopulation. The key process is the inter-patch connection function by migration (Hanski and Gilpin, 1997). Migration from one local population to another is possible, but is usually limited (Hanski and Simberloff, 1997). Extinction of local populations can occur and the persistence of a metapopulation depends on the dynamics of extinction and recolonisation of local habitat patches. Metapopulation will persist if the rate of colonization exceeds or equals the extinction rate (Taylor, 1990).

Modelling has become an important tool in population and conservation biology (Cappuccino and Price, 1995; Haefner, 1996; Hanski and Gilpin, 1997; Hilborn and Mangel, 1997; Roughgarden, 1998; Shugart, 1998). Model can be categorised into two classes: qualitative (expressed in words or diagrams) and quantitative (expressed in mathematical equations) (Wu, 1994). Many ecological theories today are represented in mathematical terms since mathematics provide the most precise language to describe complex ecological systems and is also an ideal tool for prediction in ecological systems (Tilman *et al.*, 1994; Jansen, 1995; Gyllenberg and Hanski, 1997; Hanski and Ovaskainen, 2000; Keymer *et al.*, 2000; Casagrandi and Gatto, 2002). However, mathematical formulations have limitation in that they usually force ecologists to make clear and unambiguous assumptions. Metapopulation models are always represented as analytical or simulation models.

There are three types of modelling approaches used in metapopulation studies assuming many habitat patches and local populations (Hanski and Gilpin, 1997): (i) spatially implicit approaches, often based on a critical simplification of what at first appears as a complex problem, in which the habitat patches and local populations are discrete (and are generally assumed to have independent dynamics) but are assumed to be all equally connected to each other, (ii) spatially explicit approaches in which it is assumed that local populations are arranged as cells on a regular grid (lattice), with population sizes modelled as either discrete or continuous variables and where local populations are assumed to interact only with local populations in the nearby cells, and (iii) spatially realistic approaches in which the models allow one to include in the model the specific geometry of particular patch networks, such as how many patches are there in the network, how large they are, and where exactly they are located.

Metapopulation concept has also prompted renewed research interest concerning dispersal capacities that is extremely valuable in understanding population structure (Yuttham *et al.*, 2003). Extinction and metapopulation theories emphasize that stochastic fluctuations in local populations cause extinction and that local extinctions generate empty habitat patches that are then available for re-colonization. Metapopulation persistence depends on the balance of extinction and colonization in a static environment. For many rare and declining species, Thomas (1994) argued that (i) extinction is usually the deterministic consequence of the local environment becoming unsuitable *e.g.*, through habitat loss or modification, introduction of a predator, etc., (ii) the local environment usually remains unsuitable following local extinction, so extinctions only rarely generate empty patches of suitable habitat, and (iii) colonization usually follows improvement of the local environment for a particular species (or long-distance transfer by humans). Thus, persistence depends predominantly on whether organisms are able to track the shifting spatial mosaic of suitable environmental conditions or on maintenance of good conditions locally.

With the recent development of metapopulation theory (Hanski, 1989), it has been recognized that regional-scale processes are also important for long-term survival of species in the landscape (Carroll *et al.*, 2003; du Toit *et al.*, 2004). In such cases, population viability analysis has to be performed at regional scale and should take into account both the present distribution of the species in the landscape and the number, and distribution of patches that are potentially available for re-colonization. Management of threatened populations will be necessary to guarantee long-term survival of threatened species and ecosystems (Beissinger and Westphal, 1998; Reed *et al.*, 2002). Population viability analysis (PVA) is becoming an ever more central tool in conservation biology (Beissinger and McCullough, 2002; Morris and Doak, 2002). In PVA, biological and landscape data are used to parameterize a population model which projects the dynamics, abundance and

metapopulation structure of a focal threatened species into the future. From these projections, viability is estimated and compared under different scenarios of landscape or population management to design effective conservation guidelines.

One of the widely used approaches to PVA is the demographic method which is extensively used during the last decade (Menges, 1990; Haig *et al.*, 1993; McCarthy *et al.*, 1994). While this approach may not be feasible for species which are critically endangered because of their population being scanty in nature, it however has been proved to be one of the most effective of all PVAs if the dynamics of all subpopulations is accounted for. Therefore, demographic study continues to be the most important and fundamental aspect of population biology. Several workers have highlighted the importance of demography in landscape models to study the invasive spread of species (Moody and Mack, 1988; Lavorel *et al.*, 1995; With, 2002, 2004). Other recent works have demonstrated that demographic rates might in fact be more important than dispersal ability of populations to persist in a fragmented landscape (South, 1999; With and King, 1999).

The status of populations whether declining, increasing or stable can be established from accurate demographic data (Maschinski *et al.*, 1997). Vital rates which are the main component in demography do not differ much in pattern for any given population under natural stochastic environment and these determine the growth or decline of that population. In the presence of external factors however (*e.g.*, anthropogenic interference), these underlying factors may vary within a metapopulation depending upon severity of external influence, and could adversely affect the persistence of a species. Since PVAs are also useful for comparing effects of different factors or management options (Beissinger and Westphal, 1998; Menges, 2000), stochastic simulation can therefore be used to test and compare how different populations or groups would respond under these different scenarios (Garcia, 2003) where threats (*e.g.*, predation, diseases, inbreeding, population reduction)

and management regimes are suspected to affect population growth rate (van Groenendael and Slim, 1988; Ehrlen, 1995; Nantel *et al.*, 1996; Menges, 1997; Menges and Dolan, 1998; Pfab and Witkowski, 2000; Lennartsson and Oostermeijer, 2001).

The correct identification and nomenclature of *Panax* is one of the most challenging areas of Araliaceae taxonomy. Because of the presence of several morphological intermediates, particularly in leaf and rhizome morphology, identity of Himalayan *Panax* has posed serious challenge for several decades now. During the past three-decades, the field of systematic biology has undergone several simultaneous revolutions. The three most significant changes have been in the development and refinement of systematic theory, the technical elaboration of data analysis brought out by the development of computers, and the introduction of molecular analysis. Although molecular biology is not a panacea for systematics, molecular systematists can approach many problems previously considered intractable by morphologists (Hillis, 1987; Patterson, 1987). With the emergence of new techniques such as amplification of specific regions of DNA is now possible by the Polymerase Chain Reaction (PCR) that was invented by Kary Mullis in 1983. This technique was first used to identify the gene for sickle cell anaemia. PCR has revolutionized the approaches to molecular study in many fields. Different modifications of the PCR technique are available for studying polymorphisms in DNA.

The study on diversity and phylogeny of a plant species or genus (or even higher levels) is of great significance, primarily because of its link to many branches of biological sciences. The presence of variation in a population particularly at the molecular level is important both to improve the population through selection of pre-existing variation or new mutations (Barrett and Schluter, 2008) and in quality (*et al.*, 2009) and disease control (Zhu *et al.*, 2000). Knowledge of genetic diversity within and among populations is important for conservation management, especially in the identification of genetically unique structural

units within a species and to determine the populations that needs protection (Zhuravlev *et al.*, 2010; Cruse-Sanders and Hamrick, 2004).

Furthermore, genetic diversity has a significant effect on ecological processes such as primary productivity, population recovery from disturbance, interspecific competition, community structure, and fluxes of energy and nutrients (Hughes *et al.*, 2008). The loss of genetic variation however, is detrimental to the plant population as it scales down its ability to respond to environmental change which enhances the probability of extinction or limited evolution (Aguilar *et al.*, 2008). From the phylogenetic point of view, a strongly supported phylogeny would provide a means for evaluating character evolution (Les *et al.*, 1999; Borsch *et al.*, 2008), molecular evolution (Grimm and Denk, 2007), historical biogeography (Lohne *et al.*, 2008), and global changes (Edwards *et al.*, 2007). Phylogenetics reconstructions may also help in the discovery of greater plant diversity and assist biologists in choosing areas or species to prioritize in their conservation efforts (Cameron, 2010).

Despite the large size of the nuclear genome and the large number and diversity of genes that it includes, most efforts to infer phylogeny with nuclear gene sequences have evolved the nuclear ribosomal DNA cistron (rDNA). The highly conserved coding regions (18S, 26S rDNA) are quite useful mainly for studies at the family level and above, whereas the rapidly evolving regions such as the internal transcribed spacer regions (ITS) and the intergenic spacer (IGS) are best suited for comparing species and closely related genera and at population level. Ribosomal DNA cistrons typically are located in the nucleolar organizing region (NOR) and may be present on several different chromosomes (Thompson and Flavell, 1988). The internal transcribed spacer (ITS) region of the 18S–5.8S–26S nuclear ribosomal cistron is one of the most popular sequences for phylogenetic inference at the generic and infrageneric levels in plants (Varghese *et al.*, 2003). The ITS-1 and ITS-2

regions are part of the nuclear rDNA transcript but are not incorporated into ribosomes but appear to play a role in the maturation of nuclear rRNAs, bringing the large and small subunits into close proximity within a processing domain (reviewed in Baldwin *et al.*, 1995). The need for sequence data from nuclear genome at lower taxonomic levels makes the ITS region a popular site for this study. Since the ITS region is G+C rich and prone to secondary structure, sequencing can be difficult (Baldwin *et al.*, 1995). Different protocols have been used to amplify and sequence the ITS regions (Baldwin, 1992; Wen and Zimmer, 1996; Soltis and Kuzoff, 1995).

In spite of high medicinal value, the saponin contents of Himalayan *Panax* have been little studied. Plants synthesize a vast range of organic compounds that are traditionally classified as primary and secondary metabolites. The beneficial medicinal effects of plant materials typically result from the combination of secondary products present in the plants. The medicinal actions of plants are unique to a particular species or groups and are coherent with the concept as the combinations of secondary products in a particular plant are often taxonomically distinct (Wink, 1999). This is in contrast to primary products, such as carbohydrates, lipids, proteins, chlorophylls and nucleic acids which are common to all plants and are involved in the primary metabolic processes of building and maintaining plant cells (Kaufman *et al.*, 1999; Wink, 1999).

The secondary metabolites play a pivotal role in the ecophysiology of plants (Briskin, 2000). They also play a defensive role against herbivore, pathogen attack, inter-plant competition and an attractant role as pollinators or symbionts (Kaufman *et al.*, 1999; Wink and Shimmer, 1999). Secondary metabolites have protective actions in relation to abiotic stresses which are associated with changes in temperature, water status, light levels, UV exposure and mineral nutrients and also play key roles at the cellular level as plant growth regulators, modulators of gene expression and in signal transduction (Kaufman *et al.*,

1999). Studies on plant secondary metabolites have been increasing over the last 50 years. These molecules are known to play a major role in the adaptation of plants to their environment, but also represent an important source of active pharmaceuticals which are classified as alkaloids, glycosides, tannins, phenolic compounds, volatile oils, terpenoids, saponins, steroids, resins and bitter principles. These are used as medicine, food, flavors, colours, dyes, poisons and perfumes etc.

Ginsenosides are triterpenes saponins considered to be the main bioactive principles of the most important Oriental herbal medicine "ginseng" derived from the roots and rhizomes of different *Panax* species. The most commonly used *Panax* species are *P. ginseng* (Korean or Asian ginseng), *P. quinquefolius* (American ginseng), *P. notoginseng* (Tienchi or Sanchi), *P. vietnamensis* (Vietnamese ginseng) and *P. japonicus* (Japanese ginseng). Till date most of the research has focused on Korean ginseng which has been used in Asia for more than 5000 years as a tonic and a panacea that can promote longevity (Dharmananda, 2002).

For any conservation programme, prioritization of species is fundamental, which is based on assessment and categorization of species into different threat categories. For effective conservation strategy, categorization of threatened species should be done using the data on their population abundance. Most endemic and high value medicinal plant species of north-east India are collected from the wild and are under threat due to over-exploitation, and habitat destruction as a consequence of rapid pace of developmental activities and shifting cultivation. Scientific conservation efforts focusing on threatened species are very little. Many threatened categories of species have not been evaluated because of data deficiency. Even those have been categorized, are not based on precise quantitative data for various population parameters. Subsequent to the collection of empirical data, the threat status of several species may change.

As mentioned earlier, in India, five species of *Panax* are found viz., *P. assamicus, P. bipinnatifidus, P. sikkimensis, P. sokpayensis* and *P. pseudoginseng*. The occurrence of species has been reported from a very limited area. Complete inventory of natural range of each species and distribution mapping are prerequisites for effective conservation measures. There is also a need to conclusively establish the identity of each species based on morphological and molecular profiling. Because human activities can change the level and pattern of genetic diversity, the loss of genetic diversity may ultimately reduce the evolutionary potential of species to respond to the future environmental changes.

Considering the high rate of disappearance of the species and reduction in species population size of *Panax*, it is urgent to adopt measures of conservation to prevent the species from extinction. For taking up *in situ* conservation measure, the first and foremost task is to identify the natural habitats and the growing areas of *Panax*, for demarcation and further protection measures. Most endemics have specialized niches resulting in restricted distribution. If the niche of the species is characterized it becomes easier for the *ex situ* conservation of the species. Besides, the quantification of niche helps in determining the distribution area of the species. However, very scanty work has been done on all five Himalayan ginseng. In view of this the present study was undertaken to:

1. Study the distribution pattern, niche and population characteristics of *Panax* species complex in north-eastern India;
2. Resolve the taxonomic ambiguity of Himalayan *Panax* species complex using molecular phylogeny approach; and
3. Quantify the ginsenoside contents for Himalayan *Panax* species complex and correlate it with the ecological conditions of the species.

The findings of this Ph. D. work have been divided into nine chapters. Following this introductory chapter, the review of literature chapter provides a synoptic view on the works done on *Panax* world-wide. Chapter 3 deals with the study sites that cover five states of north-eastern India. The detailed description of all *Panax* species of north-east and their phenology are given in Chapter 4. The results of the studies on niche characterisation and distribution mapping of *Panax* species of north-eastern India using ecological niche models are presented in Chapter 5. Chapter 6 deals with the establishment of taxonomic identity of *Panax* species from north-eastern India using morphological and molecular markers. The assessment of the impact of disturbance on *Panax assamicus* metapopulation was undertaken in Chapter 7 using metapopulation modelling. The inter-population variation in ginsenoside contents in *Panax* species complex has been presented in Chapter 8. Chapter 9 discusses the findings of the entire study and draws conclusion.

CHAPTER 2
REVIEW OF LITERATURE

Plants have been used as therapeutic agents since ancient times in India, as described in Susruta Samhita and the Charak Samhita (Prajapati *et al.*, 2003). They were also widely used for health care purpose by many other known cultures of the past such as Egyptian, Babylonian, Jewish, and Chinese. These herbal remedies are still the mainstay of primary health care for at least 80% of world population, mainly in developing countries (Fransworth, 1988). Over the years, medicinal plants have come up as an irreplaceable entity in the modern drug discovery and nearly 20 to 30% modern drugs are directly derived from plants while many others are synthetic analogues of natural compounds found in plants (Brower, 2008).

Traditional medicine has been an important element of health care systems in Asian countries and includes Ayurveda, Yunani, Siddha, Homeopathy and folk medicine systems which have been in practice in India since time immemorial. Even today, hundreds of millions of people, mostly in developing countries, derive a significant part of their health care and subsistence needs, and income from medicinal plants and animal products gathered from the wild (Iqbal, 1993; Walter, 2001). The demand for a wide variety of ethno-medicinal species is increasing particularly those with potential for commercial trade. With the increased recognition of the species which are being over-exploited, agencies such as WHO, IUCN and WWF are recommending that wild species be brought into cultivation systems (Lambert *et al.*, 1997). Cultivation can also have conservation impacts, which need to be better understood. Production of ethno-medicinal plants through cultivation, for example, can reduce the percentage of wild populations that are harvested. However, it may

lead to loss of genetic diversity as well as loss of incentives to conserve wild populations (Anon, 2002).

The Himalayan region is known for its diverse medicinal plants and North-Eastern region is home to a variety of them. In remote areas, the use of wild plants for medicinal purposes is quite common. Some of these plant species are over-exploited, thereby threatening their populations in the wild (Rai and Sharma, 1994). Many of these species are used only in folk medicine at the local level. Among the widely used plants, quite a number of them have not been assessed in the context of their population status in the natural habitats. Considerable efforts have been made by various institutions to document the usage of ethno-medicinal plant species. However, there is no empirical research on their actual status in the wild.

India is one of the twelve mega biodiversity countries of the world with its 45000 plant species spread over 16 different agro-climatic zones, 10 vegetative zones and 15 biotic provinces (Singh and Chowdhary, 2000; Kala and Sajwan, 2007). The country has about 15000 recognized medicinal plants which include 7000 plants used in Ayurveda, 700 in Unani, 600 in Siddha, 450 in Homeopathy and 30 in modern medicines (Das, 2008). Herbal medicines are becoming popular worldwide due to the growing recognition that natural products are cheaper and are with no side effects. Demands for medicinal plants are increasing in both developing and developed countries. In India, about 2,000 drugs used are of plant origin (Dikshit, 1999).

The Himalayan region is accredited as a treasure house of medicinal herbs, and contributes to about 30% of the endemic species found in the Indian sub-continent (Nautiyal *et al.*, 1998). The medicinal plants of the Himalayas in general are known for their high medicinal value (Anonymous, 2000). The compression of thermal life zones and the fragmentation of the landscape make the Himalayas a hotspot of medicinal plant diversity (Kaul, 2010).

Around 8000 species of angiosperms, 44 species of gymnosperms and 600 species of pteridophytes have been reported from the Indian Himalayas (Singh and Hajra, 1996). Of these, 1748 species are identified as medicinal plants (Samant *et al.*, 1998), some of which are found throughout the Himalayas (although with altitude-specificity), while some are endemic to a particular geographical location. Amongst different climatic zones, the maximum medicinal plants diversity (1717 species) has been reported in the temperate zone (Kala *et al.*, 2006). Many of these species are listed in Appendix I of CITES (Convention on International Trade in Endangered Species of Flora and Fauna) and majority of them are under different threat categories.

Himalayan medicinal plants are now under great pressure due to their excessive collection from the wild leading to the substantial loss of their populations (Kala and Sajwan, 2007). More than 90% of medicinal plant raw material for herbal industries and export is drawn from the natural habitats increasing in the degree of threat to natural populations (Dhar *et al.*, 2002). In fact today the medicinal plants those are used by human beings are in great threat (Rao *et al.*, 2004; Kala and Sajwan, 2007). Some of the species, which are in great demand from pharmaceutical companies include, *Picrorhiza kurrooa, Podophyllum hexandrum, Nardostachys grandiflora, Dactylorhiza hatagirea, Aconitum heterophyllum* and *Saussurea costus*. Most of these species have been listed in Appendix I and II of CITES (Uniyal *et al.*, 2002). Excessive anthropogenic pressure is the main cause of decline in the population and accessibility of these medicinal plants in the Himalayan region (Dhar, 2000; Kala, 2000). Several potential causes of rarity in medicinal plant species include, habitat specificity, narrow range of distribution, land use disturbance, introduction of non-natives, habitat alteration, climatic changes, heavy livestock grazing, explosion of human population, fragmentation and degradation of population, population bottleneck and genetic drift (Kala, 2005, 2000; Weekley and Race, 2001; Oostermeijer *et al.*, 2003)

2.1. Loss of medicinal plant genetic resources

The demand of botanical raw drugs annually is quite high in domestic market in India (Ved and Goraya, 2007). At the level, there are nearly two million practitioners of ISM (Indian systems of medicine) (Anonymous, 2000), 9500 registered pharmacies of ISM and a number of unlicensed small-scale herbal pharmacy units (Ved and Goraya, 2007). The increasing demand of medicinal plants has created a high pressure on the existing resources in India, particularly the temperate and alpine plants of Himalayas (Dhawan, 1997; Hazlett and Sawyer, 1998). Almost all the Himalayan medicinal plants, which are traded in high volume (>100 MT/year), have come under immense threat of depletion (Ved and Goraya, 2007). More than 90% of raw material for pharmaceutical companies is drawn from wild, as less than 20 species of plants are under commercial cultivation (Ved *et al.*, 1998; Uniyal *et al.*, 2002) and about 70% of the plant collections involve destructive harvesting because of the use of parts like roots, bark, wood, stem and the whole plant in case of herbs (Natesh, 2000). Most of the medicinal plants are endemic to the region (Chatterzee, 1939), and are more vulnerable to overharvesting. Factors like human population growth, increased pollution, deforestation, urban development and other developmental activities like hydroelectric projects and road lying also cause great loss of genetic diversity (Tandon *et al.*, 2009). Loss of natural habitats due to massive deforestation and expansion of agricultural practices have driven many endemic species to the brink of extinction in the Himalaya (Pandit *et al.*, 2007). The threat status of medicinal plants of different states of Himalayan range has been assessed by various CAMP (Conservation Assessment of Medicinal Plants) workshops, organised by Foundation for Revitalization of Local health and Traditions from time to time (Goraya, 2011).

2.2. Ginseng

The ginseng genus is one of the approximately 120 genera of flowering plants with an eastern Asian and eastern North American disjunct distribution (Wu, 1983). *Panax* consists of approximately 18 species, of which 16 are from eastern Asia and two from eastern North America (Wen and Zimmer, 1996; Wen, 2001b; Yoo *et al.*, 2001; Lee and Wen, 2004). In India Araliaceae is represented by 15 genera distributed mostly in north and northeastern region. The genus *Panax* comprises of *Panax assamicus* Ban., *P. bipinnatifidus* Seem., *P. pseudoginseng* Wall., *P. sokpayensis* Sharma and Pandit., and *P. sikkimensis* Ban. In India these species are found in Arunachal Pradesh, Meghalaya, Manipur, Nagaland, Sikkim and also in Darjeeling area of West Bengal (Pandey *et al.*, 2002, 2004, 2007, 2009; Mao *et al.*, 2009; Sharma and Pandit, 2009). Among the Asiatic species, several Himalayan taxa have been debatable due to sympatry of morphologically distinct taxa and the existence of occasional morphological intermediates (Wallich, 1829; Wen and Zimmer, 1996). The Himalayas, central and Western China is considered the centre of diversity. During the late tertiary and quaternary period, the rise of the Himalaya and the formation of mountainous regions of central and western China may have created numerous isolated habitats which is ideal for the speciation in *Panax* (Axelord *et al.*, 1998; Wen, 2001b).

High medicinal value of several Himalayan plant species including *Panax* is responsible for their extraction from the wild, which has already reduced the population sizes of these taxa in most of the natural populations (Pandit and Babu, 1998). The illegal trade in wild ginsengs has caused a drastic decline in recent years in the wild populations which leads to some species being endangered (Koren *et al.*, 2003; Cruse-Sanders and Hamrick, 2004). The Himalayan *Panax* species are already enlisted as vulnerable in the Red Data Book of India (Nayar and Sastry 1990). In CAMP (2003), *P. wangianus* was classified as an endangered species.

2.3. Medicinal uses of ginseng

Ginseng is frequently used in Asian countries as a traditional medicine and till today ginseng preparations are amongst the most popular and best-selling herbal medicines worldwide (Ernst, 2002). The most commonly used *Panax* species are *P. ginseng* (Korean or Asian ginseng), *P. quinquefolius* (American ginseng), *P. notoginseng* (Tienchi or Sanchi) and *P. japonicus* (Japanese ginseng). Most of the research has been focused on Korean ginseng which has been used in Asia for more than 5000 years as a tonic and a panacea that can promote longevity (Dharmananda, 2002). Ginseng is used mainly to increase resistance to physical, chemical and biological stress and boost general vitality (ESCOP, 2003; Kiefer and Pantuso, 2003). This activity of ginseng has been described as "adaptogenic" in most of the alternative medicine literature. Immume system modulation, anti-stress activities and anti-hyperglycemic activities are among the most notable features of ginseng in laboratory and clinical trials. Moreover, a number of investigations indicate the antitumor properties and other pharmacological activities related to cancer, but no trials have confirmed a clinically significant anticancer activity yet (Chang *et al.*, 2003). It also modulates blood pressure, metabolism and immune function (Liu and Xiao, 1992; Attele *et al.*, 1999; Spelman *et al.*, 2006; Xiang *et al.*, 2008). It has also been reported to be efficacious in the treatment of amnesia. In addition, significant improvement in learning and memory has been observed in brain-damaged rats (Zhao and MacDanields, 1998; Zhong *et al.*, 2000) and aged rats (Zhong *et al.*, 2000) after oral administration of ginseng powder. Lee (1992) and Fulder (1996) reported that ginseng has adaptogenic, stimulating, anabolic, antibiotic and anticancer activities. Ginseng extracts have been used as a commercial dietary supplement. Recent analyses suggested its use in anti-cancer, immune modulation (Christensen, 2009), antioxidant (Lee *et al.*, 2008; Ye *et al.*, 2008), anti-

inflammatory (Park *et al.*, 2005), anti-apoptotic (Lee *et al.*, 2003a), and immunostimulant (Rivera *et al.*, 2005; Yu *et al.*, 2005).

Panax ginseng, used medicinally in China, Korea, and Japan is well known as an adaptogen and a restorative tonic which is widely used in traditional Chinese medicine (TCM) and Western herbal preparations (Duke, 2000; Blumenthal, 2003). It is used mainly to increase resistance to physical, chemical and biological stress and boost general vitality, and it is often featured in traditional medicine used by cancer patients (Chang *et al.*, 2003; Kiefer and Pantuso, 2003). Other eclectic uses include treatment for infertility, liver disease, amnesia, colds, menopause and erectile dysfunction (Weiss, 1988; Duke, 2000; Blumenthal, 2003).

P. quinquefolius has been reported to have a broad range of pharmacological effects such as cardiovascular and central nervous system effects, anti-diabetes effects, anti-tumor activities, and immunomodulation (Attele *et al.*, 1999; Ang-Lee *et al.*, 2001; Court, 2000). It is also indicated for fatigue and as an immunostimulant in times of stress. Experimental studies revealed the plant to have estrogenic (Duda *et al.*, 1996), antimutagenic (Chang *et al.*, 1986a; Fujimoto *et al.*, 1991; Duda *et al.*, 1996), and hypoglycemic effects (Oshima *et al.*, 1987; Vuksan *et al.*, 2001a, b) and also to improve impaired memory and learning (Benishin *et al.*, 1991; Salim *et al.*, 1997; Li *et al.*, 1999). The therapeutic use of *Panax notoginseng* includes promotion of blood circulation, removal of blood stasis, induction of blood clotting, relief of swelling, and alleviation of pain (Lei and Chiou, 1986; Wei and Du, 1996; Cicero, 2003) and has also been used for the treatment of coronary heart disease and cerebral vascular disease (Zheng and Yang, 1994; Zheng, 2000; Chan *et al.*, 2002). The rhizomes of *Panax japonicus* have anti-ulcer action and fibrinolysis (Yamahara *et al.*, 1987; Matsuda *et al.*, 1989). In addition, it also helps in preventing obesity complications and serves as a good adjuvant in the present armamentarium of anti-obesity drugs (Han *et al.*,

2005). The dried roots are also used to smooth coughs and reduce phlegm (Chang and But, 1986).

2.4. Taxonomic history of Ginseng

Linnaeus concept of *Panax* was primarily based on the flower morphology, which are highly conserved in the ginseng family (Decaisne and Planchon, 1854; Bentham and Hooker, 1867; Philipson, 1970). The ginseng genus was thus broadly defined. De Candolle (1830) described many species belonging to *Panax.* Seeman (1868) included only the species which are closely related to *P. quinquefolius* and *P. trifolius* in *Panax,* and this concept was followed by later workers (Harms, 1898; Hara, 1970; Hoo and Tseng, 1973; Zhou *et al.*, 1975; Hoo and Tseng, 1978; Wen and Zimmer, 1996; Wen and Nowicke, 1999; Choi and Wen, 2000). Morphologically all species of *Panax* have palmately compound leaves, whorled leaf arrangement, a single terminal inflorescence, and a bi- or tri-carpellate ovary.

Panax shows high level of morphological variation. The taxonomy of *Panax* has been controversial (Hara, 1970; Zhou *et al.*, 1975; Hoo and Tseng, 1978; Yang, 1981; Wen and Zimmer, 1996) due to the existence of morphological intermediates especially in the rhizome and leaflet morphology and the lack of understanding of character variation. To delimit the species in *Panax*, several authors have used a morphological character which includes habit, nature and rhizome features, leaflets, bracts and fruits (Seemann, 1868; Burkill, 1902; Hara, 1970; Graham, 1966; Hu, 1976). Taxa (taxonomic groups, e.g., species, variety) connected by such intermediates were grouped together as a single "species" by Hara (1970). Two species were recognised from Asia): *P. ginseng* and *P. pseudoginseng* Wallich by Hara (1970) and Li (1942). *P. ginseng* is well defined whereas the circumscription of *P. pseudoginseng* is quite debatable. Except *P. ginseng* all other Asiatic species of *Panax* were at one time treated as *P. pseudoginseng* (Hara, 1970; Hoo

and Tseng, 1973, 1978; Li, 1942). This "species" was further subdivided into subspecies and variety. Hara (1970) divided *P. pseudoginseng* into two subspecies (ssp. *pseudoginseng* and ssp. *himalaicus*) and three varieties (var. *angustifolius*, var. *bipinnatifidus* and var. *himalaicus*). Hoo and Tseng (1973) followed Hara's concept at the species level, but treated seven varieties of *P. pseudoginseng* in China alone. Zhou *et al.* (1975) studied the triterpenoid and morphological variation and recognized six species and four varieties in Asia. He defined *P. pseudoginseng* narrowly, following the original description of Wallich (1829). Tanaka (1990) reported that the oleanolic acid glucuronide saponins in the roots of *P. pseudoginseng* from Central Nepal were much lower than those in all other *Panax* species examined, including Chinese, Himalayan and Japanese taxa. Wen and Zimmer (1996) found that *P. pseudoginseng* of central Nepal has a distinct internal transcribed spacer (ITS) sequence profile as well as distinct morphology (e.g., presence of stipules, upright rhizomes, red fruits and large seeds). They argued for a narrow delimitation of the species sensu Wallich (Wallich, 1829) which was supported by the chloroplast DNA restriction site dataset (Choi and Wen, 2000; Yoo *et al.*, 2001).

Zhou *et al.* (1975) had a broad definition of *P. japonicus* C.A. Meyer and submerged several taxa such as *P. bipinnatifidus* Seemann and *P. wangianus* S.C. Sun under *P. japonicus.* Recent phylogenetic analyses using the chloroplast DNA (cpDNA) restriction site variation and nuclear ribosomal ITS sequences indicate that *P. bipinnatifidus* and *P. wangianus* are distinct from *P. japonicus* (Choi and Wen, 2000; Wen and Zimmer, 1996). *P. notoginseng,* widely used in China has a debatable taxonomic history (Hoo and Tseng, 1978; Tanaka, 1990; Wen and Zimmer, 1996; Yang, 1981; Zhou *et al.*, 1975). Hoo and Tseng (1973, 1978) treated it as a variety of *P. pseudoginseng* whereas Zhou *et al.* (1975) and Wen and Zimmer (1996) recognised it as a distinct species. Wen and Nowicke (1999) found that the pollen ultrastructure of *P. pseudoginseng* sensu Wall. (=*P. pseudoginseng*

subsp. *pseudoginseng* of Hara) is different from that of Hara's *P. pseudoginseng* subsp. *japonicus* from Japan (=*P. japonicus* in Wen and Zimmer, 1996) and from China (=*P. major* and *P. sinensis* in Wen and Zimmer, 1996). The two American species, *P. trifolius* and *P. quinquefolius*, are well defined by morphological, molecular, and pollen ultrastructural data (Wen and Zimmer, 1996; Wen and Nowicke, 1999). Based on triterpenoid chemistry, Zhou *et al.* (1975) divided *Panax* of China into two evolutionary groups. Group I consists of *P. ginseng* and *P. notoginseng*, which has tetracyclic dammarane type saponins. Group II comprises of *P. japonicus, P. pseudoginseng, P. stipuleanatus* and *P. zingiberensis* which has the pentacyclic oleanane type saponins. Zhou *et al.* (1975) suggested that *P. pseudoginseng* is an evolutionary intermediate between the two groups as this taxon possesses morphological characters of group I (short rhizomes, carrot-like root, and large seeds), but its triterpenoid chemistry is similar to that of Group II. Tanaka (1990) with his collaborators (Tanaka and Kasai, 1984; Duc *et al.*, 1993, 1994) conducted extensive studies on the saponin composition of *Panax* and reported the chemogeographic correlation of the species.

Yang (1981) examined the variation of chromosome number within *Panax*. In Araliaceae, the basic number of the genus is 12. Species of *Panax* which are diploid includes *P. notoginseng, P. pseudoginseng, P. sinensis* and *P. trifolius* or tetraploid which comprises of *P. ginseng, P. japonicus* and *P. quinquefolius*. Among the tetraploid species, *P. ginseng* has the chromosome number of 2n = 44 or 48 in comparison to 48 in *P. japonicus* and *P. quinquefolius*. Yang (1981) considered the center of cytological diversity in *Panax* as southwestern China.

2.5. Ecological niche

The niche concept has been defined in several ways across the history of ecology (Schoener, 1989, 2009; Chase and Leibold, 2003). Different definitions of niche have been

proposed and amongst all Hutchinson (1957) definition is the most widespread and useful. The term 'niche' was first coined by Sir Joseph Grinnell (1917) in his classical paper '*The niche relationships of the California Thrasher*'. He emphasized that niche of a species is the sum of the ecological conditions that allows a species to persist and produce offspring. After him, Charles S. Elton (1927) proposed that the ecological niche of a species is characterized by the functional role it plays in an ecosystem. Thereafter, G.E. Hutchinson (1957) theorized ecological niche to be the activity range of a species defined by an n-dimensional hyper volume of all the environmental conditions. Hutchinson's niche concept differed from that of Grinnel and Elton in being defined as a property of a species rather than as a recess in a community (Schoener, 1989). Hutchinson (1957) made a worthy distinction between the fundamental and realised niche. The fundamental niche describes the abiotic conditions in which a species is able to persist, whereas the realised niche describes the conditions in which a species persists given the presence of other species (e.g., predators and competitors).

Several aspects of the fundamental niche can be conserved over long evolutionary time scales. For example, tens of thousands of actinopterygian fish species are confined to aquatic habitats, and many fish clades are confined to either saltwater or freshwater. The tendency of species to retain aspects of their fundamental niche overtime is called niche conservatism. Niche conservatism can be referred as a process, even though it may be caused by many factors at the population level (a feature it shares with other evolutionary processes, such as speciation and anagenesis).

Researchers have developed various metrics originating from niche theory related to niches, such as niche breadth and niche overlap, during the past decades (Hutchinson, 1959; MacArthur, 1972; Pianka, 1973; Schoener, 1974; Fox, 1981; Tokeshi, 1986). Further research has elaborate niche theory to encompass important questions about species

abundance and distribution (Pulliam, 2000), diversity–productivity relationship (Tilman *et al.*, 2001), community stability (Tilman *et al.*, 1997), ecosystem functioning (Loreau, 2000), and biological invasions (Peterson and Vieglais, 2001). Niche concept plays an important role in ecology, and understanding the different attributes of niches such as environmental space and resource use is a key factor in studying community organisation (Chase and Leibold, 2003). Species differ in their niche positions in ecological gradient space, as described by the multiple dimensions of the Hutchinsonian n-dimensional niche (Hutchinson, 1957; Whittaker *et al.*, 1973). Early studies on niches and community organisation were established on either spatial overlap in distribution or overlap in food resource use (Tokeshi, 1999; Chase and Leibold, 2003; Vazquez and Stevens, 2004).

Theoretical works of MacArthur (1972) and Roughgarden (1974) hypothesize that the two determinants of species diversity and community structure could be niche breadth of a species and niche overlap between the species. In terms of the spatial model of niche, as formalized by Hutchinson (1957) and expanded by Slobodkin (1962), Levins (1968) and MacArthur (1968), niche breadth is defined as the "distance through" a niche along some particular line in niche space. Different terms used for niche breadth include "niche width" (van Valen, 1965; McNaughton and Wolf, 1970), "niche size" (Klopfer and MacArthur 1960; Willson, 1969), and "versatility" (Maguire, 1967). Niche breadth is primarily used as an inverse measure of ecological specialisation (Colwell and Futuyma, 1971). Measures of niche breadth have been used to test hypotheses, e.g., smaller animals show greater diet specialization than larger animals (Emlen, 1973; Rotenberry, 1980) or that wide-niched species are better adapted to uncertain environment, (Levins, 1968; Slobodkin and Sanders, 1969; Rotenberry and Wiens, 1980).

A diverse number of approaches and metrics have been used to measure niche overlap (Horn, 1966; MacArthur and Levins, 1967; Schoener, 1970; Colwell and Futuyma, 1971;

May and Arthur, 1972; Pianka, 1980). Generally, these methods dates back to the period in which competition was considered to be the primary mechanism structuring ecological communities and measures of niche overlap were developed to quantify differences due to competition (Chase and Leibold, 2003). In ecological communities, species partition the available resources among themselves and is a key determinant of the diversity of coexisting species. Therefore, a community with more resource sharing or greater niche overlap will support more species than with less niche overlap. Niche overlap is simply the joint use of a resource, or resources, by two or more species. In other words, it is the region of niche space (Hutchinson, 1958) shared by two or more contiguous niches. Pianka's (1973) measure of niche overlap is shown to be the cosine of the angle between the consumption vectors of two species at equilibrium.

Furthermore, ecologists have explored for generalities in the relationships between niche characteristics, community organisation, and species richness. For example, niche overlap may decrease (Pianka, 1974) and niche separation increases with increasing richness (Fox, 1981). These findings have been evidenced in recent studies on aquatic organisms. Dole´dec *et al.* (2000) found that mean niche breadth (as defined by multiple environmental gradients) in fish communities was negatively related to species richness, indicating the presence of competitive interactions. Soininen and Heino (2007) also reported the same in diatom communities. These analyses lend support to the hypothesis that there are relationships between niche properties and species richness.

2.6. Ecological niche modelling

The analysis of species–environment relationship has always been a central issue in ecology. The importance of climate to explicate animal and plant distribution was recognized through the works of von Humboldt and Bonpland (1807) and de Candolle (1855). Climate data in combination with various environmental factors has been widely

used to address the vegetation patterns around the world (Salisbury, 1926; Cain, 1944; Good, 1953; Holdridge, 1967; McArthur, 1972; Box, 1981; Stott, 1981; Walter, 1985; Woodward, 1987; Ellenberg, 1988). The quantification of such species–environment relationships represents the core of predictive geographical modelling in ecology. The foundation of ecological niche modelling is based on Grinnell's ecological niche concept which has a single focus i.e. the environmental factors and permits model development. Eltonian, Huthinsonian and MacArthurian concepts however are more process-based which include species functions and biotic interactions, and hence are difficult to fit in a modelling framework. Ecological niche in the light of ENM can be defined as '*.....the set of ecological conditions within which the species is able to maintain its population without immigration*' (after Grinnell 1917).

These models are generally based on various hypotheses as to how environmental factors control the distribution of species and communities. An elaborate knowledge of a species' ecological and geographic distribution is fundamental for conservation planning and forecasting (Ferrier, 2002; Funk and Richardson, 2002; Rushton *et al.*, 2004), and for understanding ecological and evolutionary determinants of spatial patterns of biodiversity (Rosenzweig, 1995; Brown and Lomolino, 1998; Ricklefs, 2004; Graham *et al.*, 2006).

Biologists all around the world rely on distribution models to modify conservation strategies due to the current threats to biodiversity and the difficulty of obtaining detailed, repeated species inventories worldwide. Distribution models predict species richness (Jetz and Rahbeck, 2002), centres of endemism (Johnson *et al.*, 1998), the occurrence of particular species assemblages (Neave *et al.*, 1996) or individual species (Gibson *et al.*, 2004), and the breeding habitat (Osborne *et al.*, 2001), breeding success (Paradis *et al.*, 2000), abundance (Jarvis and Robertson, 1999) and genetic variability (Scribner *et al.*, 2001) of species.

Species distribution models attempt to provide detailed prediction of distribution by relating presence or abundance of species to environmental predictors. However, since the absence of a species from a locality is difficult to demonstrate, and false absences can decrease the reliability of predictive models, species distribution prediction based on presence-only data has been developed (Busby, 1991; Mitchell, 1991; Walker and Cocks, 1991; Carpenter *et al.*, 1993; Scott *et al.*, 1993; Stockwell and Peters, 1999; Peterson *et al.*, 1999; Hirzel *et al.*, 2001, 2002; Robertson *et al.*, 2001). Consequently, modelling techniques for predicting species distributions have attracted increasing attention (Guisan and Zimmermann, 2000; Austin, 2002). The distribution of species and their potential habitats in response to disturbance also have been modelled, particularly over the last two decades (Horn, 1985; Turner *et al.*, 1991; Bergengren *et al.*, 2001; Tilman and Lehman, 2001; He *et al.*, 2002).

One of the most former examples of strategies in modelling applying correlations between distribution of species and climate was that of Johnston (1924), who predicted the invasive spread of a cactus species in Australia. Hintikka (1963) evaluated the climatic determinants of the distribution of several European species (quoted in Pearson and Dawson, 2003). The use of computer-based predictive modelling of species distribution originates in the mid-1970s which was accelerated by the quantification of species–environment available (Austin, 1971). The earliest species distribution modelling attempt was the niche-based spatial predictions of crop species by Nix *et al.* (1977).

Further work on species distribution model was by Ferrier (1984) in the early 1980s and two seminal books were published in promoting this new approach (Verner *et al.*, 1986; Margules and Austin, 1991). These advances were mainly supported by the developments in computer and statistical sciences, and by strong theoretical support to predictive ecology as 'more rigorously scientific, more informative and more useful ecology' (Peters, 1991).

Several publications were published in the early 1990s and the first partial reviews published by Franklin (1995) and Austin (1998) appeared shortly.

Distribution models can be used to monitor declining species (Osborne *et al.*, 2001), predict range expansions of recovering species (Corsi *et al.*, 1999), estimate the likelihood of species long-term persistence in areas considered for protection (Cabeza *et al.*, 2004) and identify locations suitable for reintroductions (Joachim *et al.*, 1998). They allow biologists to identify sites vulnerable to local extinction (Gates and Donald, 2000) or species invasion (Kriticos *et al.*, 2003), and to explore the potential consequences of climate change (Peterson *et al.*, 2002). Distribution models have been shown to perform better for some taxa than for others (Venier *et al.*, 1999). Range size is one of the ecological characteristic which shows deviation from species to species, that might influence the success of distribution models (Venier *et al.*, 1999; Manel *et al.*, 2001; Stockwell and Peterson, 2002) and such influence could have ecological roots. Species with large ranges or disjunctive distributions may exhibit subspecific variation in habitat associations because of local adaptation (Stockwell and Peterson, 2002). To an automated model-fitting algorithm, such disjoint habitat preferences could interfere in model prediction. Poor performance of models for narrow-ranging species may instead have methodological roots. Their habitat associations may be perfectly consistent at fine spatial scales, but may not show evidence at the spatial grain of analysis (Fielding and Haworth 1995).

Ecological niche models have been used to study issues in evolution (Peterson, 2001; Hugall *et al.*, 2002), ecology (Anderson *et al.*, 2002 a, b), and conservation (Godown and Peterson, 2000; Sa´nchez-Cordero and Martınez-Meyer, 2000; Peterson and Robins, 2003). These methods (e.g. BIOCLIM- Nix, 1986; Busby, 1991; GARP- Stockwell and Noble, 1991; DOMAIN- Carpenter *et al.*, 1993) combine geographic locations of a particular species with environmental data to identify the parameters and then map this information to

predict the species geographic distribution. Generally, interpolated climate data (Berry *et al.*, 2002; Joseph and Stockwell, 2002); or environmental data incurred through remote sensing (Fuentes *et al.*, 2001; Oindo, 2002; Zinner *et al.*, 2002) are used to build models.

Besides its prime importance as a research tool in autecology, predictive geographical modelling recently gained importance as a tool to assess the impact of accelerated land use and other environmental change on the distribution of organisms (Kienast *et al.*, 1995, 1996, 1998; Lischke *et al.*, 1998; Guisan and Theurillat, 2000), to test biogeographic hypotheses (Mourell and Ezcurra, 1996; Leathwick, 1998), to improve floristic and faunistic atlases (Hausser, 1995) or to set up conservation priorities (Margules and Austin, 1994). An innumerable number of statistical models is currently in use to simulate either the spatial distribution of terrestrial plant species (Hill, 1991; Buckland and Elston, 1993; Carpenter *et al.*, 1993; Lenihan, 1993; Huntley *et al.*, 1995; Shao and Halpin, 1995; Franklin, 1998; Guisan *et al.*, 1998, 1999), aquatic plants (Lehmann *et al.*, 1997; Lehmann, 1998), terrestrial animal species (Pereira and Itami, 1991; Aspinall, 1992; Mladenoff *et al.*, 1995, 1999; Augustin *et al.*, 1996; Corsi *et al.*, 1999; Mace *et al.*, 1999; Manel *et al.*, 1999a), fishes (Lek *et al.*, 1996; Mastrorillo *et al.*, 1997), plant communities (Fischer, 1990; Brzeziecki *et al.*, 1993; Zimmermann and Kienast, 1999), vegetation types (Brown, 1994; van de Rijt *et al.*, 1996), plant functional types (Box, 1981, 1995, 1996), biomes and vegetation units of similar complexity (Monserud and Leemans, 1992; Prentice *et al.*, 1992; Tchebakova *et al.*, 1993, 1994; Neilson, 1995), plant biodiversity (Heikkinen, 1996; Wohlgemuth, 1998), or animal biodiversity (Owen, 1989; Fraser, 1998).

Austin *et al.* (1990) and Vetaas (2002) studied the quantification on the environmental niche of species. Testing of biogeographical, ecological and evolutionary hypothesis is carried by Leathwick (1998), Anderson *et al.* (2002) and Graham *et al.* (2004). Beerling *et al.* (1995) and Peterson (2003) published papers in assessing species invasion and

proliferation. The impact assessment of climate, land use and other environmental changes on species distribution was done by Thomas *et al.* (2004) and Thuiller (2004). Proposing unsurveyed sites of high potential of occurrence for rare species was shown from the works of Elith and Burgman (2002), Raxworthy *et al.* (2003), Engler *et al.* (2004). Pearce and Lindenmayer (1998) studied on appropriate management plans for species recovery and mapping suitable sites for species reintroduction. The use of SDM in supporting conservation planning and reserve selection was published by Ferrier (2002) and Araujo *et al.* (2004). Modelling species assemblage (biodiversity, composition) from individual species prediction was presented by Leathwick *et al.* (1996), Guisan and Theurillat (2000), and Ferrier *et al.* (2002).

Giriraj *et al.* (2008) and Irfan-Ullah *et al.* (2006) presented works on predicting the potential distributional area of species and planned targeted biodiversity survey in new areas. The identification of potential areas for species reintroduction was done by Martinez-Meyer *et al.* (2006b) and Wilson *et al.* (2011). Identification of areas having potential for species invasions was published by Peterson and Vieglais (2001) and Barik and Adhikari (2011). Warren *et al.* (2008) and Maguire and Stigall (2009) addressed questions related to species evolution and biogeography. Waltari *et al.* (2007) mapped the prehistoric distribution of species. Predicting climate change impact on the future distributional behaviour of species was studied by Peterson *et al.* (2002) and Barik and Adhikari (2011). Mapping risk areas for emerging infectious diseases was published by Peterson *et al.* (2005), Levine *et al.* (2007) and Adhikari *et al.* (2008).

2.7. Metapopulation

The study of species populations at landscape level is an increasingly focussed research area for conservation of species in patchy or fragmented environment (McCullough, 1996; Marsh and Trenham, 2001). There are different approaches towards undertaking population

studies at a regional scale. Several workers such as Johnson *et al.* (1992a and b), Wiens *et al.* (1993), Ims (1995), With and Crist (1995, 1996) and Turchin (1998) emphasized the importance of temporal habitat configuration in predicting the population dynamics of a species in fragmented landscape. Gruber and Henle (2004) studied the habitat structure and orientation in an arboreal *Gehyra variegata* and their findings suggested that species configuration lowers predation risk of the population which suggested that determining the habitats of the species and their distribution is a pre-requisite of metapopulation research. Most studies of metapopulation consider the dynamics of populations divided into a number of sub-populations that may to some extent exchange migrants and that may be subjected to local extinctions and recolonizations (Hanski and Simberloff, 1997).

Metapopulation dynamics and the applicability of metapopulation models to specific problems in conservation have contributed significant insights into conservation and have inspired field studies focused on collecting key data on demography and movement. Moreover, metapopulation concepts have had positive effects in conservation research, especially, as interest in metapopulation dynamics has prompted renewed research concerning dispersal capacities that is extremely valuable in understanding population structure (Yuttham *et al.,* 2003). The term metapopulation was first introduced by Levins (1969). Studies using metapopulation generally subdivide the general population into a series of local populations with a balance between extinctions and recolonisations of local populations that facilitates long-term persistence of the metapopulation. The key process is the interpatch connection functions by migration (Hanski and Gilpin, 1997). Metapopulation models have been widely used in the biological field including in population ecology, conservation biology, and pest control (Harrison, 1994; Wu, 1994; Hanski and Gilpin, 1997; Takagi, 1999; Fagan *et al.,* 2002). Modelling has become an important tool in population and conservation biology as evidenced by many books that

provide excellent advice on model construction and its uses (Cappuccino and Price, 1995; Haefner, 1996; Hanski and Gilpin, 1997; Hilborn and Mangel, 1997; Roughgarden, 1998; Shugart, 1998). Metapopulation models are useful and popular models that fall into the quantitative type and are always represented as analytical or simulation models.

For an in-depth metapopulation research, the known occupancy and distribution of species is required. A history of research in metapopulation biology has been narrated by Hanski and Simberloff (1997) and Hanski (1999). The core mathematical model in metapopulation theory is the Stochastic Patch Occupancy Models (SPOM). SPOMs assume a network of habitat patches, which have only two possible states, occupied by the focal species or empty. Ecologists working with conservation tend to prefer individual based metapopulation model (Akcakaya and Ferson, 1990; Lacy, 1993, 2000; Akcakaya, 2000) or population based (Sjogren-Gulve and Ray, 1996) or stage-structured (Akcakaya, 1998; Caswell, 2001; Morris and Doak, 2005) simulation models. The advantage of these models is that any process and mechanism that the researcher may wish to add to the model can be added readily. The best use of these models, as perhaps of any population models, for conservation and management is to contrast alternative scenarios that differ in only a small number of factors (Hanski, 1997; Ralls and Taylor, 1997; Beissinger and Westphal, 1998; Akcakaya and Sjogren-Gulve, 2000).

Several theoretical models have been formulated to assess species survival probabilities at the landscape level (Lahaye *et al.*, 1994; Gustafson and Gardner, 1996; With *et al.*, 1997; Hanski and Ovaskainen, 2000; Casagrandi and Gatto, 2002; Dreschler *et al.*, 2003). Generally only two types of metapopulations are possible for a given species: (i) Discrete metapopulation– characteristic feature of a true metapopulation (Freckleton and Watkinson, 2002) and (ii) Patchy metapopulation– there are populations exhibiting a rather more continuous structure which are arbitrarily defined using grid-based as suggested by Thomas

and Kunin (1999) or threshold distance criteria as followed by Kolb (2002, 2004) and Jacquemyn *et al.* (2005) for demarcating species populations. Therefore, spatial structure is an important determinant of metapopulation. Spatial structure refers to the location of individuals, which are grouped into sub- or local populations. Different sub-populations may have different demographic characteristics, such as population size (abundance), carrying capacity, and vital rates. Hanski (1997) outlined four conditions that, if satisfied, suggest that a Levins metapopulation approach will help explain regional persistence of a species: (i) suitable habitat occurs in discrete patches; (ii) all local populations have a substantial risk of extinction; (iii) habitat patches must not be too isolated to prevent recolonization; and (iv) local populations do not have completely synchronous dynamics.

Plant species which are adapted to particular disturbance regimes that impose frequent population turnover, fit metapopulation approaches. For example, Gawler *et al.* (1988) reported that populations of *Pedicularis furbishiae* were often destroyed in the short term by ice scouring and flooding, but relied on these disturbances to create suitable habitat for long-term persistence. In the xeric scrublands of central Florida, plant species are adapted to fire which is the dominant type of disturbance. While some species endure in the landscape via resprouting, others rely on seed dispersal and recruitment to recover from fire and are most likely to show measurable metapopulation dynamics. These differential responses of the species to fire together with microhabitat diversity and the competitive abilities of the constituent species, structure the heterogeneous plant communities in Florida scrub (Menges and Hawkes, 1998). Besides external influences and disturbances, internal demographic properties like births and deaths interact with both population size and life history in their effect on population persistence time and extinction risk. Stochastic demographic variability increases the risk of extinction of small populations (Shaffer, 1987). Kokko and Ebenhard (1996) showed that life history characteristics and the effective

population size were more important than total population size for determining the strength of demographic stochasticity. Variation in life history characteristics such as seed bank and clonal propagation may also affect the risk of population extinction due to environmental stochasticity (Eriksson, 1996). In a theoretical study, Lande (1993) showed that the average life time of a population subjected to environmental stochasticity or random catastrophes is more dependent on its long run population growth rate than on initial population size.

Matrix population models are used to assess the viability of structured populations (Morris and Doak, 2002). Repeated iterations of the matrix model result in the projection of a population's equilibrium growth rate and extinction risk, providing a measure of the overall performance of populations. Moreover, sensitivity and elasticity analyses of matrix models can identify the life-history stages most critical for the persistence of a species. The results of matrix model analysis and simulation are often used to evaluate the vulnerability of a population to extinction and to assess different management options (Garcia, 2003; Freckleton *et al.*, 2003; Beverly and Martell, 2004; van Mantgem *et al.*, 2004). Apart from these models are the spatially explicit metapopulation models that describe the dynamics of each population with structured demographic models, and incorporate spatial dynamics by modelling dispersal and temporal correlation among populations. One of the models is based on a regular grid, each cell of which is modeled as a subpopulation of a metapopulation (Price and Gilpin, 1996). Another approach expands spatially explicit metapopulation models by incorporating information about habitat relationships of the species and the characteristics of the landscape in which the metapopulation exists (Akçakaya and Atwood, 1997). This method uses a habitat suitability map to determine the spatial structure of the metapopulation (number and location of habitat patches in which subpopulations of the metapopulation live) and population-specific parameters. Many plant population studies are presented as sets of deterministic models developed from data for

specific years (Shaffer, 1981; Bierzychudek, 1982; Kalisz and McPeek, 1992) and only few studies have calculated a stochastic population growth rate (Caswell, 1989; Nakaoka, 1996). Efforts to introduce stochasticity into these models have often taken the form of choosing a sequence of matrices obtained for different years according to a random sampling scheme or a scheme designed to address theoretical questions (Bierzychudek, 1982; Kalisz and McPeek, 1993). More commonly, environmental stochasticity or various types of disturbance or catastrophe have been considered in Population Viability Analyses (PVAs).

Out of 21 studies that used stochastic modelling to predict extinction, 19 were reported with extinction probabilities; others reported times to extinction or both. The time periods used for projecting extinction risk vary from 25 to 1000 years (many authors used several different periods), with 50, 100 and 200 years being the most frequently used time periods; thus, comparison of extinction risk is difficult. A few studies demonstrate risk analyses with full distribution of times to extinction. These full-time distributions are recommended because they are less misleading than single results (Beissinger and Westphal, 1998). In deterministic models, many other parameters have been calculated, with elasticities (Benton and Grant, 1999) which are the most common. However, because elasticities within species vary across space and time (Horvitz and Schemkse, 1995; Oostermeijer *et al.*, 1996; Silvertown *et al.*, 1996) interpretations of elasticities need to be made with caution. In particular, elasticities of declining populations differ from those of increasing populations. Stochastic modelling and other complex approaches have been less commonly used in plant PVAs. Demographic stochasticity (Damman and Cain, 1998; Menges, 1998) is not considered as great a threat to population viability as systematic factors (such as continuing habitat loss) or other stochastic factors.

Metapopulation viability is drastically and negatively affected by the strength of the environmental stochasticity; temporal variation in population growth rate may lead several local populations and even the whole metapopulation to extinction. Doak *et al.* (2002) demonstrated that, in the absence of precise information on seed demographic rates, model predictions based on assumptions about a persistent seed bank can vary widely depending on the amount of variation in vital rates for the reproductive phases of the plant life cycle. This study reflects the need for realistic assessment of environmental variation and its impact at all life history stages. Extinction of populations is of prime evolutionary and conservation interest and stochasticity is a decisive factor in the survival or extinction of populations (Goel and Richter-Dyn, 1974; Goodman, 1987a, b).

Theoretical research on stochastic extinction of populations and the application of stochastic models to population viability analysis (PVA) have become very popular (Leigh, 1981; Soulé, 1987; Lande and Orzack, 1988; Hanski and Gilpin, 1991; Mace and Lande, 1991; Burgman *et al.*, 1993; Wissel *et al.*, 1994; Settele *et al.*, 1996; Drechsler *et al.*, 1998; Amler *et al.*, 1999). PVA approach has gained popularity in conservation biology and is reflected in the availability of several reviews (Goel and Richter-Dyn, 1974; Akçakaya and Ferson, 1990; Boyce, 1992; Lacy, 1993; Lindenmayer *et al.*, 1995; Oostermeijer *et al.*, 1996; Reich and Grimm, 1996; Groom and Pascual, 1997; Beissinger and Westphal, 1998).

The concepts of minimum viable metapopulation and minimum available suitable habitats (Hanski *et al.*, 1996) are likely to be applicable to many plant species. Data on species presence or absence in suitable habitat patches have been used to infer metapopulation dynamics in 80 species of Florida scrub plants. For 25 species, with occupancy related to patch size, isolation or fire regime, an incidence-based metapopulation model was used to infer colonization and extinction rates (Quintana-Ascencio and Menges, 1996) which indicates that, as patch size decreases, herbs are more sensitive than woody plants to

increased extinction risks. Natural variability in population dynamics is compounded by uncertainty in the population parameters due to lack of perfect information. Therefore, development of models in population studies have become an integral part for better and clear understanding of the population behaviour. Besides, the predictive capacities of the models help in species conservation. The consequent difficulty of making precise predictions has shaped the language of PVA (Shaffer, 1990). The conservation-related problems and questions that PVA addresses are usually phrased in terms of probabilities. For example, we may want to assess the probability of extinction or the chance of recovery from a population bottleneck.

The connection between metapopulation ecology and population genetics is quite significant because both are concerned with spatial population structure and rate of migration. The population geneticist Wright (1931) used a model of a subdivided population made up of demes (local populations) with gene flow (migration) among them to model changes in gene frequency due to natural selection and genetic drift. His assumption was similar as Levins (1969) that all of the populations were of the same size and equally connected. His model and subsequent population genetics models differed from metapopulation models because the local populations persist over time and gene frequencies always changes within and among them. Slatkin (1977) first combined metapopulation and population genetics models to address effects of colonization-extinction dynamics on genetic differentiation among populations. Further work was reviewed by Whitlock (2004) who investigate the effects of spatial structure on genetic drift, genetic variability, accumulation of deleterious alleles and other forms of inbreeding depression, and different types of selection. Species present in fragmented habitats, despite showing characteristics of metapopulations, do exhibit negative effects of inbreeding (Saccheri *et al.*, 1998). Even though genetic variability declines with population structuring,

the variability among local populations will be higher, and limitations to gene flow can cause genetic differentiation among local populations (Wade and McCauley, 1988) and allow local adaptation. Empirical evidences have shown that spatial subdivision causes genetic subdivision, and in some cases, local adaptation (Reznick and Ghalambor, 2001). With increasing access to molecular tools, the effect of metapopulation structure on genetics has become more sophisticated, extending to studies of evolution of quantitative traits and whole genome patterns of molecular variation. Hanski and Saccheri (2006) showed that local population growth rate in a butterfly metapopulation was associated with genetic variation of a glycolytic enzyme involved in metabolism (pgi).

The earliest plant PVA was calculated for age-structured data derived from a *Pinus sylvestris* forest (Usher, 1969). The classic study of *Ranunculus repens* brought matrix methods to the attention of plant ecologists (Sarukhán and Gadgil, 1974). Some other notable plant PVAs are those of Werner and Caswell (1977), Bierzychudek (1982), Fiedler (1987), van Groenendael and Slim (1988), Menges (1990), Burgman and Lamont (1992), Cochrane and Ellner (1992), Kalisz and McPeek (1992), Alvarez-Buylla (1994), Bullock *et al.* (1994), Erikkson (1994), Ehrlén (1995), Nantel *et al.* (1996), Valverde and Silvertown (1997), Bradstock *et al.* (1998), Damman and Cain (1998), Enright *et al.* (1998), Gross *et al.* (1998), Menges and Dolan (1998), Pascarella and Horvitz (1998) and Oostermeijer (1999).

Most plant PVAs have been carried out based on short duration data and have been performed on a single species with only a few populations. The mean, median and modal length of a PVA is about four years (Fiedler, 1998). Most studies also consider very few populations (mean 3.4, median 2.0 and mode 1.0), but because populations within species vary widely in demographic parameters, studies based on only a few populations would seem incomplete. Demographic variation over time (environmental stochasticity) is only

weakly correlated among populations (Horvitz and Schemkse, 1995; Crone and Gehring, 1998), which suggests that multiple populations need to be followed for several years. Some of the earliest PVAs used a single parameter which is time to extinction (Brigham and Schwartz, 2003).

PVAs can be used to define, given an assumption of the maximum risk to be tolerated (*e.g.*, less than 5% risk of extinction in 100 years), a Minimum Viable Population (MVP) that will forestall extinction. Deterministic and stochastic analyses, which incorporated harvesting pressure on wild ginseng (*Panax quinquefolium*) and wild leek (*Allium tricoccum*) in Quebec, Canada, were used to formulate MVPs (Nantel *et al.*, 1996). While there are a large number of both short-term and long-term studies focusing on almost all aspects of plant population biology are available for the temperate zone (Tamm, 1972; Whigham, 1984; Calvo, 1990; Leeson *et al.*, 1991; Primack and Stacey, 1998; Brzosko and Wroblewska, 2003; Wotavova´ *et al.*, 2004), the plant demography of the species-rich tropical zone, focusing on critical numerical analyses has been little studied.

2.8. Phylogeny of Araliaceae

The relict family Araliaceae comprises of 47 to 80 genera with 900 to 1350 species (Kharkevich, 1987; Plunkett *et al.*, 2004) which includes mostly large trees and shrubs. Woody climbers are also present with a few herbaceous perennials (e.g., some species of *Aralia* and *Panax*). Out of the six large genera, five inhabit the tropics and subtropics comprising of more than 50 species (Plunkett *et al.*, 2004). The small genera are found in the temperate zone, and are connected with the coastline areas of the continents (Grushvitskii, 1981; Plunkett *et al.*, 2004). The classification of Araliaceae is not precise (Zhuravlev and Kolyada, 1996). The family is subdivided into tribes (ranging from three to ten) based on a relatively small number of diagnostic characters resulting from ten classifications (Grushvitskii *et al.*, 1985; Takhtajan, 1987; Oskolskii, 1994; Plunkett *et al.*,

2004). The family includes a number of important medicinal plants such as *Panax* (ginseng) and *Eleutherococcus* (siberian ginseng) and several well known ornamentals including *Hedera* (english ivy), *Schefflera* (umbrella trees) and *Polyscias*. Araliaceae have traditionally been allied with Apiaceae on the basis of morphological (Harms 1898; Judd *et al.*, 1994) and anatomical evidence (Metcalfe and Chalk, 1950) and this treatment has been largely supported by recent molecular studies (Plunkett *et al.*, 1996, 1997). Araliaceae are characterized by large morphological diversity and homoplasy of their characters, which hampers intra and intergeneric classification (Oskolskii, 1994; Plunkett *et al.*, 2004). Therefore, identification of new species, as well as grouping and regrouping of the species are still in progress (Skvottsova *et al.*, 1994; Mitchell *et al.*, 1997; Kim and Sun, 2000; Wen *et al.*, 2003; Yunfei, 2003). Most systems of classification place Araliaceae and Apiaceae together in the order Apiales (*sensu* Cronquist, 1981, 1988; Thorne, 1992; Takhtajan, 1987, 1997) which in turn has been placed in or near subclass Rosidae (Cronquist, 1981, 1988). Recent studies indicated that Apiales should be placed in a broadly defined Asteridae (Chase *et al.*, 1993; Olmstead *et al.*, 1993, Plunkett *et al.*, 1996, Soltis *et al.*, 1997c) which is closely allied to Pittosporaceae (Plunkett *et al.*, 1996, 1997; Xiang and Soltis, 1998).

Araliaceae are characterized by conserved but very diverse vegetative features (Philipson 1970; Eyde and Tseng, 1971). Members of the family possess five sepals, 5-12 petals, which are free or form a calyptras (as in *Pentapanax* and *Tupidanthus)*, mostly 5-10 stamens (rarely to numerous, e.g., 120 in *Tupidanthus*) and a gynoecium with 2-10 (mostly 5, rarely upto 200 in *Tupidanthus*, or 1 in *Seemannaralia* R. Viguier) locules, and an inferior ovary (rarely superior as in *Dipanax* Seem.) with a nectar disk. The fruits in araliads are berries or drupaceous fruits that are dispersed by birds (Ridley, 1930). The leaves in the family vary from simple to variously lobed or divided, to palmately and/or pinnately compound.

More recently, DNA sequencing studies based on the chloroplast genes rbcL and matK (Plunkett *et al.*, 1996, 1997) have provided important insights into the evolution and diversification of Araliaceae and its relationship with Apiaceae and other closely related families. The evolutionary history of the family needs revision as evidenced from the earlier studies. Araliaceae is usually considered as a paraphyletic group belonging to the order Apiales (Thorne, 1983; Plunkett *et al.*, 2004). However, recent investigations of phylogenetic relationships between the species using molecular DNA markers proved that the group of species traditionally classified as Araliaceae was monophyletic (Plunkett *et al.*, 1997; Plunkett, 2001; Wen *et al.*, 2001; Chandler and Plunkett, 2004; Plunkett *et al.*, 2004). However, the phylogenetic relationships in Araliaceae are better understood within some genera and between the groups (Wen and Zimmer, 1996; Mitchell and Wagstaff, 1997; Wen *et al.*, 1998; Costello and Motley, 2001; Wen, 2001b).

2.9. Molecular phylogeny of *Panax*

Panax consisting of 18 species is quite useful for inferring the phylogenetic utility of the *trn*C–*trn*D region, as it has very ancient members as well as a species complex that is regarded as evolutionarily young (Wen and Zimmer, 1996; Wen, 2001a,b). The phylogeny of *Panax* was previously reconstructed with the nuclear ribosomal ITS region (Wen and Zimmer, 1996) and chloroplast DNA restriction site analysis (Choi and Wen, 2000) and these data provide a chance for comparison with the *trn*C–*trn*D dataset. The monophyly of *Panax* is confirmed by the following morphological synapomorphies: palmately compound leaves, whorled leaf arrangement, a small terminal umbellate inflorescence, and a bi- or tri-carpellate ovary (Wen and Zimmer, 1996).

Wen and Zimmer (1996) reported that *P. pseudoginseng* of central Nepal has a distinct ITS sequence profile as well as morphology (e.g., presence of stipules, upright rhizomes, red fruits, and large seeds) and therefore argued for a narrow delimitation of the species (*sensu*

Wallich 1829). This conclusion was supported by studies on chloroplast DNA restriction site data (Choi and Wen, 2000; *Yoo et al.*, 2001). Choi and Wen (2000) reported 18 restriction site mutations in this chloroplast region across 11 species of *Panax* which leads to the study on the phylogenetic utility of this region at the interspecific level. ITS sequences of nuclear rDNA of *P. bipinnatifidus* from India have been studied (Pandey *et al.*, 2004, 2009). Watanabe *et al.* (1998) examined the morphology, RAPD profile and saponin contents of *P. pseudoginseng* and other allied groups from Nepal and Japan. Their phylogenetic tree suggested that the Himalyan *Panax* is distinct from the Japanese populations. *Panax trifolius* from eastern North America is morphologically and palynologically unique (Wen and Zimmer, 1996; Wen and Nowicke, 1999) and recent studies have shown to be sister to the clade with the remaining species in the genus.

2.10. 18S ribosomal DNA as a taxonomic tool

Ribosomal characters are the most exploited source of molecular data for systematic studies of organismal relationships (Appels and Honeycutt, 1986; Troitsky and Bobrova, 1986). In the nuclear genome of eukaryotes, the ribosomal RNA gene (rDNA) occurs as multiple copies of tandemly repeated units. Each repeat consists of a transcribed region that comprises an external transcribed spacer (ETS), followed by the 18S gene, an internal transcribed spacer (ITS-1), the 5.8S gene, a second internal transcribed spacer (ITS-2) and finally the 26S gene (Hamby and Zimmer, 1992). Each such unit is separated from the next repeat by an intergenic spacer (IGS). Many studies have showed that the 18S rRNA gene contain variable and conserved regions, including several evolutionarily conserved "functional domains" (Gutell *et al.*, 1985; Soltis *et al.*, 1997c), which suggested that this gene sequence could give more essential information for reconstruction of phylogenetic relationship. The size of the 18S rDNA which is approximately 1800 bp has made it more amenable to PCR amplification and sequencing and has been used extensively in

comparison to the 26S/28S rDNA which is more than 3,000 bp, making it difficult to obtain its complete sequence (Soltis and Soltis, 1998).

The first major effort for application of 18S data to angiosperm phylogeny was undertaken in the late 1980s and based on direct RNA sequencing of portions of both 18S and 26S regions (Hamby and Zimmer, 1988, 1992; Zimmer *et al.*, 1989). Further studies (Nickrent and Franchina, 1990; Boulter and Gilroy, 1992; Bharathan and Zimmer, 1995) contributed to a growing plant small subunit database, with the trend towards complete 18S rDNA sequences.

Nickrent and Soltis (1995) examined patterns of 18S variation in 62 species of flowering plants, compared this variation with the plastid gene *rbc*L for the same taxa, and thereby point out for further 18S surveys of the angiosperms. Soltis *et al.* (1997a and b, 1998) conducted a more comprehensive survey and maximum parsimony analyses (MP) of 223 angiosperm 18S sequences, generating results largely congruent with those observed in MP analyses of rbcL representing 499 angiosperm species (Chase *et al.*, 1993). Only one 18S rDNA sequence type is typically found in an organism, excepting a few cases, where intra-individual variation has been detected. Two types of 18S rDNA are found, as in *Dugesia mediterranea* (flatworm), of which only one is transcribed although both types appear functional (Carranza *et al.*, 1996).

The first detailed analyses of molecular evolution of 18S rRNA genes in angiosperm was made possible by the availability of complete 18S rDNA sequences obtained by Soltis *et al.*, (1997c) and Nickrent and Soltis (1995). Nickrent and Soltis (1995) have described angiosperm 18S rDNA as a region comprising highly conserved segments with few or no substitutions, interspersed with highly variable regions that undergo dozens of substitutions per site. The most conserved regions of angiosperm 18S rDNA are generally 50 nucleotides

in length and occur in the stem region of the secondary structure model which also includes some small loops. The highly variable regions are located in the terminal helices and are much shorter with 5-10 nucleotides in length (Soltis and Soltis, 1998b). The ribosomal DNAs of other organisms are also typically mosaics of highly conserved and variable regions (van de Peer *et al.*, 1996).

Analyses of the entire 18S rDNA sequences have revealed the implications for the origin of land plants, monophyly of gymnosperms and sister group of the angiosperms (Kranz *et al.*, 1995; Chaw *et al.*, 1997). Recent studies have showed a number of characters used for broad-scale phylogenetic reconstruction of the angiosperms, derived from 18S rDNA (Nickrent and Soltis, 1995, Soltis *et al.*, 1997c). Even though 18S rDNA evolves at only one-third to one-half the rate of rbcL (Nickrent and Soltis, 1995), it has been used to infer relationships within specific angiosperm clades (Hoot *et al.*, 1995a, b; Kron, 1996; Soltis and Soltis, 1997; Rodman *et al.*, 1998).

2.11. Use of ITS sequence of ribosomal DNA as a taxonomic tool

The internal transcribed spacers plays a key role in the maturation of nuclear rRNAs, bringing both the small and large subunits into close proximity within a processing domain (Baldwin *et al.*, 1995) which points out that these two regions are under some evolutionary constraint in structure and sequence supported by the comparison of both the regions in angiosperms (Hershkovitz and Zimmer, 1996).

The commonly used markers for lower-level phylogenetic analysis in plants are the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (White *et al.*, 1990; Baldwin *et al.*, 1995), chloroplast *mat*K (Johnson and Soltis, 1994; Steele and Vilgalys, 1994), *ndh*F (Olmstead and Sweere, 1994), the noncoding *trn*L-F region (Taberlet *et al.*, 1991), the *atp*B–*rbc*L spacer (Ehrendorfer *et al.*, 1994; Manen *et al.*, 1994; Savolainen *et al.*, 1994,

1997; Setoguchi *et al.*, 1997), the *rps*16 intron (Downie and Katz-Downie, 1999), and a few single- or low-copy nuclear genes such as *adh* (Gaut and Clegg, 1993; Small *et al.*, 1998), GBSSI (MasonGamer *et al.*, 1998; Mason-Gamer and Kellogg, 2000; Peralta and Spooner, 2001), *ncp*GS (Doyle, 1991; Emshwiller and Doyle, 1999), the 5S spacer (Cox *et al.*, 1992; Kitamura *et al.*, 2001), and *Pgi*C (Gottlieb and Ford, 1996).

ITS sequences are proving valuable for phylogenetic reconstruction in angiosperms (Baldwin *et al.*, 1995), algae (Coleman *et al.*, 1994; Bakker *et al.*, 1995) and ferns (Stein *et al.*, 1992). They have also been used to infer phylogeny at lower taxonomic levels in a diverse array of organisms including fungi (Vilgalys and Sun, 1994) and insects (Campbell *et al.*, 1993; Fritz *et al.*, 1994; Schlotterer *et al.*, 1994; Vogler and De Salle, 1994). Recent published works (Hershkovitz and Lewis, 1996; Hershkovitz and Zimmer, 1996) indicate that both ITS-1 and ITS-2 are inherently G+C rich and portions of these regions are conserved among angiosperms. About 40% of ITS-2 is conserved across all angiosperms sequenced. Hershkovitz and Zimmer (1996) lay out an alignment in which 50% of the ITS-2 sequences is alignable above the family level in angiosperms which proves that the ITS region have high information content at lower taxonomic levels and also exhibit conserved sequence patterns with high alignability across angiosperms.

Several strategies have been applied to amplify and sequence the ITS regions (Baldwin, 1992; Wen and Zimmer 1996; Soltis and Kuzoff, 1995) but sequencing of this region can be difficult, because the region is G+C rich and prone to secondary structure (Baldwin *et al.*, 1995). Although the total length of the ITS regions together with the 5.8S gene, is fairly short and uniform across angiosperms (600-700 bp), it is much longer in Gnetales, Ginkgo, conifers and cycads (Liston *et al.*, 1996).

2.12. DNA molecular approaches using PCR-restriction fragment length polymorphism (PCR-RFLP)

PCR-restriction fragment length polymorphism (RFLP)-based analysis, also known as cleaved amplified polymorphic sequence (CAPS), is a popular technique for genetic analysis. This has its origin in the DNA rearrangements, occurring due to mutations, insertions or deletions (Burr *et al.*, 1983) and unequal crossing over (Schlotterer and Tautz, 1992). It has been applied for the detection of intraspecies as well as interspecies variation and is also an extremely valuable technique for genotyping of species-specific variations. It is the most commonly used reference standard for genotyping of Factor V Leiden and prothrombin G20210A (Emadi *et al.*, 2010). It has also been used for a variety of other purposes including detection of the JK allele associated with a Kidd-null phenotype (Horn et al., 2011), and determination of apolipoprotein E (APOE) alleles (Jiang *et al.*, 2011). Besides being valuable for the determination of intraspecies variation, the PCR-RFLP technique is very popular for species identification and differentiation. Recently, it was the preferable technique for identification and differentiation of mycobacterial species (Sankar *et al.*, 2011). Moreover, it has also been used for differentiation of game bird species by amplification of a conserved region of the mitochondrial d-loop (Rojas *et al.*, 2009). Using primers targeting the conserved region in the 12S rRNA gene, followed by restriction enzyme treatment and electrophoretic separation, closely related poultry species could be differentiated (Saini *et al.*, 2007) using the PCR-RFLP technique. A similar approach has been used to differentiate game animals such as roe deer, red deer and mouflon from domesticated ruminants (Fajardo *et al.*, 2006, 2009). The use of a single restriction enzyme may be adequate for species differentiation by PCR-RFLP (Fajardo *et al.*, 2009). Application of more than one restriction enzyme is needed to differentiate between closely related species (Rojas *et al.*, 2009). RFLP are co-dominant markers yielding highly

reproducible patterns. But the technique is time consuming and requires large quantity of good quality DNA and also good supply of probes as well (Karp *et al.*, 1996).

Ngan *et al.* (1999) and Fushimi *et al.* (1997) applied PCR-RFLP on ginseng authentication based on ribosomal ITS1-5.8S-ITS2 region and 18S rRNA gene respectively. Both studies showed promising results on identification of the two *Panax* species and their adulterants. Using this method Ngan *et al.* (1999) expanded for further authentication of many other traditional Chinese medicines (TCM). This assay method is more reproducible and reliable than DNA fingerprinting and RAPD.

PCR-RFLP analysis has been carried out for the 18S rRNA gene from nuclear rDNA digested with *Ban* II and *Dde* I on three *Panax* species, *P. ginseng*, *P. quinquefolius* and *P. japonicus* as well as their corresponding ginseng drugs (Fushimi *et al.*, 1997). The PCR-RFLP patterns of internal transcribed spacer (ITS) gene region digested with *Hinf* I, *Taq* I and *Sau* 3A were also able to differentiate between *P. ginseng* and *P. quinquefolius* and identifed the ginseng drugs from the two common adulterants *Mirabilis jalapa* and *Phytolacca acinosa* (Ngan *et al.*, 1999).

2.13. Secondary structure using 5.8S rRNA

Biomolecules display a close interaction between structure and function. Therefore, the growing number of RNA molecules with complex functions, beyond that of encoding proteins, has brought increased demand for RNA structure prediction methods. Since prediction of tertiary structure is usually impraticable, the area of RNA secondary structures is an example where computational methods have been highly successful. The first practical dynamic programming algorithms to predict the optimal secondary structure of an RNA sequence date back over 20 years (Zuker and Stiegler, 1981). Further progress has been extended to allow prediction of suboptimal structures (Zuker, 1989, Wuchty *et al.*, 1999)

and thermodynamic ensembles (McCaskill, 1990), which allow to assign a confidence level or 'well definedness' to the predictions (Zuker and Jacobson, 1995). Recently, several methods have dealt with the problem of predicting a consensus structure for a group of related RNA sequences (Gorodkin *et al.*, 1997; Hofacker *et al.*, 1998, 2002; Juan and Wilson, 1999; Knudsen and Hein, 1999; Luck *et al.*, 1999). Such conserved structures are of particular interest, since conservation of structure in spite of sequence variation implies that the structure must be functionally important. By enhancing energy rules with sequence covariation, these methods also obtain much better prediction accuracies.

Studies on the secondary structures of rRNAs and adjacent regions have increased since the beginning of DNA sequencing in the late 1970s. rRNAs occur as multiple tandem repeats in nuclear DNA. Each repeat is transcribed as a single rRNA precursor, which is subsequently cleaved by a series of nucleolar events leading to the mature small subunit rRNA (SSU), the mature 5.8S rRNA and the mature large subunit rRNA (LSU). The SSU is separated from the 5.8S rRNA by the first internal transcribed spacer (ITS1), and the second internal transcribed spacer (ITS2) is located between the 5.8S rRNA and the LSU (Perry, 1976; Maroteaux *et al.*, 1985; Lenaers *et al.*, 1989).

In eukaryotes the large subunit of cytoplasmic ribosomes contain a 5.8S rRNA component which is hydrogen bonded to the high-molecular-weight rRNA (Pene *et al.*, 1968; Weinberg and Penman, 1968). The first complete nucleotide sequence of a eukaryotic 5.8S rRNA (yeast) was determined by Rubin (1973) who also proposed a model for its secondary structure. The 5.8S rRNA gene is rarely used to infer phylogeny because it is highly conserved (Troitsky and Bobrova, 1986; Troitsky *et al.*, 1991; Hamby and Zimmer, 1992; Suh *et al.*, 1993; Hershkovitz and Lewis, 1996) and also because of its small size which corresponds to only 164-165 bp (Hershkovitz and Lewis, 1996).

2.14. Phytochemicals in *Panax*

Plants produce a vast and diverse range of organic compounds, the great majority of which do not participate directly in growth and development. These substances, traditionally referred to as secondary metabolites, often are differentially distributed among few taxonomic groups within the plant kingdom. Secondary metabolites are classified as: alkaloids, glycosides, tannins, phenolic compounds, volatile oils, terpenoids, saponins, steroids, resins and bitter principles. These are used as medicine, food, flavours, colours, dyes, poisons and perfumes. It is estimated that ¼th of prescription drugs contains at least one chemical originally identified from plants. Secondary metabolites are low molecular weight compounds occurring within the plant kingdom. More than 100,000 structures have already been described (Buckingham, 1994), and many more are yet to be discovered with an estimate of the total number in plants which exceeds 500,000 (Mendelsohn and Balick, 1995). Albrecht Kossel, a plant physiologist was the first to designate the term "secondary" for these seemingly nonfunctional compounds (Kossel, 1891).

There is immense scope for new drug discoveries based on traditional medicinal plant use throughout the world (Farnsworth, 1984; Tyler, 1986; Cox *et al.*, 1989; Phillipson and Anderson, 1989; Schultes and Raffauf, 1990; Farnsworth and Soejarto, 1991; Moerman, 1991; Turner and Herbda, 1990). Recently, Lewis (1992) outlined several hundred plants by medical categories which are currently used in modern medicine and pharmacy, exemplifying the recent selections of natural products and their incorporation into modern pharmacopeias. The therapeutic importance of ginseng has led to the development of various analytical methods for the determination of the total saponin content, group-specific analysis, and target compound determination. The pharmacokinetics and metabolism of different ginseng saponin compounds have been analyzed in both animals and humans.

Ginsenosides are considered to be the main bioactive principles of ginseng, associated with the pharmacologic activity and are used as marker compounds for quality control (Shibata *et al.*, 1985; Huang, 1993; Chuang *et al.*, 1995; Sticher, 1998; Attele *et al.*, 1999). They fall within a group of approximately 30 plant saponins present in the roots, leaves, berries, and other parts of the ginseng plant (Chuang and Sheu, 1994). More than 40 ginsenosides have been identified, isolated and characterised (Teng *et al.*, 2003). Ginsenosides are secondary plant metabolites and used as part of the defensive strategy to thwart microorganisms and discourage herbivores (Wink, 2008). Ginsenosides, the dammarane-type triterpene saponin are classified based on the attachment position of various water soluble sugar moieties to the non-polar aglycone. Based on their aglycone moieties, ginsenosides can be mainly divided into two categories: 20(S)-protopapaxdiol with two sugar moieties (ginsenoside Rb1, Rb2, Rb3, Rc, Rd and Rh2) and 20(S)-protopapaxtriol containing three sugar moieties (ginsenoside Re, Rf, Rg1, Rg2 and Rh1) (Kim *et al.*, 2007). Other ginsenosides are also identified, such as oleanolic acids (Ro, Rhs, R1, F4) and pseudoginsenoside F11 (Wang *et al.*, 2005; Leung *et al.*, 2007). The pharmacological activity of ginsenosides is based primarily upon the structural aspects of the molecule, which is a glycosylated steroidal triterpene.

The various compounds present have been widely investigated for their effects on disturbances of the central nervous system, hypothermia tumor metastasis, antioxidant, antidiabetes, antiaging radioprotective activities (Chang *et al.*, 1998). Ginsenosides have played as important resources in the development of new drugs (Attele *et al.*, 1999).The major ginsenoside Rb1, exhibited remarkable effects on the central nervous system (Chang *et al.*, 1998; Saito, 1989) and drug-induced memory impairment (Xie *et al.*, 2002). In addition, the regulation of ChAT, NGF and trkA mRNA expression by ginsenoside Rb1 in the rat brain was observed (Saito, 1989). Rb1 have been shown to act as phytoestrogens in

breast cancer cells via activation of the estrogen receptors (Lee *et al.*, 2003b). Rc and Rh2 have demonstrated inhibitory effects on the proliferation of human breast cancer cells (Rice *et al.*, 2000; Murphy *et al.*, 2001), while ginsenoside Rg1 serve as a functional ligand of the glucocorticoid receptor (Lee *et al.*, 1997) and as a suppressor of oxidative stress (Xiao-Chun *et al.*, 2003). Ginsenoside Rh1 isolated from *P. ginseng* root with many pharmacological effects such as anti-allergic and anti-inflammatory activities (Park *et al.*, 2004), anti-angiogenic effects in tumour progression (Usami *et al.*, 2008) has been given attention in further research. It is also an important intermediate metabolite of ginsenoside Rg1 and Re which can reach the systemic circulation in humans after oral administration (Tawab *et al.*, 2003). Saponins of *P. notoginseng* have been reported to increase the blood flow of the coronary arteries (Zhang *et al.*, 1980; Huang *et al.*, 1999) and to decrease the consumption of oxygen by heart muscles (Chen *et al.*, 1983).

Reverse C18 column was usually employed to separate ginsenosides (Shangguan *et al.*, 2001; Wan *et al.*, 2006a, b; Wood *et al.*, 2006; Zhang *et al.*, 2006; Chen *et al.*, 2007). Polyvinyl alcohol-bonded stationary phase was also used to separate ginsenosides (Quiming *et al.*, 2007). HPLC has been extensively used for the analysis of ginsenosides in the last two decades (Ma *et al.*, 1996; Zhai *et al.*, 2001; Jiang *et al.*, 2007; Zhou *et al.*, 2005). Microwave-assisted extraction (MAE) procedures have been also used for the extraction of ginsenosides from *P. ginseng* root and the degradation of ginsenosides in aqueous solution (Kwon *et al.*, 2003; Ren and Chen, 1999; Shu *et al.*, 2003).

Panax species have their own characteristic ginsenosides which are not present in other species of the genus and their relative amounts can be used to differentiate between the species. The most abundant ginsenosides in *P. ginseng* and *P. quinquefolius* are Rb_1, Rb_2, Rc, Rd, Re and Rg_1 (Ji *et al.*, 2001). More than 30 ginsenosides have been reported from *P. ginseng* and most exhibit four types of aglycone moieties: protopanaxadiol,

protopanaxatriol, ocotillol and oleanolic acid types (Fuzzati *et al.*, 1999; Fuzzati, 2004). The presence of ginsenoside Rf in *P. ginseng* and 24-(R)-pseudoginsenoside F11 in *P. quinquefolius* can be used for the identification of both species if LC-MS is used for the final detection (Chan *et al.*, 2000; Li *et al.*, 2000). The amounts of Rb1, Re and Rd in *P. quinquefolius* are higher compared to *P. ginseng* while the amount of Rg1, Rb2 and Rc are greater in *P. ginseng* than *P. quinquefolius* (Wang *et al.*, 2007). *P. quinquefolius* has little or no ginsenoside Rf, has a lower ratio of ginsenoside Rg1 to Rb1 than *P. ginseng* (van Breemen, 1995; Li *et al.*, 1996) and consistent with the pharmacology of ginsenosides Rg1 and Rb1 (Shibata *et al.*, 1985), is considered to be more balanced and less stimulating than *P. ginseng* (Hobbs, 1996).

In *P. notoginseng* (Sanchi ginseng), Rg1 and Rb1 ginsenosides are the most abundant (Dan *et al.*, 2008; Wang *et al.*, 2009; Chen *et al.*, 2010). The unique notoginsenosides fingerprints which include, R1, -R2, -R4, -Fa, -Q, -S, -Fc,-H, -A, -B, -C, -D, -E, -F, -G, -H, -I, -J has made P. *notogingseng* to be easily differentiated (Yoshikawa *et al.*, 1997; Ng, 2006; Dan *et al.*, 2008) from *P. quinquefolius* and *P. ginseng*. Total ginsenoside content of *P. notoginseng* is higher than that of *P. ginseng* and *P. quinquefolius* (Dong *et al.*, 2003).

Currently, over 20 different saponins have been identified in *P. notoginseng* root (Gan and Zhen, 1992; Li and Fitzloff, 2001) including ginsenosides, notoginsenosides, and gypenosides. Among these saponins, ginsenoside Rg1, Rb1, Rd and notoginsenoside R1 are considered to be the major components (Gan and Zhen, 1992). *Panax vietnamensis* (Vietnamese ginseng) ginsenoside includes Rb1, Rb2, Rd, Re ,Rh5, Rg1, Rh1, Rh4 and vinaginsenosides R1, R2, R10, R25, pseudo-ginsenoside RT4 and majonoside R2 (Tran *et al.*, 2001). Tanaka (1990) reported that the oleanolic acid glucuronide saponins in the roots of *P. pseudoginseng* from central Nepal were much lower compared to Chinese, Himalayan, and Japanese taxa.

From the above review of literature, it is evident that very little work has been done for Indian *Panax* species. Therefore, a comprehensive study covering taxonomic, ecological, genetic and phytochemical aspects of all the available *Panax* species in India has been carried out during this Ph. D. programme.

CHAPTER 3
STUDY SITES

In India, the species of *Panax* are found only in north-eastern region. However, none of the species has been reported from Assam, Mizoram and Tripura. Hence, the survey was conducted in five north-eastern states viz., Arunachal Pradesh, Manipur, Meghalaya, Nagaland and Sikkim.

The species distribution map was prepared based on presence data i.e. GPS location, collected through field survey, for *Panax assamicus* in Meghalaya and Manipur, *P. variabilis* in Manipur, *P. bipinnatifidus* in Arunachal Pradesh and Sikkim, *Panax* sp. (not yet identified, could be a new species) in Arunachal Pradesh and Nagaland, and *P. pseudoginseng* in Nagaland (Fig. 3.1). The data were used to predict the potential distribution area for each species through ENM. Based on model prediction, further field surveys were undertaken to locate new populations. These predicted areas were also confined to the above mentioned five states. Niche characterization studies based on certain climatic factors and soil physico-chemical properties of the habitats of *Panax* spp. were also conducted in the above mentioned five states. Molecular phylogeny studies using 18S rRNA gene and 18-28S rDNA region and PCR-RFLP were carried out for all the species of *Panax* except for *P. pseudoginseng* due to scarcity in the number of individuals. Population dynamics studies were conducted in Meghalaya for *P. assamicus* populations only. The inter-population variation in ginsenoside content was analysed for selected populations representing *P. assamicus, P. bipinnatifidus, P. haridasanii, P. pseudoginseng* and *P. variabilis* (Table 3.1). The selection was based on adequate availability of rhizomes for phytochemical analysis.

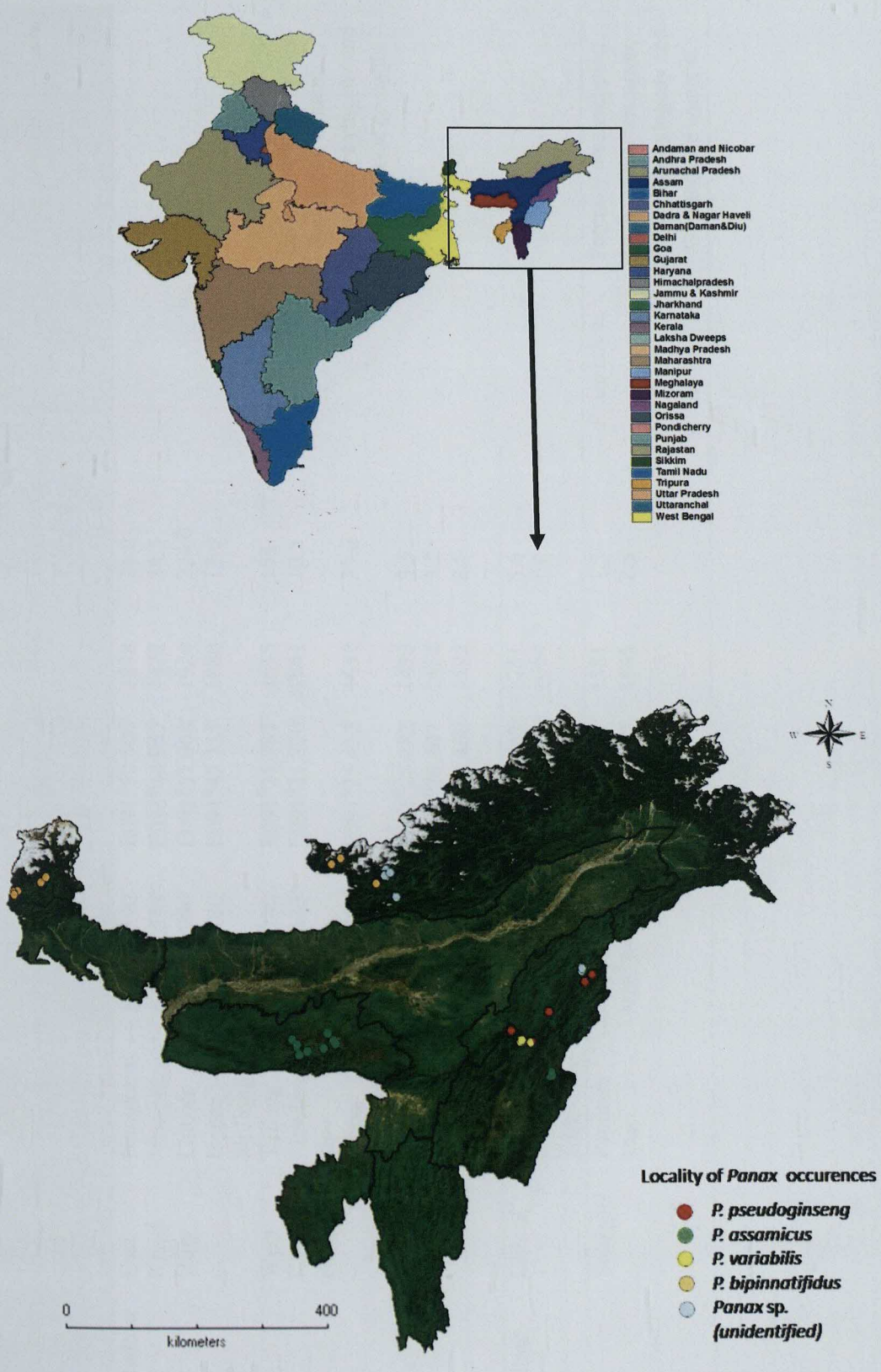


Fig. 3.1: Occurrence of different species of *Panax* in north-east India.

Table 3.1: Study sites for different species of *Panax* in north-eastern India and the aspects of study undertaken for each species.

State	District	Locality	Latitude	Longitude	Altitude (m)	Estimated Extent of Occurrence (ha)	Species	Aspects of the study covered
Meghalaya	East Khasi Hills	Nongkrem	N25°29.580'	E091°52.506'	1838	21.0	*P. assamicus*	Potential area mapping, Niche characterization, Population dynamics, Molecular phylogeny and Ginsenoside contents
		Mawphlang	N25°26.598'	E091°44.967'	1622	38.5		
		Laitkor	N25°31.996'	E091°51.064'	1924	224.0		
		Upper Shillong	N25°37.120'	E091°47.111'	1880	13.0		
	West Khasi Hills	Tyllang	N25°33.280'	E091°21.987'	1635	153		
		Mawlang khar	N25°33.133'	E091°21.800'	1841	10.5		
		Upper Laitkseh	N25°29.266'	E091°25.453'	1632	37.0		
		Marngor	N25°27.804'	E091°25.282'	1490	3.5		
		Mawkyrwat	N25°22.078'	E091°26.807'	1654	3.2		
		Jakrem	N25°24.426'	E091°32.450'	1592	2.5		
Manipur	Ukhrul	Shirui Hills	N25°07.037'	E094°26.743'	2251	5.6		
		Phangrei Peak	N25°08.636'	E094°27.555'	1935	1.8		
	Senapati	Paomata	N25°29.366'	E094°12.847'	1581	1.5	*P. variabilis*	Potential area mapping, Niche characterization, Molecular phylogeny and Ginsenoside contents
		Mao	N25°31.331'	E094°06.070'	1945	2.5		

State	District	Locality	Latitude	Longitude	Altitude (m)	Estimated Extent of Occurrence (ha)	Species	Aspects of the study covered
Nagaland	Kohima	Dzellikii	N25°38.027'	E093°58.804'	1883	3.2	*P. pseudo ginseng*	Potential area mapping, Niche characterization, Molecular phylogeny and Ginsenoside contents
	Phek	Khezha keno	N25°30.090'	E094°13.556'	1810	1.6		
	Zunhe boto	Mtsugho	N25°50.671'	E094°26.247'	1855	4.5		
	Tuensang	Chendang	N26°11.492'	E094°52.251'	1744	4.1	*Panax* sp.	Potential area mapping, Niche characterization, Molecular phylogeny and Ginsenoside contents
		Waoshou	N26°16.531'	E094°57.534'	1787	3.2		
		Bhumbak	N26°21.695'	E094°50.514'	1988	3.5		
		Nakshou	N26°19.097'	E094°49.969'	1838	1.6		
Arunachal Pradesh	West Kameng	Dibbin	N27°26.632'	E092°30.814'	1557	20.0	*Panax* sp.	Potential area mapping, Niche characterization, Molecular phylogeny and Ginsenoside contents
		Utung	N27°29.83'	E092°30.875'	1500	11.0		
	Tawang	Dirang	N22°20.460'	E092°22.962'	1516	40.0	*P. bipinna tifidus*	Potential area mapping, Niche characterization, Molecular phylogeny and Ginsenoside contents
		Tsachu	N27°43.761'	E092°00.176'	3306	1.2		
		Gomkang	N27°33.780'	E091°51.059'	2000	15.5		
Sikkim	West	Ngom-Phedi, (KBR)	N27°22'	E085°05'	3200	80.0	*P. bipinna tifidus*	Potential area mapping
	North	Lachung	N27°44.253'	E088°46.133'	3210	25.0		

The north-eastern region of India assumes a special significance for *Panax* species, as the species are found only in this region in India. Besides *Panax,* the region is also important from floristic point of view with its diverse range of flora that has been attributed to its unique location and varying eco-climatic conditions. It is located at the confluence of Indian peninsula, Indo-Malayan and Indo-Chinese biogeographical zones (Rao, 1994). The region is part of two global biodiversity hotspots viz., Himalayas and Indo-Burma, out of 34 recognized biodiversity hotspots of the world (Mittermeier *et al.*, 2011). The undulating topography, high rainfall and varied altitudes are main factors that have contributed to its rich ecosystem and habitat diversity. The region represents about 50% of the floristic wealth of India and contains about 8000 species of flowering plants including several representatives of primitive or ancient angiosperms (Takhtajan, 1969). A brief description of the state where the species of *Panax* occur is described below.

3.1. ARUNACHAL PRADESH

Arunachal Pradesh is situated between 26°30' N and 29°30' N latitude and 91°30' E and 97°30' E longitude and has an area of 83,743 km^2. The state is located in the extreme eastern corner of the country. The territory falls in the eastern Himalayas. It experiences wide variation in topographical conditions, vegetation and wild life along the altitudinal gradient that ranges from 60 m to over 7300 m asl (GORICHEN peak in Tawang district).

Geology

Arunachal Pradesh is bound by Bhutan in west, China in north and north-east and Myanmar in east. The Tertiary and Quaternary sediments of the Naga-Patkoi belt extend north-eastward in Changlang and Tirap districts of south-eastern Arunachal Pradesh. Eight domains have been identified and these are: Namdhapa Crystalline Complex which occupies the highest tectonic level, consisting of well foliated, mesocratic, biotite granitoid gneiss which is divisible into three textural types viz., augen gneiss, homophanous gneiss

and streaky gneiss. The Tilung Formation which includes quartz-chlorite/mica schist with intercalation of quartzite. Disang Group comprises of thick succession of unfossiliferous and the shale to arenaceous facies that has typical miogeosynclinal depositional facies. Rocks of Barail Group occur in two different sedimentological environments i.e., geosynclinal facies and shelf or platform facies which is coal-bearing. The Naharkatia Group occurs in the form of elongated wedges and comprises of Tipam and Girujan Formation. The Namsang Formation includes the mottled clay, sandstone, gritty to conglomeratic sandstone with pebble, coal (derived from Barail) and minor lignite lenses. Dihing Formation is the uppermost Tertiary sequence in Tirap and Changlang districts and comprises boulder to pebble-sizedclasts of quartzite and gneiss embedded in a matrix of loose sand and clay, and sand rock with very soft greenish and bluish clayey beds, carbonized wood fragments and small lenses of lignite. The Older Alluvium is characterised by oxidation of the sediments.

Soil

The type of soil in Arunachal Pradesh is difficult to generalise due to the varied terrain with numerous rivers. However, considering the sand stone rocks, the granite and gneiss formation interspersed with calcareous limestone and slate and other minerals tempered largely by swift flowing rivers. The broad group could possibly be (i) Soils of the hills, (2) Soils in the valleys and mid hills and (3) Soils in the foot hills. The soils in the valleys are rich in organic content and clayey. The soils in Arunachal Pradesh have high acidity which may be ascribed to the high rainfall and heavy runoff. The colour ranges from red to black and white (pure sand). Due to heterogeneity of basic rock and sedimentary formation of soils, there is great variation in the nature and composition of soils even at close intervals.

Climate

The climate of Arunachal Pradesh varies with elevation. Areas that are at a very high elevation in the Upper Himalaya close to the Tibetan border have an alpine or tundra climate. Below the Upper Himalayas are the Middle Himalayas, where people experience a temperate climate. Areas at the sub-Himalayan and sea-level elevation generally experience humid, sub-tropical climate with hot summers and mild winters. The higher regions of Arunachal Pradesh witnesses snowfall during the winter. The state receives heavy rainfall of 2,000 to 4,100 mm annually, most of it between May and September.

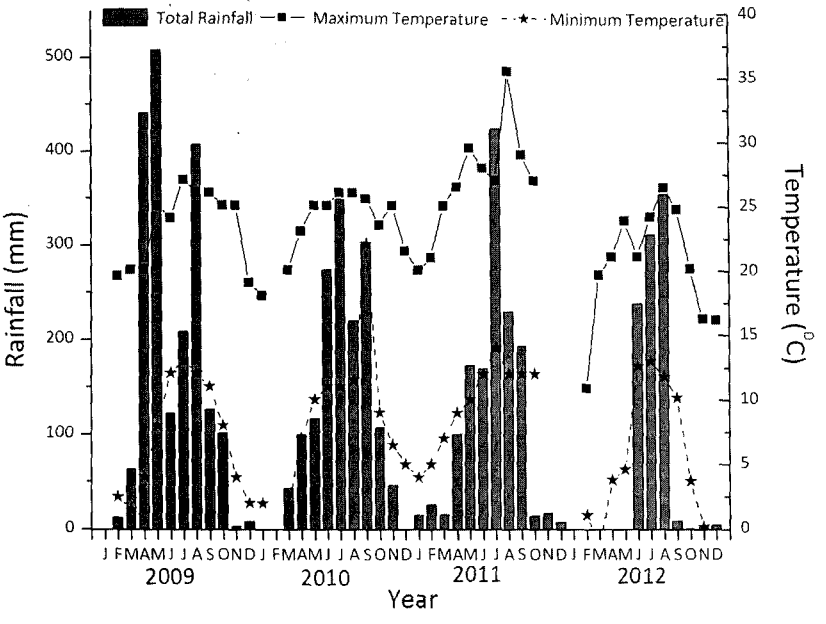


Fig. 3.2: Monthly rainfall and mean monthly minimum and maximum temperature at Bomdila of Arunachal Pradesh during the study period i.e., 2009-2012.

The meteorological data for Arunachal Pradesh was collected from Bomdila, West Kameng meteorological station and from the National Research Centre on Yak (ICAR) at Dirang. During the study period, the annual recorded was 2001 mm during 2009, 1559 mm in 2010, 1385 during 2011, and 924 mm during 2012. About 69-99% of rainfall was received during the rainy season. The monthly rainfall during the rainy season ranged from 117 mm which

is minimum in the month of May up to a maximum of 425 mm in July. The mean monthly temperature varied from a maximum of 35°C which is shown in the month of August to a minimum of (-) 2°C in the month of December (Fig. 3.2). The average relative humidity varied between 72 - 94%.

Forest types

The forests of Arunachal Pradesh as per Champion & Seth's classification (1968) have been divided into sixteen forest types which are grouped in to 10 broad forest types, and in order of area, these are: viz., tropical semi-evergreen (68.75%), sub-alpine forest(10.30%), himalayan moist temperate (7.43%), tropical moist deciduous (5.35%), subtropical broadleaved hill (3.35%), tropical wet evergreen (1.48%), himalayan dry temperate (1.51%), moist alpine scrub (0.92%), subtropical pine forests (0.85%), dry alpine scrub (0.02%). The recorded forest area in Arunachal Pradesh is 67,410 km^2, which works out to be 80.50% of the geographical area of the state. The Reserved Forest constitutes 20.46%, Protected Forests 18.49% and Unclassed Forests 61.05% (Forest survey of India, 2011).

In Arunachal Pradesh, *Panax* species are found in temperate forest of Tawang district (Fig. 3.3, a) and subtropical broadleaved forest of West Kameng district (Fig. 3.3, b). The temeperate forests extend between altitudes 1800 m to 2800 m asl. The dominant tree species include *Quercus griffithii, Rhododendron campanulatum, Rhododendron maddeni, Rhododendron nerifolium.* Other species present are: *Albizia lucida, Alnus nepalensis, Betula alnoides, Cryptomeria japonica, Cupressus* sp., *Erythirina arborescens, Ilex* sp., *Juglans regia, Lyonia ovalifolia, Macaranga denticulate, Morus* sp., *Populus gamblei, Salix* sp., and *Schima khasiana.* The common shrubs are *Artemisia nilagirica, Cotoneaster* sp., *Debregaesia longifolia, Drynaria propinqua, Elaeagnus* sp., *Gaultheria fragrantissima, Mesea indica, Mussaenda roxburghii, Neillia thyrsiflora, Plectranthus coetsa, Prinsepia utilis, Rhus javanica, Rubus ellipticus, Rubus hypergyrus, Rubus rugosus,*

Saccharum spontaneum, Spiraea canescens and Viburnum erubescens. Herbs include *Aconogonum* sp., *Anemone vitifolia, Bidens pilosa, Centella asiatica, Gerardinia heterophylla, Gnaphalium* sp., *Houttuynia cordata, Inula cappa, Lycopodium clavatum, Plantago major, Polygonum capitatum, Potentilla fulgens, Pouzolzia* sp., *Pteridium aquilinum, Ranunculus scleratus, Rubia cordifolia* and *Rumex nepalensis.*

The subtropical forests occur between altitudes 900 m-1900 m asl and are essentially evergreen and dense in nature. The forests are rich in species diversity and dominated by *Brassaiopsis glomerulata, Castanopsis purpurella, Engelherdtia spicata, Quercus griffithii.* The other species include, *Actephila excels, Caryota urens, Castanopsis* sp., *Cinnamomum obtusifolia, Drymicarpus racemosus, Eleocarpus* sp., *Ficus roxburghii, Grewia* sp., *Lithocarpus fenestrate, Lyonia ovalifolia, Macaranga denticulata, Macropanax disperma, Myrica esculenta, Oroxylum indica., Pithicellobium monodelphium, Prunus acuminata, Rhododendron arboreum, Rhus acuminata, Saurauria nepalensis, Schifellera hypoleuca, Schima khasiana, Spondias pinnata* and *Talauma hodgsonii.*

Shrubs include *Eupatorium odoratum, Piper* sp., *Plectranthus striatus, Clerodendron coolebrokianum, Debregessia longifolia, Desmodium* sp., *Ilex* sp., *Indigofera* sp., *Inula cappa, Mesea indica, Oxospora paniculata, Polygola axillata, Rubus ellipticus, Rubus* sp., *Smilax* sp., *Solanum nigrum, Solanum xanthocarpum, Vernonia volkemerifolia,* and *Zanthoxylum* sp.

Drymaria cordata, Elatostemma sessile, Pilea umbrosa, and *Nephrolepis cordifolia* dominated the herb layer. Other herbaceous species are: *Abelmoschus moschatus, Aeginetia indica, Aglaomorpha coronansi, Anthogonium gracile, Asplenium nidus, Begonia* sp., *Chirita articifolia, Chirita pumila, Commelina pedulosa, Costos speciosus, Dicranopteris* sp., *Drymeria cordata, Elsholtzia blanda, Ophiopogon intermedius, Oplimanus* sp.,

Panicum montanum Paris polyphylla, Phrynium capitatu, Piper thomsonii, Polygonum capitatum, Polypodium sp., *Rhynoglossum obliquim, Rubia cordifolia, Selaginella* sp., *Thysanolaena maxima* and *Trichosanthes* sp.

(a)

(b)

Fig. 3.3 (a-b): An overview of the temperate forest (a) Tawang/Zimithang in Tawang district and (b) subtropical broadleaved forest at Dibbin in West Kameng district of Arunachal Pradesh.

3.2. MANIPUR

Manipur lies between 23°83′ and 25°68′ N latitude and 93°03′ and 94°78′ E longitude. It has a total area of 22,347 km^2. The state is divided into two distinct physical regions: (i) an outlying area of rugged hills and narrow valleys, and (ii) the inner area of valley or plain. The altitude ranges from 40 m asl at Jiribam to 2,994 m asl at Mt. Iso Peak near Mao Songsong.

Geology

Manipur is bordered by the states of Nagaland in north, Assam in west, Mizoram in south and Myanmar in southeast. The geology of Manipur including Indo-Burma range along its eastern frontier is closely linked with the evolution of Neogene Surma basin, Inner Palaeogene fold belt and Ophiolite suture zone. The ophiolite belt occurring along Indo-Myanmar border in Manipur forms a part of Naga-Arakan Yoma flysch trough of Upper Cretaceous-Middle Miocene age. Geological data collected so far mainly relates to Ophiolite zone and adjoining terrain. Available information brings forth a geological picture depicting the spread of Tertiary rocks over the entire state with small patches of Quaternary sediments in the central part (e.g. Imphal valley) and a long narrow N-S trending ophiolite belt towards the eastern margin of the state. Thus, geotectonically three distinct domains exist which are Neogene Surma basin, Inner Palaeogene fold belt and Ophiolite zone associated with Late Mesozoic-Tertiary sediments.

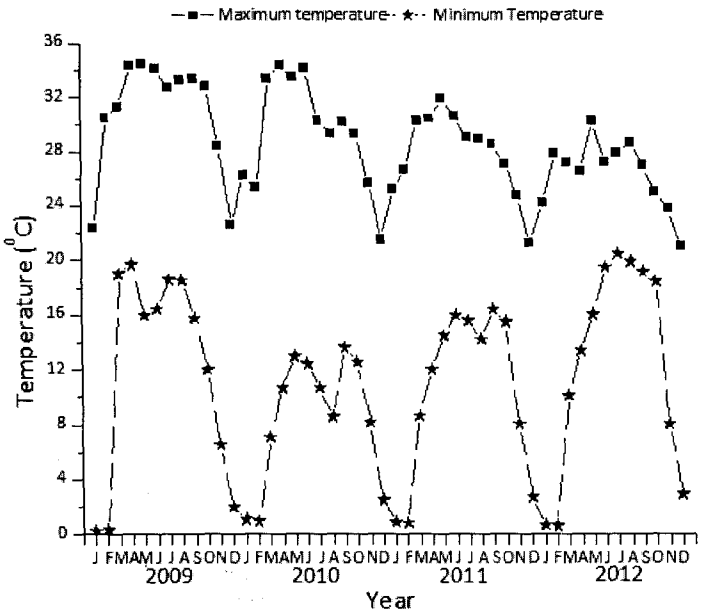
Soil

The soil of Manipur can be broadly divided into two groups, viz. the red ferrogenous soil in the hills and the alluvium in the valley. The soil generally contains small rock fragments, sand and sandy clay. The top soils on the steep slopes are very thin. In the plain areas, especially flood plains and deltas, the soil is of considerable thickness. Soil on the steep hill

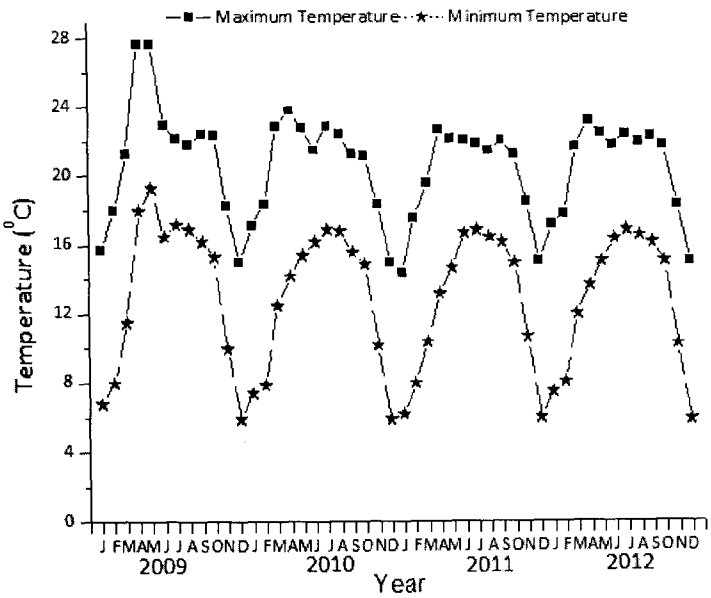
slopes is subjected to high erosion resulting into formation of sheets and gullies and barren rock slopes. The normal pH ranges from 5.4 to 6.8.

Climate

Manipur experiences moderate climate throughout the year. Depending on the altitude, the climate ranges from tropical to temperate. Though the state has three distinct seasons viz., summer, winter, and rainy, precipitation dominates the valley for 5 months. Summer starts from the month of March and continues till mid-May, when temperature reaches the maximum i.e. 36°C. Rainy season starts from mid-May and continues up to September. Manipur receives an average annual rainfall of 2593 mm. The months of October and November, more or less, remain dry. The winter season extends from December to February, when the temperature usually drops down to 0°C.



(a)



(b)

Fig. 3.4 (a-b): Monthly rainfall and mean monthly minimum and maximum temperature of Manipur during the study period i.e. 2009-2012 (a-Senapati, b-Ukhrul district).

The meteorological data collected from Krishi Vigyan Kendra-Sylvan Hengbung, Senapati meteorological station which is the closest station for Mao, a natural habitat of *Panax*. The data collected from ICAR, Ukhrul have been presented to relate the *Panax* populations in Ukhrul. The annual rainfall data for both the places could not be obtained. In Senapati district, the mean monthly temperature varied from a maximum of 34°C which is shown in the month of April, May and June to a minimum of 0.26°C in the month of January (Fig. 3.4, a). The relative humidity ranged from 33-86%. In Ukhrul district, the mean monthly temperature varied from a maximum of 28°C which is shown in the month of April and May and to a minimum of 5.9°C in the month of December (Fig. 3.4, b). The relative humidity ranged from 36-98%.

Forest types

According to Forest Survey of India, 2011 the forest cover of Manipur is 17,418 km^2 which is 77.40% of the total geographical area of the state. The Reserved Forest constitutes 8.42%, Protected Forests 23.95% and Unclassed Forests 67.63% of the recorded forest area (Forest survey of India, 2011). Based on Champion & Seth's classification (1968), the state has ten different forest types. The five forest groups in order of geographical area coverage are: subtropical broadleaved (52.94%), tropical semi-evergreen (24.82%), montane wet temperate (10.46%), subtropical pine forests (8.47%) and tropical moist deciduous (3.05%). In Manipur, *Panax* species are found in temperate forests of Senapati and Ukhrul districts (Fig. 3.5, a-b). Oaks are the dominant trees of the top canopy. These forests are situated at an altitude of 1,800 to 2,400 m asl that receives the annual rainfall of about 1,750 - 3,500mm. These forests are *Quercus-Magnolia-Acer-hylium* formations. The other common species are: *Rhododendron arboreum*, *Michelia manipurensis*, *Magnolia cambellii*, *Pinus kesiya*, *Castanopsis* spp. and *Phoebe hainesiana*. Other top storey trees are: *Alnus nepalensis*, *Betula alnoides*, *Carpinus viminea*, *Exbucklandia populnea*, *Prunus*

sp., *Pyrus* sp., *Persea macrantha*, *Celtis tetrandra*, *Cinnamomum* sp., *Pentapanax* sp., *Toona ciliate* and *Ficus* sp. Bamboo species viz., *Bambusa tulda* and evergreen shrubs such as *Rubus* sp. and *Rosa* sp. are present. Herbs include *Boehmeria macrophylla*, *Pilea umbrosa*, *Tetrastigma serrulata Viola betonicifolia Piper caninum Polyura geminate*, *Hedera nepalensis*, *Rubia sikkimensis*, *Carex* sp., *Oplismenus* sp., *Floscopa scandens Aristolochia tagala*, *Aeschynanthus parasiticus*, *Crotalaria juncea*, *Thalictrum foliolosum*, *polygonum chinense*, *Crinum latifolium*, *Pouzolzia* sp., *Dendrobium formosum* and *Vitis* sp.

(a)

(b)

Fig. 3.5 (a, b): An overview of the Temperate forests (a) Mao in Senapati district and (b) Shirui hills in Ukhrul district of Manipur.

3.3. MEGHALAYA

Meghalaya lies between 25°02′ and 26°07′ N latitude and 89°49′ an d 92°50′ E longitude and has an area of 22, 429 km^2. Being a part of the north-eastern India, it constitutes the meeting place of paleo-arctic, Indo-Malayan and Indo-Chinese bio-geographic realms. The topography of the state is variable and the elevation ranges from 50-2,040 m asl.

Geology

The Shillong plateau comprises rocks of Achaean basement complex in the central and northern parts comprising gneiss, quartzite and schist. The basement rocks are overlain by Shillong group of rocks, occurring in the central and eastern part and comprises quartzite, schist, granite and conglomerate traversed by acid and basic intrusive in Sung valley. The rocks of Gondwana in the western part of Garo Hills contain pebble beds, sandstone and shale. The volcanic eruption about 200 m years back in Jurassic time is spread over E-W direction trending narrow belt in southern part of Khasi Hills and is termed as Sylhet Traps which include basalt and rhyolites. These traps are overlain by the Cretaceous-Tertiary sediments in southern parts of the state. Main rock types belong to Khasi, Jaintia and Garo group which include conglomerate, sandstone shale, silt with coal seams and limestone. The quaternary deposits constituting thick alluvium overlay the Cretaceous-Tertiary sequence in separate patches and are composed of assorted pebbles, with thick coarse and sand and brown coloured clay. The newer alluvium lies in the river valleys of the northern and southern foothills of Garo and Khasi hills and along the western border of Garo hills comprising fine silty sand and light to dark coloured clay with sandy pockets.

Soils

Soils of Meghalaya are derived from the underlying gneisses, schists and granites. They have been grouped under latosols (Oxisol) type (Pascoe, 1950). The soils of the hills are derived from gneissic complex parent materials which are dark brown to dark reddish-

brown in colour, varying in depth from 50-200 cm. The texture of soils varies from loamy to fine loamy. The soils of the alluvial plains adjacent to the northwest and southern plateau are very deep, dark brown to reddish-brown in colour and sandy-loam to silty-clay in texture. Meghalaya soils are rich in organic carbon, deficient in available phosphorous and medium to low in available potassium. The soil pH varies from acidic (pH 5.0 to 6.0) to strongly acidic (pH 4.5 to 5.0). Most of the soils occurring on higher altitudes under high rainfall belt are strongly acidic due to intense leaching. Soils are not suitable for intensive cultivation due to their poor base (35%) saturation (Singh 1996). Acidic soils of Meghalaya are low in available Boron (B) and Molybdenum (Mo). Total Zinc, Copper and Manganese contents of these soils vary from 10.00 to 17.25, 17.00 to 71.00 and 110 to 770 ppm (parts per million), respectively and DIPA (Diethylene Triamine Penta Acetic Acid) extractable zinc, copper and manganese contents of these soils range from 0.72 to 3.20, 0.6 to 2.8 and 3.0 to 162.0 ppm, respectively.

Climate

The State enjoys a temperate climate. It is directly influenced by the south-west monsoon and the northeast winter wind. The four seasons of Meghalaya are: Spring- March and April, Summer/Rainy- May to September, Autumn- October and November, and Winter- December to February. The Monsoon usually starts by the third week of May and continues up to the end of September and sometimes till the middle of October. Maximum rainfall occurs over the southern slopes of the Khasi Hills, i.e., over Sohra and the Mawsynram platform, which receives the heaviest rainfall in the world. The average annual rainfall in the State is 12,000 mm.

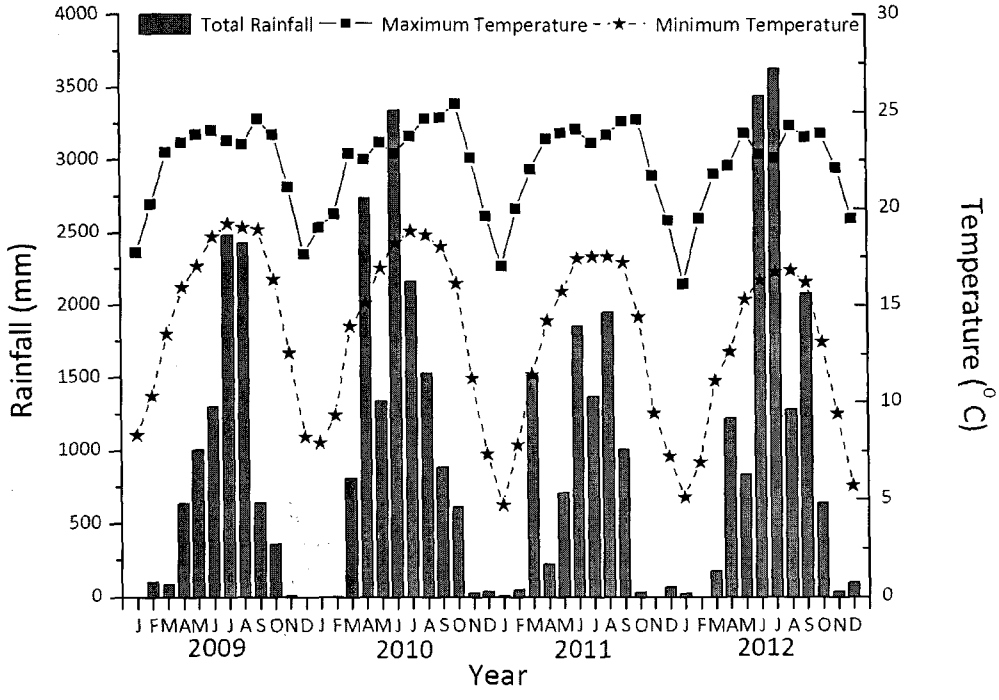


Fig. 3.6: Monthly rainfall and mean monthly minimum and maximum temperature at Cherrapunjee during the study period i.e. 2009-2012.

The meteorological data presented were collected from Cherrapunjee meteorological station. During the study period, the annual rainfall recorded was 9,038 mm in 2009, 13474 mm in 2010, 8788 in 2011, and 13,461 mm during 2012. About 70-80% of rainfall was received during the rainy season. The monthly rainfall during the rainy season ranged from 710 mm which is minimum in the month of May up to a maximum of 3624 mm in July. The mean monthly temperature varied from a maximum of 24°C in the months of June, august and September to a minimum of 5.7°C in the month of December (Fig. 3.6). The relative humidity ranged from 58 - 92%.

Forest types

The state of Meghalaya has eight forest types as per Champion and Seth's classification (1968). The forest areas under the major five forest groups are in the sequence of: tropical moist deciduous (61.62%), subtropical broadleaved (17.71%), tropical wet evergreen (10.45%), subtropical pine forests (8.29%) and tropical semi-evergreen (1.93%). The recorded forest area of the state is 9,469 km^2 which is about 42.21% of its geographical area. The Reserved Forest constitutes 11.72%, Protected Forests 0.13% and Unclassed Forests 88.15% (Forest Survey of India, 2011).

Panax assamicus populations are found in subtropical broadleaved forest (Fig. 3.7, a-e), in the state which occurs above 1200 m asl where annual rainfall ranges between 300 and 500 cm. The tree species include *Aesculus indica, Albizia mollis, Alnus nepalensis, Betula alnoides, Camellia indica, Castanopsis* spp., *Daphniphyllum himalayense, Elaeocarpus* sp., *Eleagnus latifolia, Eurya* spp., *Grevia multiflora, Ilex excelsa, Leucoseptrum cannum, Lindera caudate, Lithocarpus dealbatus, Lithocarpus fenestratus, Litsea elongata, Lyonia ovalifolia, Maesa indica, Myrica esculenta, Phyllanthus glaucus, Pinus kesiya, Quercus griffithii, Rhododendron arboreum, Rhus javanica, Schefflera hypoleuca, Schima khasiana, Schima wallichii, Viburnum coriaceum* and *Zanthoxylum armatum.* The common shrub species in the forest include *Agapetes setigera, Clerodendron* sp., *Elaeagnus pyriformis, Elsholtzia blanda, Ficus gasperriniana, Inula cappa, Jasminum dispermum, Lantana camara, Polygonum rude, Rosa moschata, Rubus rugosus, Sarcandra glabra* and *Urena lobata.* The forest floor is covered with herbs which include *Anaphalis adnata, Anaphalis timmua, Arisaema consanguineum, Begonia thomsonii, Clintonia* sp., *Dicranopteris linearis, Eupatorium adenophorum, Eupatorium riparium, Gentiana tenella, Globba clarkei, Hedera nepalensis, Hedychium coronarium, Hydrocotyl* sp., *Lygodium japonicum, Lycopodium clavatum, Selaginella* sp., *Valeriana hardwickii* and *Viola sikkimensis.*

(a)

(b)

Fig. 3.7 (a,b): An overview of the habitats of *Panax assamicus* in subtropical broadleaved forests in Meghalaya (a-Nongkrem, b-Upper Shillong).

(c)

(d)

Fig. 3.7 (c,d): An overview of the habitats of *Panax assamicus* in subtropical broadleaved forests in Meghalaya (a) (c-Laitkor, d-Laitkseh).

(e)

Fig. 3.7 (e): An overview of the habitats of *Panax assamicus* in subtropical broadleaved forests in Meghalaya at Tyllang.

3.4. NAGALAND

Nagaland, situated at the extreme north-eastern end of India, lies between 25°6' N and 27°4' N latitude and 93°20' E and 95°15'E longitude and covers an area of 16, 527 km^2 area. About 94% of the total geographical area falls under hilly and rugged terrain and only 6% land is plain, especially those bordering Assam plains along western boundary of the state. The elevation ranges from 750 m (Japukong range) to 3826 m asl (Saramati peak).

Geology

Nagaland is located in the northern extension of the Arakan Yoma ranges representing orogenic upheavals in this part of the country during Cretaceous and Tertiary periods. Geotectonically four distinct have been identified in the Naga Hills, which are framed between the foreland spur of Shillong and Mikir Massifs to the west and central Myanmar basin to the east. These are: Assam Shelf, Schuppen Belt, outer belt of imbricate, anastomising thrusts (Evans and Mathur, 1964), the Inner Palaeogene Fold Belt, comprising thick folded sequence of Disang and Barail rocks, and Ophiolitic Complex occurring further east, close to Indo-Myanmar border, associated with Late Mesozoic-Tertiary sediments.

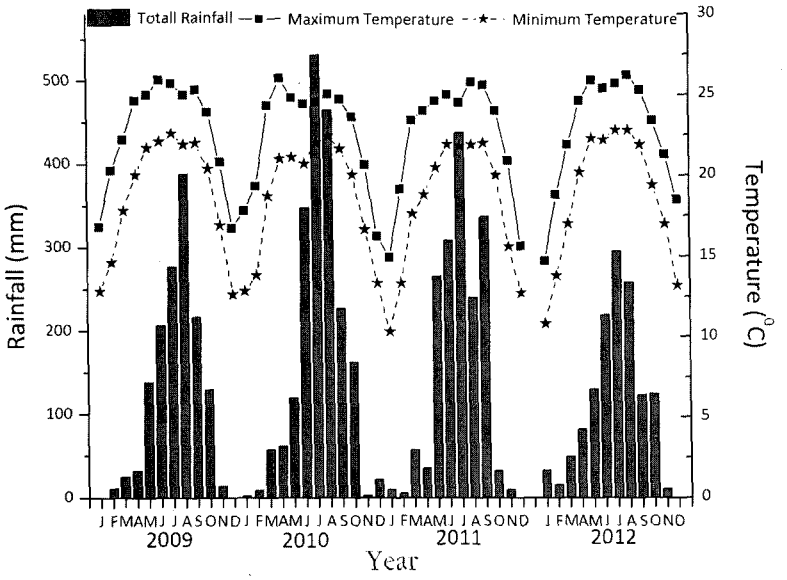
Soil

The soils of Nagaland are derived from tertiary rocks belonging to Barail and Disang series. Though the state is small, due to large variation in topography and climate, the following types of soils occur in the state: (i) Alluvial soils which consist of recent alluvium (Entisol), Old alluvium (Oxizols and Ultisol) and Mountain valley soil (Entisol) and (ii) Residual soils (soilsa) which include laterite soils (Oxizols and Ultisol), brown forest soils (Mollisols and Inceptisols) and podzolic soils (Spodesols). Soils are acidic, rich in organic carbon but poor in available phosphate and potash content. The pH ranges between 4.80-6.80. While

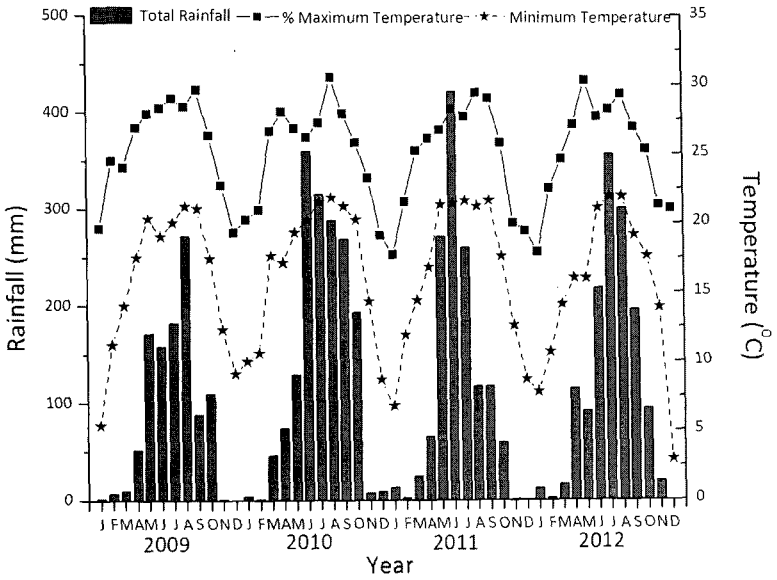
the organic carbon content may be as high as 2.9%, the average available phosphate and potash contents are 20 kg/ha and 120 kg/ha, respectively.

Climate

Nagaland experiences monsoon climate with high humidity levels round the year. The temperature during the summer season ranges from 15°C to 30°C. The torrential monsoon rain continues during the months of May, June, July, August and September. Heavy rainfall occurs between the months of May and August. The recorded average annual rainfall of the state ranges from 1300 mm-2500 mm. During winter, the temperature drops as low as 4°C, and sometime the higher altitudes are covered with frost/snow. Strong north-west wind blows across Nagaland during the months of February and March.

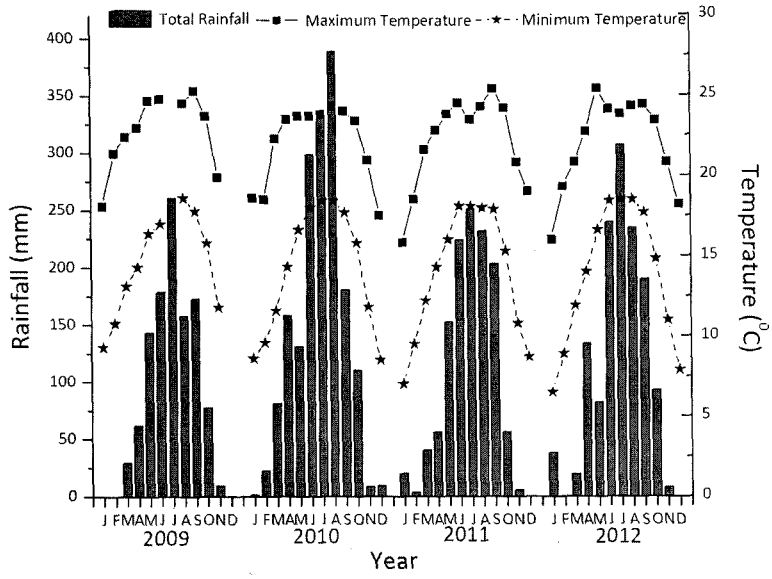


(a)

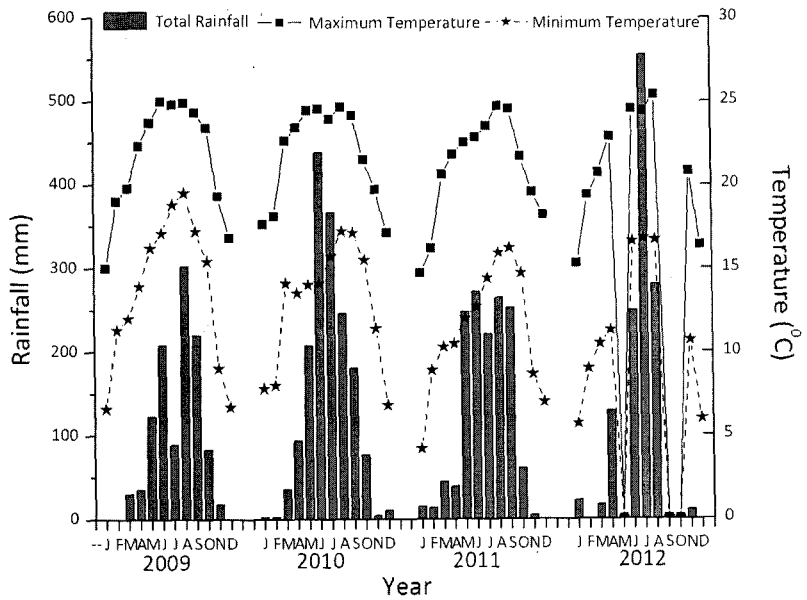


(b)

Fig. 3.8 (a-b): Monthly rainfall and mean monthly minimum and maximum temperature in Nagaland (a-Kohima, b-Phek) during the study period, i.e., 2009-2012.



(c)



(d)

Fig. 3.8 (c-d): Monthly rainfall and mean monthly minimum and maximum temperature in Nagaland (a-Tuensang, b-Zunheboto) during the study period, i.e., 2009-2012.

The meteorological data were collected from Department of Soil and Water Conservation, at Kohima for Kohima, Phek, Zunheboto and Tuensang district, the *Panax* growing areas of the state. During 2009-2012 study period, the annual rainfall in Kohima recorded was 1436 mm in 2009, 2001 mm in 2010, 1736 mm in 2011 and 1341mm in 2012. During the rainy

season about 77-91% rainfall was received. During the rainy season, the monthly rainfall ranged from 138 mm recorded in May to 531 mm obtained in July. The mean monthly temperature varied from a maximum of 26°C in the month of August to a minimum of 10°C in the month of February (Fig. 3.8, a). The average relative humidity ranged from 38% - 88%.

In Phek district, the annual rainfall recorded was 1050 mm in 2009, 1692 mm in 2010, 1350mm in 2011 and 1421 mm in 2012. About 82-88% of rainfall was received during the wet season. The monthly rainfall ranges from 92 mm in May to 359mm in June. The mean monthly temperature varied from 30°C in August which is maximum and a minimum of 3°C in December (Fig. 3.8, b). The average relative humidity ranges from 48-89%.

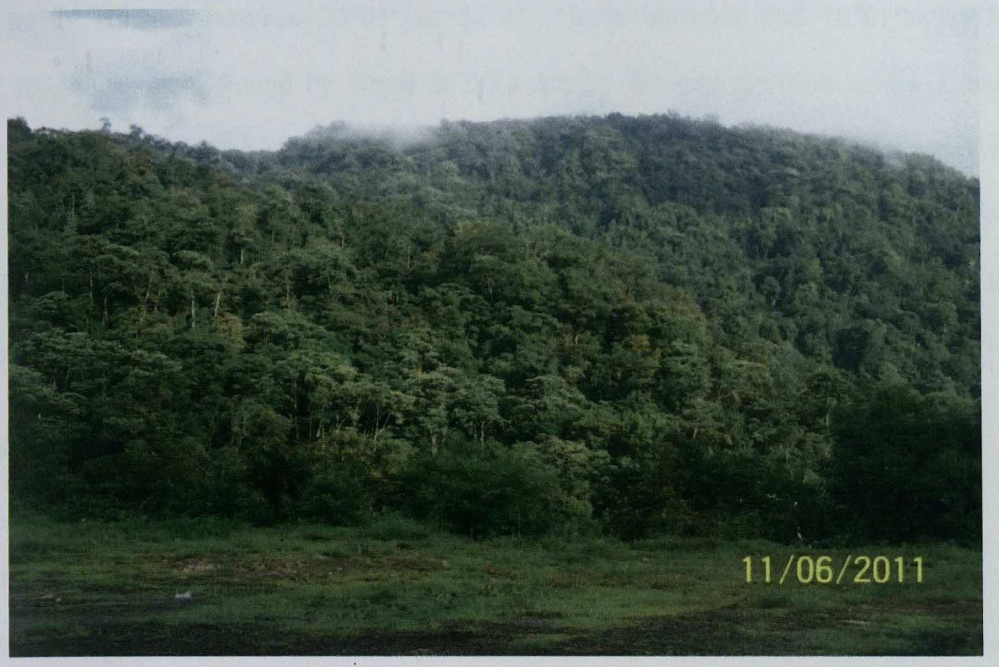
The annual rainfall for Zunheboto district recorded were 1109 mm in 2009, 1663 mm in 2010, 1435 mm in 2011 and 1264 mm in 2012. During the period, the district receives about 85-88% of rainfall. The monthly rainfall during the rainy season ranged from 123 mm which is minimum in the month of May up to a maximum of 438 mm in July. The mean monthly temperature varied from a maximum of 25°C in June and August to a minimum of 6°C in December (Fig. 3.8, c). The average relative humidity ranged from 51-88%.

The annual rainfall in Tuensang district recorded were 1089 mm in 2009, 1722 mm in 2010, 1242 mm in 2011 and 1342 mm in 2012. The district receives about 77-84% of rainfall during the wet season from May-September. The monthly rainfall recorded during the rainy season ranged from 82 mm in May up to 388 mm in August. The mean monthly temperature varied from a maximum of 25°C in September to a minimum of 8°C in December (Fig. 3.8, d). The average relative humidity varied from 52 - 93%.

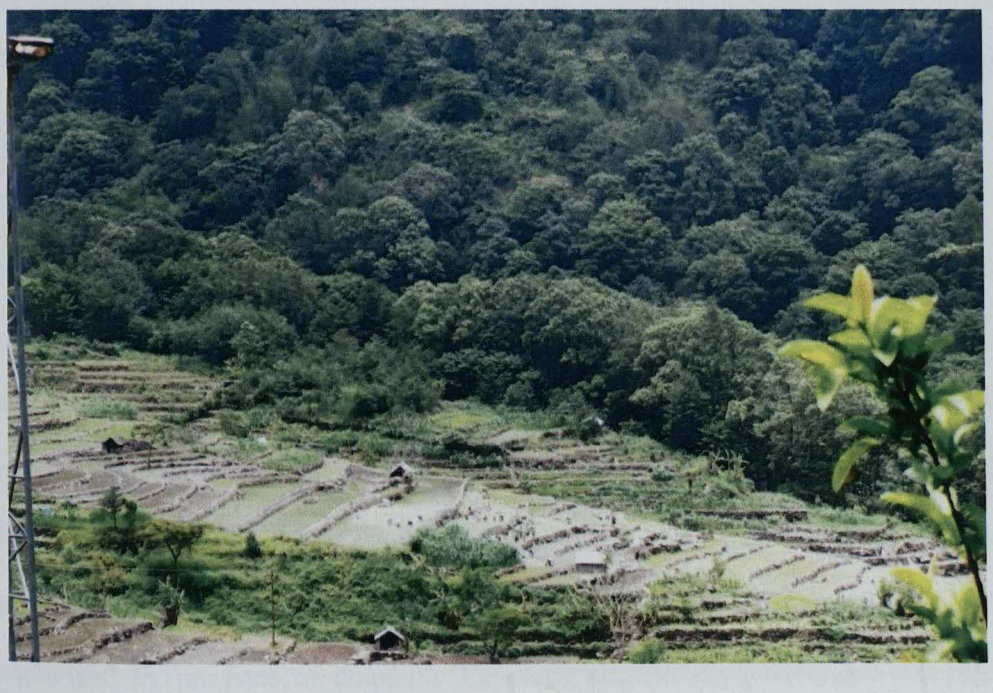
Forest types

Nagaland has seven forest types as per Champion & Seth's classification (1968). The area under six forest groups are in order of: tropical moist deciduous (47.43%), tropical semi-evergreen (16.34%), subtropical broadleaved (15.56%), montane wet temperate (12.69%), subtropical pine forests (7.49%) and tropical wet evergreen (0.49%). The recorded forest area in Nagaland is 9,222km^2, which is 55.62% of the geographical area of the state. Reserved, Protected and Unclassed forests constitute 0.93%, 5.51% and 93.56% of the forest area, respectively (Forest Survey of India, 2011).

Panax species are found mainly in the wet temperate forests classified under Naga hills wet temperate forest according to Champion and Seth (Fig. 3.9, a-b). It is distributed along the Assam/Burma border from 1,800 m asl upwards with rainfall ranging from 1,500 – 6,250 mm. The top canopy trees include *Magnolia* sp., *Manglietia* sp., *Michelia* sp. and *Quercus* sp., being the dominant ones. Other trees included are *Acer* sp., *Actinodaphne obovata, Alnus nepalensis, Betula alnoides, Carpinus viminea, Exbucklandia populnea, Litsea* sp., *Pyrus* sp., *Sterculia villosa* and *Syzygium tetragonum.* Shrubs like *Acacia* sp., *Clerodendron serratum, Rubus* sp., etc. Herbs consist of *Aeschynanthus* sp., *Arisaema tortuosum, Begonia* sp., *Elastostema platyphylla, Eupatorium* sp., *Pilea umbrosa, Polygonum fimbriatum, Rhaphidophora calophyllum, Strobilanthes anisophyllus.* Climbers include *Rhaphidophora calophyllum* and *Smilax lanceifolia.*



(a)



(b)

Fig. 3.9 (a-b): An overview of the Temperate forests (a) Bhumbak in Tuensang district and (b) Khonoma in Kohima district of Nagaland.

3.5. SIKKIM

Sikkim is situated between 27°05' to 28°07' North latitudes and 88°01'to 88°55' East longitudes. It is bound by Nepal in the west, by the vast stretches of the Tibetan plateau in the North and by Bhutan and Chumbi Valley of Tibet in the east. Darjeeling district of West Bengal stretches along its southern boundary. The State of Sikkim has a total area of 7096 sq. km. and is stretched over 112 km from North to South and 64 km from East to West.

Geology

Sikkim-Darjeeling Himalayas are techno-stratigraphically defined by four domains with characteristic stratigraphic and structural attributes. From south to north the mountains can be divided into: foot hill belt, inner belt, axial belt and trans-axial belt. The state is mostly covered by precambrian metamorphites of low to medium grade (Daling Group), high grade gneisses (Darjeeling Gneiss and Kanchendzonga Gneiss), Chungthang Formation (quartzite, calc-silicate rocks, marbles, graphite schist's and occasionally amphibolites) with intrusive granites (Lingtse granite gneiss) and Phanerezoic rocks including Gondwana and Tethyan sedimentaries. The Paleozoic and Mesozoic (Tethyan) sequence in the northern and north-western part of Sikkim are fossileferous. The Gondwana super Group consists of sandstone, shale and carbonaceous shale with occasional thin bands of coal and pebbly shale horizon. Daling group of rocks can be classified into three formations viz., Gorubathan formation which is characterized by quartz-chloride-sericite schists, phyllite and quartzite's, the Reyang formation characterized by quartzite's (occasionally calcareous), phyllite interbanded with carbonaceous slate and Buxa formation which is characterized by presence of dolomitic limestone occasionally interbanded with phyllite and development of organo sedimentary structure (stromatolites).

Soil

Natural variation of climatic conditions and wide range of parent materials involved in the soil formation of Sikkim, have resulted acidic soils of diverse nature. Among the four districts of Sikkim, the frequency of soil samples having pH less than 5 are 50% in North Sikkim and in other districts is below 12% (Bhutia *et al.*, 1985). These soils have been mapped, described, analyzed, characterized and classified under 5 broad physiographic units. In accordance with the physiographic sequence of the terrain features, 78 soil families were identified in Sikkim and mapped into 69 mapping units. The soils of Sikkim belong to 3 orders, 7 suborders, 12 great groups and 26 subgroups. It is observed that Inceptisols are dominant (42.84%) followed by Entisols and Mollisols occupying 42.52% and 14.64% respectively.

Climate

Sikkim is located in montane subtropical, temperate, alpine and tundra climatic zones due to high Himalayas. Elevation plays a key role in shaping the climatic types in the state. Sikkim is the most humid region in the whole range of Himalaya due to its proximity to Bay of Bengal. The region is under the influence of South-west monsoon from first week of June and it rains up to middle of October. The region receives heavy to moderate rainfall from both South-East and South-West monsoon. The annual rainfall of the state varies from 3000 mm in the southern parts to 5000 mm in the northern part. The lower hills below 1500 m asl experience sub-tropical humid type climate. The hot humid summer day may experience temperature up to 35°C and during cool winter, the minimum temperature approaches 0°C. Mean summer temperature reaches s high as 38°C in hot valleys and mean winter temperature goes down to -30°C in higher altitudes. Mean relative humidity ranges between 80-100% in rainy season and for the rest of the year remains above 70% depending upon other climatic conditions.

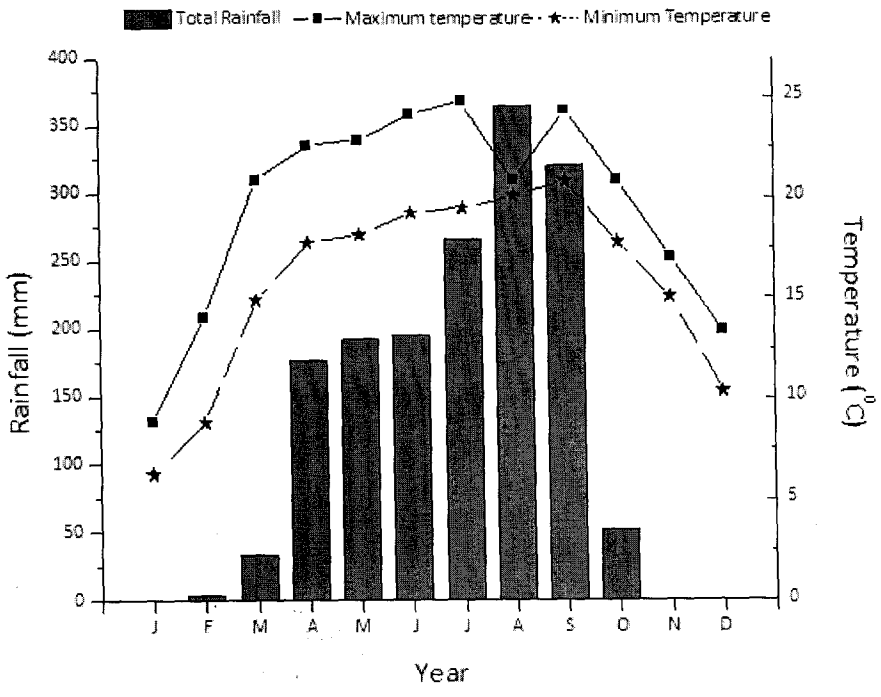


Fig. 3.10: Average monthly rainfall and mean monthly minimum and maximum temperature at Sikkim during 2004-2009.

The meteorological data presented were collected from Sikkim meteorological station during the period 2004-2009, the average annual rainfall recorded was 1603 mm during 2004-2009. About 70-85% of rainfall was received during the rainy season. The monthly rainfall during the rainy season ranged from 191 mm which is minimum in the month of May up to a maximum of 365 mm in August. The mean monthly temperature varied from a maximum of 24.85°C in the months of July, to a minimum of 6.3°C in the month of January (Fig. 3.10).

Forest types

The forests of Sikkim, as per Champion & Seth's classification, have been divided into ten forest types which are grouped in to 6 major forest types viz., sub-alpine forest (27%), subtropical broadleaved hill (25.15%), montane wet temperate forests (24.78%), moist alpine scrub (9.49%), Himalayan moist temperate (6.45%) and tropical moist deciduous (5.15%). The recorded forest area of Sikkim is 5,841 km^2, which constitutes 82.31% of state's geographical area. The Reserved Forest constitutes 93.34% and Protected Forests amounts to 6.66% (Forest Survey of India, 2011).

As per the classification of Champion and Seth (1968), the forest at an elevation of 1,000-2,000 m asl is classified as East-Himalayan subtropical wet-hill forest ($8BC_1$) under the group subtropical broad leaved hill forests. The forest at an elevation of 2,300-3,000 m asl is classified as East-Himalayan moist temperate mixed coniferous forest (12C3a) under the group Himalayan moist temperate forest. The forest at an elevational range of 3,000-3,050 m asl is classified as East-Himalayan subalpine-birch/fir forest ($14C_2$) under the group subalpine forest. These three montane forests are referred to as subtropical, temperate and subalpine forests, based on their group name. *Panax* species are found in the temperate and subtropical forest of Sikkim.

In the temperate forest, rainfall is estimated to be between 1,250-2,000 mm (Fig. 4.1, a). Most of the trees are hung with epiphytic mosses and other epiphytic growth. The forest is dominated by *Quercus lamellosa, Q. lineata* intermixed with deciduous trees such as *Acer campbellii, A. nepalensis, Betula alnoides, Lithocarpus pachyphylla, Magnolia campbellii.* Other trees include *Litsea elongate, Macaranga* sp., *Rhododendron arboreum* and *Symplocus* sp. Shrubs include, *Arundinaria maling, Berberis* spp., *Daphne bholua* and *Rubus* sp. Herbs include, *Ainsliaea aptera, Anaphalis triplinervis, Anisadenia saxatilis,*

Arisaema griffithii, Cyanotis vaga, Dryopteris barbigera, Elatostema sp., *Stellaria sikkimensis, Viola biflora* and *Senecio wallichii.*

In the subtropical or lower montane, the precipitation is generally over 2,000 mm and the dominant trees include, *Alnus nepalensis, Castanopsis hystrix*, *Elaeocarpus lanceaefolius, Engelhardtia spicata, Eurya acuminata, Quercus lineate* and *Rhus javanica* (Fig. 4.1, b). Other tree species include *Lyonia ovalifolia, Prunus cerasoides* and *Viburnum cylindricum.* Shrubs consist of *Boehmeria platyphylla, Dicranopteris linearis, Melastoma normale* and *Thysanolaena maxima.* Herbs include *Achyranthes aspera, Anaphalis* sp., *Bidens biternata, Desmodium multiflorum, Elsholtzia* spp. and *Hydrocotyle nepalensis.*

(a)

(b)

Fig. 3.11 (a-b): An overview of the Upper Temperate/Alpine forest in (a) West Sikkim and (b) Lower Temperate/subtropical forest in North Sikkim of Kanchenjunga Biosphere Reserve.

CHAPTER 4

DESCRIPTION AND INVENTORY OF *PANAX* SPECIES IN NORTH-EASTERN INDIA

4.1. INTRODUCTION

For centuries mankind has been using various plants as nutrient, beverage, cosmetics, dye and medicine to maintain health and improve the quality of life. Ginseng, a medicinal herb, has long been used in the Far East, in particular Korea and China as herbal medicine in maintaining physical vitality. *Panax* was first described by Linnaeus in 1735 by naming two herbaceous species, *P. quinquefolius* L. (American ginseng) and *P. trifolius* L. (dwarf ginseng) from eastern North America (Linnaeus, 1735, 1753). The genus name *Panax* (Pan= all + axos= medicine) means 'cure all' in Greek. The herbal root is named as ginseng, because it is shaped like a man (Fig. 4.1) and is believed to symbolise his three essences (i.e. body, mind and spirit) and is known as the lord or king of herbs (Hu, 1976). Ginseng had been used mainly as a tonic to rejuvenate weak bodies, but only rarely as a curative medicine. However, in the *Bancao Gangmu* (Encyclopedia of Herbs) written by Li Shizhen of China in 1596 A.D., *Panax* is an ingredient in several formulations for curing 23 diseases (Li Schizhen, 1596).

4.1.1. Classification of the Genus *Panax*

According to George Bentham and J.D. Hooker (1862-1883), *Panax* was classified as follows:

Kingdom- Plantae

Division-Magnoliophyta

Class- Magnoliopsida

Order- Apiales

Family- Araliaceae

Genus- *Panax*

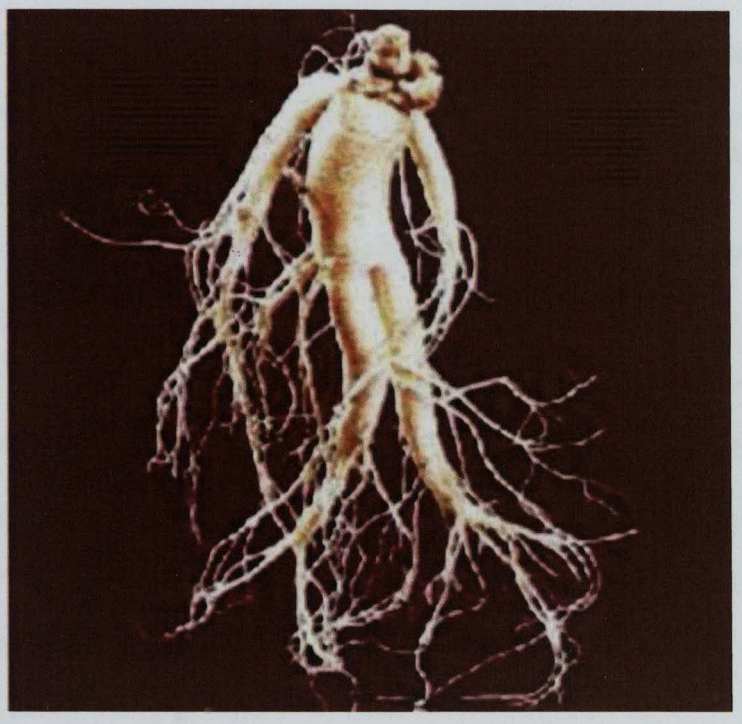


Fig. 4.1: *Panax ginseng* C.A. Meyer shaped like a person.

4.2. TAXONOMIC DESCRIPTION OF THE GENUS *PANAX*

Perennial herbs found in moist rich woods. Rhizome short or elongated, branched, fascicled, or simple, tuberous; base of the stem clasped by membranaceous, deciduous or persistent scales. Stem unbranched, erect, glabrous. Leaves 1-5, generally 3 in a single whorl at the summit of stem, digitately compound, membranaceous, exstipulate; leaflets 3-7, mostly 5, petiolate or sessile, glabrous or with sparse to dense setae along the veins on the dorsal surface, elliptic to obovate, sometimes linear, the base acute to rounded, the apex acute to long acuminate, the margin serrated, uni- or biserrate. Inflorescence a single short or long peduncled, terminal umbel of many flowers, the pedicels subtended by lanceolate bracts, sometimes more than one flowers on single pedicels. Flowers bisexual or unisexual or polygamodioceous. Floral tube obconical or cylindrical, articulated in flowers. Sepals 5, cup-shaped or united at the base, 5 toothed, green. Petals 5, triangular or ovate or linerarly oblong. Stamens 5, filament fleshy; anthers oblong or ovate to elliptic, bilobed, lobes sometimes angular possibly it marks the longitudinal dehiscence. Ovary 5-celled, style 2-3 united at the base, or free, reflexed, persistent, inserted at the middle of the concave or convex smooth or corrugated disc. Fruits flat or globose, ribbed.

Table 4.1: Species under genus *Panax*

Species	Resolved synonyms	Common Name	Distribution
Panax ginseng C. A. Meyer	*Panax quinquefolius* L.var. *ginseng* (C.A. Meyer) Regel &Maack apud *P. quinquefolius* L.a. *coreensis* Siebold *P. schin-seng* T. Nees *Aralia ginseng* (C.A.Meyer) Baillon *A. quinquefolia* (L.) Decaisne & Planchon var. *ginseng* (C.A. Meyer)	Korean, oriental, chinese, manchurian ginseng, ren seng and renshen	China, Korea, eastern Russia, Japan, United States, Canada.
Panax japonicus C. A. Meyer	*P. quinquefolia* b. *japonica* Siebold *P. schin-seng* Nees var. *japonica* Nees *P. repens* Maxim. *Aralia repens* (Maxim.) Makino *A. quinquefolia* var. *repens* (maxim.) Burkill	Japanese ginseng, tochiba-ninjin, satsuma-ninjin, chikusetu ninjin	Japan
Panax notoginseng F. H. Chen	*Aralia quinquefolia* (L.) Decaisne & Planchon var. *notoginseng* Burkill *Panax pseudoginseng* wallich var. *notoginseng* (Burkill)	Sanchi ginseng, radix notoginseng, san qi and tiangi	China
Panax quinquefolius L.	-	American, occidental ginseng, ginseng and sang	Canada and United States
Panax stipuleanatus H. T. Tsai & K.M. Feng	-	Ye sanqi, tu sanqi, bai sanqi and zhu jie qi	China and northern Vietnam
Panax trifolius L.	-	Dwarf ginseng and groundnut ginseng	Canada and United States
Panax variabilis	-	-	China
Panax vietnamensis Ha et Grushv.	*Panax omeiensis* J. Wen *P. sinensis* J. Wen	Vietnamese, bamboo ginseng, cu ngai rom con, zhu gen qi, omei sanqi, omei qi, zhu bian qi, zhu bian sanqi, zhu jie shen, sanchi and ye sanqi	Central to eastern Vietnam and China (Central and Western)

Species	Resolved synonyms	Common Name	Distribution
Panax wangianus Sun.	*Panax pseudoginseng* wallich var. *wangianus* (S.C. Sun) Hoo & Tseng	Narrow leaved ginseng (Xia ye jia renshen), Wang's sanqi	South-western China
Panx zingiberensis C.Y. Wu & K.M. Feng	-	Jiang zhuang sanqi, ginger-like sanqi	China
Indian *Panax*			
Panax assamicus Banerjee	-	Shensheng	Meghalaya, Manipur and Darjeeling hills of West Bengal
Panax bipinnatifidus Seemann	*Aralia quinquefolia* (L.) Decaisne & Planchon var. *elegantior* Burkill *A. quinquefolia* (L.) Decaisne & Planchon var. *major* Burkill *A. bipinnatifida* (Seem.) C. B. Clarke *A. pseudoginseng* (Wallich) Benth. Ex C.B. Clarke *A. quinquefolia* var. *pseudoginseng* (Wallich) Burkill *P. japonicus* C. A. Meyer var. *major* (Burkill) C.Y, Wu & K.M.Feng *P. japonicus* C. A. Meyer var. *bipinnatifidus* (Seem.) C.Y, Wu & K.M.Feng *P. major* Ting ex Pei *et al.* *P. pseudoginseng* Wall. var. *bipinnatifidus* (Seem.) Li *P. pseudoginseng* Wall. var. *major* (Burkill) Li *P. pseudoginseng* Wall. var. *elegantior* (Burkill) Hoo & Tseng *P. pseudoginseng* Wall. var. ssp. *himalaicus* Hara	Pearl ginseng, ge da qi and zhu zi shen	China, Arunachal Pradesh, Nepal and Sikkim, Darjeeling hills of West Bengal

Species	Resolved synonyms	Common Name	Distribution
Panax bipinnatifidus Seemann var. *angustifolius* (Burkill) J. Wen	*Aralia quinquefolia* (L.) Decaisne & Planchon var. *angustifolia* Burkill *A .pseudoginseng* (Wallich) Benth. Ex C.B.Clarke var. *angustifolia* (Burkill) Craib *P. pseudoginseng* Wallich var. *angustifolius* (Burkill) Li *P. repens* Maxim. var. *angustifolium* (Burkill) F.H.Chen *P. japonicus* C.A.Meyer var. *angustifolius* (Burkill) Cheng &Chu *P. sikkimensis* Banerjee *P. assamicus* Banerjee		Tibet of China, Northern India, Nepal
Panax pseudoginseng Wallich	*Aralia pseudoginseng* (Wallich) Benth. Ex C.B.Clarke *A. quinquefolia* var. *pseudoginseng* (Wallich) Burkill	Jia renshen, Himalayan, Nepal ginseng and pseudoginseng	Nepal, India
Panax sikkimensis Ban.	-	ginseng	Sikkim
Panax sokpayensis Sharma & M.K.Pandit	-	ginseng	Sikkim and Darjeeling hills of West Bengal

4.3. METHODS

4.3.1. Inventory of *Panax* in North-Eastern India

Secondary data collection through earlier herbarium records at Central National Herbarium, Kolkata (CAL), Itanagar (ARUN), Shillong (ASSAM), and Chinese National Herbarium, Institute of Botany, Beijing (PE), floras, published reports and primary information from local people and traditional healers on distribution were collected. Extensive field surveys were undertaken in the entire north-eastern region to validate the secondary data and primary information and Secondary data were converted into coordinates through field survey and additional data points (coordinates) were collected for *Panax* locations. The data was used for Ecological Niche Modelling of which some data were used in testing and training. After the model predicted the potential areas, *Panax* survey was carried out based on model predictions after dividing the potential predicted area into 1 x 1 km^2 grid areas and new populations were identified. The populations were present in Meghalaya, Manipur, Nagaland and Arunachal Pradesh. The overall survey was done during 2009-2012.

4.3.2. Phenology

Phenological studies are as important to our understanding of species interactions and community function as the spatial aspects are. Phenology was studied over a period of 3 years from 2010-1012 in Meghalaya, Manipur, Arunachal Pradesh and Nagaland. Phenological events, e.g., emergence and establishment of seedlings, flowering time, fruiting time and seed set were recorded.

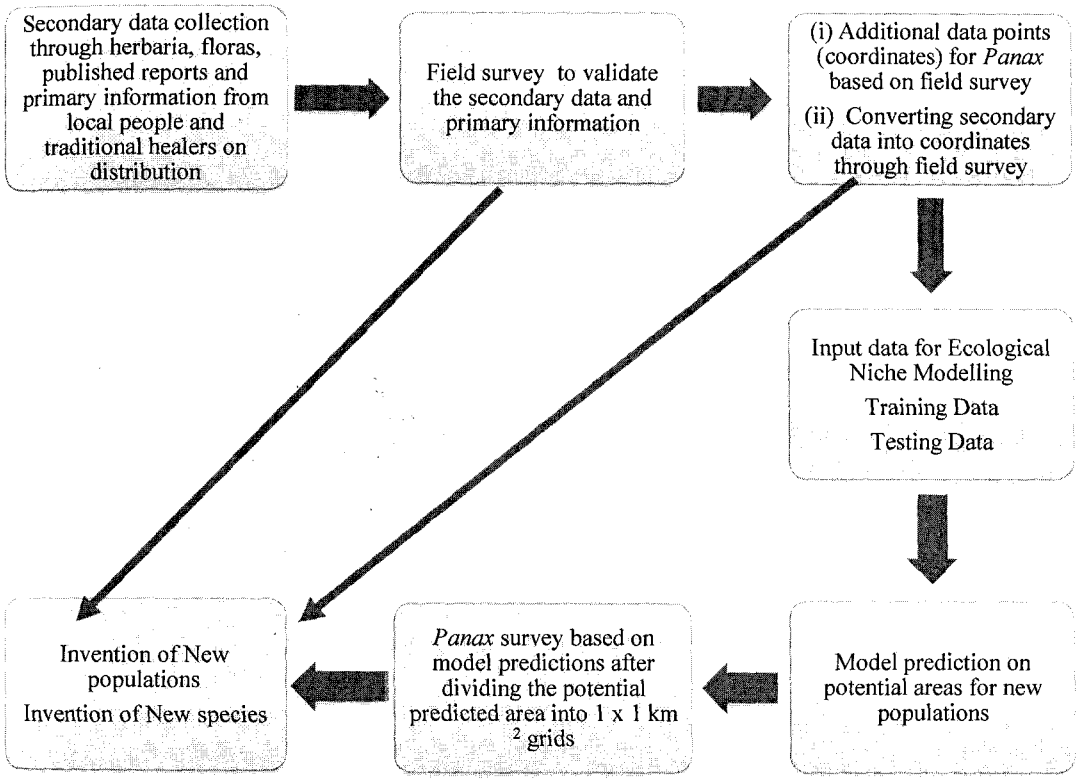


Fig. 4.2: Flowchart representing the protocol for *Panax* inventory.

4.4. RESULTS

4.4.1. Taxonomic description, ecology and phenology of *Panax* species from North-eastern India

4.4.1.1. *Panax assamicus* Banerjee

Tall, stout herb of 50-125 cm tall. Tuber horizontal, creeping and elongated with thick internodes, unbranched. Stem straw coloured, stout, erect, glabrous. Leaves whorled at the summit of the stem, exstipulate, digitately 5-7 foliate, 12-36 cm long, petiolate; petioles glabrous, stout, angular, 7-18 cm long; leaflets petiolulate, narrowly lanceolate, linear to broadly elliptic, 6-18 cm long, 1.2-3.0 cm wide, long acuminate, acumination up to 2 cm long, both surfaces setose on veins and midribs, base rounded, rarely attenuate, minutely uniformly serrate, midrib regular, rarely oblique, petiolules 0.3-1.8 cm long, lateral ones shorter than the rest. Inflorescence a terminal umbel, sometimes 2-6 umbels, glabrous, 10-38 cm long, pedunculate, glabrous, stout, sometimes whorled at the top of the rachis; umbels 40-60 flowered, 2-4 cm in diameter; bracteoles linear, persistent, glabrous, 1.2 cm long. Flowers greenish-white in bud, bisexual, 1.2 cm long. Calyx green, cuplike obscurely toothed, teeth less than 1mm long, alternate to petals. Corolla polypetalous; petals 5, glabrous, oblong one-nerved, inflexed apiculate. Stamens 5, alternate with petals, filaments filiform, 2 mm long; anthers oblong, bilobed, dorsifixed; ovary inferior. Stigma 2-4, rounded. Fruit red, globose with a black tip (Fig. 4.3, 4.4).

Ecology

Elevation: 1632-2251m asl.; ***Forest type:*** Subtropical broadleaved and temperate forest.; ***Soil texture and reaction:*** Loamy sand-sandy and prefers acidic soil; ***Habitat:*** Prefers shady and moist areas; ***Associated species***: Grows mostly along with ferns (Fig. 4.3 a, 4.4 a), herbs which includes *Hedera helix*, *Oplismenus burmanii*, *Impatiens* sp., *Tetrastigma*

serrulatum, *Viola sikkimensis*, *Arisaema* sp., *Clintonia* sp., *Hydrocotyl asiatica*, *Hedychium coccineum*, *Ophiopogon* sp., *Crassocephalum* sp., *Pilea umbrosa*, *Elatostema* sp. etc.

Phenology

The re-emergence of seedling took place during early-April up to late-June. The seedling establishment occurred between early-May and early-September. Flowering occurred during mid-June and continued up to late August. The fruiting period started from late-July and extends up to mid-October. Seed setting started during late-August and continued till early-October. Most of the plants withered during late-September to October. The phenology in both the populations from Meghalaya and Manipur differed in their phenophase timings by 15-30 days (Table 4.2, 4.3).

Fig. 4.3: *Panax assamicus* (Meghalaya): Whole plant (a); herbarium specimen (b); Inflorescence (c); rhizome (d); fruit (e).

Table 4.2: Phenology of *Panax assamicus* from Meghalaya (e = Early; m = Mid; l = Late).

	Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL CYCLE	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
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E																									■	■	■										

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

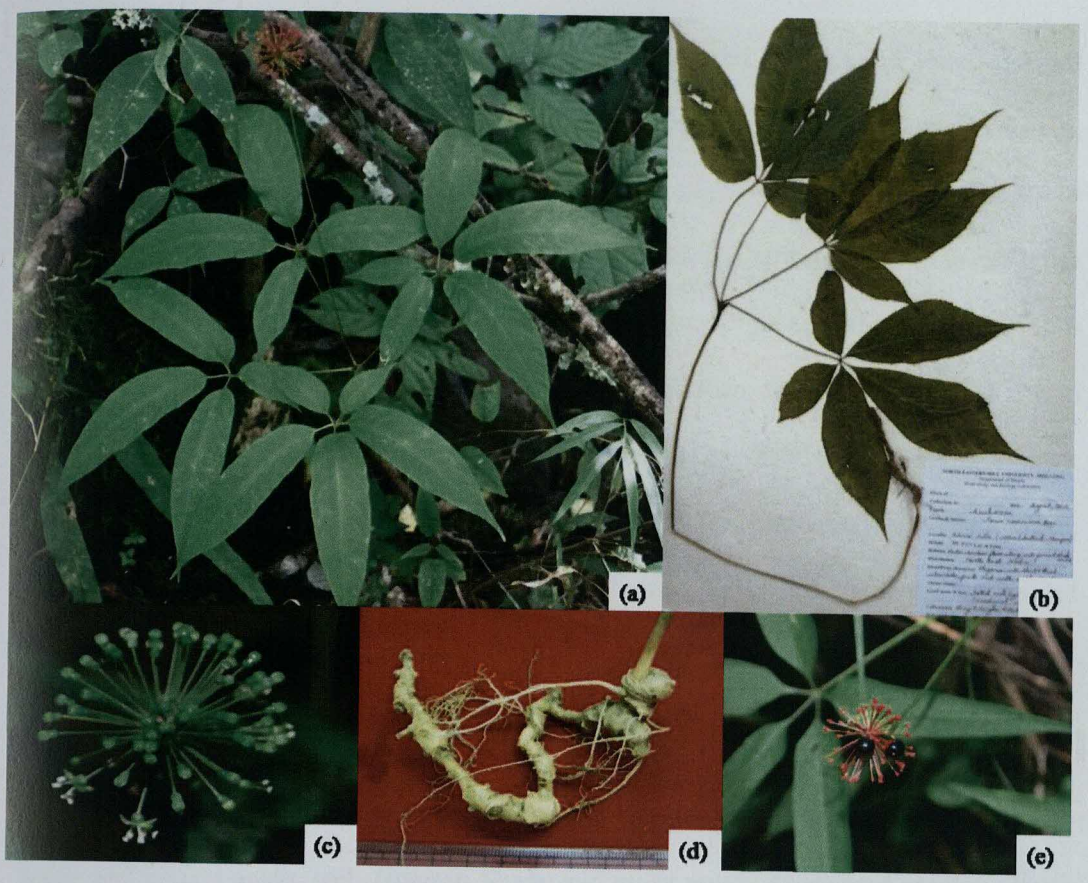

Fig. 4.4: *Panax assamicus* (Manipur): Whole plant (a); herbarium specimen (b); Inflorescence (c); rhizome (d); fruit (e).

Table 4.3: Phenology of *Panax assamicus* from Manipur (e = Early; m = Mid; l = Late).

	Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL CYCLE	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
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C																■	■	■	■	■	■	■	■	■													
D																						■	■	■	■	■	■	■	■	■							
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(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.4.1.2. *Panax bipinnatifidus* Seeman

Perennial herb, 30-50 cm tall. Rhizome creeping and elongated, with slender internodes and subglobose nodes. Stem straight, slender, terete, glabrous, bearing whorl of 3-5 leaves, leaflets narrowly elliptic to broadly elliptic, sometimes slightly cleft to bipinnatifid, 12-19 cm long, petiolate, petioles glabrous, terete 5- 13 cm long, lanceolate acuminate, lobes serrated. Inflorescence terminal, solitary, simple, bearing single umbel at the top or 2-5 clustered, 25-45 flowered, peduncles 8-14 cm long, glabrous, terete, articulated. Flowers green, bisexual, actinomorphic, bracteate, bracts caducous. Petals 5, triangular, Calyx cupular, sepals obscurely toothed; teeth 5, caducous, valvate. Stamens 5, anthers bilobed, dorsifixed, Ovary inferior, styles 2-3, erect. Fruits berries, red with a black tip, 1-2 seeded (Fig. 4.5).

Ecology

Elevation: 1500–3210 m asl.; ***Forest type:*** Temperate forest; ***Soil texture and reaction:*** Sandy and prefers acidic soil; ***Habitat:*** Shady and moist areas; ***Associated species***: Grows mostly along with ferns and herbs viz., *Oplismenus compositus, Anaphalis margaritaceae, Elsholtzia strobilifera, Fragaria nubicola, Calanthe tricarinata, Pilea umbrosa* etc.

Phenology

The re-emergence of seedlings took place during late-march up to late-May. The seedling establishment occurred between late-April and early-August. Flowering occurred during early-June and continued upto late-July. The fruiting period started from early-July and extended up to early-September. Seed setting started during early- August and continued till late-September. Most of the plants withered during late-September to October (Table 4.4).

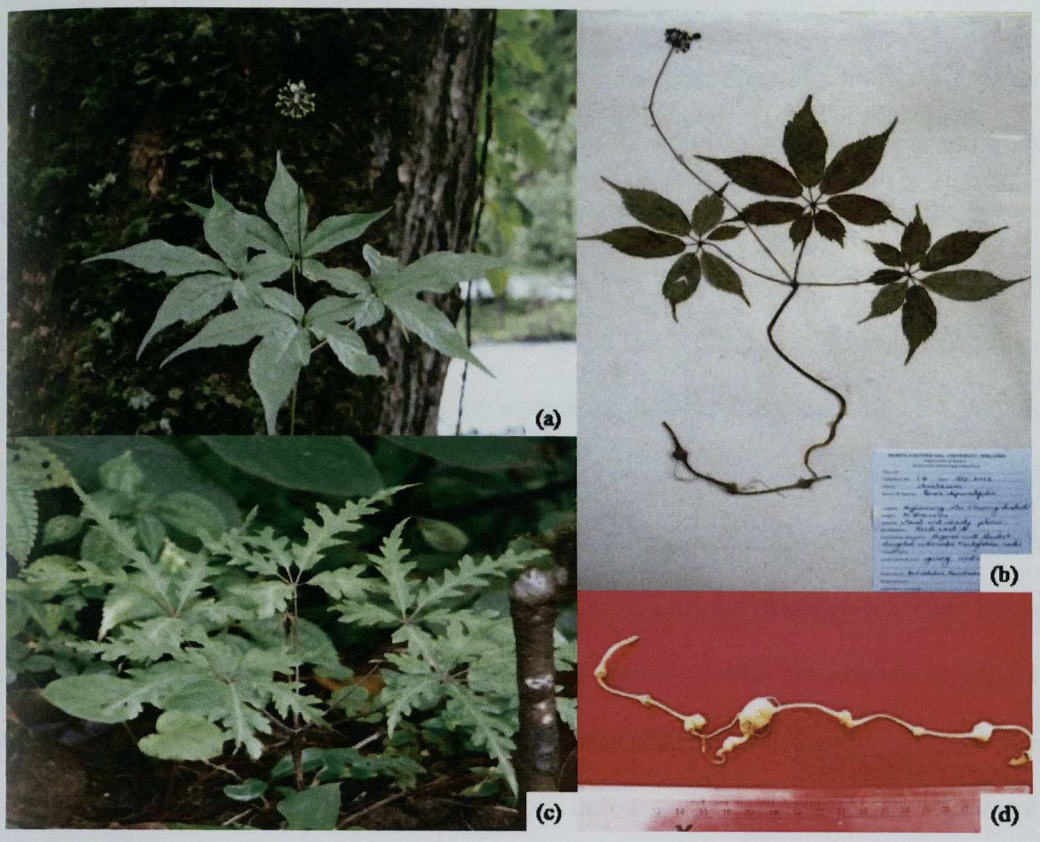

Fig. 4.5: *Panax bipinnatifidus* (Arunachal Pradesh): Whole plant (a,c); herbarium specimen (b); rhizome (d).

Table 4.4: Phenology of *Panax bipinnatifidus* from Arunachal Pradesh (e = Early; m = Mid; l = Late).

	Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL CYCLE	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
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(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.4.1.3. *Panax* sp. (possibly a new species to be confirmed)

A perennial herb, 30-70 cm tall. Rhizome horizontal, bamboo like upto 20-30 cm long and 2-5 cm broad with prominent stem scars and ring at the nodes. Internodes short and thick; dark brown coloured roots tuberous decayed or detached at later stage. Adventitious roots reaching up to 17 cm deep. Stem cylindrical, erect, dark greenish coloured, glabrous, stout, 23 -55 cm long, greenish membranous scales at the base, deciduous. Leaves exstipulate, palmately compound, whorled towards the stem apex, 3-4 per stem, digitately 5-9 foliate , 9-26 cm long, petiolate, petioles glabrous, stout, 6 -14 cm long. Leaflets petiolulate rarely sessile, membranaceous, oblong-obovate, lanceolate, sometimes narrow to broadly elliptic, 7-14 cm long and 2-4 cm wide, apex long narrowly acuminate, acumination upto 1.7 cm long, base slightly rounded, attenuate and oblique, densely setose on adaxial surface and lesser on the abaxial surface, margins biserrated, unicostate reticulate venation. Petiolules 0.6 -3 cm long, lateral ones shorter than the rest. Inflorescence a terminal umbel, glabrous, peduncles longer than petiole 7-16 cm umbels. Flowers 60-70 per umbel, greenish white in bud, bracteate, actinomorphic, epigynous, 0.6-1.2 cm long, pedicellate, pedicels glabrous, 1.4-2 cm long. Sepals 5, lanceolate, glabrous, green, cup like obscurely toothed, alternate to petals, gamosepalous; petals 5, polypetalous, lanceolate, ca. 2-3 mm long, darkish to light green in colour, glabrous, one nerved; stamens 5, alternate with petals, 3-4 mm long, filaments free, anthers oblong, dorsifixed, bilobed; Carpels 2 free, ovary inferior, stigma bifid. Fruit a berry, 2-3 lobed in transverse section, subglobose 4-5mm in diameter, lower portion red, upper portion black when matured. Seeds 2-3, kidney-shaped, creamy white, 8-10 mm long and 4 mm wide (Fig. 4.6, 4.7).

Ecology

Elevation: 1500 – 1988 m asl; ***Forest type:*** Temperate and subtropical broadleaved forest; ***Soil texture and reaction:*** Loamy sand-sandy and prefers acidic soil; ***Habitat:*** Shady and moist areas; ***Associated species***: Grows mostly along with ferns like *Dicranopteris* sp., and herbs viz., *Commelina benghalensis, Elatostema* sp., *Piper thomsonii, Musa* sp., *Rubia* cordifolia, *Arisaema tortuosum, Vitis* sp., *Phrynium capitatum, Athyrium* sp., *Asplenium nidus, Costus speciosus,* etc.

Phenology

The re-emergence of seedling took place during late-March up to late-May. The seedling establishment occurred between late-April and early-August. Flowering occurred during late-May and continued up to late-July. The fruiting period started from early-July and extended up to late-September. Seed setting started during mid-August and continued till early-October. Most of the plants withered during late-September to October. The plants from both the populations (Nagaland and Arunachal Pradesh) differed in their phenophase timings by 15 days (Table 4.5, 4.6).

Fig. 4.6: *Panax* sp. (Nagaland): Whole plant (a); herbarium specimen (b); Inflorescence (c); seed (d); rhizome (e).

Table 4.5: Phenology of *Panax* sp. from Nagaland (e = Early; m = Mid; l = Late).

	Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
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(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

Fig. 4.7: *Panax* sp. (Arunachal Pradesh): Whole plant (a); herbarium specimen (b); inflorescence (c) rhizome (d) fruit (e).

Table 4.6: Phenology of *Panax* sp. from Arunachal Pradesh (e = Early; m = Mid; l = Late).

	Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
CYCLE A																																				
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(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.4.1.4. *Panax pseudoginseng* Wallich

Tall, stout herb of 30-60 cm tall. Rhizome fusiform, short, elongated with thick internodes, unbranched, tubers single or fascicled. Stem, stout, erect, glabrous Base of aerial stem with persistent glabrous and membranaceous scales. Leaves 3-4 verticillate at apex of stem, exstipulate, digitately 5-7 foliate, 14-21 cm long, upper surface with bristly hairs along veins and veinlets, lower surface less, petiolate; petioles glabrous, stout, angular, 7-11 cm long, leaflets petiolulate, lanceolate to narrowly elliptic, 6-10 cm long, 1.5-3.0 cm wide, long acuminate, acumination up to 1.2 cm long, base attenuate, margins biserrated, midrib regular, rarely oblique, petiolules 0.5-1.0 cm long, lateral ones shorter than the rest. Bases of petiole and petiolules with numerous lanceolate, stipulelike appendages, obovate-elliptic to obovate-oblong. Inflorescence a terminal umbel with 1-3 umbel, glabrous, pedunculate, 7-11 cm long glabrous. Flowers bisexual each 40-60 flowered. Calyx green, cuplike obscurely toothed, teeth less than 1mm long, alternate to petals. Corolla polypetalous; petals 5, glabrous, oblong one-nerved, inflexed apiculate. Stamens 5, alternate with petals, anthers oblong, bilobed, dorsifixed; ovary inferior, 2-carpellate. Stigma 2-4, rounded. Fruit red, globose, 1-2 seeded (Fig. 4.8).

Ecology

Elevation: 1744 – 1883m asl; ***Forest type:*** Temperate forest; ***Soil texture and reaction:*** Loamy sand-sandy and prefers acidic soil; ***Habitat:*** Shady and moist areas; ***Associated species***: Grows mostly along with ferns and herbs viz., *Begonia* sp., *Arisaema tortuosum, Eupatorium* sp., *Pilea umbrosa,* etc.

Phenology

The re-emergence of seedling took place during mid-April up to early-June. The seedling establishment occurred between mid-May and mid-July. Flowering occurred during late-May and continued up to mid-July. The fruiting period started from late-June and extended up to late-August. Seed setting started during mid-July and continued till mid-September. Most of the plants withered during late-September to October (Table 4.7).

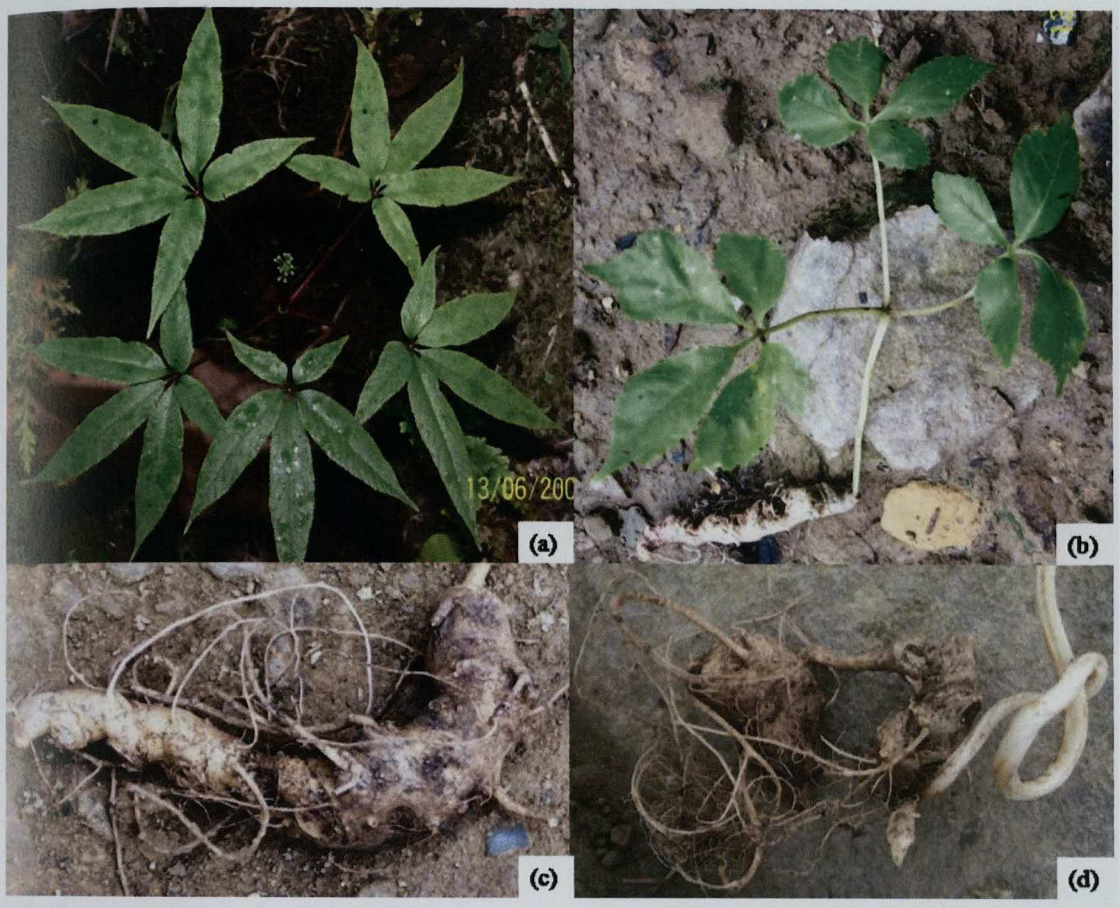

Fig. 4.8: *Panax pseudoginseng* (Nagaland): Whole plant (a,b); rhizome types (c,d).

Table 4.7: Phenology of *Panax pseudoginseng* from Nagaland (e = Early; m = Mid; l = Late).

	Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
CYCLE A																																				
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C																																				
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E																																				

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.4.1.5. *Panax variabilis* J. Wen

Tall, stout herb of 30-65 cm tall. Horizontal tuber, elongated with thick internodes, unbranched. Stem green coloured, erect and glabrous. Leaves whorled at the summit of the stem, exstipulate, digitately 5-6 foliate, 15-30 cm long, petiolate; petioles glabrous, stout, angular, 7-14 cm long; leaflets petiolulate, obovate to broadly elliptic, 7-13 cm long, 1.5-2.6 cm wide, long acuminate, acumination up to 2 cm long, base rounded to cuneate, sometimes oblique, midrib regular, biserrated, both surfaces setose on veins and midribs; petiolules 0.5-1.7 cm long, lateral ones shorter than the rest. Inflorescence terminal, solitary, simple, bearing single umbel at the top or 2-3 clustered, 45-60 flowered; pedunculate, glabrous, stout, 13-24 cm long, bracteoles linear, persistent, glabrous. Flowers greenish-white in bud, bisexual, 1.2 cm long. Calyx green, cuplike obscurely toothed, alternate to petals. Corolla polypetalous; petals 5, glabrous, oblong one-nerved, inflexed apiculate. Stamens 5, alternate with petals, anthers oblong, bilobed, dorsifixed; ovary inferior. Fruit red with a black tip, globose (Fig. 4.9).

Ecology

Elevation: 1581-1945m asl.; ***Forest type:*** Temperate forest; ***Soil texture and reaction:*** Sandy and prefers acidic soil; ***Habitat:*** Shady and moist areas; ***Associated species***: Grows mostly along with ferns which includes *Hedera helix*, *Tetrastigma serrulatum*, *Oplismenus compositus*, *Impatiens* sp., *Pesicaria chinensis.*, *Boehmeria* sp., *Pilea umbrosa* etc.

Phenology

The re-emergence of seedling took place during late-March up to late-May. The seedling establishment occurred between late-April and mid-July. Flowering occurred during late-May and continued up to early-July. The fruiting period started from late-June and continued up to mid-August. Seed setting started during late-July and continued till mid-September. Most of the plants withered during late-September to October (Table 4.8).

Fig. 4.9: *Panax variabilis* (Manipur): Whole plant (a); herbarium specimen (b); Inflorescence (c); rhizome (d).

Table 4.8: Phenology of *Panax variabilis* from Manipur (e = Early; m = Mid; l = Late).

	Jan			Feb			Mar			Apr			May			Jun			Jul			Aug			Sept			Oct			Nov			Dec		
ANNUAL CYCLE	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l	e	m	l
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B													■	■	■	■	■	■	■	■																	
C																■	■	■	■	■																	
D																			■	■	■	■	■														
E																						■	■	■	■	■											

(A= Emergence of seedlings; B= Establishment of seedlings; C= Flowering; D= Fruiting; E= Seed set)

4.4.1.6. *Panax sokpayensis* Sharma & M.K. Pandit

A perennial herb, 80-130 cm tall. Rhizome simple, horizontal, 20-60 cm long with prominent stem scars and rings at nodes; internodes short and thick; tuber single, globose in each rhizome which often decays in old individuals. Stem cylindrical, erect, stout, 50-90 cm long, scales at base, deciduous. Leaves exstipulate, palmately compound, 4-5 per stem, whorled towards the stem apex, 20-38 cm long, petiolate; petioles glabrous, stout, 10-20 cm long; leaflets 5, oblanceolate to narrowly elliptic, 7-16 cm long, 2.5-5 cm broad; petiolules, 1-2.5 cm long; apex caudate, upto 4 cm long; base rounded, attenuate or oblique; margin serrulate, some biserrulate; densely setose on adaxial surface. Inflorescence a terminal umbel; peduncles stout, 15-30 cm long. Flowers 50-65 per umbel, bracteates, andromonoecious, actinomorphic, epigynous, 3-4 mm long; bracts large, leafy or linear up to 0.5-2.0 cm long, pedicellate; pedicels, 1-2.5 cm long, glabrous; sepals 5, lanceolate, glabrous, alternate to petals, green; petals 5, polypetalous, lanceolate, ca. 3.0 mm long and ca. 2.5 mm wide, glabrous, deciduous, white; stamens 5, 3-4 mm long, filament free; anther oblong, ca. 3 mm long and ca. 2 mm wide, dorsifixed, bilobed; carpels 2-3, free; ovary inferior. Fruit a berry, 2-3 lobed in transverse section, subglobose, ca 0.7 cm long and ca. 0.6 cm wide, lower portion red, upper portion black; seeds 2-3, ovoid, ca. 0.4 mm long and 0.3 mm wide (Fig. 4.10).

Ecology

Elevation: 1700 - 2300 m asl; ***Forest type:*** Sub-montane forest; ***Habitat:*** Shady and moist areas; ***Associated species***: Grows mostly along with ferns and herbs which includes *Viburnum erubescens, Daphne cannabina, Urtica parviflora, Gerardiana heterophylla* etc.

Phenology

Seedling emergence and establishment starts in the month of March which ends in May. The plant starts flowering in the month of April and extends to May, fruiting starts from August and continues till September. Seed setting starts during August till October. The plants withered during September to October.

4.5. DISCUSSION

Araliaceae members are distributed mainly in the tropics and subtropics especially in eastern Asia, southern Asia, southeastern Asia and the Pacific islands, with some genera occurring in the temperate zone which includes North America (Wen *et al.*, 2001). The species growing in North America are distributed with an altitudinal range of 120 - 1,300 m. In eastern Asia, *Panax* species are found in the range of 800 - 4,000 m asl. In southern Asia which includes northeastern India, the Himalayan ginseng are distributed at higher altitudinal range i.e. 1,516 – 3,210 m.

All the species of *Panax* recorded from North-eastern India viz., *P. assamicus, P. bipinnatifidus, Panax* sp. (possibly a new species), *P. pseudoginseng, P. variabilis, P. sokpayensis* and *P. sikkimensis* have restricted distributions and are mostly restricted to North-East India only. *P. pseudoginseng* extended its distribution to Nepal. *P. assamicus* is distributed in two states of the region i.e. Meghalaya and Manipur. *Panax* sp. is in Arunachal Pradesh and Nagaland. These species are quite distinct in their morphological characters but bears high resemblance in many morphological characters. *Panax* demonstrates a high level of morphological variation, which was also observed in all the Himalayan species. This has created serious problem in the past in establishing the taxonomic identity within the genus in Himalayas.

All *Panax* species in Himalayas are medicinal and have a ready high value market in nearby countries. Due to this property, most of the matured individuals have been harvested from the wild during the past few years. During the present study, over-harvesting, deforestation, forest fragmentation and other anthropogenic activities were identified as factors responsible for making the species threatened. The plants from the forests of northeastern India are reported to be exported to Bhutan, Myanmar and China.

Species of *Panax* vary in terms of persistency of tap roots. For example, the roots of *P. japoinicus*, *P. vietnamensis* and *P. wangianus* are decayed or detached at a later stage. The shape of roots also varies among the species. Three types of rhizomes are recognizable based on the works of Hara (1970). The type 1 rhizome consists of upright type (< 5cm long) and includes *P. ginseng*, *P. pseudoginseng*, *P. quinquefolius* and *P. trifolius*. The type 2 rhizome consists of horizontally elongated rhizomes with thick and short internodes which includes *P. japonicus*, *P. stipuleanatus*, *P. vietnamensis*, *P. wangianus* and *P. zingiberensis*. Horizontally elongated rhizomes with slender and elongated internodes and subglobose nodes falls under type 3 rhizomes which includes *P. bipinnatifidus*. All the three types of rhizomes are illustrated by Hara (1970). These species also vary in the number of leaves, presence or absence of stipules, leaflet shape, pubescence, leaflet division, as well as the number of flowers per inflorescence. The peduncle of *P. quinquefolius* is shorter than its petioles which is different from other species of *Panax* where it is longer than their petioles. Wen (2001a) and Shu (2007) reported that the number of prongs was 1-5 in *P. quinquefolius* and ranges from 3-7 prongs in other species. In *P. quinquefolius,* the maximum number of leaflets was 20 (Anderson *et al.*, 1993). In *P. ginseng, P. japonicus, P. zingiberensis*, *P. notoginseng* and *P. pseudoginseng*, it ranged from 9–42 leaflets (Shu 2007; Wen, 2001a). *P. ginseng, P. notoginseng* and *P. quinquefoilus* bears hermaphrodite flowers; *P. trifolius* is sex-changing and dioecious plant in which male and female umbel consists of 15–30 flowers and 3–14 flowers respectively (Wen 2001a). *P. bipinnatifidus, P. pseudoginseng, P. vietnamensis* and *P. wangianus* are polygamo-monoecious. Shu (2007) and Wen (2001a) reported that the numbers of flowers per plant ranged from 20 to 100 in *P. ginseng*, *P. japonicus*, *P. notoginseng* and *P. pseudoginseng*. The ovary is 2-locular in *P. ginseng* and *P. quinquefolius*, 3-locular in *P. trifolius* and 2-3 locular in other species of *Panax*. Fruits of *P. ginseng, P. notoginseng, P.*

pseudoginseng and *P. quinquefolius* are bright red; in *P. trifolius* the fruits are yellow; but in other species the fruits are red with a black tip.

Ginseng is best described as a widespread but scarce understory plant. For plants which have been harvested heavily, particularly in areas where it was formerly abundant exhibit such distribution and this has important implications for management of the species. *P. quinquefolius* have a somewhat unusual form of rarity in that they have a broad distribution, can occur in a variety of forest community types, aspects and elevations, and yet they are not abundant anywhere (Rabinowitz, 1981).

In *P. quinquefolius,* Lewis and Zenger (1983) observed that four-prong and five-prong plants represented the oldest individual of a population. Similar observations were reported by using the combination of leaf area and leaf number which are good indicators of age (Furedi, 2004; McGraw and Furedi, 2005). Lewis and Zenger (1982), Carpenter and Cottam (1982); Lewis (1984); Anderson *et al.* (1993) and Gagnon (1999) reported that the height of aerial shoot, the number of leaves and leaflet and leaflet area increased with age. Therefore, the number of bud scars on the rhizome was a good predictor of age in *P. quinquefolius*.

The phenological events in Himalayan *Panax* species extended over a short period of time starting from late March and continued till October. There was overlapping period in all the phenophases but multiple comparison reveals the difference of 15-30 days interval in the onset or ending of a particular phenophase especially with plants from different geographical regions. The phenological events are also studied in *P. quinquefolius* (Proctor *et al.*, 2003) which revealed similar events in all the phenophases.

CHAPTER 5

NICHE CHARACTERISATION AND DISTRIBUTION MAPPING OF *PANAX* SPECIES OF NORTH-EASTERN INDIA USING ECOLOGICAL NICHE MODELS

5.1. INTRODUCTION

The niche concept has been used both as a tool for systematically describing the major environmental variables influencing the distribution and abundance of single species (Maguire, 1973; James *et al.,* 1984), and as a device for understanding inter-specific interactions and community structure (Pianka, 1981). Several aspects relating to niche concept such as niche breadth and niche overlap have been formulated and studied by researchers during the past decades (Hutchinson, 1959; MacArthur, 1972; Pianka, 1973; Schoener, 1974; Fox, 1981; Tokeshi, 1986).

In the recent years, research studies have expanded the context of niche theory to address important questions about species abundance and distribution (Pulliam, 2000), diversity–productivity relationship (Tilman *et al.*, 2001), community stability (Tilman *et al.*, 1997), ecosystem functioning (Loreau, 2000), and biological invasions (Peterson and Vieglais, 2001).

Niche has been mostly characterized as measures of resource utilization (Pianka, 1983; Giller, 1984). More recent perspectives on niche concept highlight its vital importance in understanding community organisation (Chase and Leibold, 2003). Characterization of the environment where known populations occur can partially describe a species' niche (Anderson *et al.*, 1993). It is well-known that species differ in their niche positions in ecological gradient space as depicted through Hutchinsonian n-dimensional niche concept (Hutchinson, 1957; Whittaker *et al.*, 1973). Despite this recognition, many earlier studies

on niche and community organisation were based on either spatial overlap in distribution or overlap in food resource use (reviewed by Tokeshi, 1999; Chase and Leibold, 2003; Vazquez and Stevens, 2004).

Research has become increasingly focused on the extrapolation of species distribution from incomplete data to obtain reliable distribution maps most efficiently (Mitchell, 1991; Pereira and Itami, 1991; Buckland and Elston, 1993; Iverson and Prasad, 1998; Manel *et al.*, 1999 a,b; Parker, 1999; Peterson *et al.*, 1999; Pearce and Ferrier, 2000; Vayssie`res *et al.*, 2000; Hirzel *et al.*, 2001; Guisan *et al.*, 2002; Hortal and Lobo, 2002; Ferrier *et al.*, 2002). By processing environmental information and presence/absence data, several statistical methods can provide estimates of the probability of occurrence of a given species (Guisan and Zimmermann, 2000). The factors governing the distribution of species have been developed using habitat distribution modeling or ecological niche modeling (ENM) (Guisan and Zimmermann 2000; Elith *et al.*, 2006; Kozak *et al.*, 2008). ENM considers environmental factors as ecological conditions e.g., temperature, precipitation, soil, vegetation and land cover, and uses the datasets from Geographic Information System (GIS) databases such as www.worldclim.org and www.diva-gis.org.

Availability of high resolution satellite imageries, downscaling tools for environmental variables, and interpolated spatial datasets on climate and vegetation has increased the accuracy of model prediction. ENM alleviates interpolation as well as extrapolation of species distributions in geographic space across different time periods. This has made it possible to develop species distribution maps with high level of statistical confidence and identify areas suitable for reintroduction of threatened species (Irfan-Ullah *et al.*, 2006; Martinez-Meyer *et al.*, 2006; Papes, 2006; Kumar and Stohlgren, 2009; Ray *et al.*, 2011; Moran-Ordonez *et al.*, 2012).

through ecological niche modelling. It uses computer algorithms to generate predictive maps of species distribution in a geographic space by correlating the point distributional data with a set of environmental raster data. ENM is widely used in many ecological applications (Elith *et al.*, 2006; Peterson *et al.*, 2006) including modelling of species distribution in terrestrial, freshwater and marine environments (Elith and Leathwick, 2009). Through ENM approach, it is possible to identify areas that are suitable for the conservation of a species (Irfan-Ullah *et al.*, 2006; Papes, 2006), discovery of new sister species (Raxworthy *et al.*, 2003) as well as reintroduction of threatened category of species (Martinez-Meyer *et al.*, 2006). Prediction and mapping of potential suitable habitat for threatened species is critical for restoration of their declining native populations in their natural habitat, artificial introductions, or selecting conservation sites, and conservation and management of their native habitat (Gaston, 1996). Habitat modeling approach is difficult due to the sparse and clustered distribution data on threatened species (Ferrier *et al.*, 2002; Engler *et al.*, 2004).

The main objectives in the present study are:

1. Characterization of ecological niche of different species of *Panax*
2. To map the potential distributional areas of each species
3. To use ecological niche modelling as a tool to seggregate the species of *Panax*

5.2 METHODS

5.2.1. Niche characterization

5.2.1.1. Characterization of niche through measurement of forest microclimatic factors

The microclimatic factors studied were: relative humidity, air temperature, soil temperature and light intensity, which were measured 1 m above the forest floor on the particular day. The measurements were taken at 30 minutes interval at five random points in each area on

the measurement day. Light intensity was measured by a Digital Luxmeter (TES 1332A), atmospheric temperature and relative humidity by a Thermohygrometer Th-103 (Mex-therm), and soil temperature was measured using digital soil thermometer (Multi Thermometer).

5.2.1.2. Characterization of niche through measuring soil physico-chemical properties

In order to characterize the niche, microclimatic and edaphic conditions of the habitats of *Panax* were studied during the study period i.e. 2008-2012.

Soil sampling and processing

Soil samples were collected from each study site during the pre-monsoon and post-monsoon seasons for three years during 2009-2011. For one time analysis, soil samples were also collected from all the places of North-east where *Panax* species were present. Five replicate soil samples were randomly collected from each site using a steel corer (6.5 cm diameter) from the surface layer (0-10 cm depth). The replicated soil samples were mixed thoroughly to obtain one composite sample for each site. The analyses were performed within 24 hours of sample collection using a portion of this soil sample for those parameters which need fresh samples for analysis. These parameters were, soil moisture content and pH. The remaining portion of the samples was then air dried and sieved through 2.0 mm sieve, and stored in polythene bags for analyses of other physico-chemical parameters.

The soil physical parameters studied were: soil moisture content (SMC), soil texture, water holding capacity (WHC), bulk density (BD) and porosity. Soil chemical properties include, soil pH, soil organic carbon (SOC), soil organic matter (SOM), available phosphorus (Avail. P), exchangeable potassium (Exchng. K) and total Kjeldahl nitrogen (TKN). The

macro elements studied were, calcium (Ca), magnesium (Mg) and Iron (Fe); and micro elements viz., total manganese (Mn), zinc (Zn) and copper (Cu).

Soil Physical Properties

Soil texture

Soil texture was determined by Buouyoucos hydrometer method (Allen *et al.*, 1974). Fifty grams of air dried sieved soil sample was taken in a 500 ml conical flask. Twenty-five ml of 5 % sodium hexametaphosphate was added to it. Then 400 ml of tap water was added and the flask was put on a shaker for two hours at 90-100 rpm. The suspension was then transferred into 1000 ml graduated cylinder, and the volume was made up to the mark. The first reading was recorded with hydrometer after one minute of stirring. First reading of the hydrometer was for silt and clay in the suspension. The soil suspension was allowed to stand undisturbed for five hours, and second hydrometer reading was recorded which corresponds to the amount of clay. If the suspension temperature varied from the room temperature, i.e., 25°C, 0.36 was added to every one degree rise in temperature from room temperature. Similarly 0.36 was subtracted for every degree fall in temperature. Textural class was determined by using the International Society of Soil Science triangle. Soil texture was calculated using the following formulae:

$$\text{Clay (\%)} = \frac{\text{A g}^{-1} \times 100}{\text{50 - moisture weight of soil (g)}} - 1$$

$$\text{Silt +Clay (\%)} = \frac{\text{B g}^{-1} \times 100}{\text{50 - moisture weight of soil (g)}} - 1$$

$$\text{Silt (\%)} = (\text{Silt+Clay})(\%) - \text{Clay (\%)}$$

$$\text{Sand (\%)} = 100 - (\text{Silt + Clay})(\%)$$

(Where, B= Hydrometer readings after 4min 48 seconds; A= Hydrometer readings after 5 hours; 1= Calgon correction factor).

Soil moisture content

Soil moisture content (SMC) was determined by gravimetric method (Allen *et al.*, 1974). Ten g of fresh soil sample was taken in a petri-plate and oven dried at 105^0C till constant weight. Samples were removed from the oven, and when cooled, weights were recorded. Soil moisture content was determined using the following formula:

$$\text{SMC (\%)} = \frac{\text{Fresh soil (g) - Oven dried soil (g)}}{\text{Oven dried soil(g)}} \times 100$$

Bulk density

Bulk density (BD) was estimated by gravimetric method (Allen *et al.*, 1974). Core sampler was pressed into the soil to fill the inner metal cylinder. The sampler was removed ensuring an intact sample. Soil extending beyond each end of the inner cylinder was trimmed, and the remaining soil was pushed from the cylinder into the petriplate. The petriplate was weighed before and after oven drying at 105^0C till constant weight. Bulk density of soil was determined using the formula:

$$\text{BD}(\text{g cm}^{-3}) = \frac{\text{Dry weight of soil (g) - fresh weight of soil (g)}}{\text{volume of metal corer (cm}^{-3})}$$

Porosity

Porosity was calculated using bulk density and particle density value as 2.65 g cm^{-3} (Allen *et al.*, 1974). This value is internationally assumed value since the density of the soil particle in most mineral soil is about 2.6–2.7 g cm^{-3}. Porosity of soil was calculated using the formula:

$$\text{Porosity (\%)} = 1 - \frac{\text{Bulk density}}{\text{Particle density}} \times 100$$

Water Holding Capacity

Water Holding Capacity (WHC) was determined by Keen's box method (Piper, 1942), using copper cups of 5.6 cm internal diameter and 1.6 cm height. Water holding capacity was calculated as follows:

$$\text{WHC (\%)} = \frac{\text{Amount of water in soil}}{\text{Dry weight of soil (g)}} \times 100$$

$$\text{WHC (\%)} = \frac{\text{W2-W3-W4}}{\text{W3-W1}} \times 100$$

(Where, W1=Weight of Keen's cup+dry filter paper; W2= Weight of Keen's cup + saturated filter paper+saturated soil; W3= Weight of Keen's cup+ saturated soil after oven drying; W4= Weight of Keen's cup + saturated filter paper).

Soil chemical properties

Soil pH

A digital pH meter (Professional Meter, PP-20, Sartorius) was used to determine the pH of soil. Fresh soil sample (15 g) was mixed with deionized water in the ratio 1:2.5 w/v. The solution was stirred in a magnetic stirrer for about 15-20 minutes and left overnight. The pH reading was recorded the next day.

Soil organic carbon

Soil organic carbon (SOC) was determined by calorimetric method (Anderson and Ingram, 1993). One gram of air dried, sieved soil was mixed with 10 ml of 5% potassium dichromate solution in a 100 ml conical flask. Twenty ml of concentrated H_2SO_4 was added and kept for 30 minutes for cooling. After cooling, 50 ml of 0.4% $BaCl_2$ was added, mixed thoroughly and allowed to stand overnight so as to leave a clean supernatant. One reagent blank and standard solutions were prepared similarly without adding soil samples.

Transmittance was recorded at 600 nm using Spectrophotometer (Lambda-35, UV/VIS, Perkin Elmer, USA). Soil organic carbon was calculated using the formula:

$$SOC\ (\%) = \frac{K \times 0.1}{W \times 0.74}$$

Where, K= concentration of carbon obtained from standard graph; W = soil sample dry weight.

Soil organic matter (SOM) content was obtained by multiplying the soil organic carbon content by 1.724, assuming that the SOM contains 58% of carbon.

Available phosphorus

Available phosphorus (Avail. P) was determined after extracting soil phosphorus following Bray and Kurtz method (Bray and Kurtz, 1945). Two grams of soil were taken in a 50 ml conical flask and 20 ml of extracting solution (0.025 M HCl in 0.03 M NH_4F) was added to it. The flasks were shaken for five minutes in an Incubator Orbital Shaker (Bangalore Genei, India) at 200 rpm at room temperature (24-27°C). The filtrates were filtered through Whatman No.42 filter paper. Activated charcoal was added for coloured samples to obtain a colourless filtrate and finally filtered through Whatman No.42 filter paper.

Available P was determined following ammonium-molybdate blue method (Allen *et al.*, 1974). Five ml of the aliquot was added into a 100 ml beaker and 10-15 ml of distilled water was added along with 2 ml of ammonium molybdate solution and 2 ml of stannous chloride reagent. The total volume was made up to 50 ml by adding distilled water. One reagent blank and standard solutions were prepared without adding soil samples. Transmittance was recorded at 700 nm after 30 minutes using Spectrophotometer (Lambda-35, UV/VIS, Perkin Elmer, USA). Available phosphorus in soil was calculated using the formula:

$$\text{Avail. P (\%)} = \frac{\text{C (mg) x Solution volume (ml)}}{\text{10 x Aliquot (ml) x soil sample weight (g)}}$$

Where, C = Concentration of P (mg) obtained from standard graph.

Exchangeable potassium

Exchangeable potassium (Exchng. K) was determined by extracting soil in ammonium acetate extractant at pH 7, by adding few drops of acetic acid or ammonium solution (Jackson, 1973). Ten grams of air dried sieved (0.2 mm) soil sample was taken into a 500 ml conical flask and 250 ml of ammonium acetate extract was added. The contents were shaken for six hours on a gyratory shaker at 90-100 rpm, after which it was kept overnight. The contents were shaken again for five minutes the next day and then filtered through Whatman No.44 filter paper. The first 20-30 ml of the filtrate was rejected. Reagent blanks and standard solutions were prepared accordingly without adding soil samples. The samples were analysed using Flame Photometer (Model- 1381E, ESICO). Exchangeable potassium in soil was calculated using the formula:

$$\text{K (\%)} = \frac{\text{C (ppm)} \times \text{Solution volume (ml)}}{10^4 \times \text{Soil sample weight (g)}}$$

Total Kjeldahl nitrogen

Total nitrogen was determined by Kjeldahl digestion method followed by colorimetric analysis (Allen *et al.*, 1974). Ammonium gas-diffusion technique was applied (application notes, AN-5222) for estimation of total nitrogen, using the automated Spectrophotometric Flow Injection Analyser (FIAstar, Model 5000-Analyser, 5027-Sampler, AB, FOSS, Hoganas, Sweden). One gram of air dried sieved soil samples was added with 10 ml concentrated sulphuric acid using FOSS Kjeldahl tablets CU/3.5 (3.5 g K_2SO_4/0.4 g $CuSO_4$ X $5H_2O$) as catalyst, and digested at 360°C for three hours on a Tecator Digestor (Model-20, FOSS, Hoganas, Sweden). Reagent blanks and standard solutions for analysis were

prepared as per the manual. Each batch of samples for digestion contained at least one reagent blank i.e., reagents only, without soil sample. The digested mixture was filtered through Whatman No.1 filter paper and made up to 100 ml volume using distilled water. The samples were analyzed following the application notes for the element as per the manual. Total nitrogen in soil was calculated using the formula:

$$\text{Total N (\%)} = \left(\frac{C \times 0.001 \times 0.1}{W}\right) \times 100$$

Total element

For determination of total element concentrations such as magnesium (Mg), calcium (Ca), iron (Fe), manganese (Mn), Copper (Cu) and zinc (Zn), soil samples were subjected to tri-acid digestion (Allen *et al.*, 1974). One gram of soil sample was transferred to each digestion tube and 15 ml of the tri-acid mixture, prepared in 1:5:0.5 ratio i.e., $HClO_4$ (60%): HNO_3 (conc.): H_2SO_4 (conc.), was added to each digestion tube. The tubes were kept at room temperature overnight until the vigorous reaction phase was over. This was followed by digestion on a block digestor at 150°C for one hour. When all the traces of HNO_3 evaporated (characterized by cessation of emission of brown coloured fumes), the temperature was raised to 250°C for about one hour. The digestion was continued for another 30 minutes until the dense white fumes of $HClO_4$ disappeared from the digestion tube. The digestion was considered completed when clear and colourless digests were obtained. The tubes were allowed to cool at room temperature and the contents were allowed to filter through Whatman No.1 filter paper and made up to 100 ml volume with double distilled water. Each batch of samples for digestion contained at least one reagent blank i.e., only reagents without soil sample.

Aliquots of the digests were taken for analyses of Mg, Ca, Fe, Mn, Cu and Zn using Atomic Absorption Spectrometer (AAS) (AAnalyst, Model-200, Perkin Elmer, USA). Reagent

blanks and standard solutions for each element were prepared according to the instrument manual. All soil analyses were carried out in triplicate and the results presented are mean values of triplicate samples. Dilution factors were applied wherever necessary for every sample dilution and concentration. The results were expressed on a dry weight basis. Mg, Ca, Fe, Mn, Cu and Zn contents in soil were calculated using the formula:

$$\text{Element (\%)} = \frac{\text{C (ppm)} \times \text{Solution Volume (ml)}}{10^4 \times \text{Soil sample weight (g)}}$$

5.2.2. Distribution mapping using Ecological Niche Modelling

The term 'niche' was first coined by Sir Joseph Grinnell (1917) in his classical paper '*The niche relationships of the California Thrasher*'. He emphasized that niche of a species is the sum of the ecological conditions that allows a species to persist and produce offspring. After him, Charles S. Elton (1927) proposed that the ecological niche of a species is characterized by the functional role it plays in an ecosystem. Thereafter, G.E. Hutchinson (1957) theorized ecological niche to be the activity range of a species defined by an n-dimensional hyper volume of all the environmental conditions. The foundation of ecological niche modelling is based on Grinnell's ecological niche concept which has a single focus i.e. the environmental factors, that permits model development. Eltonian, Hutchinsonian and MacArthurian concepts are however more process based which include species functions and biotic interactions, and hence are difficult to fit in a modelling framework. Ecological niche in the light of ENM can be defined as '*.....the set of ecological conditions within which the species is able to maintain its population without immigration*' (after Grinnell 1917).

Species across an evolutionary time scale tend to be in a dynamic equilibrium with its environment and retain aspects of their fundamental niche, a phenomenon called 'niche conservatism' (Wiens and Graham, 2005). The predictive attribute of ENM is based

basically on this assumption. Studies on numerous species across the globe have confirmed that ecological niche modelling of the climatic characteristics of the native range of a species can predict its introduced range. Hence, this attribute of the ENM can be used to identify potential areas for reintroduction of species which are already threatened.

Several ecological niche modelling packages/algorithms are available in the web and most of them can be freely downloaded. Some of the commonly used programs are MAXENT, GARP, DIVA, BIOMAPPER, BIOCLIM, DOMAIN, MODECO, FLORAMAP, Support Vector Machine, Artificial Neural Networks, Generalized Linear Models, Generalized Additive Models, Maximum Likelihood, Classification & Regression Trees, Boosted Regression Trees, Random Forests, Bayesian Methods, and Relative Environmental Suitability.

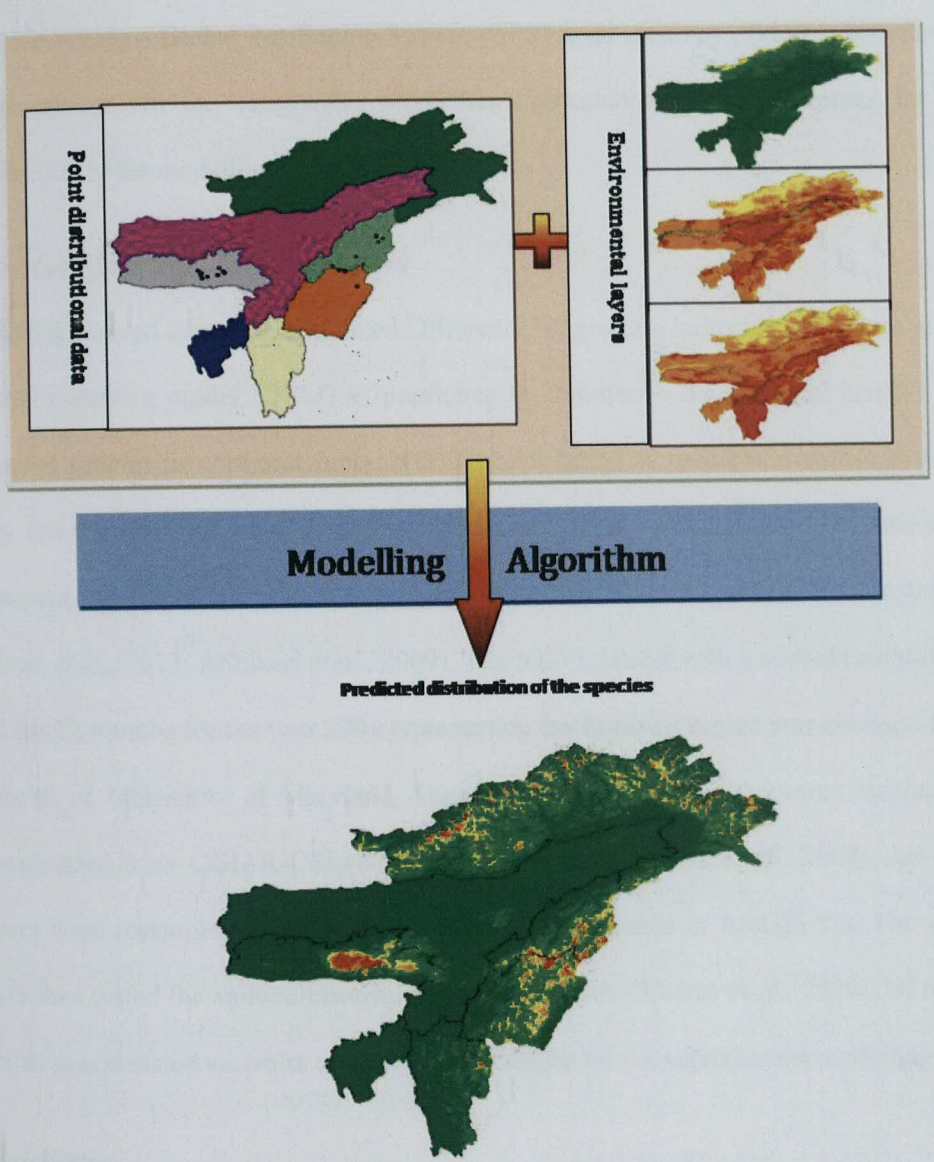


Fig 5.1: Ecological Niche Modelling frame-work.

5.2.2.1. Species occurrence data

The primary distributional records for *P. assamicus*, *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis*, *P. sikkimensis* and *Panax* sp. (new species) were obtained through repeated field surveys in northeast India based on secondary herbarium records and information provided by knowledgeable villagers. The geographic coordinates of the species occurrences were recorded using a Global Positioning System (GPS) to an accuracy of ≈10 metres during the field survey. All the coordinates were then converted to decimal degrees for use in ecological niche modelling.

Selection of environmental predictors

AVHRR derived data on Normalized Difference Vegetation Index (NDVI) were used with digital elevation model (DEM) as predictors to summarize the potential habitats for the selected species in northeast India. NDVI was selected as predictor variable because: (i) they are the manifestations of ecological changes taking place in a region, and (ii) they represent the conditions related to presence of the species more accurately at a finer scale (Singh *et al.*, 2013; Adhikari *et al.*, 2009). The NDVI dataset with a spatial resolution of ≈1 km for 12 months for the year 2003 representing the Eurasian region was obtained from the website of University of Maryland. Digital elevation data of 90 metres resolution was downloaded from CGIAR-CSI (http://srtm.csi.cgiar.org, Jarvis *et al.*, 2008). All the data layers were resampled to a spatial resolution of 500 metres in ArcGIS 9.3. The variables were then tested for multicollinearity using ENM Tools (Warren *et al.*, 2010). Of these, the sets of uncorrelated variables ($r < 0.8$) were retained for ecological niche modelling.

Modelling

Maximum entropy program (MaxEnt version 3.3.3e, Phillips *et al.*, 2006) was used to model the potential distribution of the *Panax* species in northeast India. MaxEnt estimates the maximum entropy probability distribution function to predict the geographic location of

a species based on environmental variables and reconstructs the boundaries of the ecological niche by constraining the probability distribution based on the environmental parameters of the grid-cell presence record (Phillips *et al.,* 2006). MaxEnt was preferred over other algorithms, as it holds a strict mathematical definition, gives a continuous probabilistic output, can simultaneously handle both continuous and categorical environmental data, can investigate variable importance through jackknife procedure, has the capacity to handle low sample sizes, simplicity for model interpretation, and facilitates replicated runs to allow cross-validation, bootstrapping and repeated sub-sampling to test model robustness (Phillips *et al.,* 2006; Pearson *et al.*, 2007; Elith *et al.*, 2011).

Validation of model robustness

For each of the species, we executed 100 replicated model runs through employing cross validation. Here, samples were divided into replicate folds and each fold was used for test data. Other parameters were set to default as the program is already calibrated on a wide range of species datasets (Phillips and Dudik, 2008). From the replicated runs, average, maximum, minimum, median and standard deviation were generated. Model quality was evaluated based on AUC value and the model was graded as follows: poor (AUC<0.8), fair (0.8<AUC<0.9), good (0.9<AUC<0.95) and very good (0.95<AUC<1.0) (Thuiller *et al.,* 2005). Areas with a probability level>0.7 was classified as suitable for occurrence of the species.

5.2.3. Niche breadth and Niche differentiation

5.2.3.1. Levins niche breadth (1968)

The niche breadth (B) was calculated for each parameter following Levins (1968):

$$B = \frac{1}{\sum_{i=1}^{R} Pi^2}$$

where, p_i is the proportion of resource i used and R is the total number of the resource states.

Niche breadth of the selected species and the niche overlap between them was estimated using ENM Tools 1.4.3 (Warren *et al.*, 2010). Niche overlap between different species pairs was measured using Schoener's D statistic (Schoener, 1968), as it is best suited to compare niche overlaps in a geographical space based on species distribution modelling (Rödder and Engler, 2011). The niche overlap values were categorized as: 0-0.2 (limited overlap), 0.2-0.4 (low overlap), 0.4-0.6 (moderate overlap), 0.6-0.8 (high overlap) and 0.8-1.0 (very high overlap) (Rodder and Engler, 2011).

5.2.3.2. Niche differentiation in ecological space

Niche differentiation of the selected species in ecological space was analyzed using principal component analysis (PCA). Here, the averaged ecological niche models for each species were first converted from probabilistic to binary maps distinguishing suitability and unsuitability. Regions with probability level > 0.7 (conservative estimate) was classified as suitable and the rest as unsuitable. These binary maps were then combined with the NDVI layers for 12 months in ArcGIS software. Corresponding NDVI data for each species were extracted from the predicted suitable regions in northeast India.

5.2.4. Statistical Analyses

Statistica version 6, available at www.statsoft.com, Origin Version 7 usable at www.OriginLab.com were used to analyse the data. ANOVA was carried out to test the variation in microclimatic and edaphic factors among different places from where *Panax* species were collected. Multiple stepwise forward regression analysis was carried out to key out the most statistically significant variables that contribute to the presence of *Panax* species. Principal Component Analysis was performed using PAST version 2.17c (Hammer *et al.*, 2001) downloadable at www.softpedia.com and between-groups PCA was run for all the soil data. The environment data used for modeling were also imported and grouped species-wise and between-groups PCA was run for variance-covariance matrix of the NDVI

data for niche visualization. The box-and-whisker plot (or box plot) is an excellent exploratory graph for summarizing the distribution of one continuous variable, possibly broken up into several categories. It is very useful for picking up key aspects of the distribution of samples of modest to very large size.The solid line depicts the median (50^{th} quantile), box depicts the interquartile (25-75^{th} quantiles) range, whiskers depict the range of the data from minimum to maximum value, isolated points or the outliers, and depict the "extreme" values.

5.3. RESULTS

5.3.1. Ecological niche characterization

5.3.1.1. Micro-environmental characteristics

Air temperature (°C)

The range of air temperature for *P. assamicus* was 18-28°C, and that for *Panax* sp. was 23.2-26.6°C and for *P. pseudoginseng* it was 20- 24.9°C. The species with a narrow range of air temperature were, *P. variabilis* with 18-18.6 ^{0}C and *P. bipinnatifidus* with 13.1-14°C (Fig. 5.2, a). One-way ANOVA showed significant variation in air temperature both due to species and study sites ($p< 0.001$) (Table 5.3).

Relative humidity (%)

P. pseudoginseng exhibited a relative humidity range of 76-99%, and the range for *P. variabilis* was 86-96%. *P. assamicus* grows in the range of 58.5-94.9% and *Panax* sp. had a range of 62-79.4%. The lowest relative humidity range was for *P. bipinnatifidus* i.e., 52-60% (Fig. 5.2, b). The variation in relative humidity range among the species ($p<0.001$) and sites ($p<0.05$) was significant (Table 5.3).

Soil temperature (°C)

The soil temperature range was highest for *P. assamicus* (14.8-21.1°C) and *Panax* sp. grows in the range of 16.7-19.2°C. *P. pseudoginseng* had a temperature range of 15.9-8.4°C and the range for *P. variabilis* was 14.8-15.6°C. *P. bipinnatifidus* grows within a narrow range of 10.2-11.4°C (Fig. 5.2, c). The soil temperature varied significantly among the species ($p<0.001$) and sites ($p<0.05$) (Table 5.3).

Light intensity (Lux)

P. pseudoginseng grows in the light intensity range of 650-2,900 Lux while for *P. assamicus* the range was 800-2,500 Lux. *P. bipinnatifidus* and *P. variabilis* occur almost in the same light intensity range of 700-1300 Lux and *Panax* sp. grows in the range of 600-1,640 lux (Fig. 5.2, d). One-way ANOVA showed significant variation in light intensity among the species ($p<0.01$) and study sites ($p<0.001$) (Table 5.3).

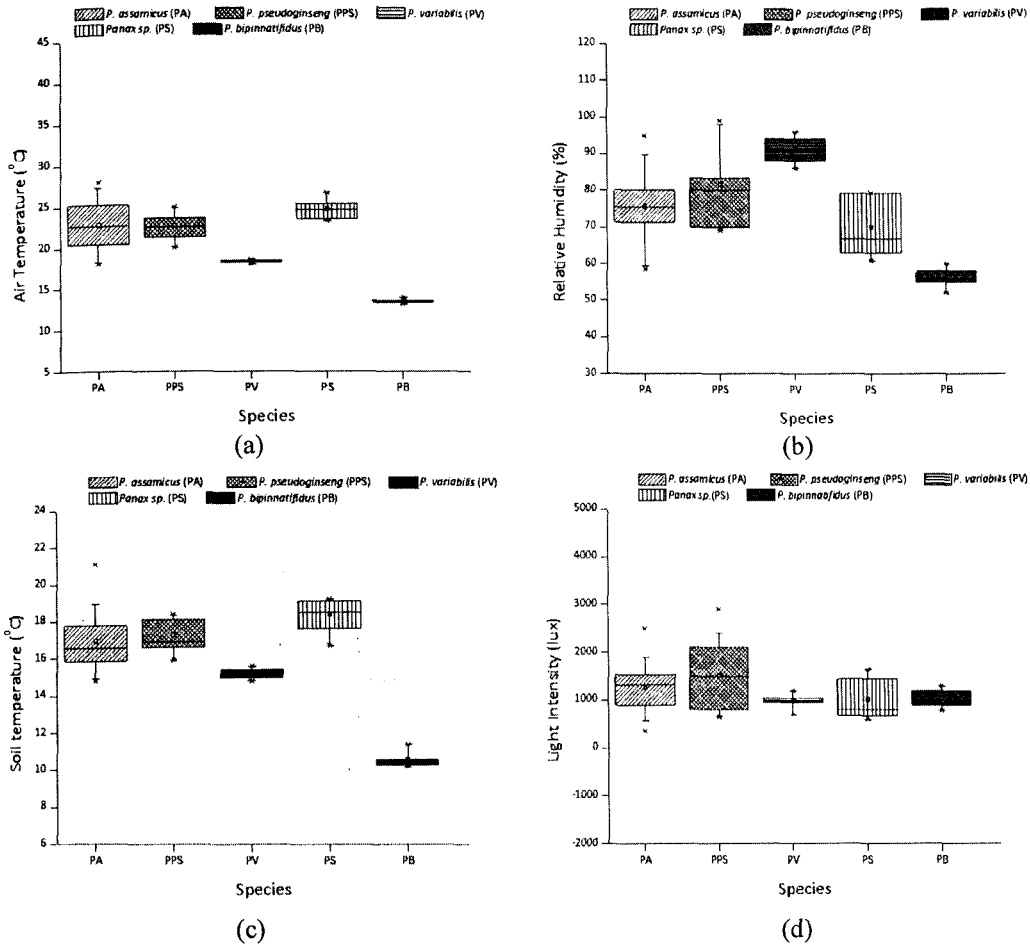


Fig. 5.2 (a-d): Micro-environmental factors in the study sites of *Panax* species. (a) Air temperature (°C); (b) Relative Humidity (%); (c) Soil temperature (°C); (d) Light intensity (Lux). *Box* interquartile range; *upper horizontal line (bar)* uppermost value; *lower horizontal line* lower most value; *solid line within the box* median; *asterisk outside the box* outlier.

5.3.1.2. Soil physico-chemical properties

Soil texture

The texture of the soils where *Panax assamicus* grows in Meghalaya showed a range of textural class from loamy sand to sandy based on the composition of sand (85.5-93.67%), silt (5-10.5%), and clay (1.3-7.7%). For *P. assamicus* from Manipur, the soil texture was sandy with a proportion of sand (91-92%), silt (5–6%), and clay (2–3%). In *P. variabilis*, the soil texture was sandy with sand (92%), silt (5.2–6.4%), and clay (1.8-3%) and in *P.*

pseudoginseng it varies from loamy sand to sandy in texture with sand (86.6-92.6%), silt (4.8–8%), and clay (2.2–2.8%). The soil texture in *Panax* sp. ranges from sandy loamy to sandy with sand (84.6-93%), silt (4.8–10 %), and clay (2.2–5.4%) and in *P. bipinnatifidus*, sand (92.6-95%), silt (3.2–4.4%), and clay (1.8–3%) and textural class being sandy (Table 5.1). One-way ANOVA showed significant variation in the amount of clay, silt and sand due to species ($p<0.05$) and study sites ($p<0.000$) (Table 5.2).

Table 5.1: Soil textural range for different *Panax* species.

Locations	Clay (%)	Silt (%)	Sand (%)	Textural Class
***P. assamicus* (Meghalaya)**				
Nongkrem	4 ±1	10.5±1.89	85.5±0.96	Loamy sand
Upper Shillong	1.67±0.67	6.67±0.67	91.67±0.67	Sand
Laitkor	7.00±1.15	7.33±0.67	85.5±0.67	Loamy sand
Upper laitkseh	1.93±0.38	5.87±0.46	92.20±0.61	Sand
Tyllang	5.67±0.67	8 ±1.15	86.33±0.67	Loamy sand
Mawlangkhar	6.33±0.67	7.33±0.67	86.33±0.67	Loamy sand
Marngor	1.67±0.67	5.33±0.67	93 ±1.15	Sand
Mawlamwir	7.67±1.33	7.33±0.67	85 ±1.15	Loamy sand
Jakrem	5.67±0.67	9.33±0.67	85 ±1.15	Loamy Sand
Mawphlang	1.33±0.28	5 ±0.38	93.67±0.36	Loamy sand
***P. assamicus* (Manipur)**				
Shirui Hills	3.20±0.36	5.00±0.68	91.80±0.80	Sand
Chorcheng	2.20±0.49	6.40±0.75	91.40±0.75	Sand
***P. pseudoginseng* (Nagaland)**				
Waoshu	2.60±0.97	6.80±0.63	90.60±0.97	Sand
Khezhakeno	5.40±0.40	8±1.10	86.6±0.75	Loamy sand
Dzellikii	2.20±0.80	5.20±0.80	92.60±0.75	Sand
Mtsugho	2.80±0.47	4.80±0.33	92.40±0.52	Sand
***P. variabilis* (Manipur)**				
Mao	1.80±0.49	6.40±0.75	91.80±1.02	Sand
Paomata	3.00±0.89	5.20±0.49	91.80±1.02	Sand
***Panax* sp.**				
Bhumbak (Nagaland)	5.40±0.75	10.00±0.89	84.60±0.75	Loamy sand
Dibin (Arunachal Pradesh)	2.20±0.80	4.80±0.80	93±0.89	Sand
***P. bipinnatifidus* (Arunachal Pradesh)**				
Gomkhang	3.00±0.63	4.40±0.40	92.60±0.75	Sand
Dirang	1.80±0.49	3.20±0.49	95±0.63	Sand

Table 5.2: One-way ANOVA showing effect of species and sites on soil textural class.

Parameters	Variation due to	df	F	*P* value
Clay (%)	Species	4	2.705	0.034
	Sites	11	3.510	0.000
Silt (%)	Species	4	5.731	0.000
	Sites	11	2.536	0.007
Sand (%)	Species	4	6.370	0.000
	Sites	11	4.240	0.000

Water holding capacity (WHC)

Water holding capacity of the soil in *P. assamicus* growing areas was in the range of 31-76.8%. The corresponding ranges for *P. bipinnatifidus, P. pseudoginseng, Panax* sp. and *P. variabilis* were 46.8-53.8%, 31.3-51.9%, 28.6-51.7% and 43.3-51.2%, respectively (Fig. 5.2, e). One-way ANOVA showed significant variation in WHC among the species ($p<0.001$) and among the studied sites ($p<0.01$) (Table 5.3).

Bulk density

Soil bulk density ranges for *P. bipinnatifidus, Panax* sp., *P. pseudoginseng, P. assamicus* and *P. variabilis* were 0.7-1.2 g cm^{-3} , 0.5-1g cm^{-3}, 0.3-1 g cm^{-3}, 0.4-0.8 g cm^{-3}, 0.5-0.6 g cm^{-3}, respectively (Fig. 5.2, f). One-way ANOVA showed significant variation ($p<0.001$) among the species and study sites (Table 5.3).

Porosity

Soil porosity ranges for *P. pseudoginseng, P. assamicus, P. variabilis*, *Panax* sp. and *P. bipinnatifidus* were 62.4-87.5%, 69.1-86.2%, 76.3-81.3%, 62.9-81% and 55.3-75.6%, respectively (Fig. 5.2, g). One-way ANOVA showed significant variation ($p<0.001$) among the species and study sites (Table 5.3).

Soil moisture content (SMC)

The SMC range for soils of *P. assamicus* growing areas was 22.1-47.7%. The corresponding figures for *Panax* sp., *P. pseudoginseng, P. variabilis* and *P. bipinnatifidus* were 30.6-47.5%, 31.1-46%, 44.1-46.7% and 29.5-31.45% (Fig. 5.2, h). One-way ANOVA showed significant variation in soil moisture content among the species and study sites ($p<0.001$) (Table 5.3).

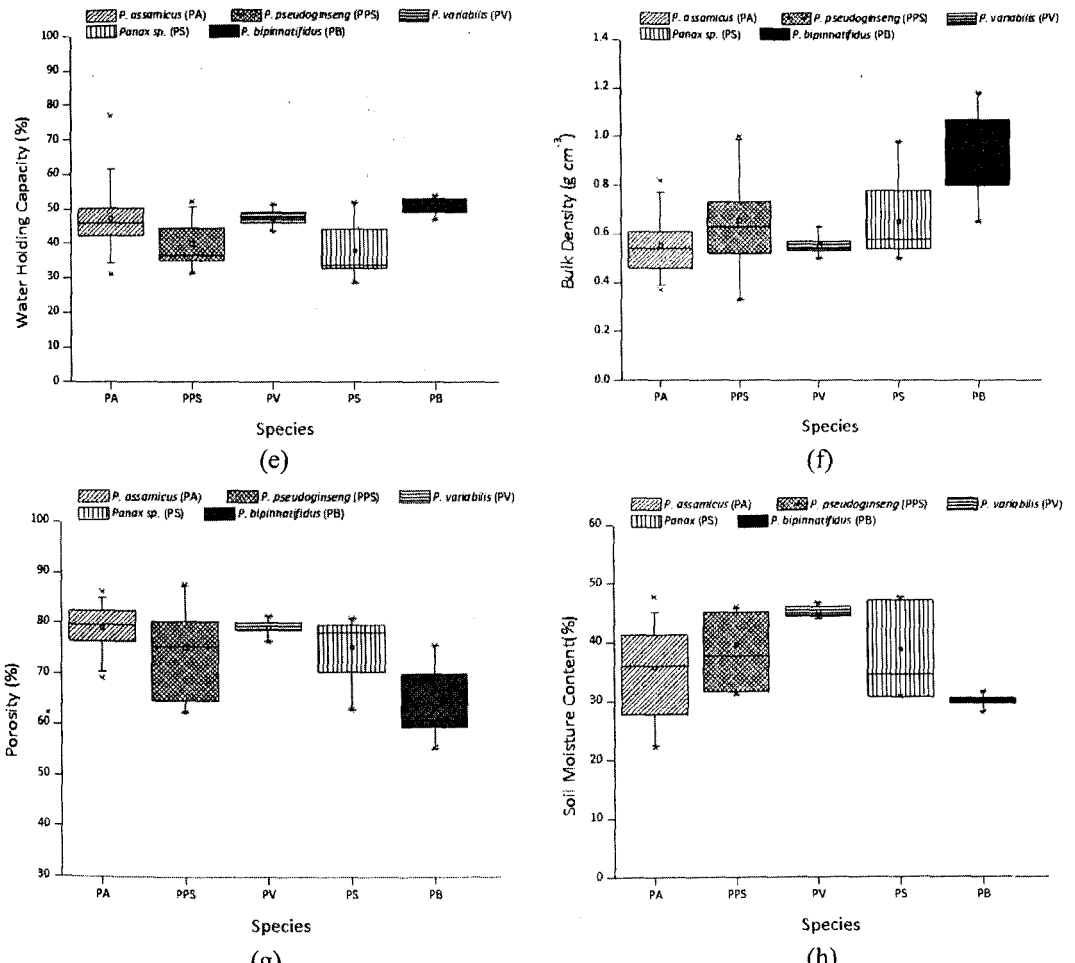


Fig. 5.2 (e-h): Soil physical properties in the study sites of *Panax* species. (e) Water holding capacity (%); (f) Bulk density (g cm $^{-3}$); (g) Porosity (%); (h) Soil moisture content (%). Box interquartile range; *upper horizontal line (bar)* uppermost value; *lower horizontal line* lower most value; *solid line within the box* median; *asterisk outside the box* outlier.

Soil pH

The soil pH was acidic ranging from 4.4 to 6.0 in *P. assamicus* habitats, 4.8 to 5.9 in *P. variabilis*, and 4.3 to 4.8 in *P. bipinnatifidus*. *P. pseudoginseng* and *Panax sp.* had a wider pH ranging from 4.9-7.1 and 5.2-7.3 (Fig. 5.2, i). The soil pH varied significantly among the species ($p<0.001$) and sites ($p<0.05$) (Table 5.3).

Total Kjeldahl nitrogen (TKN)

TKN values were higher in *P. pseudoginseng* (0.4-1.0%) and *Panax* sp. (0.5-1.25%) growing areas followed by *P. assamicus* (0.4-1.0%) and *P. variabilis* (0.6-0.7%). It was lowest in *P. bipinnatifidus* (0.4-0.5%) (Fig. 5.2, j). One-way ANOVA showed significant variation among the species ($p<0.01$) and the sites ($p<0.001$) (Table 5.3).

Soil organic carbon (SOC)

Soil organic carbon values were higher in *P. variabilis* (5.2-6.2%) and *P. assamicus* (4.1-5.8%) followed P. *pseudoginseng* (3.9-5.5%). It was lowest in *Panax* sp. (2.5-4.4%) and *P. bipinnatifidus* (4.1-4.2%) (Fig. 5.2, k). One-way ANOVA showed significant variation among the species ($p<0.001$) and the sites ($p<0.01$) (Table 5.3).

Available phosphorus (P)

Available phosphorus values were higher in *Panax* sp. (22.8-143.3 $\mu g\ g^{-1}$) and *P. pseudoginseng* (23.8-78 $\mu g\ g^{-1}$) followed by *P. variabilis* grows within the range (59-65.2 $\mu g\ g^{-1}$). It was lowest in *P. assamicus* (16-25.9 $\mu g\ g^{-1}$) and *P. bipinnatifidus* (17.3-18 $\mu g\ g^{-1}$) (Fig. 5.2, l). One-way ANOVA showed significant variation among the species ($p<0.001$) and the sites ($p<0.01$) (Table 5.3).

Exchangeable potassium (K)

Exchangeable K values were higher in *Panax* sp. (138-440 μg g^{-1}) followed by *P. pseudoginseng* (192.5-360 μg g^{-1}), *P. assamicus* (78.9-362.5 μg g^{-1}) and *P. variabilis* (135-275 μg g^{-1}). It was lowest in *P. bipinnatifidus* (165.5-178 μg g^{-1}) (Fig. 5.2, m). One-way ANOVA showed significant variation among the species ($p<0.001$) and the sites ($p<0.001$) (Table 5.3).

Magnesium (Mg)

Total magnesium values were higher in *P. assamicus* (1.7-11 mg g^{-1}) followed by *Panax* sp. (3.1-5.7 mg g^{-1}), *P. pseudoginseng* (2.9-4.9 mg g^{-1}) and *P. variabilis* (2.7-3.6 mg g^{-1}). It was lowest in *P. bipinnatifidus* (3.2-3.6 mg g^{-1}) (Fig. 5.2, n). One-way ANOVA did not show significant variation among the species ($p>0.05$) but was significant among the sites ($p<0.001$) (Table 5.3).

Calcium (Ca)

Total calcium values were higher in *P. assamicus* (0.03-11.09 mg g^{-1}) followed by *Panax* sp. (0.05-1 mg g^{-1}), *P. pseudoginseng* (0.01-0.6 mg g^{-1}) and *P. variabilis* (0.1-0.2 mg g^{-1}). It was lowest in *P. bipinnatifidus* (0.1-0.2 mg g^{-1}) (Fig. 5.2, o). One-way ANOVA did not show significant variation among the species ($p>0.05$) but was significant among the sites ($p<0.001$) (Table 5.3).

Iron (Fe)

Total iron values were higher in *P. assamicus* (27.3-83.6 mg g^{-1}) followed by *Panax* sp. (29.5-59.2 mg g^{-1}), *P. pseudoginseng* (24.5-37 mg g^{-1}) and *P. variabilis* (23.5-31.6 mg g^{-1}). It was lowest in *P. bipinnatifidus* (27.7-28.6 mg g^{-1}) (Fig. 5.2, p). One-way ANOVA showed significant variation among the species ($p<0.001$) and the sites ($p<0.001$) (Table 5.3).

Manganese (Mn)

Total manganese values were higher in *P. assamicus* (0.1-1.4 mg g^{-1}) followed by *P. pseudoginseng* (0.2-0.3 mg g^{-1}). *P. variabilis* and *Panax* sp. occurs in the same range (0.2-0.5 mg g^{-1}). It was lowest in *P. bipinnatifidus* (0.1-0.3 mg g^{-1}) (Fig. 5.2, q). One-way ANOVA did not show significant variation among the species ($p>0.05$) but was significant among the sites ($p<0.001$) (Table 5.3).

Zinc (Zn)

Total zinc values were higher in *P. assamicus* (40.4-101 µg g^{-1}) and *P. pseudoginseng* (55.8-93.6 µg g^{-1}). *Panax* sp. and *P. variabilis* grows within the range (53.9-69.5 µg g^{-1}) and (42.8-53.8 µg g^{-1}). It was lowest in *P. bipinnatifidus* (70.7-73.8 µg g^{-1}) (Fig. 5.2, r). One-way ANOVA showed significant variation among the species ($p<0.001$) and the sites ($p<0.001$) (Table 5.3).

Copper (Cu)

Total copper values were higher in *Panax* sp. (32.3-52.4 µg g^{-1}). *P. assamicus* and *P. pseudoginseng* occurs within the same range with (20.1-48.1 µg g^{-1}) and (24-47.2 µg g^{-1}). *P. bipinnatifidus* occurs in the range (29.2-32.4 µg g^{-1}) and it was lowest in *P. variabilis* (27.8-28.2 µg g^{-1}) (Fig. 5.2, s). One-way ANOVA showed significant variation among the species ($p<0.001$) but was not significant among the sites (Table 5.3).

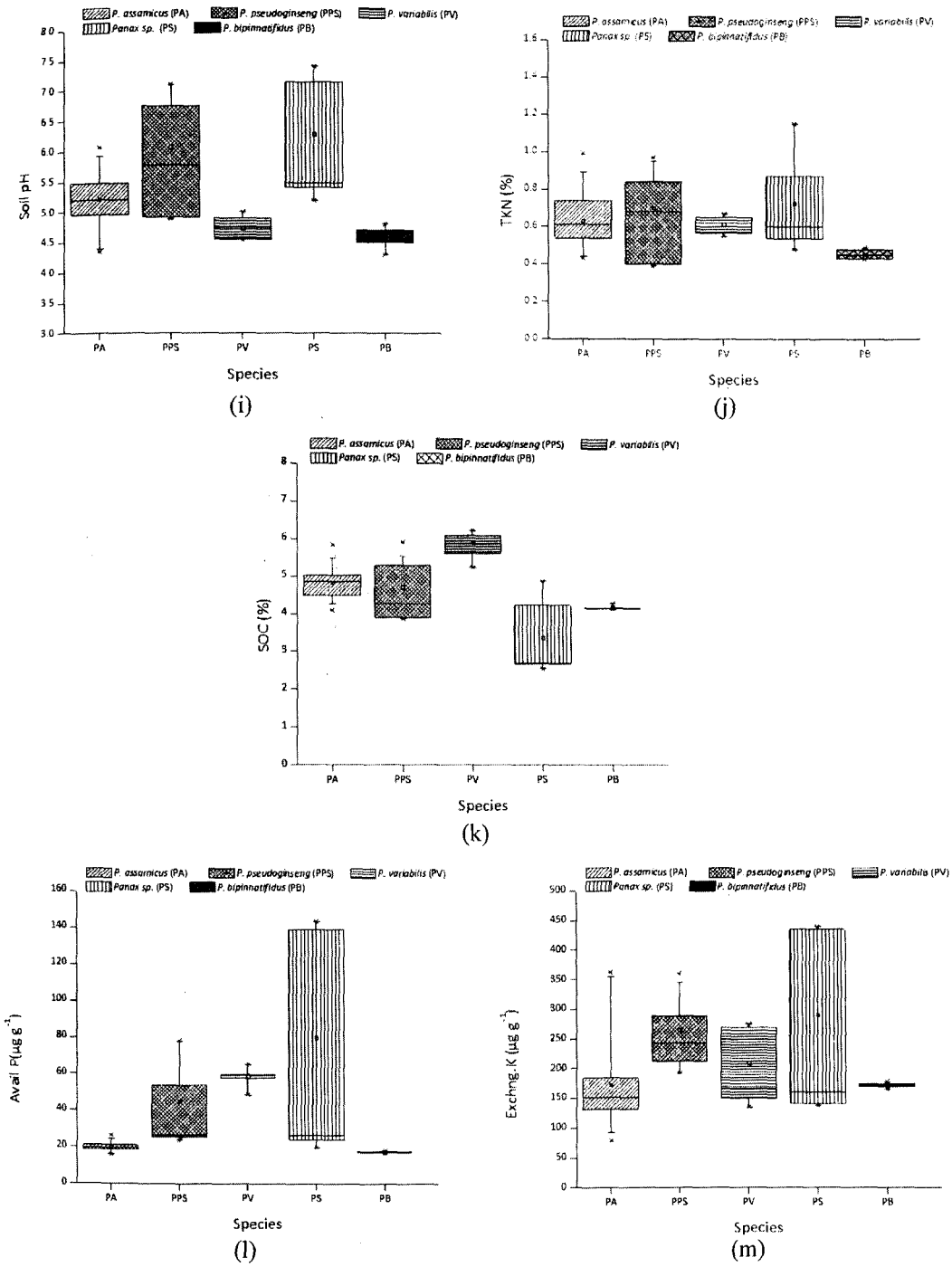


Fig. 5.2 (i-m): Soil chemical properties in the study sites of *Panax* species. (i) Soil pH; (j) Total Kjeldahl nitrogen (%); (k) Soil organic carbon (%); (l) Available phosphorus (µg g^{-1}); (m) Exchangeable potassium (µg g^{-1}). *Box* interquartile range; *upper horizontal line (bar)* uppermost value; *lower horizontal line* lower most value; *solid line within the box* median; *asterisk outside the box* outlier.

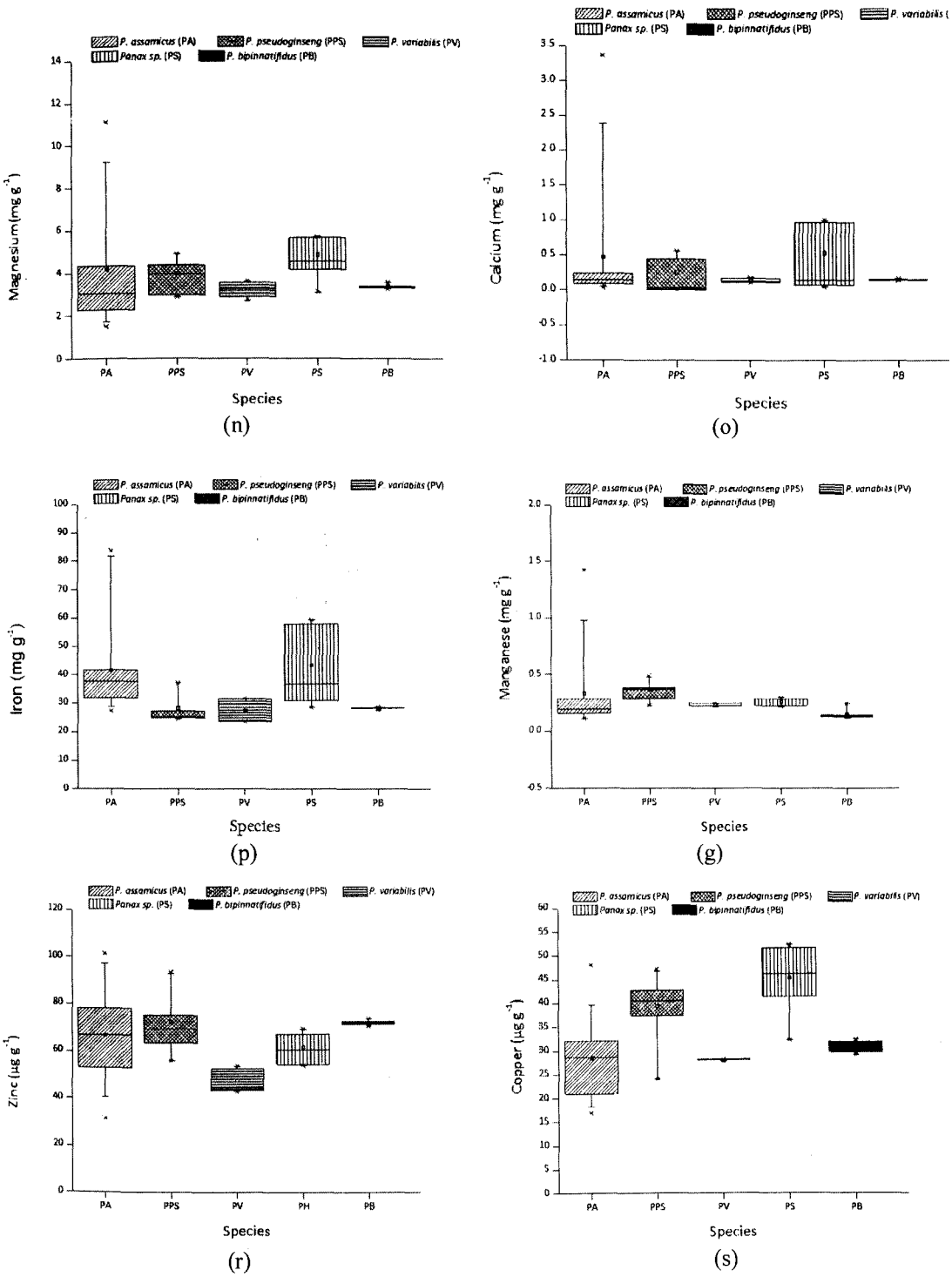


Fig. 5.2 (n-s): Soil chemical properties in the study sites of *Panax* species. (n) Magnesium (mg g^{-1}); (o) Calcium (mg g^{-1}); (p) Iron (mg g^{-1}); (q) Manganese (mg g^{-1}); (r) Zinc (µg g^{-1}); (s) Copper (µg g^{-1}). Box interquartile range; *upper horizontal line bar* Uppermost value; *lower horizontal line* lower most value; *solid line within the box* median; *asterisk outside the box* outlier.

Table 5.3: One way ANOVA showing effect of species and sites on microenvironmental variables and soil physico-chemical properties in all the study sites (*p<0.05, **p<0.01, ***p<0.001).

Parameters	Variation due to	df	F	*p* value
Micro-environmental variables				
Air temperature (°C)	Species	4	47.73***	0.000
	Sites	11	4.16***	0.000
Relative Humidity (%)	Species	4	27.17***	0.000
	Sites	11	2.11*	0.027
Soil Temperature (°C)	Species	4	73.48***	0.000
	Sites	11	2.30*	0.015
Light intensity (Lux)	Species	4	3.77**	0.007
	Sites	11	9.26***	0.000
Soil physical properties				
Water Holding Capacity (%)	Species	4	7.87***	0.000
	Sites	11	2.93**	0.002
Porosity (%)	Species	4	18.50***	0.000
	Sites	11	4.22***	0.000
Bulk Density (g cm^{-3})	Species	4	18.50***	0.000
	Sites	11	4.22***	0.000
Soil Moisture Content (%)	Species	4	8.87***	0.000
	Sites	11	3.46***	0.000
Soil chemical properties				
Soil pH	Species	4	22.26***	0.000
	Sites	11	2.02*	0.034
Soil organic carbon (%)	Species	4	35.33***	0.000
	Sites	11	2.95**	0.002
Total Kjeldahl Nitrogen (%)	Species	4	5.16**	0.001
	Sites	11	10.87***	0.000
Exchangeable Potassium (µg g^{-1})	Species	4	9.90***	0.000
	Sites	11	6.88***	0.000
Available Phosphorus (µg g^{-1})	Species	4	27.67***	0.000
	Sites	11	2.59**	0.006
Magnesium (mg g^{-1})	Species	4	**1.15**	**0.336**
	Sites	11	28.99***	0.000
Iron (mg g^{-1})	Species	4	9.22***	0.000
	Sites	11	26.17***	0.000
Calcium (mg g^{-1})	Species	4	**1.36**	**0.253**
	Sites	11	44.78***	0.000
Manganese (mg g^{-1})	Species	4	**2.00**	**0.100**
	Sites	11	64.62***	0.000
Copper (µg g^{-1})	Species	4	21.54***	0.000
	Sites	11	**1.66**	**0.095**
Zinc (µg g^{-1})	Species	4	5.82***	0.000
	Sites	11	5.55***	0.000

5.3.2. Niche breadth and Niche differentiation

5.3.2.1. Levin's niche breadth

The relative utilization of the micro-environmental parameters by *Panax* species revealed that *Panax* sp. had the broadest niche breadth in respect of air temperature (2.00), soil temperature (1.00), relative humidity (2.00) and light intensity (2.00). *P. assamicus* on the other hand, had an intermediate niche breadth for air temperature (1.86), soil temperature (1.10), relative humidity (1.40) and light intensity (2.80). *P. bipinnatifidus* and *P. variabilis* had similar niche breadth of 1.00 in all the micro-environmental parameters studied. *P. pseudoginseng* had the same niche breadth of 1.00 for the above variables stated except for light intensity (2.70) (Table 5.4).

The relative utilization of the soil physical parameters by the five species clearly shows that *P. assamicus* had the broadest niche breadth for soil moisture content (2.40), bulk density (3.90), water holding capacity (2.00), porosity (2.10), clay (2.13), silt (1.26) and total sand (2.00) followed by *P. pseudoginseng* for soil moisture content (1.60), bulk density (4.00), water holding capacity (1.60), porosity (2.67), clay (1.60), silt (1.60) and total sand (2.00) (Table 5.4).

Panax sp. had medium niche breadth for soil moisture content (2.00), bulk density (2.00), water holding capacity (2.00), porosity (1.00), clay (2.00), silt (2.00) and total sand (2.00). *P. bipinnatifidus* and *P. variabilis* had the narrowest niche breadth of 1.00 for all the soil physical parameters (Table 5.4).

The relative usage of the soil chemical parameters showed that *P. assamicus* had the broadest niche breadth for soil pH (1.70), soil organic carbon (1.70), soil organic matter (2.40), Ttotal Kjeldahl nitrogen (1.91), exchangeable potassium (2.80), available phosphorus (1.40), magnesium (2.90), iron (2.50), calcium (1.30), manganese (2.70),

copper (2.50) and zinc (2.90) followed by *P. pseudoginseng* for soil pH (1.00), soil organic carbon (2.70), soil organic matter (2.60), total Kjeldahl nitrogen (2.67), exchangeable potassium (2.70), available phosphorus (2.70), magnesium (1.00), iron (1.60), calcium (1.60), manganese (2.70), copper (1.60) and zinc (2.70). *Panax* sp. had an intermediate niche breadth of 2.00 for all the parameters except for magnesium and manganese with a niche breadth of 1.00. *P. bipinnatifidus* and *P. variabilis* showed the narrowest niche breadth of 1.00 for all the soil chemical parameters studied (Table 5.4).

The mean Levin's niche breadth for all the environmental parameters clearly revealed that *P. assamicus* had the broadest niche breadth of 2.2 followed by *P. pseudoginseng* with a niche breadth of 2.0. *Panax sp.* had an intermediate niche breadth of 1.7 and both *P. bipinnatifidus* and *P. variabilis* showed the same niche breadth of 1.0 (Table 5.4).

Table 5.4: Levins niche breadth for five *Panax* species of north-east India.

Parameters	*P. assamicus*	*P. bipinnatifidus*	*P.pseudo ginseng*	*P. variabilis*	*Panax* sp.
Micro-environmental variables					
Air temperature (°C)	1.86	1.00	1.00	1.00	2.00
Soil temperature (°C)	1.10	1.00	1.00	1.00	1.00
Relative Humidity (%)	1.40	1.00	1.00	1.00	2.00
Light Intensity (Lux)	2.80	1.00	2.70	1.00	2.00
Soil physical properties					
Soil Moisture Content (%)	2.40	1.00	1.60	1.00	2.00
Bulk Density (g cm^{-3})	3.90	1.00	4.00	1.00	2.00
Water Holding Capacity (%)	2.00	1.00	1.60	1.00	2.00
Porosity (%)	2.10	1.00	2.67	1.00	1.00
Clay (%)	2.13	1.00	1.60	1.00	2.00
Silt (%)	1.26	1.00	1.60	1.00	2.00
Total Sand (%)	2.00	1.00	2.00	1.00	2.00
Soil chemical properties					
Soil pH	1.70	1.00	1.00	1.00	2.00
Soil organic carbon (%)	1.70	1.00	2.70	1.00	2.00
Soil organic matter (%)	2.40	1.00	2.60	1.00	2.00
Total Kjeldahl nitrogen (%)	1.91	1.00	2.67	1.00	2.00
Exchangeable potassium (μg g^{-1})	2.80	1.00	2.70	1.00	2.00
Available phosphorus (μg g^{-1})	1.40	1.00	2.70	1.00	2.00
Magnesium (mg g^{-1})	2.90	1.00	1.00	1.00	1.00
Iron (mg g^{-1})	2.50	1.00	1.60	1.00	2.00
Calcium (mg g^{-1})	1.30	1.00	1.60	1.00	1.00
Manganese(mg g^{-1})	2.70	1.00	2.70	1.00	1.00
Copper(μg g^{-1})	2.50	1.00	1.60	1.00	2.00
Zinc (μg g^{-1})	2.90	1.00	2.70	1.00	1.00
Mean Niche breadth	**2.2**	**1.0**	**2.0**	**1.0**	**1.7**

5.3.2.2. Principal Component Analysis (PCA)

The PCA yielded 18 components which correspond to the 18 variables used in the analysis (Table 5.5). Projection of the data of the five studied species on first two principal axes showed the separation of samples into five groups at 80% concentration ellipse level (Fig. 5.3). A total variance of 74.42% was contributed by the first two principal axes. The component loadings of eighteen variables along the three axes are given in Table 5.5. The first axis (PC1) explained 49.53% of the total variance in which total Kjeldahl nitrogen (%),

soil temperature (°C), air temperature (°C) and clay (%) showed significant values in differentiating the components to segregate the five *Panax* species. The second axis (PC2) explained 24.89% of the total variance in which zinc (μg g^{-1}), copper (μg g^{-1}) and bulk density (g cm^{-3}) had significant values in differentiating the species. In the third axis (PC3), which accounted for 15.83% of the total variance, available phosphorus (μg g^{-1}), exchangeable potassium (μg g^{-1}) and copper (μg g^{-1}) were significant in separating the species. In the PCA scatter plot, five distinct groups were formed i.e. *P. assamicus, P. pseudoginseng, Panax sp., P. variabilis* and *P. bipinnatifidus.* Three species viz., *P. assamicus, P. pseudoginseng* and *Panax sp.* showed overlapping and stretched out group indicating a wide range of variation. The two other groups were distinct and were formed separately at a distance from the earlier three groups. These two groups were *P. variabilis* and *P. bipinnatifidus.* In both the groups the samples were clumped which shows that these species have a narrow range of variation.

Table 5.5: Summary of PCA on 18 variables used to segregate the five *Panax* species.

Component loadings	PC1	PC2	PC3
Soil pH	0.294	-0.083	0.252
SMC (%)	0.153	-0.418	-0.004
SOC (%)	-0.076	-0.408	-0.208
TKN (%)	0.322	-0.047	0.153
Av. P (μg g^{-1})	0.188	-0.182	0.393
K (μg g^{-1})	0.255	0.149	0.320
Mg (mg g^{-1})	0.271	0.192	-0.233
Ca (mg g^{-1})	0.250	0.187	-0.307
Fe (mg g^{-1})	0.219	0.181	-0.288
Zn (μg g^{-1})	0.012	0.386	-0.127
Mn (mg g^{-1})	0.233	-0.022	-0.370
Cu (μg g^{-1})	0.216	0.215	0.369
RH (%)	0.146	-0.415	-0.087
ST (°C)	0.325	-0.111	0.026
AT (°C)	0.334	-0.026	0.029
LI (lux)	0.119	0.004	0.062
BD (g cm^{-3})	-0.229	0.280	0.253
Clay (%)	0.300	0.176	-0.127

5.3.2.3. PCA scatter biplot

The PCA biplot applying density with other environmental variables yielded 17 components which correspond to the 17 variables used in the analysis. Projection of the data of the five studied species on first 2 principal axes showed the segregation of samples with 80% concentration ellipse level (Fig 5.4). A total variance of 66.20% was contributed by the first two principal axes. The first axis (PC1) explained 40.53% of the variance in which total Kjeldahl nitrogen (%), soil temperature (°C) and air temperature (°C) were positively correlated and formed one group with same vector length whereas bulk density was negatively correlated. The second axis (PC2) explained 24.89% of the total variance and magnesium, calcium and zinc were positively correlated with same vector length and soil organic carbon, soil moisture content and relative humidity were negatively correlated (Fig. 5.4).

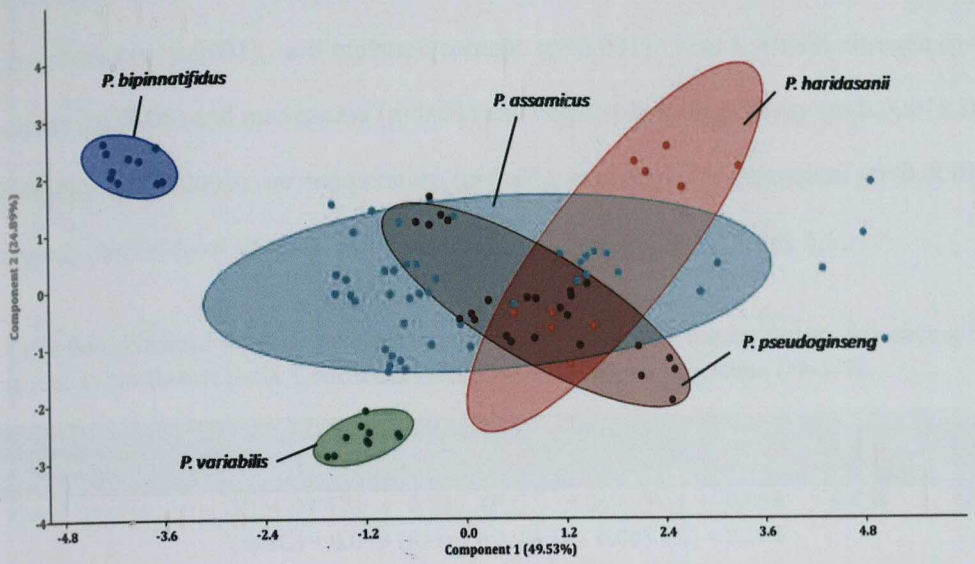


Fig. 5.3: PCA ordination plot of the first two Principal Components of soil physical, chemical and micro-environmental variables of *Panax* species.

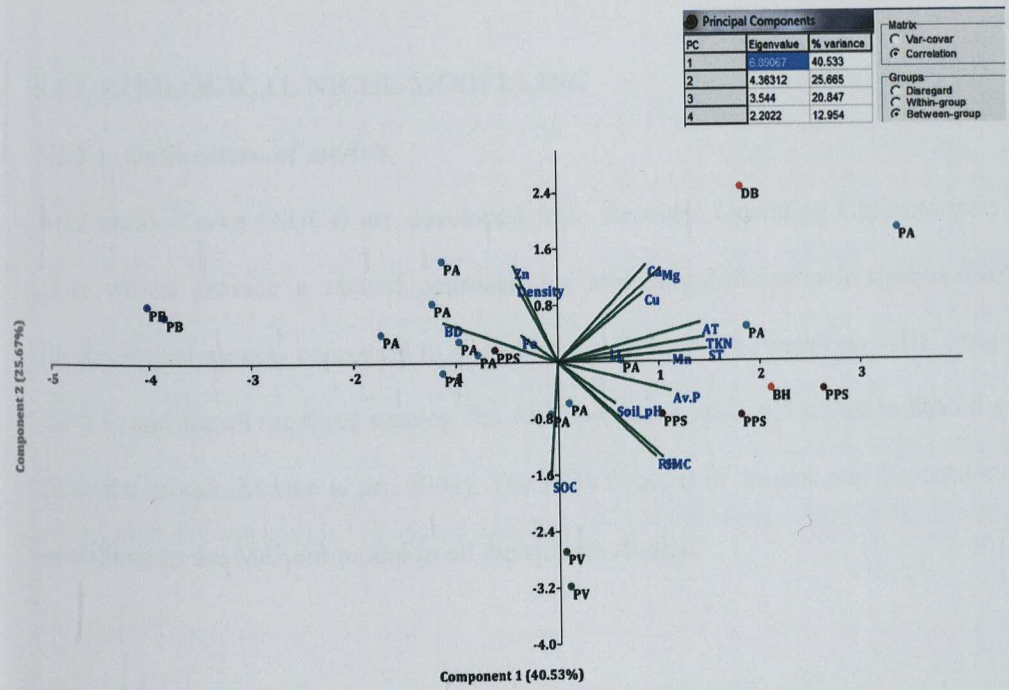


Fig. 5.4: PCA scatter biplot of the first two Principal Components using density and 16 environmental variables.

5.3.2.4. Factors influencing the presence of *Panax* species in north-east India

The presence of *Panax* species in all the study sites was positively correlated with available phosphorus ($p<0.0001$), soil moisture content ($p<0.001$), total Kjeldahl nitrogen ($p<0.01$), copper ($p<0.05$) and manganese ($p<0.01$) and negatively with porosity ($p<0.0001$), relative humidity ($p< 0.0001$), air temperature ($p<0.01$), exchangeable potassium ($p<0.0001$), soil organic carbon ($p<0.05$), soil pH ($p<0.0001$) and iron ($p<0.01$) (Table 5.6.)

Table 5.6: Forward stepwise multiple regression equations showing the factors influencing *Panax* species in North-east India. Coefficients represent B values for prediction (N=110).

Dependent variable (Y)	Regression equation	Adjusted R^2	*p*-value
Panax species	Y = 21.771 + 0.043 (P_{av}) - 0.069 (Po) + 0.058 (SMC) - 0.059 (RH) - 0.1 (AT) - 0.005 (K) - 0.294 (SOC) + 1.685 (TKN) - 0.930 (pH) + 0.034 (Cu) + 1.629 (Mn) - 0.034 (Fe)	0.854	<0.0000

Note: P_{av} = available phosphorus; Po = porosity; SMC = soil moisture content; Rh = relative humidity; AT = air temperature; K = exchangeable potassium; SOC = soil organic carbon; TKN = total kjeldahl nitrogen; pH = soil pH, Cu = copper; Mn = manganese; Fe = iron.

5.3.3. ECOLOGICAL NICHE MODELLING

5.3.3.1. Calibration of models

Area under Curve (AUCs) are developed from Receiver Operating Characteristic (ROC) plots which provide a ranked approach for assessing differences in species distribution for developed models compared to a random distribution. The resulting AUC ranged from 0.0-1.0, and for all the three species, the AUC produced was >0.5 which indicated a perfect fit to the model (Menke *et al.*, 2009). The NDVI values of August and September did not contribute to the MaXent model in all the species studied.

P. assamicus

The model calibration test for *P. assamicus* yielded satisfactory results with mean training AUC of 0.99 and mean AUC test of 0.98-0.99 which is 'very good' (Table 5.7). Amongst the input environmental variables, in *P. assamicus* from Meghalaya, elevation was the most influential and contributed 63.9% to the MaxEnt model whereas in *P. assamicus* from Manipur, the NDVI values of January and March contributed to 63.3% to the model. Twelve layers of NDVI jointly contributed 36.1% to the model for the Meghalaya populations of which NDVI of March had maximum contribution (8.9%), while NDVI of February, June and November contributed to 5.7-6.6%. The NDVI values of January, April, May, July and December jointly contributed to 8.9%. In Manipur populations, NDVI contributed to 88.9% of which January and March added the maximum with 28.7% and 34.6% followed by May with 13.7%. A contribution of 11.9% was made by February, April, June, October, November and December NDVI values (Table 5.8).

P. bipinnatifidus

The mean training AUC for *P. bipinnatifidus* is 0.99 and mean test AUC is 0.98 which indicate 'very good' result (Table 5.7). The NDVI values from January to December contributed the maximum accounting for 94.6% to the model. From the input environmental variables, the NDVI values of December and July contributed to 29.2 % and 26.4%, followed by May contribution of 21.8% which played an important role in the efficacy of the MaxEnt model. The NDVI values of January, February, March, April, June, November and elevation collectively contributed to 22.6% to the model (Table 5.8).

P. pseudoginseng

The mean training AUC and mean test AUC is 0.99 which indicate 'very good' result (Table 5.7). The twelve layers of NDVI values contributed 88.4% to the MaXent model. The NDVI value of January contributed the highest about 39.5% and is the most important

from all the variables. March and May values added to 18.7% and 20.5% to the model but no contribution from June and July. The NDVI values of February, April, October, November, December and elevation together contributed to 21.4% to the model (Table 5.8).

P. variabilis

In *P. variabilis*, a satisfactory result was obtained from the model with mean training AUC and mean test AUC of 0.99 which is 'very good' (Table 5.7). The NDVI values of twelve months contributed to 92% to the model with March value accounting for 53.9% followed by December with 16.1%. A total of 30% was contributed by January, February, April, May, June, July, November and October elevation values (Table 5.8).

***Panax* sp.**

The mean training AUC is 0.98 and mean test AUC is 0.97 which show 'very good' result (Table 5.7). The twelve layers of NDVI values contributed 89% to the MaXent model. The NDVI value of July contributed the maximum up to 40.9% and the corresponding October and May values were 22.2% and 15.8%, respectively. January and March did not show any contribution. The NDVI values of February, April, June October, November, December and elevation together contributed 21.2% to the model (Table 5.8).

Table 5.7: Results of the replicated model runs using cross validation procedure.

Species	Mean AUC_{train} (±SD)	Mean AUC_{test} (±SD)
P. assamicus Meghalaya	0.993±0.003	0.988±0.022
P.assamicus Manipur	0.999±0.00	0.998±0.003
P. bipinnatifidus	0.991±0.002	0.985±0.017
P. pseudoginseng	0.997±0.0002	0.995±0.003
P. variabilis	0.998±0.0006	0.996±0.007
Panax sp.	0.986±0.0007	0.977±0.011

Table 5.8: Relative contribution of the environmental variables for each species resulted from MaXent distribution model.

Environmental variables	Percent contribution to the habitat model					
	P. assamicus Meghalaya	*P.assamicus* Manipur	*P. bipinnatifidus*	*P. pseudoginseng*	*P. variabilis*	*Panax sp.*
Physiography						
Digital Elevation Model (DEM)	63.9	11.1	5.4	11.7	8	11
Total	63.9	11.1	5.4	11.7	8	11
Normalized Difference Vegetation Index (NDVI)						
January	0.1	28.7	4.4	39.5	2.5	0
February	5.7	5.1	1	0.1	2	1.1
March	8.9	34.6	2.1	18.7	53.9	0
April	1.7	0.3	6.1	0.1	0.1	4.7
May	1.2	13.7	21.8	20.5	6.5	15.8
June	6.0	0.3	0.1	0	0.5	1.3
July	2.8	0	26.4	0	9.8	40.9
August	-	-	-	-	-	-
September	-	-	-	-	-	-
October	0	2.3	0	4.7	0.2	22.2
November	6.6	0.5	3.5	4.3	0.5	0.4
December	3.1	3.4	29.2	0.5	16.1	2.7
Total	**36.1**	**88.9**	**94.6**	**88.4**	**92**	**89**

5.3.3.2. JACKNIFE TEST

The importance of each environmental predictor variable was assessed using jackknife operation (Yost *et al.*, 2008). Jackknife operates by sequentially excluding one environmental variable out of the model and running a model using the remaining variables. It also runs a model using only the excluded variable in isolation. As a result, the gain contribution of each variable to the total gain of the model (inclusive of all variables) can be calculated. The most important variables were identified as: (1) the variable which decrease the total gain of the model when it is excluded from the environmental dataset during the calibration process, and (2) the variable which contributes to the highest gain when used alone.

P. assamicus

In *P. assamicus* (Meghalaya), the jackknife test predicted the environmental variable with the highest gain when used in isolation is elevation, which therefore appears to have the most useful information by itself but when omitted, decreases the gain the most. In *P. assamicus* (Manipur), the NDVI value of March plays an important role when used in isolation and contains more information than that in any of the other variables (Fig. 5.5 a,b).

P. bipinnatifidus

The environmental variable predicted from jackknife test in *P. bipinnatifidus* with highest gain when used in isolation is NDVI of December, which is most useful amongst the rest. The variable that decreases the gain the most when it is omitted is NDVI of July which shows to contain the most information that is not present in other variables (Fig. 5.5, c).

P. pseudoginseng

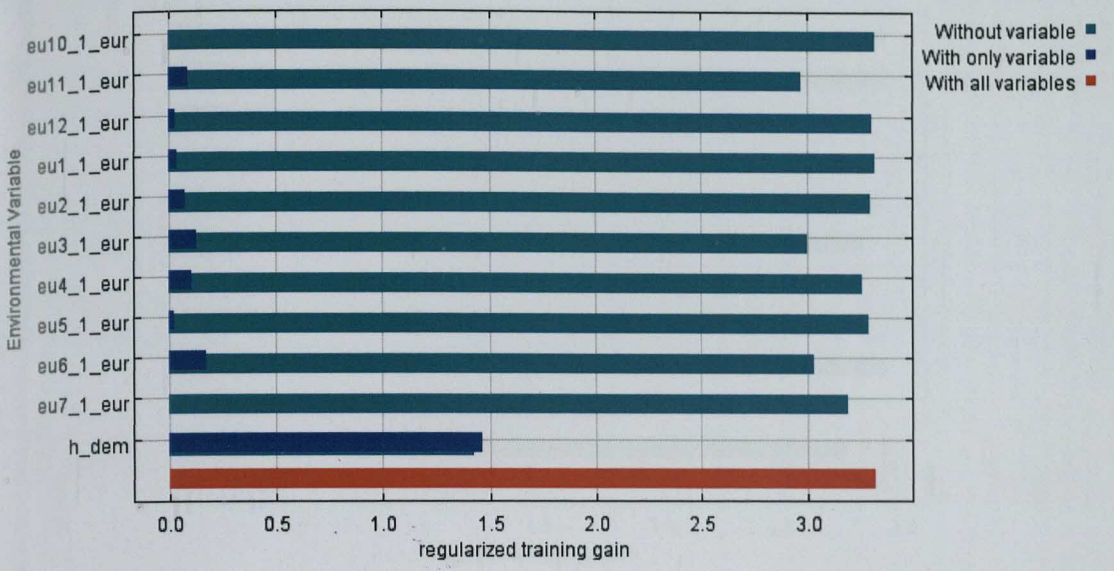
The jackknife test result showed the environmental variable with highest gain when used in isolation is January NDVI value which has the most useful information but decreases the gain the most when it is omitted (Fig. 5.5, d).

P. variabilis

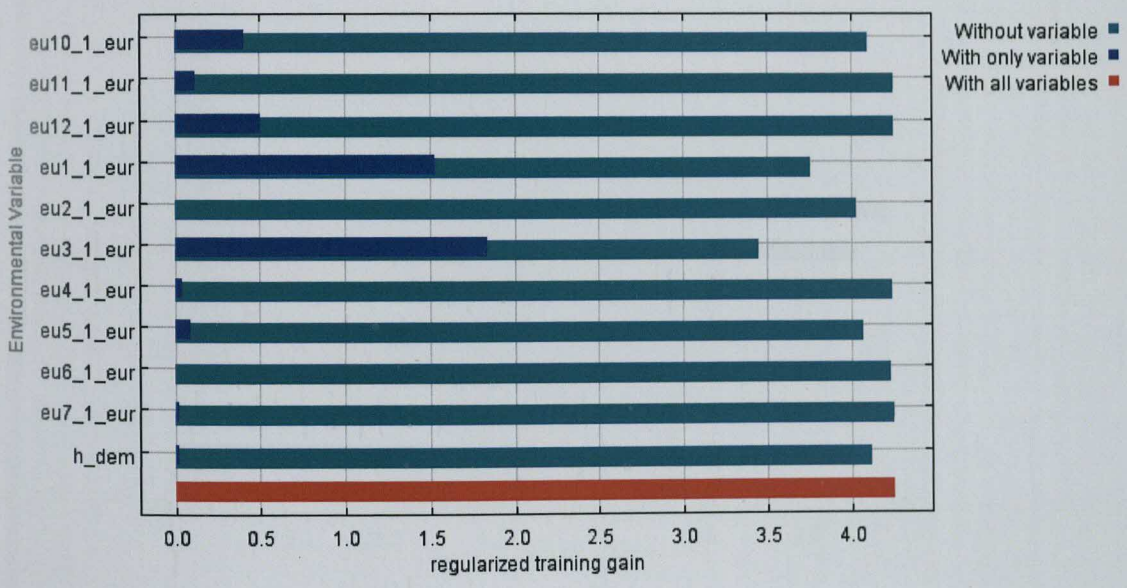
In *P. variabilis*, the environmental variable with highest gain is NDVI value of March when used in isolation through jackknife test and when excluded decreases the gain the most which appears to have the most information that is not present in the other variables (Fig. 5.5, e).

***Panax* sp.**

The jackknife test showed the environmental variable which is most important and with highest gain when used in isolation is elevation but when NDVI value of July is omitted, it decreases the gain the most in the model and clearly shows that most information is present in this particular variable (Fig. 5.5, f).



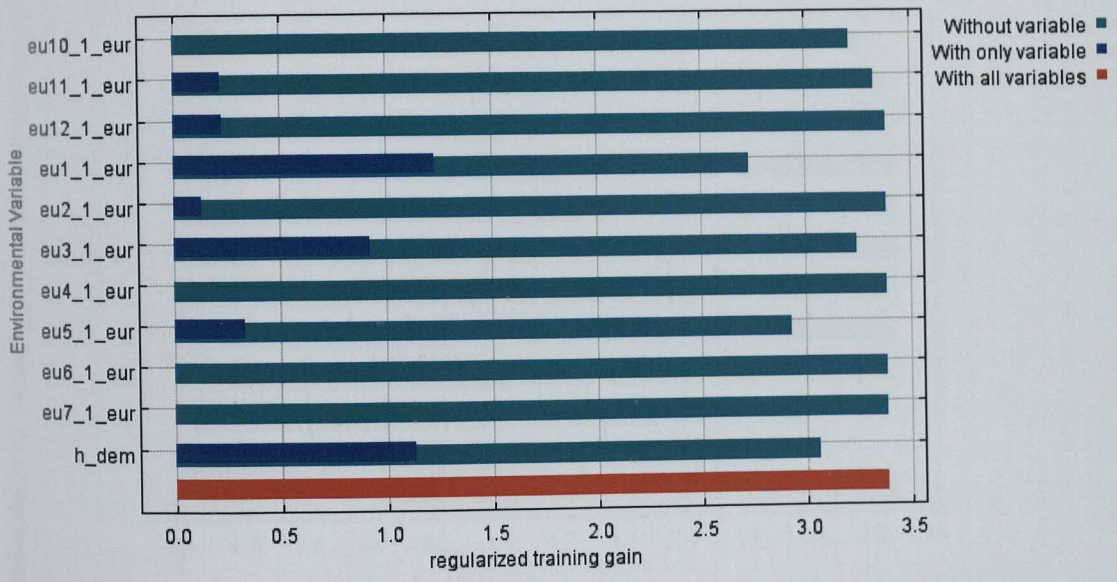
(a)



(b)

Fig. 5.5 (a-b): Result of jackknife test for evaluating the relative contribution of the predictor environmental variables to the species distribution model of *P. assamicus* in (a) Meghalaya and (b) Manipur.

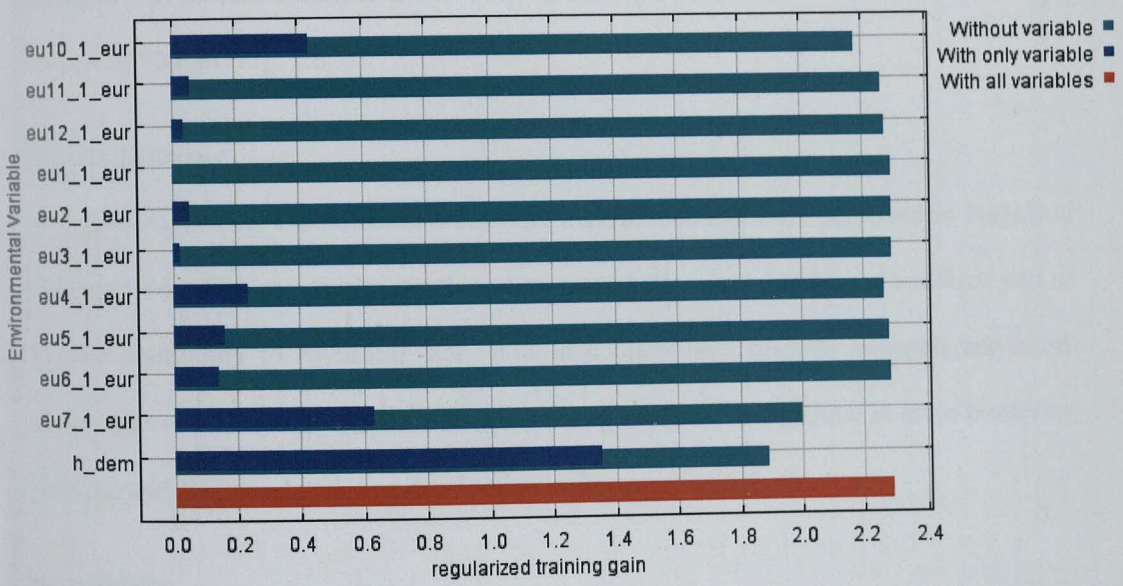
(c)



(d)

Fig. 5.5 (c-d): Result of jackknife test for evaluating the relative contribution of the predictor environmental variables to the species distribution model of (c) *P. bipinnatifidus* in Arunachal Pradesh and Sikkim and (d) *P. pseudoginseng* in Nagaland.

(e)



(f)

Fig. 5.5 (e-f): Result of jackknife test for evaluating the relative contribution of the predictor environmental variables to the species distribution model of (e) *P. variabilis* in Arunachal Pradesh and (f) *Panax* sp. in Arunachal Pradesh and Nagaland.

5.3.3.3. Potential habitat distribution

P. assamicus

In *P. assamicus*, the potential areas of distribution with very high probabilities were predicted in Manipur district of Ukhrul, Tamenglong and areas bordering Senapati and Kohima district of Nagaland. The species is also predicted towards Myanmar bordering Tuensang district of Nagaland. In Meghalaya the species is predicted in East Khasi Hills and West Khasi hills districts with very high probability (Fig. 5.6, a).

P. bipinnatifidus

The species is predicted with continuous distribution from Sikkim extending to Bhutan and Arunachal Pradesh. The species is predicted with very high probability in Tawang, West Kameng, Papum Pare, lower Subansiri, Dibang and lower Dibang, Tirap and Changlang district of Arunachal Pradesh. Distribution is also shown in Myanmar and West Bengal bordering Sikkim (Fig. 5.6, b).

P. pseudoginseng

In *P. pseudoginseng,* the potential areas with high probability were predicted in Nagaland which includes Kohima district, south eastern part of Tuensang district and southern part of Kiphire continuing to Myanmar side. It is also distributed towards Senapati and north eastern part of Ukhrul in Manipur. The species is spread across Myanmar in areas bordering Manipur and Nagaland with few distribution in Bhutan (Fig. 5.6, c)

P. variabilis

The potential areas of distribution for *P. variabilis* showed a restricted distribution confined only to the northern part of Senapati district of Manipur and areas in southern part of Kohima district of Nagaland bordering Senapati. Small patches are also predicted in Phek district of Nagaland and Ukhrul district of Manipur and also in Myanmar (Fig. 5.6, d).

***Panax* sp.**

In *Panax* sp., the potential habitats showed a continuous distribution pattern in Arunachal Pradesh with high concentration towards the southern part of West and East Kameng, Papum Pare, southern part of Lower Subansiri and West Siang districts. The other areas of predicted distribution with high suitability include Kurung Kumey, Upper Subansiri, West Siang, and with medium suitability in Lower Dibang, Changlang, and Tirap districts. In Nagaland, *Panax* sp. is distributed only in Tuensang district. The species is also predicted towards the southern part of Bhutan. It is also predicted towards Tibet, Myanmar and areas adjoining Sikkim, west Bengal and Nepal (Fig. 5.6, e).

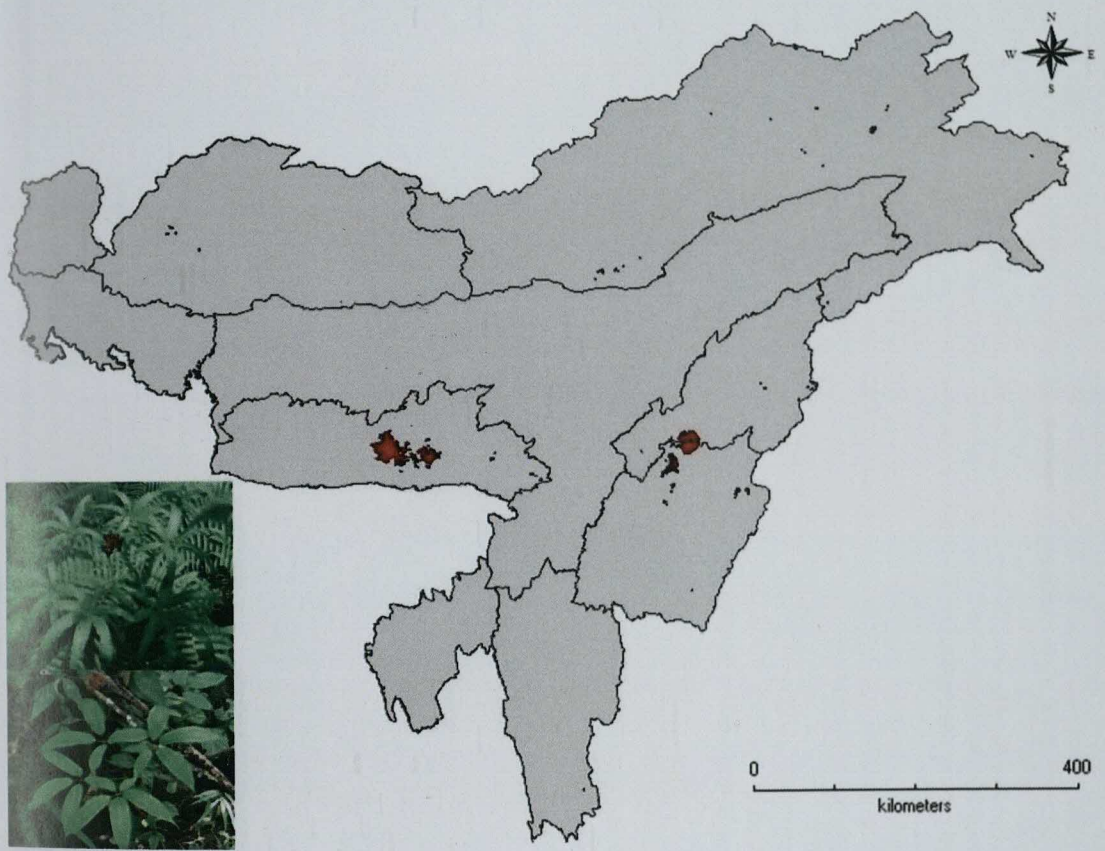


Fig. 5.6 (a): Map showing the probability of occurrence of *Panax assamicus* in Meghalaya and Manipur. Mean AUC=0.99 (Red colour shows very high 0.7-1.0 probability of species presence).

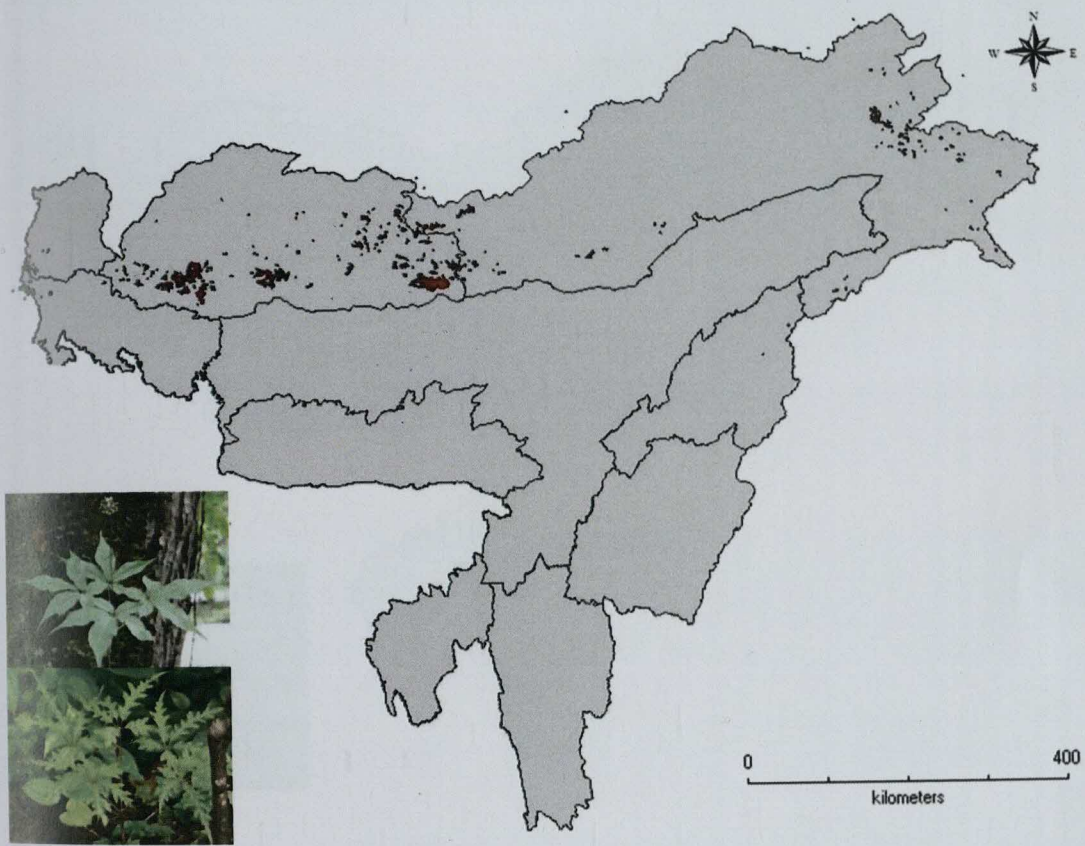


Fig. 5.6 (b): Map showing the probability of occurrence of *Panax bipinnatifidus* in Arunachal Pradesh and Sikkim. Mean AUC=0.98 (Red colour shows very high 0.7-1.0 probability of species presence).

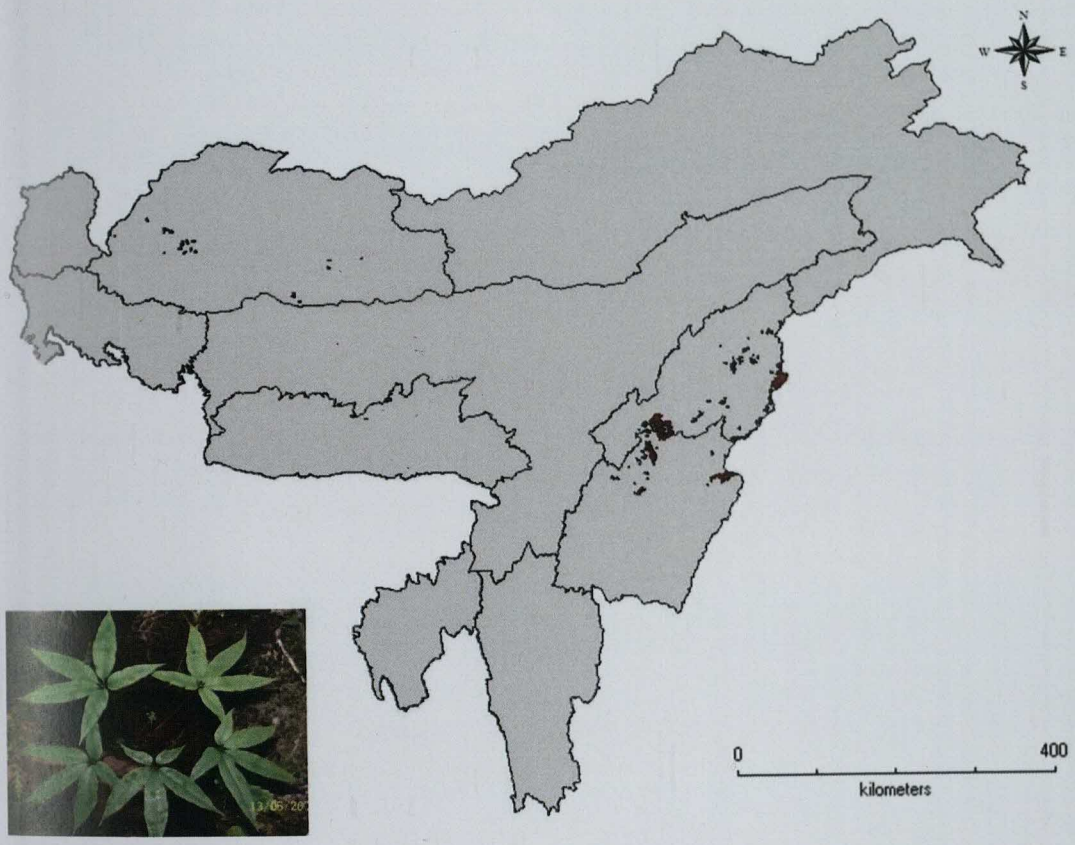


Fig. 5.6 (c): Map showing the probability of occurrence of *Panax pseudoginseng* in Nagaland. Mean AUC=0.99 (Red colour shows very high 0.7-1.0 probability of species presence).

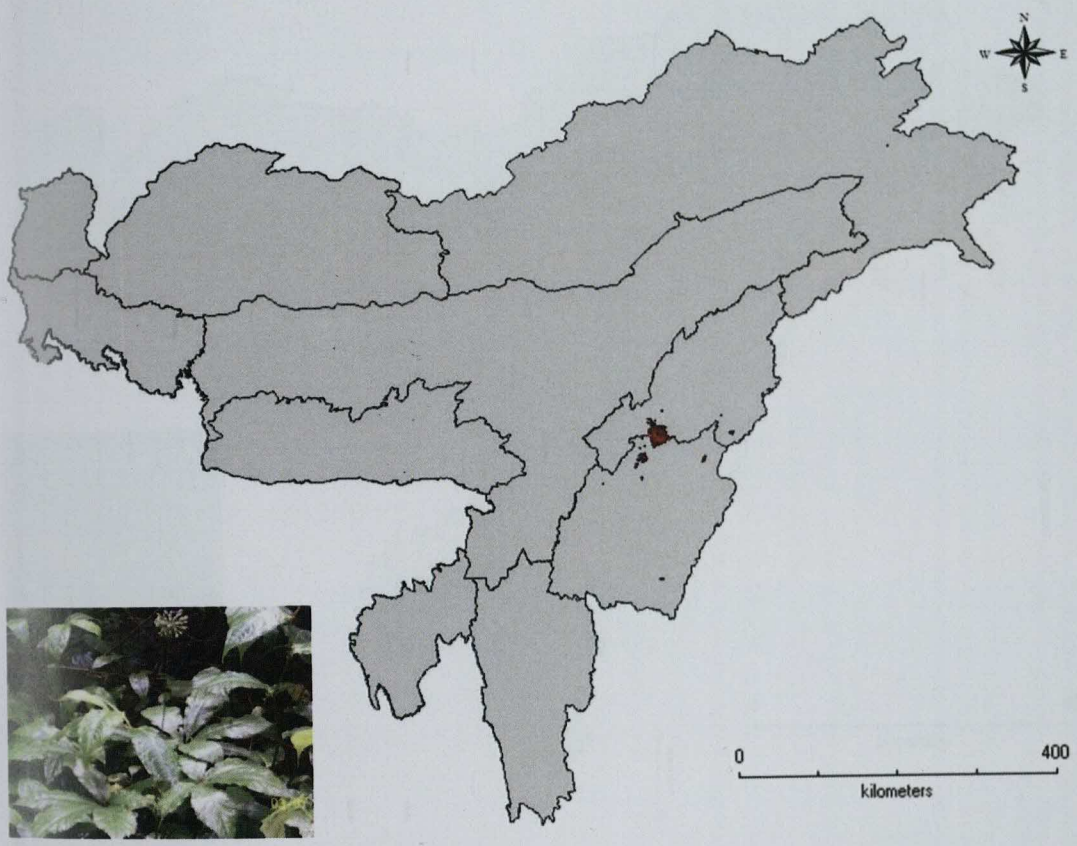


Fig. 5.6 (d): Map showing the probability of occurrence of *Panax variabilis* in Manipur. Mean AUC=0.99 (Red colour shows very high 0.7-1.0 probability of species presence).

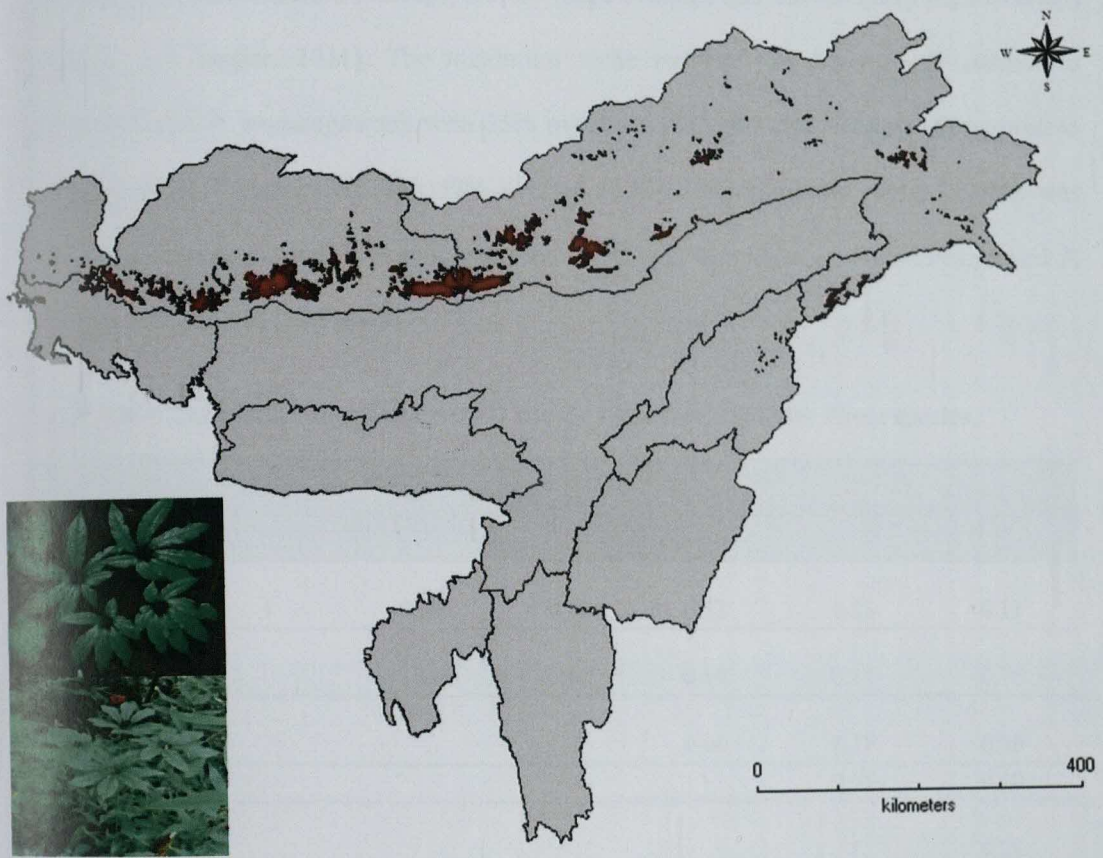


Fig. 5.6 (e): Map showing the probability of occurrence of *Panax* sp. in Arunachal Pradesh and Nagaland. Mean AUC=0.97 (Red colour shows very high 0.7-1.0 probability of species presence).

5.3.3.4. Niche breadth and niche overlap based on species distribution modelling

The niche overlap values were categorized as: 0-0.2 (limited overlap), 0.2-0.4 (low overlap), 0.4-0.6 (moderate overlap), 0.6-0.8 (high overlap) and 0.8-1.0 (very high overlap) (Rodder and Engler, 2011). The maximum niche overlap was between *P. assamicus* (Manipur) and *P. pseudoginseng* with 55% overlap (0.55) and also between *P. assamicus* (Manipur) and *P. variabilis* with 53% overlap (0.53). The minimum niche overlap was observed between *P. variabilis* and *Panax* sp. (0.10) and also between *P. assamicus* and *P. bipinnatifidus* (0.08) (Table 5.9)

Table 5.9: Niche overlap using Schoener's D statistic (Schoener, 1968) for *Panax* species.

Species	*P. assamicus* (Manipur)	*P. assamicus* (Meghalaya)	*P. bipinnatifidus*	*P.variabilis*	*P. pseudo ginseng*	*Panax* sp.
P. assamicus (Manipur)	1	0.09	0.08	0.53	0.55	0.11
P. assamicus (Meghalaya)		1	0.11	0.09	0.15	0.35
P. bipinnatifidus			1	0.06	0.17	0.30
P. variabilis				1	0.42	0.10
P.pseudo ginseng					1	0.22
Panax sp.						1

5.3.3.5. Principal Component Analysis (PCA) using NDVI values

The PCA analysis using only NDVI values yielded 12 components which correspond to the 12 layers of variables used (Table 5.10). The projected data on the five species of *Panax* on first two principal axes showed the grouping of the samples into five groups at 95% concentration ellipse (Fig. 5.7). A total variance of 92.39% was contributed by the first two principal axes. The component loadings of 12 variables along the three axes are given in Table. The first axis (PC1) describes 75.19 % of the variance in which NDVI values of

January, October and December showed significant values in differentiating the components to segregate the five *Panax* species. The second axis (PC2) explained 17.21% of the variance and the NDVI value of March is the most significance. In the third axis (PC3), which accounted for 4.95 % of the variance, February, July and November NDVI values were significant in separating the species. In the PCA scatter plot, the samples within the genus formed five distinct groups i.e., *P. assamicus, P. pseudoginseng, Panax* sp., *P. variabilis and P. bipinnatifidus.* The samples of *P. bipinnatifidus* and *Panax* sp. showed overlapping and more condensed with less variation. *P. variabilis* and *P. pseudoginseng* also showed overlapping and the samples are stretched out like an ellipse indicating a wide range of variation. *P. assamicus* on the other hand showed overlapping with *P. variabilis* and *P. pseudoginseng* and is broadly stretched out indicating a lot of variation.

Table 5.10: Summary of PCA on 12 NDVI variables used to segregate the five *Panax* species.

Component loadings	PC1	PC2	PC3
January	0.347	0.088	0.109
February	0.143	-0.230	0.360
March	0.238	0.660	0.031
April	0.041	0.021	-0.192
May	0.117	-0.469	0.156
June	0.173	-0.281	0.136
July	-0.471	-0.103	0.488
August	-0.049	0.189	0.137
September	0.025	0.282	0.206
October	0.393	-0.207	-0.435
November	0.235	0.161	0.453
December	0.568	-0.098	0.292
Total	**1.760**	**0.014**	**1.739**

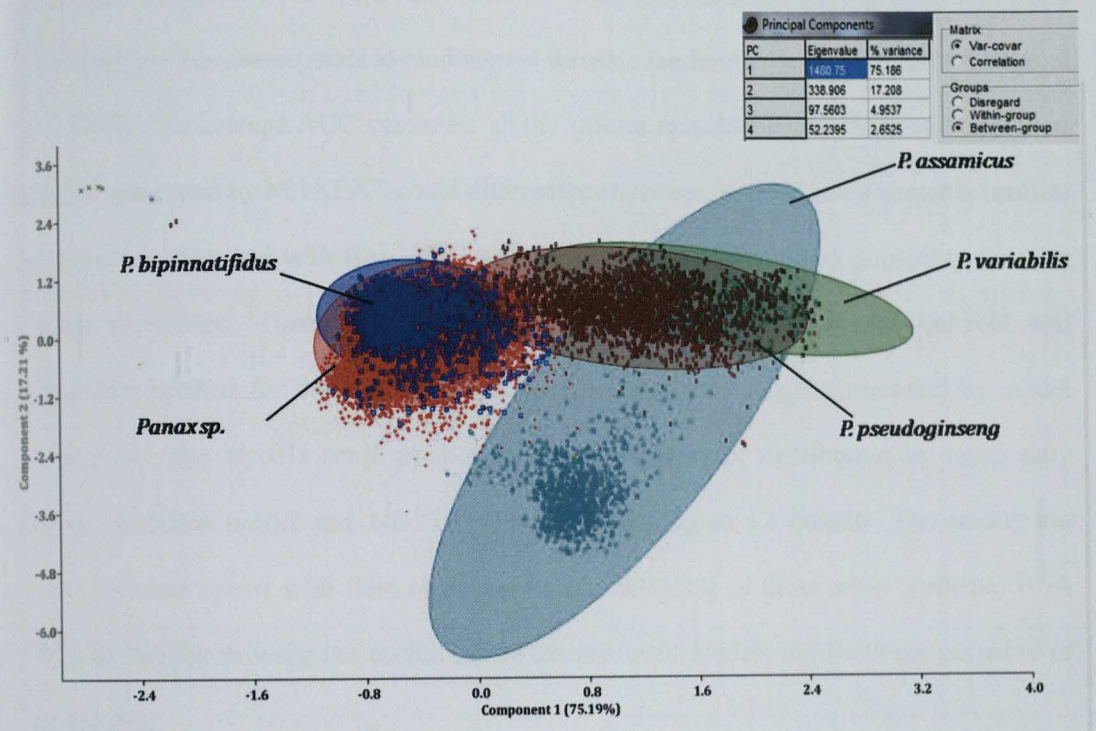


Fig. 5.7: PCA ordination plot of the first two Principal Components of 12 layers of NDVI values from January-December in segregating the five *Panax* species.

5.4. DISCUSSION

The fitting of the model parameters on the habitat distribution of *Panax* species from Northeast was fairly high and accurate. Since the 'presence only' data was used, the maximum achievable AUC was less than 1.0 (Rodríguez-Sánchez and Arroyo, 2008) and values 0.5 or less corresponds to randomness thereby, rendering the model unfit (Phillips *et al.*, 2006). The average AUC values for all the species revealed that the habitat distribution models generated by MAXENT could differentiate between suitable and unsuitable habitats of *Panax* species and with their AUC very close to 1.0 indicates their capability for very strong prediction. Therefore, MAXENT successfully distinguished the suitable and unsuitable habitats for the selected species within the native range as revealed by model calibration. The models could predict the potential areas of distribution by using only digital elevation model and NDVI layers corresponding to 12 months. The results are consistent and robust with field observations and sampling of those areas predicted from MaXent thereby proving the usefulness of the predicted models in in-situ conservation of the species.

NDVI played a key role in determining the distribution of potential habitats of *P. assamicus* (Manipur), *P. bipinnatifidus, P. pseudoginseng, P. variabilis* and *Panax* sp. in its native range but on the other hand elevation is the most important factor for *P. assamicus* (Meghalaya) distribution. NDVI layers provide good explanation on the fundamental role of other environmental factors which determined the habitat suitability of the species. Various environmental factors such as geology, soil and climate have a debatable influence on vegetation indices of a given place at a given time (Soleimani *et al.*, 2008). The effects of such underlying environmental factors are reflected through the spatial and temporal variation in the vegetation indices such as NDVI and also with Enhanced Vegetation Index (EVI). The greater contribution of some of the NDVI layers of January, March, May, July,

October and December to the overall habitat model shows the importance played by these factors in defining habitat suitability. Interestingly, the NDVI layers of January, March, May and July which contributed the most to the habitat model correspond to the period of germination, vegetative growth, flowering and fruiting of the species. The relatively high importance of vegetation parameters such as herbaceous cover, NDVI and EVI in predicting distribution of species was also reported by Stohlgren *et al.* (2001) who used NDVI, EVI and other physiographic predictors, in predicting the distribution of African honey bees. Variation in individual contributions by the NDVI layers to the modeled potential habitat was observed within and between the species. The niche overlaps in all the *Panax* species ranges from limited to moderate overlap which indicates that these species share very few resources for their survival.

P. assamicus, which is found in Meghalaya and Manipur, and *Panax* sp. from Arunachal Pradesh and Nagaland, have broad ecological niches which are geographically spread into two states. Brown (1984) suggested that species with broad ecological niches should be geographically widespread as well as being locally abundant and is achieved because a broad niche allows a species to persist in a wide range of different habitat types, while a narrow niche restricts a species to the few places where its niche requirements are met (Gaston, 1993; Kunin and Gaston, 1997).

Levin's niche breadth analysis using site soil and climatic data as well as Principal Component Analysis (PCA) confirmed the niche modeling result on the classification of *Panax* species into narrow and broad niched groups. Relatively broad-niched species can avail more areas as their suitable habitats and have a correspondingly large potential geographical range (Lawton, 1995) as can be seen in *P. assamicus* and *Panax* sp. *P. pseudoginseng* can also be regarded as a relatively broad-niched species within the genus *Panax* because it has a broader niche and is spread over three districts of Nagaland. On the

other hand, *P. bipinnatifidus* and *P. variabilis* had narrow niches and therefore can be regarded as specialists.

Brown (1984) suggested that the species which utilize a wider range of resources should be able to occupy a larger geographical area, leading to a positive relationship between niche breadth and range size. In their comprehensive review, Gaston and Blackburn (2000) suggested that niche position is more important than niche breadth in determining range size. Under this hypothesis, widespread species are those that utilize the most common and widespread resources (Hanski, 1993).

CHAPTER 6

ESTABLISHING TAXONOMIC IDENTITY OF *PANAX* SPECIES FROM NORTH-EASTERN INDIA

6.1. INTRODUCTION

Since determination of most Asian *Panax* plants remained controversial due to large variation in morphological characters and the existence of intermediate forms, it is required to take a multipronged approach such as morpho-metrical, biochemical, ecological and molecular level confirmation to arrive at a conclusion on species identity.

Although species-level systematics provide a framework for studying evolutionary patterns and processes, the application of plant molecular systematics to unreveal the evolutionary trends of the species has been rather limited due to lack of variable markers for species-level analyses. Commonly used molecular markers for lower-level phylogenetic analysis in plants are the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA, the chloroplast *mat*K, *ndh*F, the noncoding *trn*L-F region, the *atp*B–*rbc*L spacer, the *rps*16 intron, and a few single- or low-copy nuclear genes such as *adh, ncp*GS, the 5S spacer and *Pgi*C.

DNA sequences from ribosomal genes (rDNA) are the most widely exploited nuclear molecular markers in angiosperm systematics and have revolutionised the studies on angiosperm evolution. The most significant role of the rDNA sequences is that they exhibit a broad range of phylogenetic signals. Recent analyses have enhanced our understanding of phylogenetic relationships among flowering plants (Chase *et al.*, 1993; Soltis *et al.*, 1997a; Nandi *et al.*, 1998; Savolainen *et al.*, 2000a, b). All of these studies, based on plastid *rbc*L (Chase *et al.*, 1993), *18S* rDNA (Soltis *et al.*, 1997a), *rbc*L with morphology, chemistry,

and other non-DNA characters (Nandi *et al.*, 1998), and combined plastid *atp*B and *rbc*L (Savolainen *et al.*, 2000b), included hundreds of species and more than 1000 characters, placing them among the largest phylogenetic analyses so far conducted.

The *18S* rRNA gene which encodes the RNA of the small ribosomal subunit is taxonomically the most applied sequence in molecular phylogenetics. The gene responsible for small ribosomal subunit RNA in higher life forms, has been used for phylogeny reconstruction within many groups of eukaryotes, including most major groups of plants, e.g., green algae (Buchheim and Chapman, 1991; Chapman and Buchheim, 1991), bryophytes (Waters *et al.*, 1992; Mishler *et al.*, 1994; Capesius, 1995; Hedderson *et al.*, 1996, 1998), gymnosperms (Chaw *et al.*, 1993, 1995, 1997) and angiosperms (Soltis *et al.*, 1997c).

While *18S* rRNA gene has higher order of conservancy, internal transcribed spacer (ITS) regions of 18S-28S nuclear rDNA harbour greater variability and has become a major focus of comparative sequencing at the specific and generic levels in angiosperms (Baldwin *et al.*, 1995). This region includes three components: the 5.8S gene, an evolutionarily highly conserved sequence and two spacers designated ITS-1 and ITS-2. The ITS region is highly repeated in the plant nuclear genome and the entire nrDNA repeat unit is present in many thousands of copies arranged in tandem repeats at a chromosomal locus or at multiple loci (Rogers and Bendich, 1987; Hamby and Zimmer, 1992). The high copy number promotes detection, amplification, cloning and sequencing of nrDNA. From the point of phylogenetic reconstruction, this region undergoes rapid concerted evolution via unequal crossing-over and gene conversion (Arnheim *et al.*, 1980; Zimmer *et al.*, 1980; Appels and Dvorak, 1982; Arnheim, 1983; Hillis *et al.*, 1991). This attribute promotes intragenomic uniformity of repeat-units, in some cases even between nrDNA loci on non-homologous chromosome (Arnheim *et al.*, 1980; Arnheim, 1983; Hillis *et al.*, 1991; Wendel *et al.*, 1995), and in

general promotes accurate reconstruction of species relationships from these sequences (Hamby and Zimmer, 1992; Sanderson and Doyle, 1992). The small size of the ITS region (< 700 bp in angiosperms) and the presence of highly conserved sequences flanking each of the two spacers make this region easy to amplify, for example even from herbarium material, using the universal primers designed by White *et al.* (1990).

The present chapter attempts to establish *Panax* species identity by using certain morphometric and molecular markers through analysis of the respective phylogenetic trees.

The markers used are:

Morphometric markers

Five qualitative and ten quantitative characters showing clear distinction between the species were considered. The qualitative characters are rhizome, bract and leaflet type, leaflet margins and leaflet shape and quantitative characters are plant height, length of petiole, petiolule, peduncle and leaf, middle leaflet length and breadth, ratio of leaf /petiole length and peduncle/leaf length (Burkill, 1902; Banerjee, 1968; Hara, 1970).

Molecular markers

1. *18S* rRNA gene: Small-subunit sequences of rRNA such as *18S* rRNA contain variable and conserved regions, including several evolutionarily conserved functional domains, therefore, *18S* rRNA gene sequence gives more essential information for the phylogenetic relationship at generic level.
2. The internal transcribed spacer (ITS) region of the 18S–5.8S–28S nuclear ribosomal operon is one of the most popular sequences for phylogenetic inference at the generic and infra-generic levels in plants.

3. Secondary structure forms an important intermediate level of description of nucleic acids that encapsulates the dominating part of the folding energy, and is often well conserved in evolution, Hence it is routinely used to support experimental findings.
4. PCR-RFLP could be used as marker to distinguish among the *Panax* species based on specific profiles.

6.2. METHODS

6.2.1. MORPHOMETRIC ANALYSIS OF *PANAX* SPECIES

6.2.1.1. Measurement of morphometric markers

Plant height was measured from stem base to the top of flower or fruit. The longest petiole length in a plant was measured to obtain the petiole length. For leaf length, the longest leaf was measured from the petiole base to the tip of the middle leaflet. Mean lengths of petiolules and acumination were determined based on the measurement of three randomly selected leaves. The leaflet length and breadth of three middle leaflets were measured to obtain the mean values. The leaflet breadth was measured at the widest point on the lamina. Ratios of characters viz., leaf length/petiole length and peduncle length/leaf length were calculated for further analyses. Variants of each qualitative parameter were assigned a numerical score (1-3) which were used in multivariate analyses. The numerical scores assigned to different traits are: bract type (1=lanceolate, 2=caducous); leaflet type (1=undivided, 2=bipinnatifid); leaflet shape (1=linear to broadly elliptic, 2=narrowly lanceolate to broadly linear, 3=oblong obovate to broadly elliptic, 4=obovate-lanceolate to narrow elliptic, 5=lanceolate to narrowly elliptic, 6=obovate to broadly elliptic, 7=narrow elliptic to broad); Leaflets margin (1=single serration, 2=double serration); Rhizome type (1=short and thick internodes, 2=slender and elongated internodes and subglobose nodes).

For scoring morphological data, we randomly selected 8 matured individuals from each population, considering the extremely small size of the populations.

Cluster analysis

Cluster analysis (CA) was carried out using PAST version 2.17c (Hammer *et al.*, 2001) downloadable at www.softpedia.com. Cluster analysis is an exploratory technique that can be use to visualize patterns by grouping sources or nodes that share similar attribute values. Agglomerative method was followed wherein all the samples (columns) start as separate groups that are joined together one at a time until a single group is formed. At each iteration, Ward's method (Also termed minimum variance or error sums of squares clustering) was applied. All possible pairs of groups were compared and the two groups chosen for fusion were those which produced a group with the lowest variance.

ANOVA and Tukey's Honest Significant Difference (HSD) test

ANOVA (fixed effect model) was performed on species data followed by Tukey's HSD test to clarify which groups among the samples in specific had significant differences.

Principal Component Analysis

Principal Component Analysis was undertaken to reduce dimension and to project the variables onto a new set of axes, such that the maximum variance is projected or extracted along the first few axes. The maximum variation uncorrelated with axis 1 is projected on the second axis, the maximum variation uncorrelated with the first and second axis was projected on the third axis, using PAST version 2.17c (Hammer *et al.*, 2001) downloadable at www.softpedia.com and between-groups PCA was run for all the analyses.

6.2.2. MOLECULAR PHYLOGENY

6.2.2.1. Taxon Sampling

The rhizomes of *Panax* spp. were collected from Arunachal Pradesh, Meghalaya, Manipur and Nagaland and brought to the laboratory for further analysis. Five individuals from each population were collected since the population sizes were very small. In places where sub-

populations were reported, a minimum of three individuals were collected from each. The rhizomes were thoroughly washed in running water and then with distilled water. They were packed in aluminium foil and properly labelled and stored at -80^{0}C.

6.2.2.2. Isolation of DNA from rhizome

DNA isolation was performed using the modified CTAB method of Doyle & Doyle (1987) and Wallace & Costa (1996). The steps for extraction were as follows:

1. Two grams of rhizome were first washed with distilled water and then surface sterilized in 30% H_2O_2 for 2-3 minutes followed by repeated washing with double distilled water.
2. Rhizomes were crushed in liquid nitrogen using mortar and pestle in a cold room.
3. Crushed rhizomes were transferred into a 1.5 ml microcentrifuge tube containing 750 µl of warm extraction buffer [Tris Base (100mM), EDTA (200mM), NaCl (1.4M), CTAB (2% w/v), PVP (3% w/v)].
4. Fifty µl of 10% SDS (sodium dodecyl sulphate) was added into the tube.
5. Ten µl of of 2-mercaptoethanol was added into the tube.
6. The tubes were vortexed for 2 minutes and then kept in a water bath for one hour at 65°C.
7. To the tube, 500 µl of chloroform-isoamyl alcohol (24:1, v/v) was added and mixed gently.
8. The tube was then centrifuged at 13,000 rpm (16,060g) for about 15 minutes at room temperature.
9. The aqueous phase was transferred to another fresh tube and an equal volume of chloroform-isoamyl alcohol (24:1, v/v) was added to it. The tube was centrifuged again at 13,000 rpm for 15 minutes at room temperature to remove impurities like proteins etc.

10. The upper aqueous layer was transferred to another fresh tube and 2/3rd volume of pre-chilled isopropanol was added and kept at -20°C overnight for precipitation.
11. Precipitated DNA was centrifuged at 13,000 rpm at 4^0C for 15 minutes. Isopropanol was discarded and pellet was washed with 450 µl of 70% ethanol twice for 10 minutes each.
12. The collected DNA was vacuum dried, dissolved in 25 µl of double distilled water and kept at -20°C for further use.

6.2.2.3. Polymerase Chain Reaction

Amplifications of the isolated DNA was carried out using the thermal cycler Applied Biosystems GeneAmp 9700.

The annealing temperature for the primers was calculated using the formula;

$$Tm = [4 (G+C) + 2 (A+T)] - 5$$

The annealing temperatures were either increased or decreased for optimising the results. To avoid nonspecific amplifications, the annealing was carried out at a lower temperature and gradually increased until a single amplification band of expected size was obtained for the target regions.

The organization of the two regions of nuclear rDNA which includes the *18S* rRNA gene and the internal transcribed spacer is shown in Fig. 6.1.

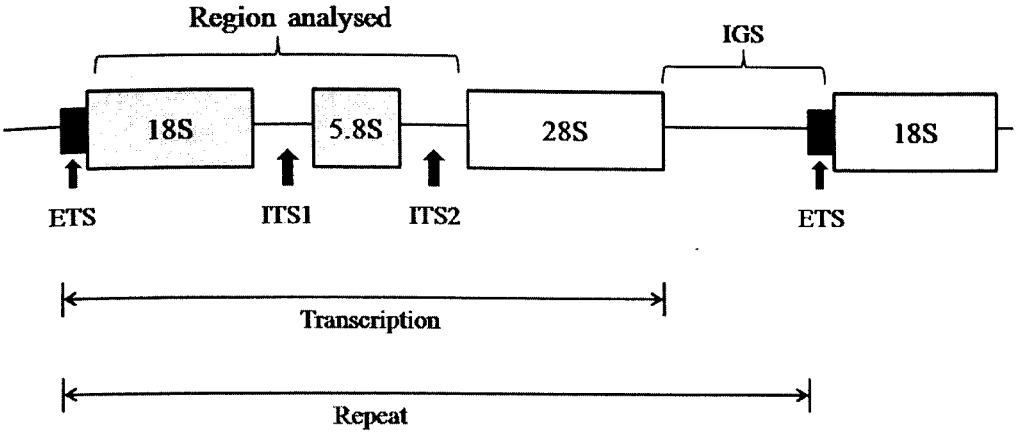


Fig. 6.1: Schematic diagram of rDNA in plants: 18S, 5.8S and 28S refer to ribosomal RNA genes. ITS1 and ITS2 are the two internal transcribed spacer regions, IGS is the intergenic spacer, ETS is the external transcribed spacer. (Region analysed includes the 18S rRNA gene and the internal tanscribed spacer region).

6.2.2.4. Amplification of *18S rRNA* gene

Amplification was carried out using two primer pairs (Table 6.2). Both the primer pairs yielded an approximately band size of 900 bp each. Each reaction mix contained 2 µl of $MgCl_2$ (25mM), 2.5µl of 10X Taq buffer (15mM), 2 µl each of the dNTP (2 mM), 0.5 µl each of the primer (2µM), 0.15 µl of *Taq* polymerase (5 Units/µl). A final volume of 25 µl was made up by adding ultra pure water. A total of 35 cycles were run for each reaction. An initial denaturation step of 5 minutes at 94°C preceded 35 cycles of denaturation at 94°C for 1 min, annealing at 66°C for 1 min, nucleotide extension at 72°C for 1 min, followed by a post-elongation step at 72°C for 10 mins at the end of the cycle.

Table 6.1: Primers used for amplification of *18S* rRNA gene.

CODE	SEQUENCE	REFERENCE
18S1FP	5'-AACCTGGTTGATCCTGCCAG-3'	Present study
18S1RP	5'-ATCATTACTCCGATCCCGA-3'	Present study
18S2FP	5'-TACGTTGGCCTTCGGGAT-3'	Present study
18S2RP	5'-GCAGGTTCACCTACGGAAAC-3'	Present study

6.2.2.5. Amplification of 18S-28S rDNA ITS region

The forward primer ITSI (White *et al.,* 1990) located at the distal part of the *18S* rRNA gene and the reverse primer ITS4 (White *et al.,* 1990) located at the initial part of the 28S rRNA gene were used to amplify the 18S-28S rDNA Internal Transcribed Spacer (ITS) region which yielded a band of approximately 700 bp in length (Table 6.2). Each reaction mix contained 2 µl of $MgCl_2$ (25mM), 2.5µl of 10X Taq buffer (15mM), 2 µl each of the dNTP (2 mM), 0.5 µl each of the primer (2µM), 0.15 µl of *Taq* polymerase (5 Units/µl). A final volume of 25 µl was made up by adding ultra pure water. A total of 35 cycles were run for each reaction. An initial denaturation step of 5 minutes at 94°C preceeded 35 cycles of denaturation at 94°C for 1 min, annealing at 57.5°C for 1 min, nucleotide extension at 72°C for 1 min, followed by a post-elongation step at 72°C for 10 mins at the end of the cycle.

Table 6.2: Primers used for amplification of 18S-28S rDNA ITS region.

CODE	SEQUENCE	REFERENCE
ITS1	5'- GTCCACTGAACCTTATCATTTAG-3'	White *et al.*, 1990
ITS4	5'-TCCTCCGCTTATTGATATGC-3'	White *et al.*, 1990

Primer stock solution

The primers used for amplification were procured in lyophilized, desalted and PAGE purified condition from M/s Sigma-Aldrich, India. The lyophilized primers were first dissolved in ultra pure water to prepare a stock solution of 100μM. From the stock solution a working solution of 2 μM was prepared by further dilution and stored at -20°C.

Deoxyribonucleoside triphosphate solutions

Deoxyribonucleoside triphosphate (dNTP) solutions were procured from M/s Bangalore Genei in 100 mM stock solutions. dATP, dCTP, dGTP and dTTP were mixed in equal quantities to prepare a final working solution of 2 mM concentration of each and stored at -20°C.

Taq DNA polymerase and Assay Buffer

Taq DNA Polymerase is a 94 kD thermostable enzyme isolated from the bacterium *Thermus aquaticus*. Taq DNA polymerase is used in PCR because it can withstand repeated exposure to high temperatures. The enzyme lacks 3' to 5' exonuclease (proof reading) activity but has an inherent 5' to 3' exonuclease activity. *Taq* DNA polymerase and buffer E [Triton X-100 and 15mM $MgCl_2$] were procured from M/s Bangalore Genei. Additional

$MgCl_2$ (25mM) was also procured. They were stored in a deep freezer at -20°C for further use.

Nucleotide sequencing

DNA sequencing was done for the 18S-28S ITS region and *18S* rDNA. Prior to sequencing, the PCR products were purified using QIAquick PCR purification kit (Microcentrifuge and Vacuum protocol) from QIAGEN. Sixty µl (~100 ng) of the amplified product was packed in a 1.5 ml eppendorf tube and sent for sequencing to M/s Macrogen, Korea. The sequencing was done using the Standard-seq service which is the most typical single primer extension protocol where the plasmid or PCR product template was read with the designated primer.

PCR-restriction fragment length polymorphism (PCR-RFLP)

The amplified 18S-28S rDNA ITS region was subjected to restriction digestion using different restriction endonucleases. The enzymes were selected using the DS Gene version 1.5 software available at ds-gene.software.informer.com, wherein a known sequence was subjected to digestion with different restriction endonucleases. The endonucleases were shortlisted based on the cutting sites and the fragments generated and three were selected (Table 6.3). The digestion mixture was prepared in a 1.5 mL microcentrifuge tube containing (12 µl) of the amplicon, 2 µl of the enzyme, 2 µl o f the buffer and the volume was made upto 20 µl with ultra pure water. The tube was sealed with parafilm and incubated overnight in a water bath at the optimal temperature according to the manufacturer's instructions for different restriction endonucleases.

Table 6.3: List of restriction enzyme used.

Sl. No.	Restriction Enzyme	Cutting site	Source Organism	Buffer (Incubation Temperature)
1	BstN1	CC↓WGG GGW↑CC	*Bacillus stearothermophilus*	*1X NEBuffer 2 (60°C)
2	MspI	C↓CGG GGC↑C	*Moraxella* sp.	*NEBuffer 4 (37°C)
3	PspG1	↓CCWGG GGWCC↑	*Pyrococcus* sp.	*NEBuffer 4 (75°C)

(* New England Biolabs)

Agarose gel electrophoresis

Genomic DNA, PCR products and digested fragments were separated on ethidium bromide (0.5 µg/mL) stained agarose gel using horizontal electrophoresis in 1X TBE (Tris Borate EDTA) buffer and visualized on 0.8% and 2% agarose gels respectively. For genomic DNA, 5 µl of DNA was mixed with 2 µl of loading buffer (Bromophenol 6X) and loaded into the agarose gel wells. For detection of PCR amplicons, 10 µl of the product was mixed with 3 µl of the loading buffer separately and loaded into the gel wells. Electrophoresis was carried at 60 V for 1 hour. One of the wells was loaded with 3 µl of λ DNA *Hind* III/ *Eco*R1 double digest marker (for genomic DNA) or 100 bp DNA ladder (for PCR amplicons) purchased from Bangalore Genei.

Restriction digested amplicons were electrophoresed in a 4% agarose gel and 20 µl of restriction product was mixed with 3 µl of loading buffer and was then loaded into the well of the thick gel and electrophoresed for about 6-7 hours at 50 V. A 100 bp molecular weight marker was also loaded in one of the lanes which served as a standard for estimating the band sizes of DNA fragments. Observation and quantification of the restriction fragments was done using BIO-RAD GelDoc EZ imager or KODAK GEL LOGIC 1500 IMAGING SYSTEM.

Sequence alignment

The sequences of the 18S-28S ITS and *18S* rDNA regions were subjected to Multiple Sequence Alignment. Each sequence was separately used as a query sequence for BLAST search for related sequences in GenBank (http://www.ncbi.nlm.nih.gov). The retrieved published ITS sequences were used to determine the boundaries of these regions in the species presently studied. The sequences thus obtained, were aligned using Clustal X program (Thompson *et al.*, 1997) available at ftp://ftpigbmc.u-strasbg.fr/pub/ClustalX/. A default setting with a fixed gap penalty of 15, a floating gap penalty of 6.66 and DNA transition weight of 0.5 in the multiple alignment parameter option were selected for alignment using the Clustal X program.

Sequence characteristics

Sequence characteristics of all genomic regions were computed using Seqstate Version 1.4.1 (Müller, 2005) usable at http://bioinfweb.info/Software/SeqState and DnaSP version 5.10.1 (Librado and Rozas, 2009) available at http://www.ub.edu/dnasp.

Phylogenetic analyses

Models of the DNA substitution process are fundamental to statistical phylogenetic inference. The use of a particular substitution model may change the outcome of the

phylogenetic analysis and statistical model selection has become an essential step for the estimation of phylogenies from DNA sequence alignments. The jModelTest 0.1 (Pasoda, 2008) was applied for both the data set. There are 88 models currently implemented in jModelTest, including 11 substitution schemes, equal or unequal base frequencies (+F), a proportion of invariable sites (+I) and rate variation among sites with a number of rate categories (+G). The Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were analyzed for comparing the models. The final model was selected on the basis of higher free parameters. The selected model for the *18S* rDNA was GTR+G (General Time Reversible + Gamma) and for 18S-28S ITS region the model selected was GTR+G+I (General Time Reversible + Gamma +invariable sites).

The phylogenetic relationships were estimated using Neighbour Joining and Maximum Likelihood methods conducted in MEGA version 5 (Tamura *et al.*, 2011) available at www.megasoftware.net and Bayesian analysis using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) usable at mrbayes.sourceforge.net applying the individual data sets, i.e. 18S rDNA and 18S-28S ITS regions separately. In Neighbour Joining method, Kimura-2-parameter model with gamma distribution was used for the analysis. In Maximum Likelihood method, the bootstrap analysis (Felsenstein, 1985) was carried out for 1000 replicates to check the relative level of support for individual clades on the cladograms in both the methods. Bayesian inference (BI) of phylogeny was conducted for both the data sets using the selected model above. BI analyses were performed for 1,000,000 generations applying the default settings (MCMC, two runs with four chains each, heating temperature 0.2, saving one tree every 100 generations). The tree created by MRBAYES was viewed with the program Fig tree v1.4.0 (Rambaut, 2012) available at http:// *tree.bio.ed.ac.uk /software/figtree/.*

6.2.2.6. Secondary structure

The secondary structure of 5.8S rRNA was predicted using the programme ViennaRNA Package version 2.1.6. The Vienna RNA secondary structure server provides a webinterface to the most frequently used functions of the Vienna RNA software package for the analysis of RNA secondary structures. It offered prediction of secondary structure from a single sequence, prediction of the consensus secondary structure for a set of aligned sequences and the design of sequences that fold into a predefined structure. All three services could be accessed via the Vienna RNA webserver at http:// rna.tbi.univie.ac.at/. (Hofacker, 2003).

The RNAfold provided both the most basic and most widely used function. The server predicted only the minimum free energy (mfe) structure of a single sequence using the classic algorithm of Zuker and Stiegler (1981). In addition to mfe folding the server could calculate equilibrium base pairing probabilities via McCaskill's partition function algorithm (Mc Caskill, 1990).

Cluster analysis

Cluster analysis or clustering is the task of grouping a set of objects in such a way that objects in the same group (called a cluster) are more similar (in some sense or another) to each other than to those in other groups (clusters). Cluster analysis can either be agglomerative or divisive. Agglomerative method begins with many clusters which are united step by step, until a single cluster is formed. Whereas the divisive methods start with a single cluster and split (partition) them into smaller clusters.

Cluster dendrogram was generated from the banding patterns from PCR-RFLP analysis using NTSYS pc version 2.02k available at ntsyspc.software.informer.com. The method SAHN (Sequential Agglomerative Hierarchical Nested cluster analysis) was used based on

Jaccard's coefficient of similarity (Jaccard, 1908) which is used to estimate the level of similarity for multivariate data. Presence or absence of bands was scored for all the individual samples so that a descending pattern of values was obtained.

The Jaccard's coefficient value for each sample was calculated using the formula

Jaccard's coefficient = Nab/ [(Na+Nb) – Nab]

Where, Nab = Number of common bands in both samples

Na = Total number of bands present in the first sample

Nb = Total number of bands present in the second sample

6.3. RESULTS

6.3.1. Morphometric analysis of *Panax* species

Cluster analysis using morphometric markers

Fifteen morphological characters of 56 specimens (7 species x 8 individuals each) were used in Cluster Analysis (CA) taking each individual specimen as the Operational Taxonomic Unit (OTU). Cluster analysis (CA) in the data set produced two major clusters in the dendrogram. The first cluster comprised of *P. assamicus* with a few individuals at lower distance measure. The second cluster was formed at greater distance and divided into sub-clusters which included *P. assamicus, P. bipinnatifidus, P. variabilis, P. pseudoginseng* and *Panax* sp. *P. bipinnatifidus* formed a subcluster of its own but all the other specimens were intermixed with each other and did not give a clear distinction in all the species (Fig. 6.2).

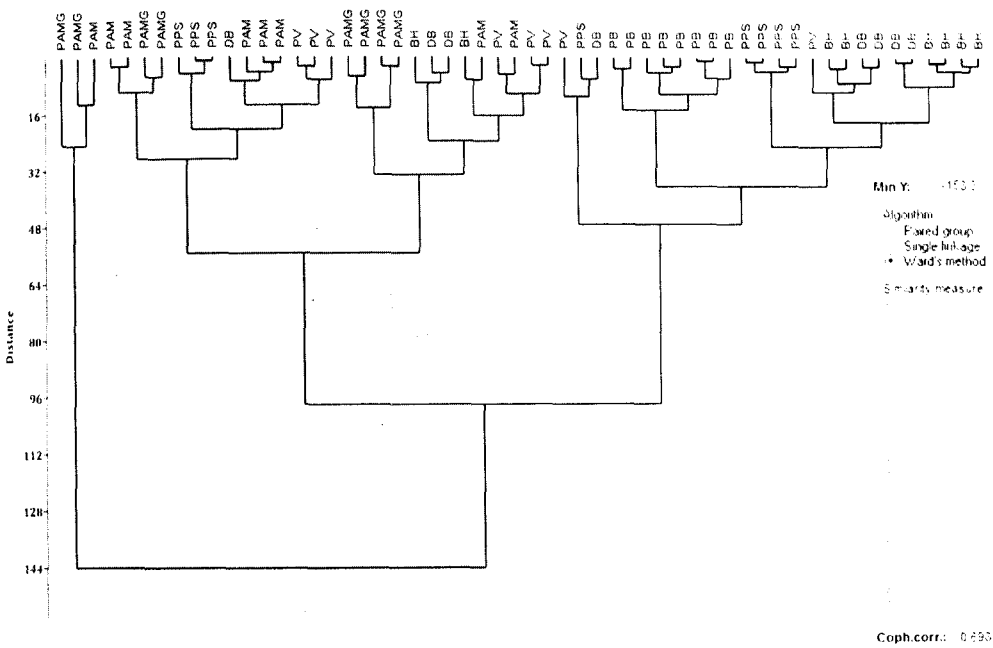


Fig. 6.2: Cluster analysis based on Ward's method using PAST version 2.17. (PAMG- *P. assamicus* from Meghalaya, PAM- *P. assamicus* from Manipur, PB- *P. bipinnatifidus*, PV- *P. variabilis*, PPS- *P. pseudoginseng*, DB- *Panax* sp. from Arunachal, BH- *Panax* sp. from Nagaland).

Variation in morphometric markers among the Panax species

Plant height was significantly greater (Tukey's HSD test, $p<0.05$) in *P. assamicus* of Meghalaya than Manipur. The plant height did not vary among the species ($p>0.05$). The petiole length and ratio of leaf length/petiole length did not vary among the species. However, peduncle length significantly varied among *P. assamicus, P. variabilis* and *Panax* sp. (Tukey's HSD test, $p<0.05$) except in *P. bipinnatifidus* and *P. pseudoginseng* (Tukey's HSD test, $p<0.05$). Leaf length in *P. assamicus* and *Panax* sp. was significantly greater than *P. bipinnatifidus, P. variabilis* and *P. pseudoginseng* (Tukey's HSD test, $p<0.05$). The ratio of leaf length/petiole length did not vary significantly among the species (Tukey's HSD test, $p>0.05$). The petiolule length did not vary significantly between *P. assamicus* (Manipur) and *P. assamicus* (Meghalaya). *P. variabilis* and *Panax* sp. had significantly longer (Tukey's HSD test, $p<0.05$) petiolule than *P. pseudoginseng, P. assamicus* and *P. bipinnatifidus.* The middle leaflet length varied significantly (Tukey's HSD test, $p<0.05$) between *P. bipinnatifidus* and *P. pseudoginseng,* and *P. variabilis, P. assamicus* and *Panax* sp. *P. assamicus* and *Panax* sp. did not vary significantly between their respective states (Tukey's HSD test, $p>0.05$). The middle leaflet breadth varied significantly (Tukey's HSD test, $p<0.05$) between their respective states both in *P. assamicus* and *Panax* sp. The ratio of peduncle length/leaf length varied significantly (Tukey's HSD test, $p<0.05$) among the species. The ratio in *P. assamicus* varied significantly (Tukey's HSD test, $p<0.05$) among the populations but did not vary among the *Panax* sp. populations. Acumination in leaf structure varied significantly among the species as well as between the two states in *P. assamicus* and *Panax* sp. populations (Tukey's HSD test, $p<0.05$) (Table 6.4)

Table 6.4: Morphological quantitative characters for the different species of *Panax* species (n=56).

Morphological characters	*P. assamicus*		*P. bipinnatifidus*	*P. pseudoginseng*	*P. variabilis*	*Panax* sp.	
	Meghalaya	Manipur				Arunachal Pradesh	Nagaland
Plant height	76.33±9.00^{a}	59.23±5.31^{ab}	46.50±1.46^{b}	45.88±3.58^{b}	52.88±4.53^{b}	49.75±4.50^{b}	49.84±3.28^{b}
Petiole length	10.36±1.00^{a}	9.59±0.93^{a}	8.64±0.80^{a}	8.99±0.36^{a}	10.85±0.74^{a}	9.40±0.40^{a}	10.19±0.53^{a}
Peduncle length	27.60±2.29^{a}	20.17±2.11^{b}	11.64±0.80^{d}	8.79±0.45^{d}	18.13±1.49^{bc}	12.63±0.92^{cd}	14.84±0.51^{bcd}
Leaf length	22.91±2.25^{ab}	21.99±1.41^{ab}	17.26±0.67^{b}	18.46±0.72^{b}	25.05±1.15^{b}	21.37±1.86^{ab}	21.86±0.66^{ab}
Ratio of leaf length/petiole length	2.23±0.10^{a}	2.38±0.17^{a}	2.10±0.15^{a}	2.06±0.03^{a}	2.34±0.06^{a}	2.24±0.14^{a}	2.17±0.07^{a}
Petiolule length	0.50±0.08^{c}	0.87±0.20^{abc}	0.85±0.09^{abc}	0.80±0.07^{bc}	1.33±0.07^{bc}	1.47±0.28^{c}	1.09±0.10^{abc}
Middle leaflet length	11.70±1.15^{a}	12.99±0.30^{ab}	7.80±0.22^{d}	8.05±0.31^{cd}	11.30±0.15^{ab}	10.14±0.34^{bc}	10.71±0.40^{b}
Middle leaflet breadth	1.95±0.18^{b}	4.01±0.34^{a}	2.28±0.06^{b}	2.44±0.12^{b}	2.36±0.04^{b}	3.35±0.31^{a}	2.44±0.02^{b}
Ratio of peduncle length/leaf length	1.25±0.10^{a}	0.97±0.15^{ab}	0.69±0.07^{bc}	0.48±0.03^{c}	0.72±0.04^{bc}	0.60±0.03^{c}	0.68±0.03^{bc}
Acumination	0.96±0.10^{bc}	1.33±0.09^{ab}	1.50±0.09^{a}	0.89±0.08^{c}	1.66±0.07^{a}	1.35±0.09^{a}	0.68±0.09^{c}

Note: Within a row, values followed by same superscripts are not significantly different while those followed by different superscripts are significantly different from each other (Tukey HSD test, $p < 0.05$). All measurements are in cm.

The PCA using morphometric markers that included 10 quantitative characters and 5 qualitative characters yielded 15 components. The five species of *Panax* were grouped on first two principal axes at 95% concentration ellipse (Fig. 6.3). A total variance of 90.65 % was explained by the first two principal axes. The first axis (PC1) explained 79.59 % of the variance in which plant height and peduncle length showed significant values in differentiating the components to separate the five *Panax* species. The second axis (PC2) explained 11.05 % of the variance in which leaf length and leaf shape showed significant values of taxonomic importance. In the third axes which explained 7.08 % of the variance, only the peduncle length revealed significant taxonomically differentiating the character to discriminate between the *Panax* species studied. In the PCA scatter plot, the samples within the genus formed five distinct groups, i.e., *P. assamicus, P. pseudoginseng, Panax* sp., *P. variabilis and P. bipinnatifidus.* The samples of *P. bipinnatifidus* and *Panax* sp. showed overlapping and more condensed indicating less variation. *P. variabilis* and *P. pseudoginseng* also showed overlapping and the samples are stretched out like an ellipse indicating a wide range of variation. *P. assamicus* on the other hand showed overlapping with *P. variabilis* and *P. pseudoginseng* and is broadly stretched out indicating a wide range of variation. *P. bipinnatifidus* is narrowly stretched with less morphological variation.

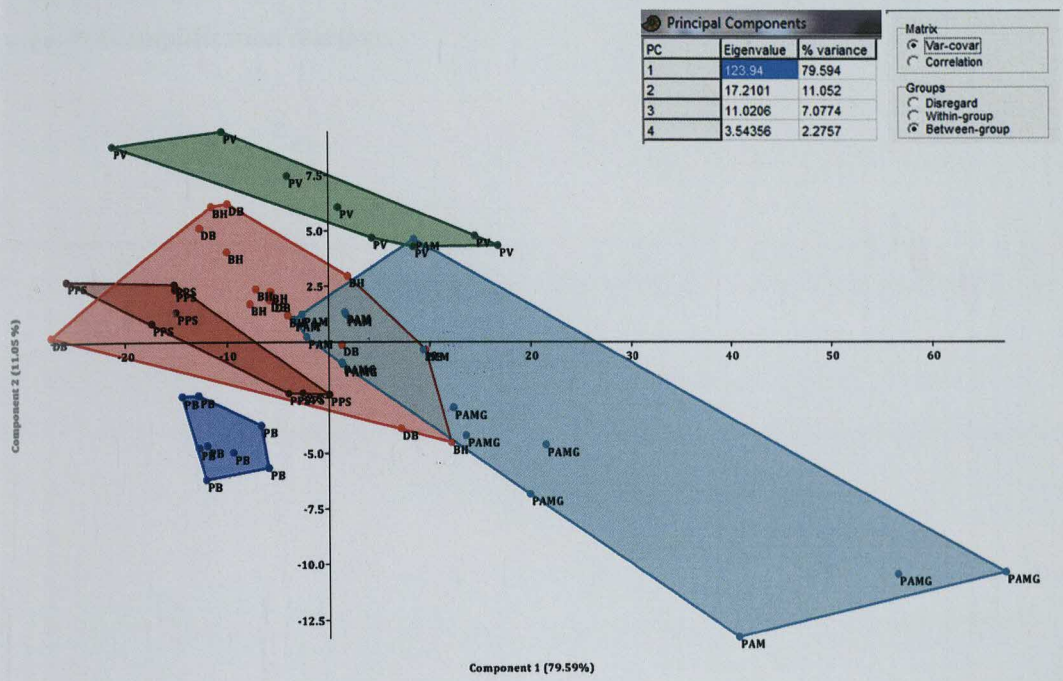
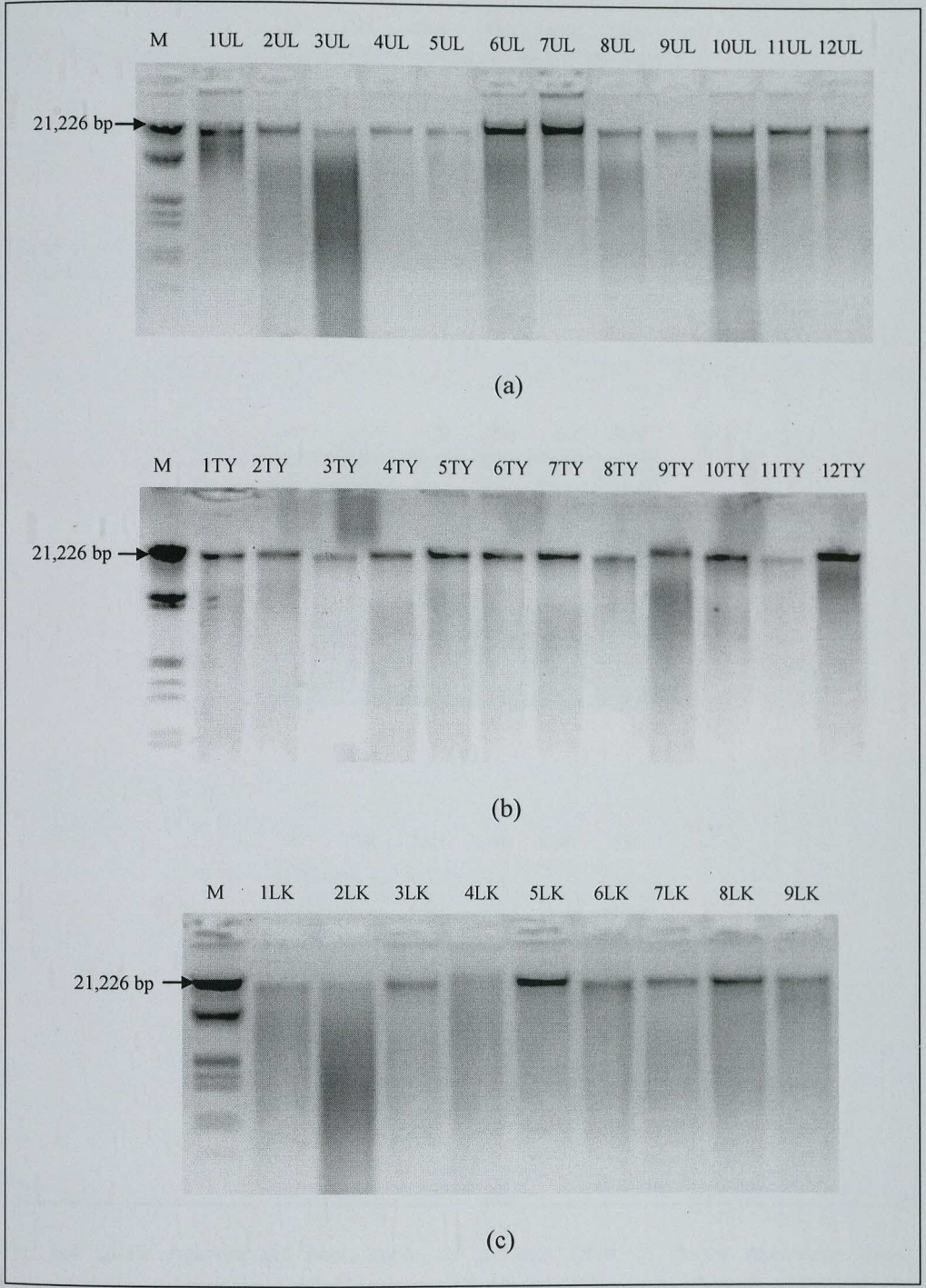


Fig. 6.3: Scatter plot of the first two components from principal component analysis (PCA) based on 15 variables of 56 specimens of *Panax* L. taxa; (PAMG- *P. assamicus* from Meghalaya, PAM- *P. assamicus* from Manipur, Pb- *P. bipinnatifidus*, PV- *P. variabilis*, PPS- *P. pseudoginseng*, DB- *Panax* sp. from Arunachal, BH- *Panax* sp. from Nagaland) showing group distances among the specimens.

6.3.2. Molecular phylogeny

6.3.2.1. DNA isolation

Approximately 21 kb genomic DNA bands were seen on electrophoresis in 0.8% agarose gel for all the samples (Fig. 6.4). The purity of the isolated DNA was assessed spectrophotometrically using the absorbance ratio of A_{260}/A_{280} which ranged between 1.8 - 2.0 indicating that all the samples were free from contaminating proteins and were suitable for PCR amplification reactions.



*(The code 1-3 represents sub-population1, 4-6=sub-population 2, 7-9=sub-population 3, 10-12= sub-population 4)

Fig. 6.4 (a-c): Agarose gel photographs of genomic DNA of *Panax assamicus* from Meghalaya (UL- Laitkseh, TY-Tyllang, LK-Laitkor) (M= λ DNA *Hind* III *Eco*R1 double digest marker).

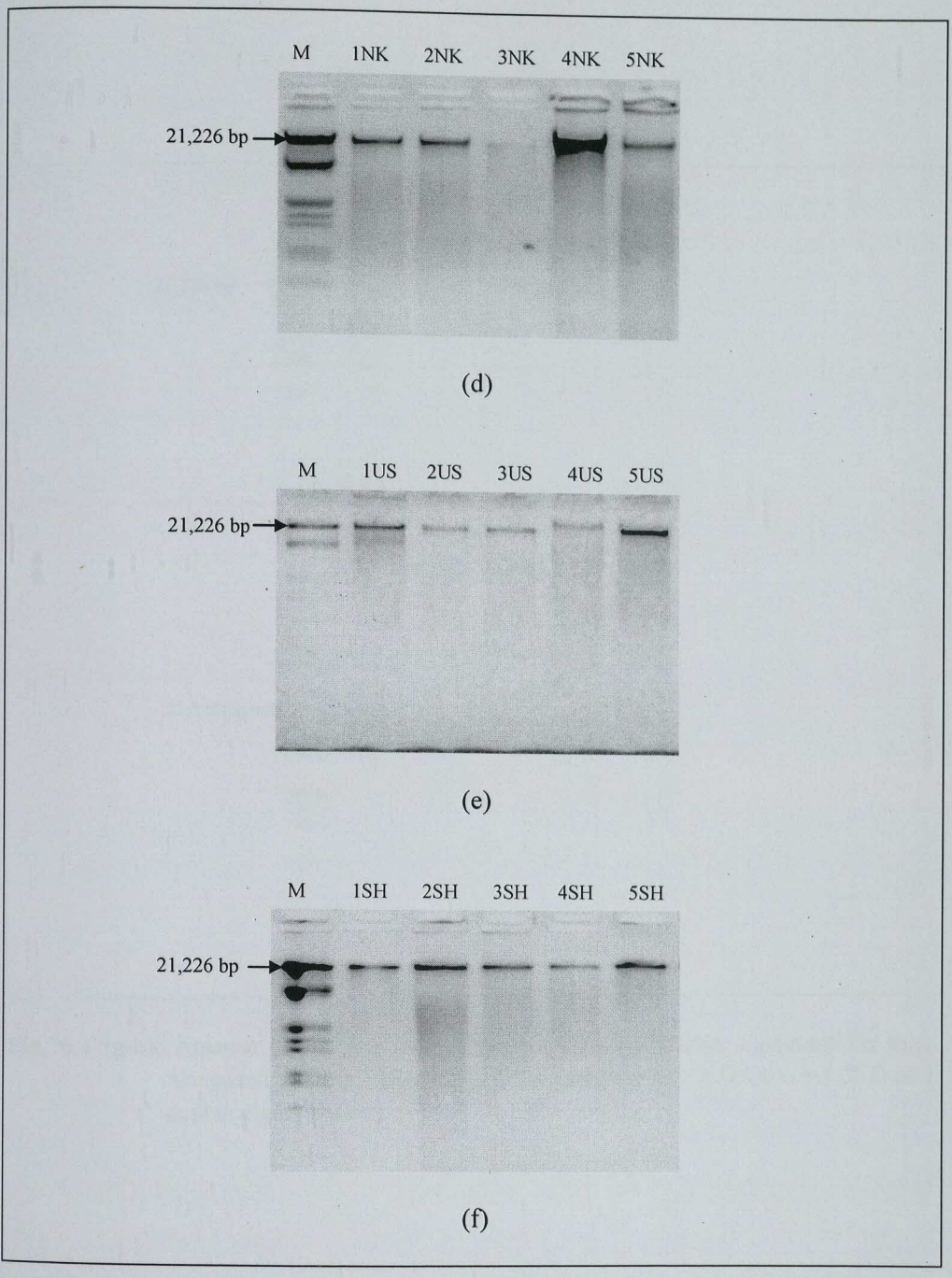
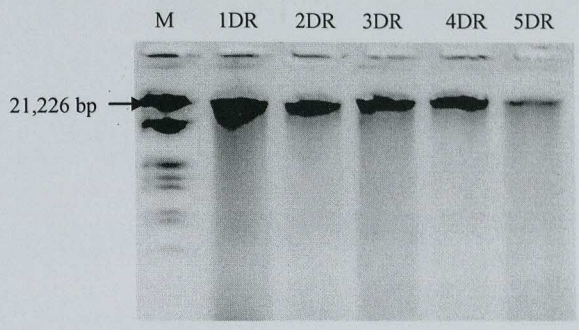
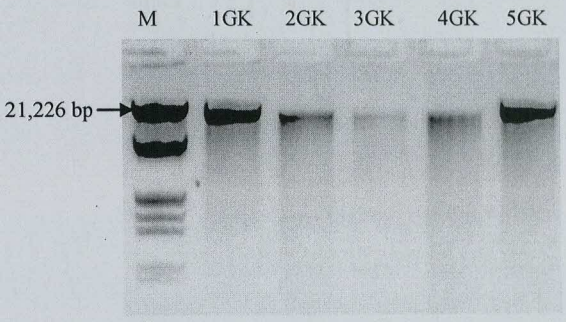


Fig. 6.4 (d-f): Agarose gel photographs of genomic DNA of *Panax assamicus* from Meghalaya (Nk-Nongkrem, US-Upper Shillong) and Manipur (SH) (M= λ DNA *Hind* III *Eco*R1 double digest marker).



(g)



(h)

Fig. 6.4 (g-h): Agarose gel photographs of genomic DNA of *Panax bipinnatifidus* from Arunachal Pradesh (DR-Dirang, GK-Gomkang) (M= λ DNA *Hind* III *Eco*R1 double digest marker).

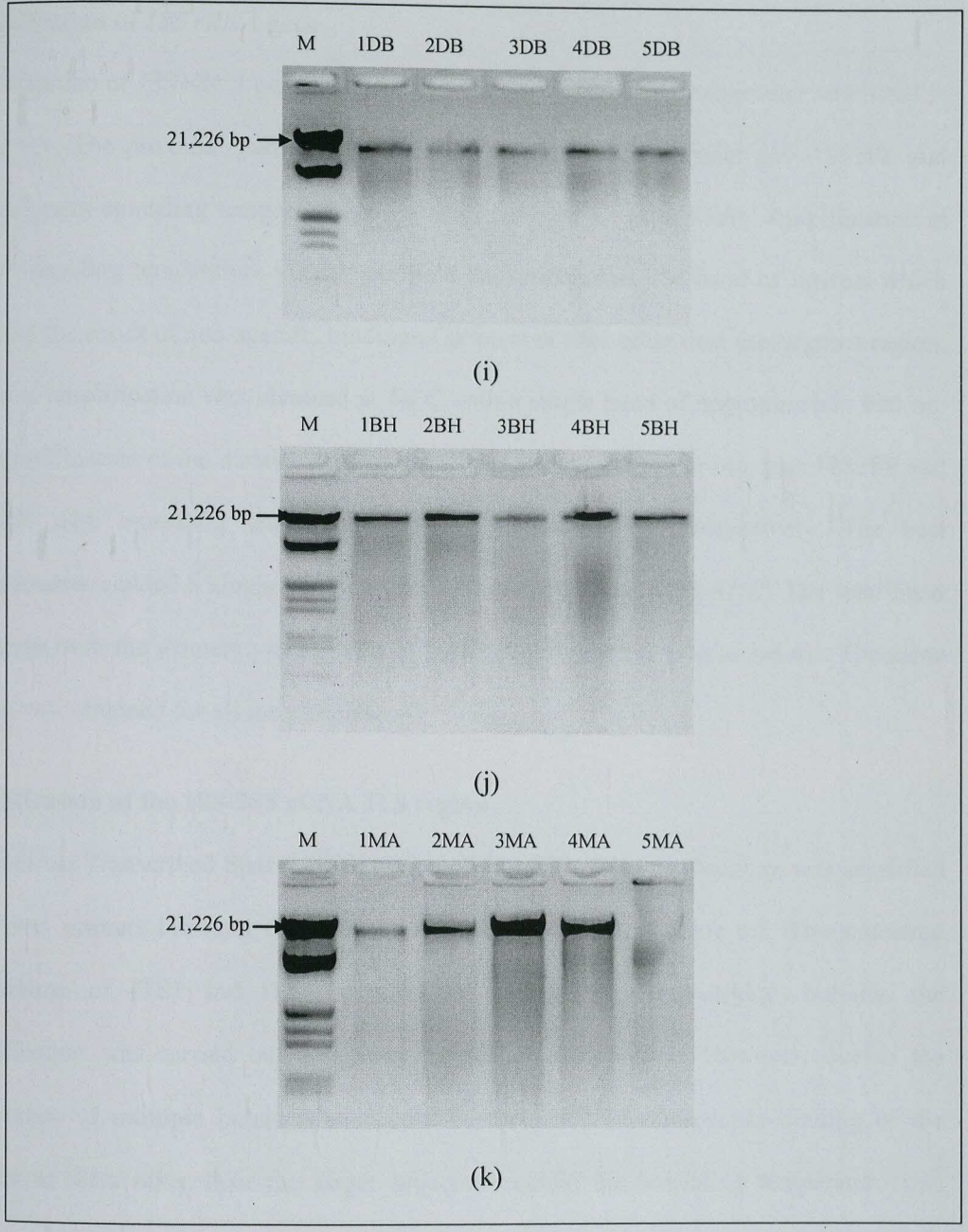


Fig. 6.4 (i-k): Agarose gel photographs of genomic DNA of *Panax* sp. from Arunachal Pradesh (DB); and Nagaland (BH) and *Panax variabilis* from Manipur (MA) (M= λ DNA *Hind* III *Eco*R1 double digest marker).

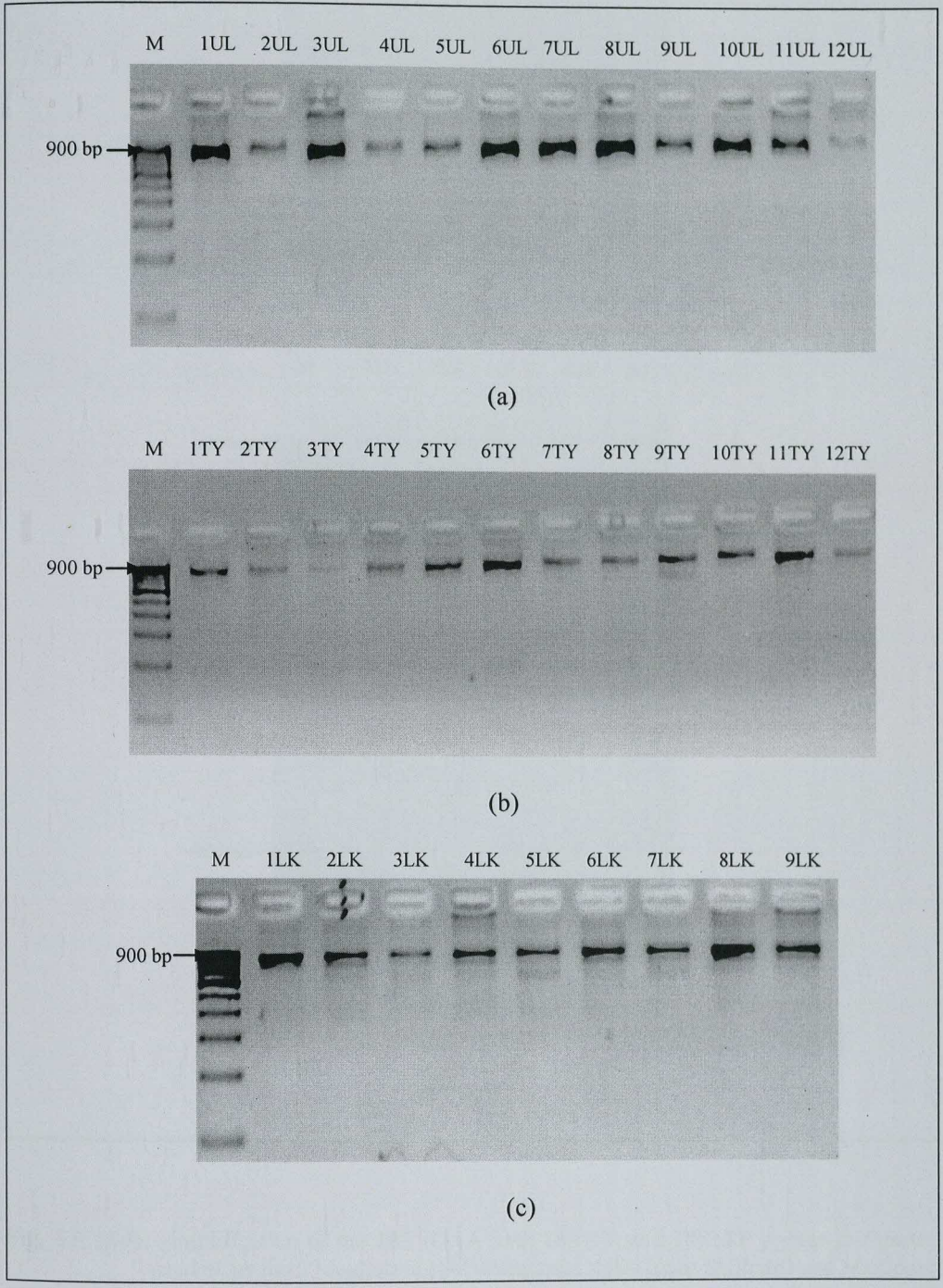
6.3.2.2. DNA amplification

Amplification of *18S rRNA* gene

Amplification of *18S rRNA* gene in *Panax* sp. was performed using two primer sets listed in Table 6.1. The proximal part of the gene was amplified using primer pair 18S1FP and 18S1RP with annealing temperatures of 66.3°C and 61.8°C respectively. Amplification at lower annealing temperature yielded multiple bands including the band of interest which could be the result of non-specific binding of primers at sites other than the targeted region. The best amplification was obtained at 66°C with a single band of approximately 900 bp. The amplification of the distal part of the gene was achieved using primer pair 18S2FP and 18S2RP with annealing temperature of 65.3°C and 63.4°C respectively. The best amplification yielded a single band of size 900 bp approximately at 67°C. The total band size from both the primers yielded size of 1.8Kb approximately (Fig. 6.5-6.6). The same profile was obtained for all the samples.

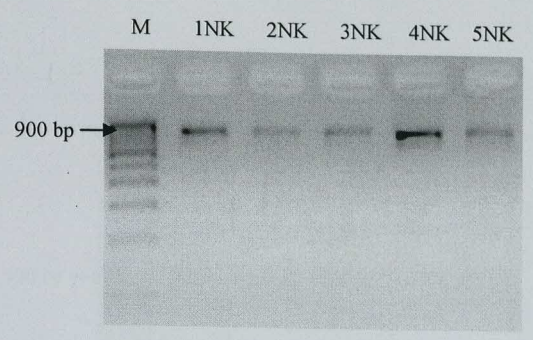
Amplification of the 18S-28S rDNA ITS region

The Internal Transcribed Spacer (ITS) region of 18S-28S rDNA in *Panax sp.*was amplified using two primers ITS1and ITS4 (White *et al.*, 1990) listed in Table 6.2. The annealing temperature of ITS1 and ITS4 were 58.2°C and 61.5°C respectively. Initially, the amplification was carried out at a lower annealing temperature. However, due to the appearance of multiple bands which could be the result of nonspecific binding of the primers at sites other than the target sequence region, the annealing temperature was subsequently increased. The best amplification result was obtained at annealing temperature of 57.5°C where a single band of approximately 700 bp was amplified (Fig. 6.7). The amplifications for the rest of the samples were carried out at this specified temperature.

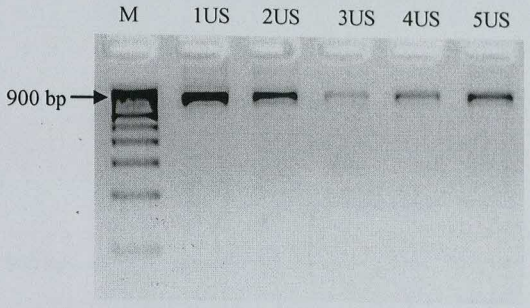


*(The code 1-3 represents sub-population1, 4-6=sub-population 2, 7-9=sub-population 3, 10-12= sub-population 4)

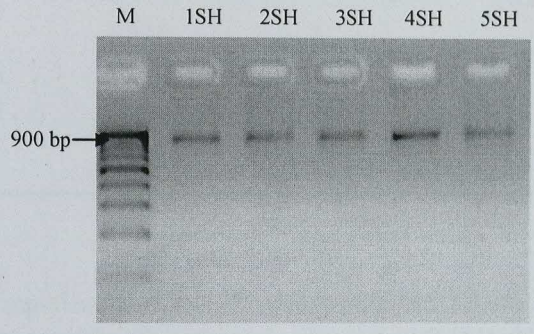
Fig. 6.5 (a-c): Amplification of the 18S rDNA with 18SFP1 and 18S1RP primer in *Panax assamicus* from Meghalaya (UL- Laitkseh, TY- Tyllang, LK- Laitkor) (M = 100 bp DNA ladder).



(d)



(e)



(f)

Fig. 6.5 (d-f): Amplification of the 18S rDNA with 18S1FP and 18S1RP primer in *Panax assamicus* from Meghalaya (NK-Nongkrem, US-Upper Shillong) and Manipur (SH) (M = 100 bp DNA ladder)

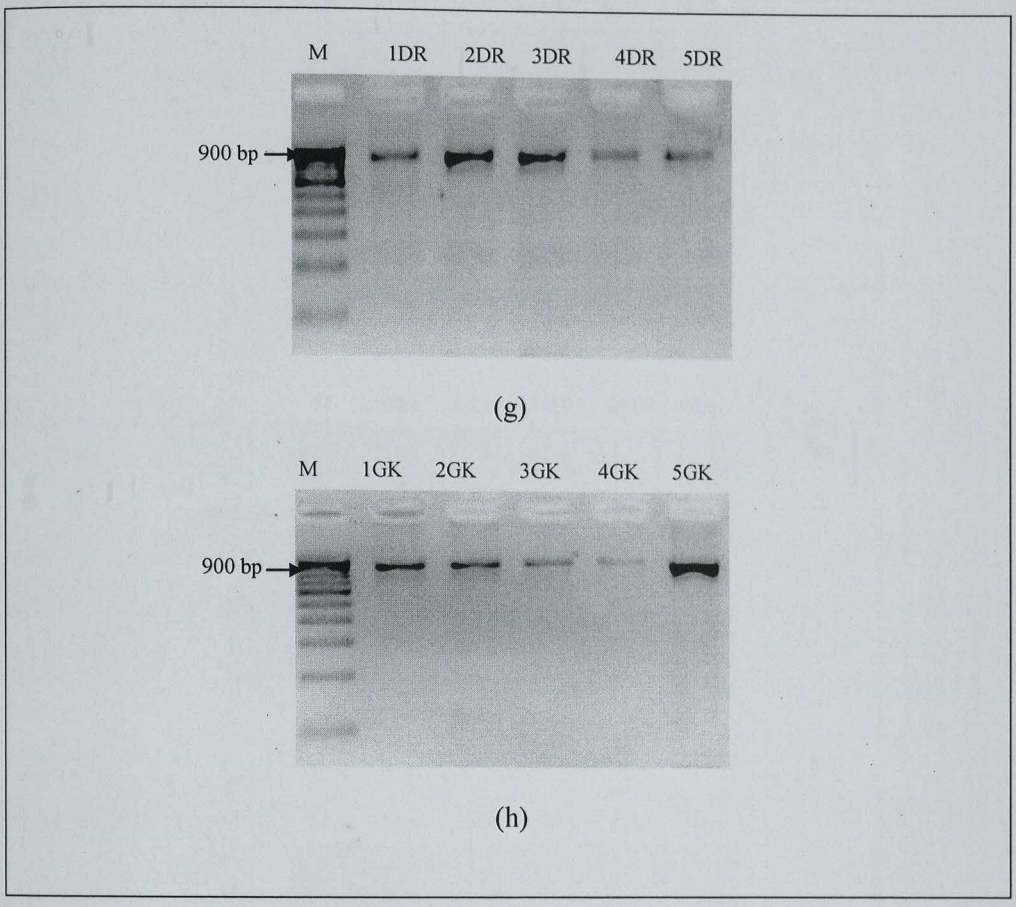


Fig. 6.5 (g-h): Amplification of the 18S rDNA with 18S1FP and 18S1RP primer in *Panax bipinnatifidus* from Arunachal Pradesh (DR-Dirang, GK-Gomkang) (M = 100 bp DNA ladder).

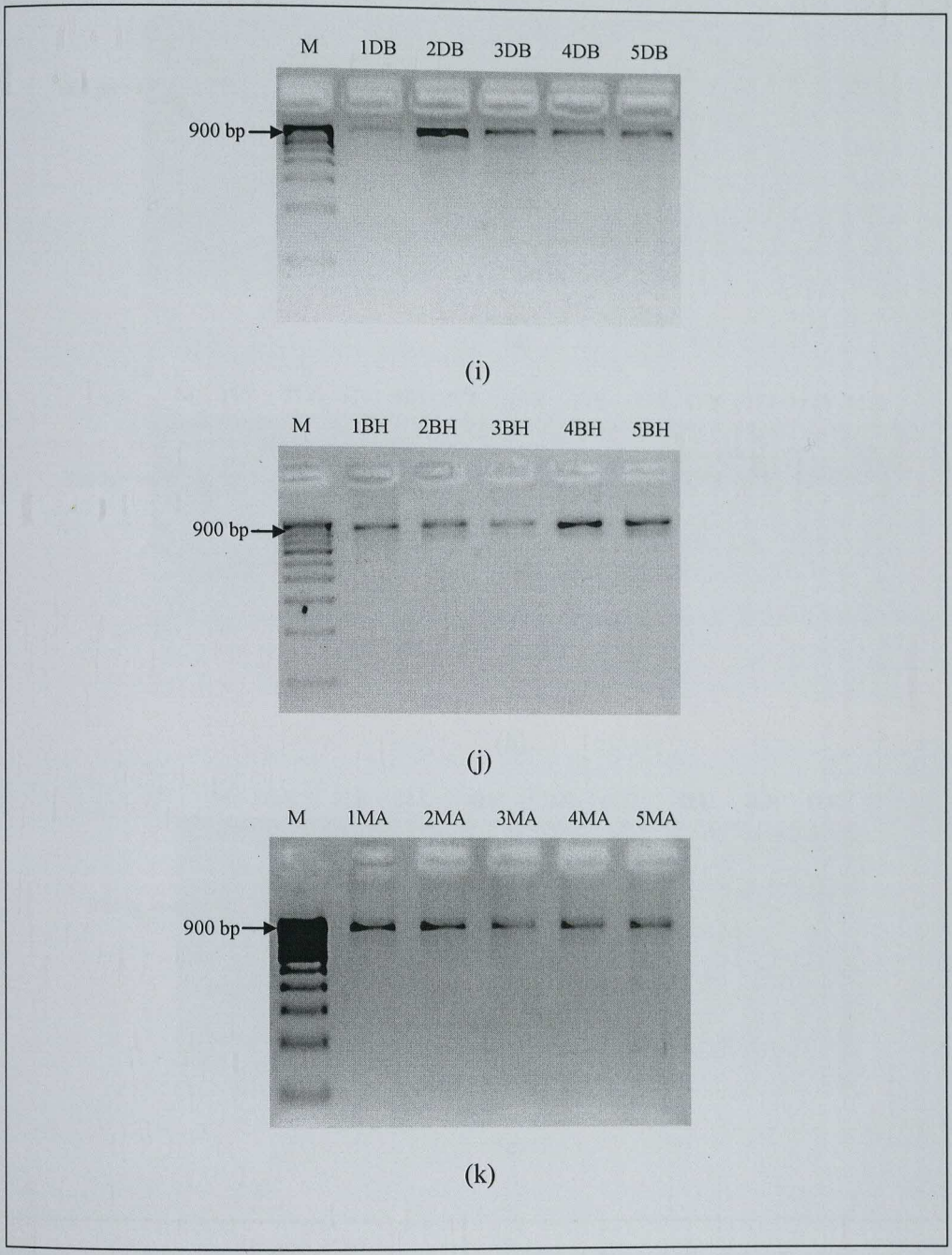
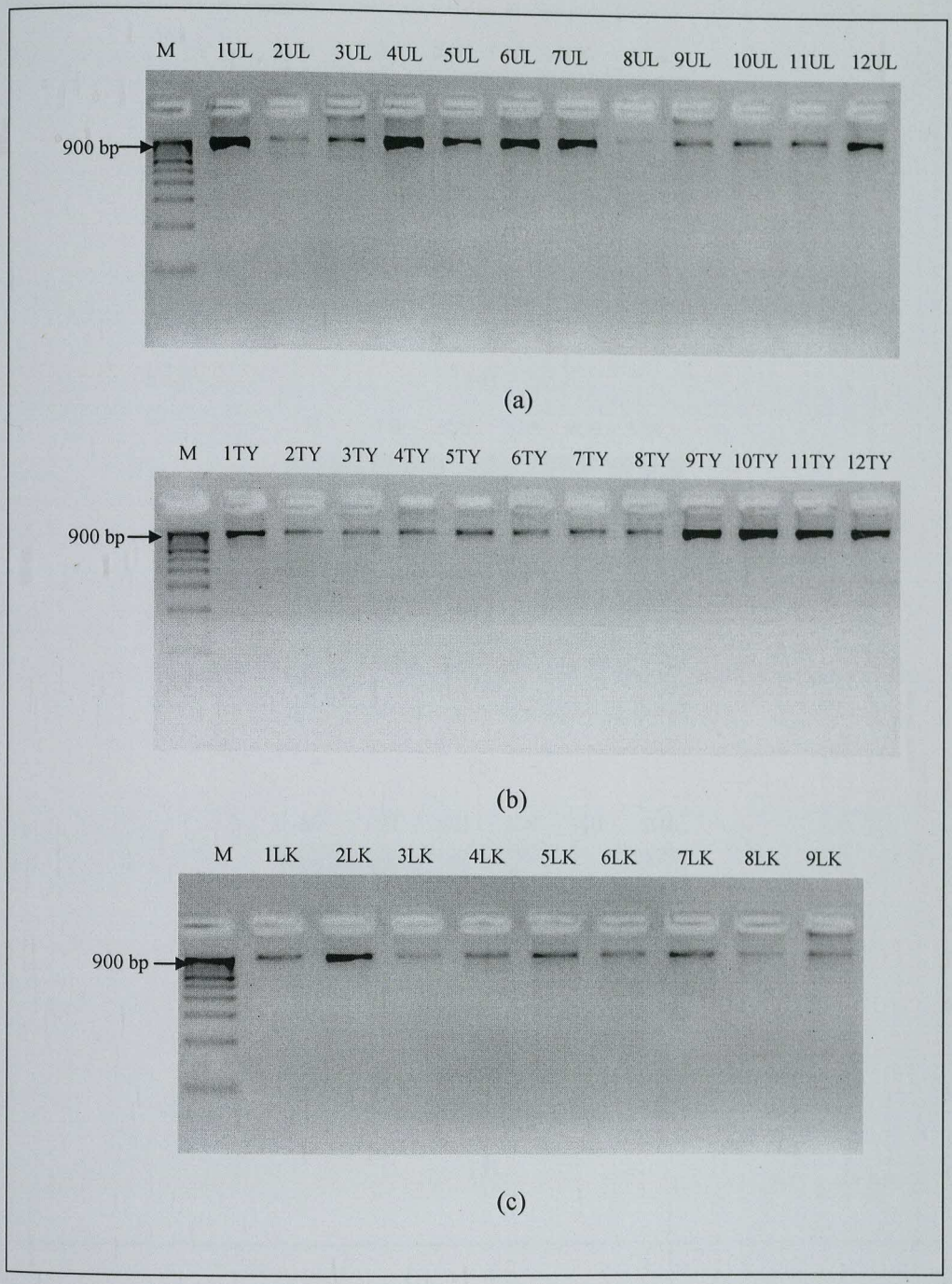


Fig. 6.5 (i-k): Amplification of the 18S rDNA with 18S1FP and 18S1RP primer in *Panax* sp. from Arunachal Pradesh (DB) and Nagaland (BH) and *Panax variabilis* from Manipur (MA) (M = 100 bp DNA ladder).



*(The code 1-3 represents sub-population1, 4-6=sub-population 2, 7-9=sub-population 3, 10-12= sub-population 4)

Fig. 6.6 (a-c): Amplification of the 18S rDNA with 18SFP2 and 18S2RP primer in *Panax assamicus* from Meghalaya (UL-Laitkseh, TY-Tyllang, LK-Laitkor) (M = 100 bp DNA ladder).

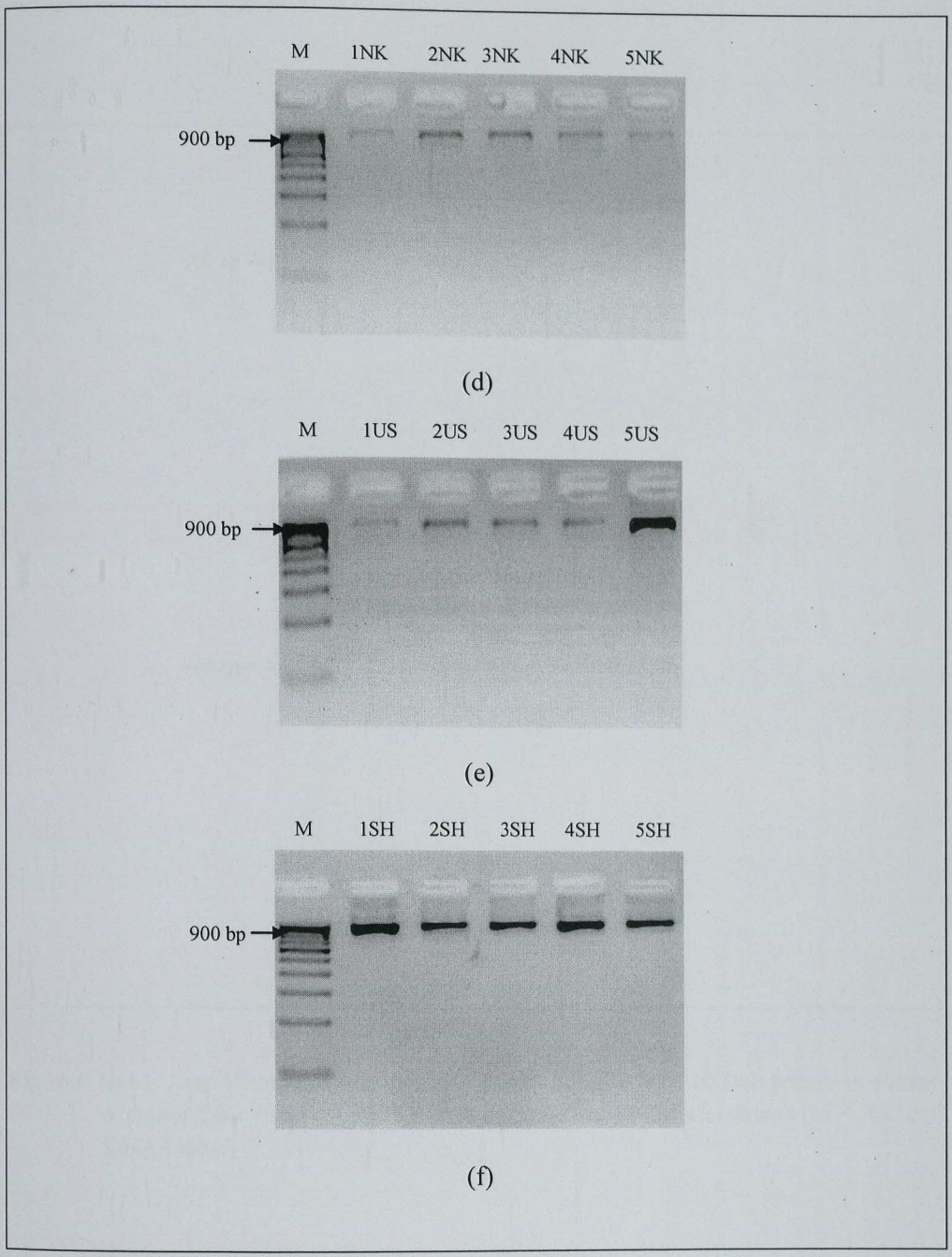


Fig. 6.6 (d-f): Amplification of the 18S rDNA with 18S2FP and 18S2RP primer in *Panax assamicus* from Meghalaya (NK-Nongkrem, US-Upper Shillong) and Manipur (SH) (M = 100 bp DNA ladder)

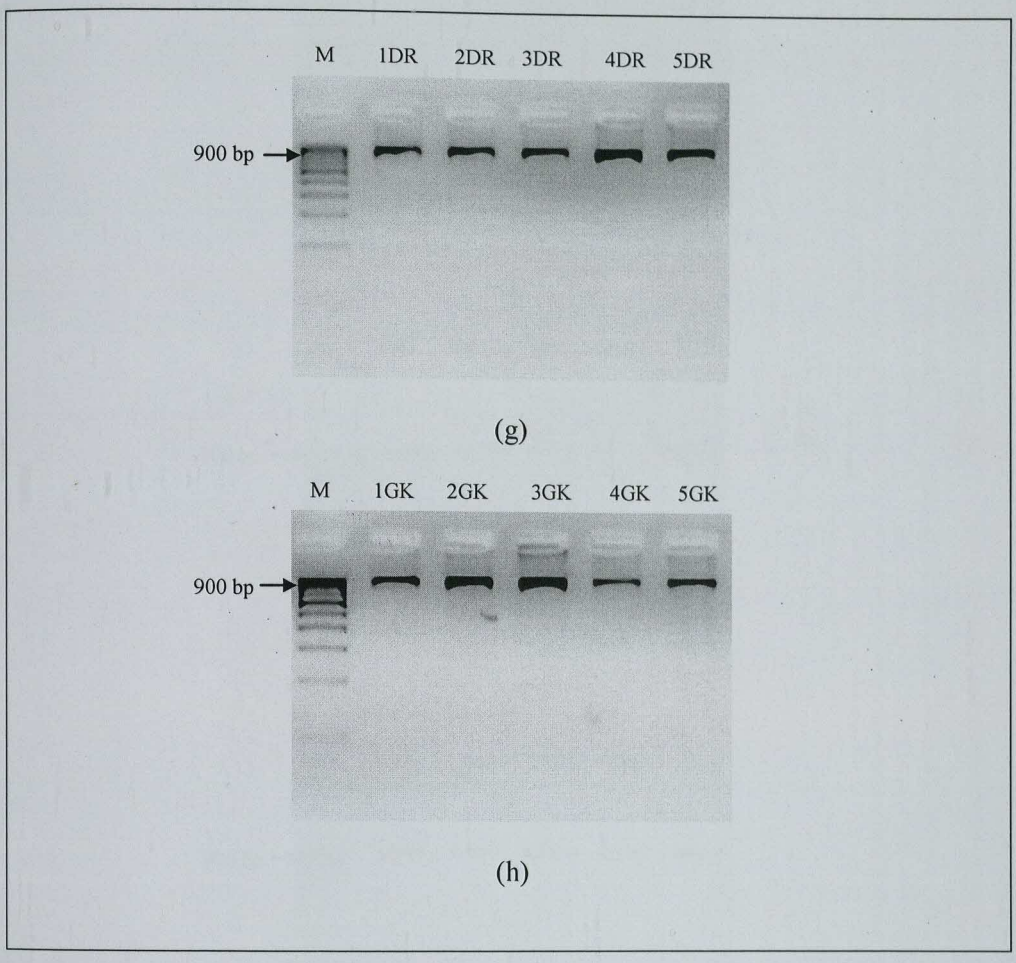


Fig. 6.6 (g-h): Amplification of the 18S rDNA with 18S2FP and 18S2RP primer in *Panax bipinnatifidus* from Arunachal Pradesh (DR-Dirang, GK-Gomkang) (M = 100 bp DNA ladder)

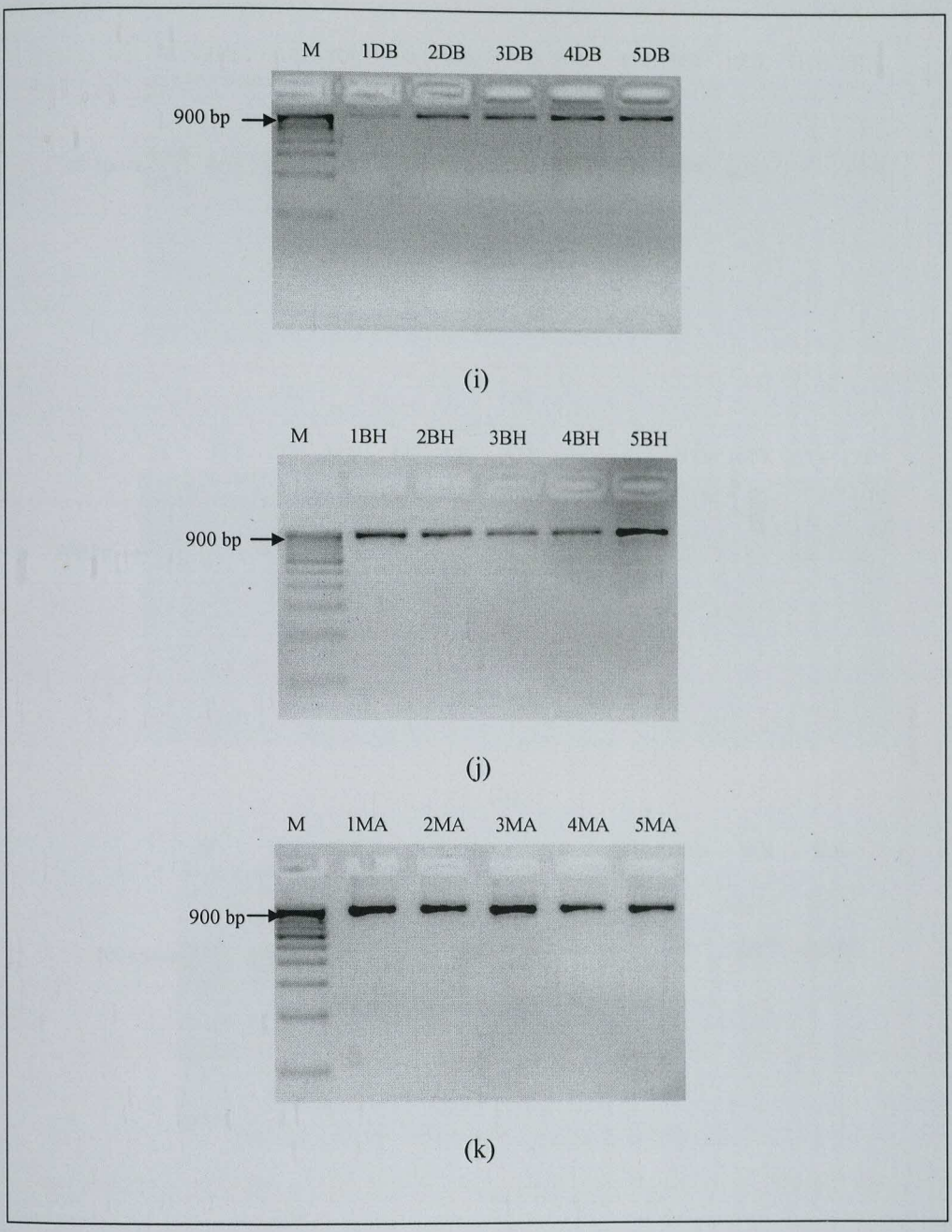
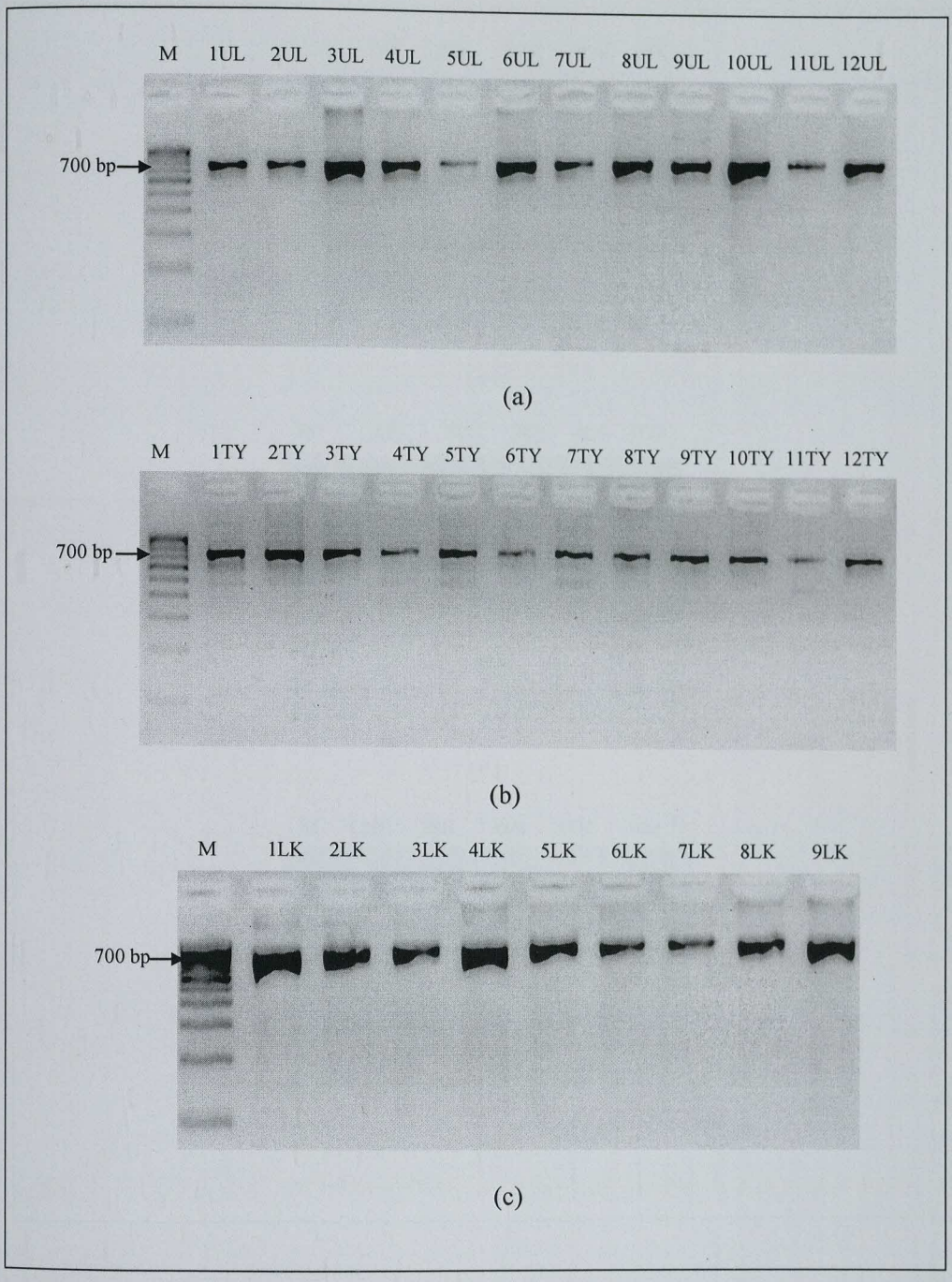


Fig. 6.6 (i-k): Amplification of the 18S rDNA with 18S2FP and 18S2RP primer in *Panax* sp. from Arunachal Pradesh (DB) and Nagaland (BH) and *Panax variabilis* from Manipur (MA) (M = 100 bp DNA ladder).



*(The code 1-3 represents sub-population1, 4-6=sub-population 2, 7-9=sub-population 3, 10-12= sub-population 4)

Fig. 6.7 (a-c): Amplification of the 18S-28S rDNA ITS region in *Panax assamicus* from Meghalaya (UL-Laitkseh, TY-Tyllang, LK-Laitkor, NK-Nongkrem) (M = 100 bp DNA ladder).

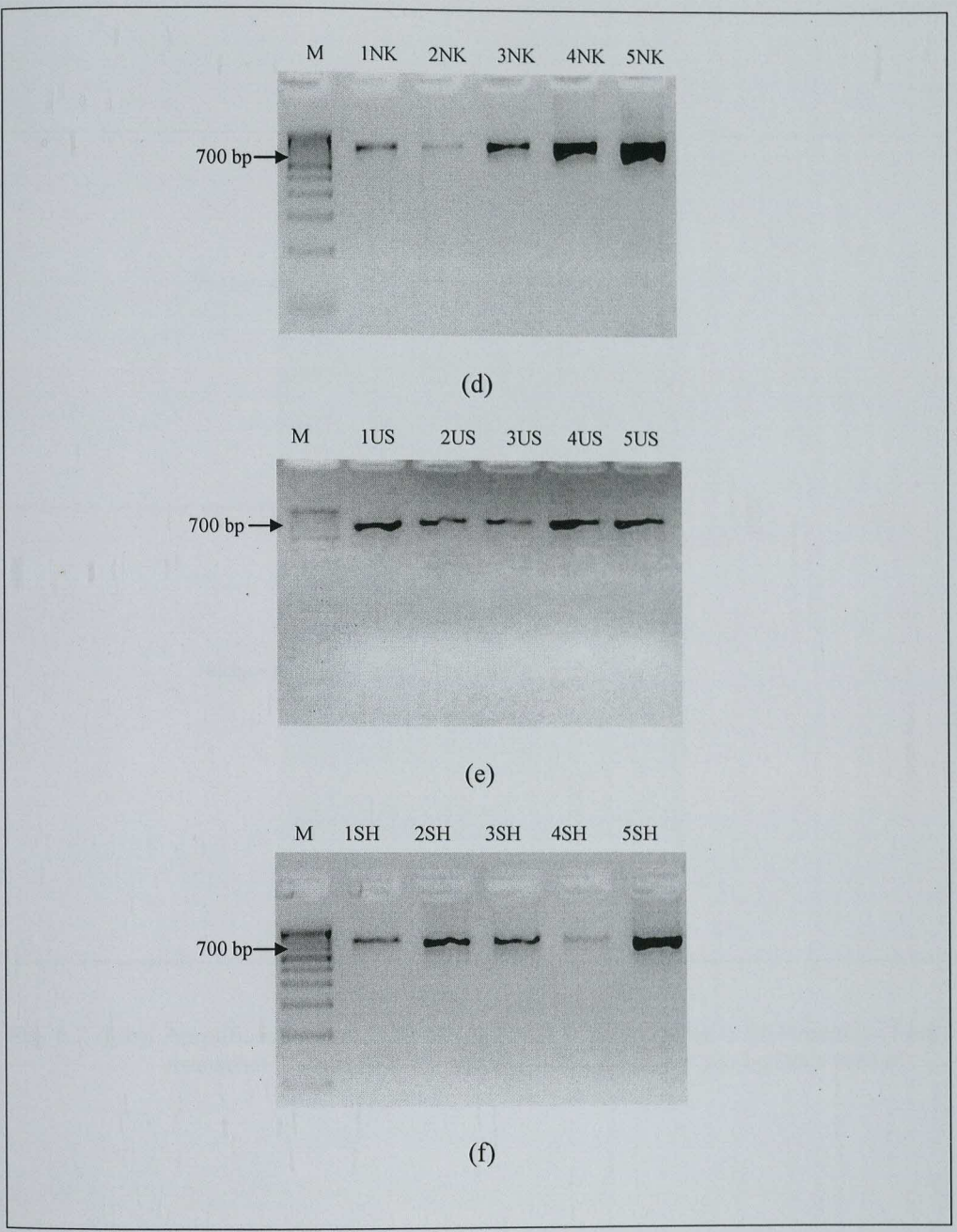


Fig. 6.7 (d-f): Amplification of the 18S-28S rDNA ITS region in *Panax assamicus* from Meghalaya (NK-Nongkrem, US-Upper Shillong) and Manipur (SH) (M = 100 bp DNA ladder).

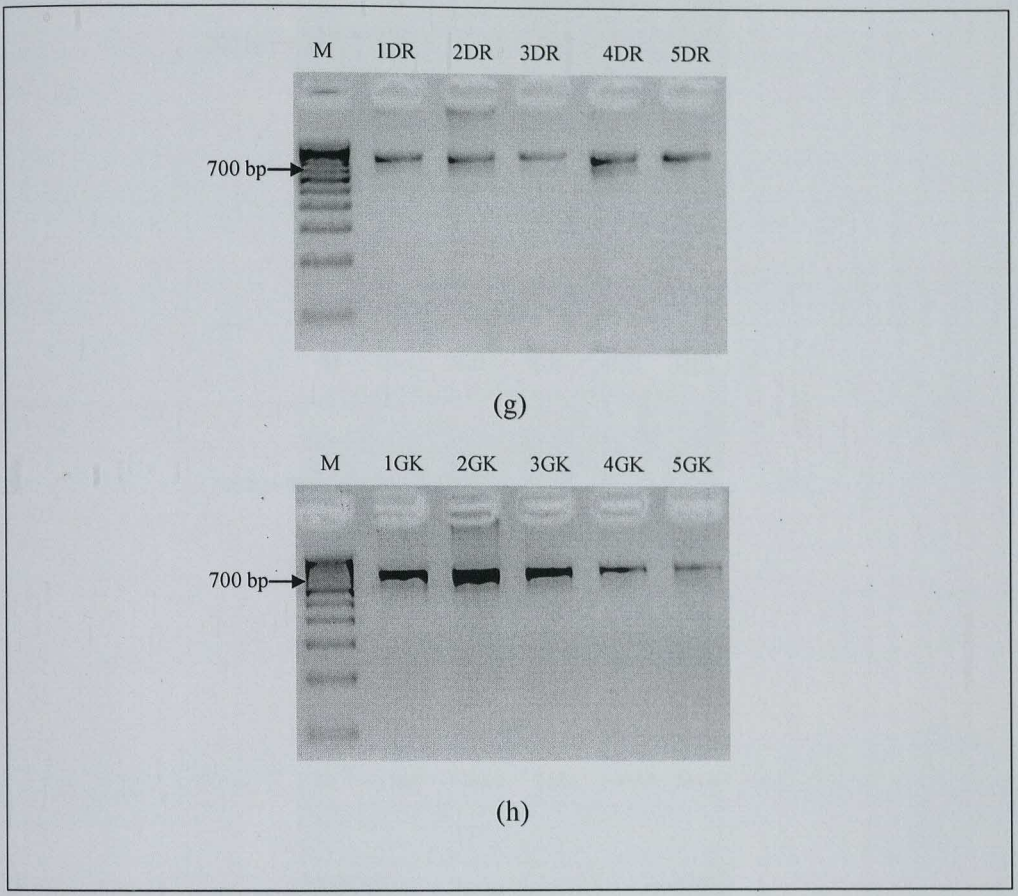


Fig. 6.7 (g-h): Amplification of the 18S-28S rDNA ITS region in *Panax bipinnatifidus* from Arunachal Pradesh (DR-Dirang, GK-Gomkang) (M = 100 bp DNA ladder).

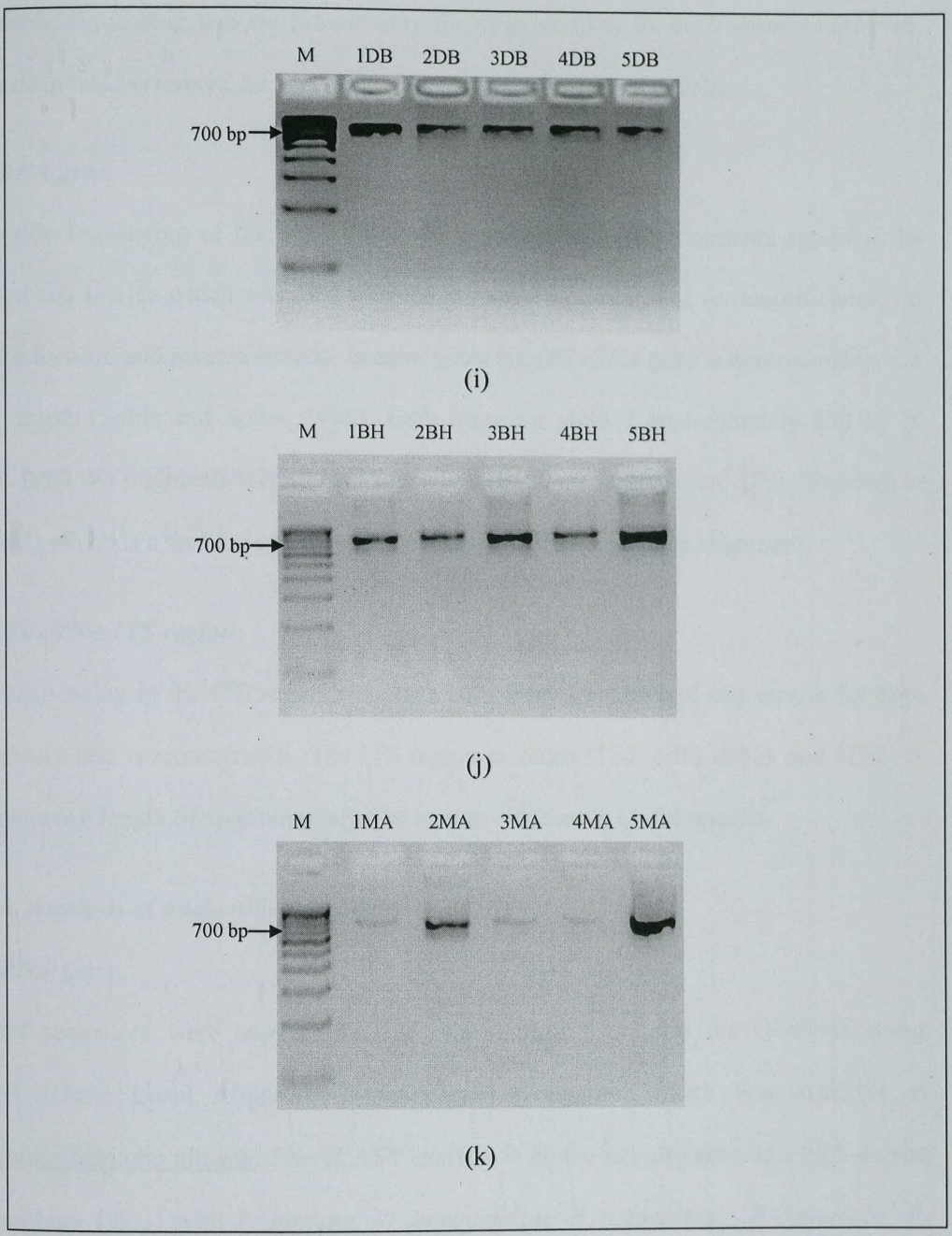


Fig. 6.7 (i-k): Amplification of the 18S-28S rDNA ITS region in *Panax* sp. from Arunachal Pradesh (DB) and Nagaland (BH) and *Panax variabilis* from Manipur (MA) (M = 100 bp DNA ladder).

6.3.2.3. Nucleotide sequence analysis

Nucleotide sequencing was carried out only for three samples for each species collected. Sequencing was performed for two different regions of the genomic DNA.

18S rRNA gene

Nucleotide sequencing of the 18S rDNA was done for both the fragments applying the standard-seq service which was read with the designated primer used for amplification for both the forward and reverse strands. In eukaryotes the *18S rRNA* gene is approximately 1.8 kb in length (Soltis and Soltis, 1998). Each fragment yielded approximately 850 bp in length. Both the fragments were joined using GeneDoc software version 2.7.0 (Nicholas *et al.*, 1997) which is a tool for editing and annotating multiple sequence alignment.

18S-28S rDNA ITS region

DNA sequencing of the ITS region was done employing the standard-seq service for both the forward and reverse strands. The ITS region includes ITS1, 5.8S rRNA and ITS2. A total sequence length of approximately 620 bp was obtained in all the species.

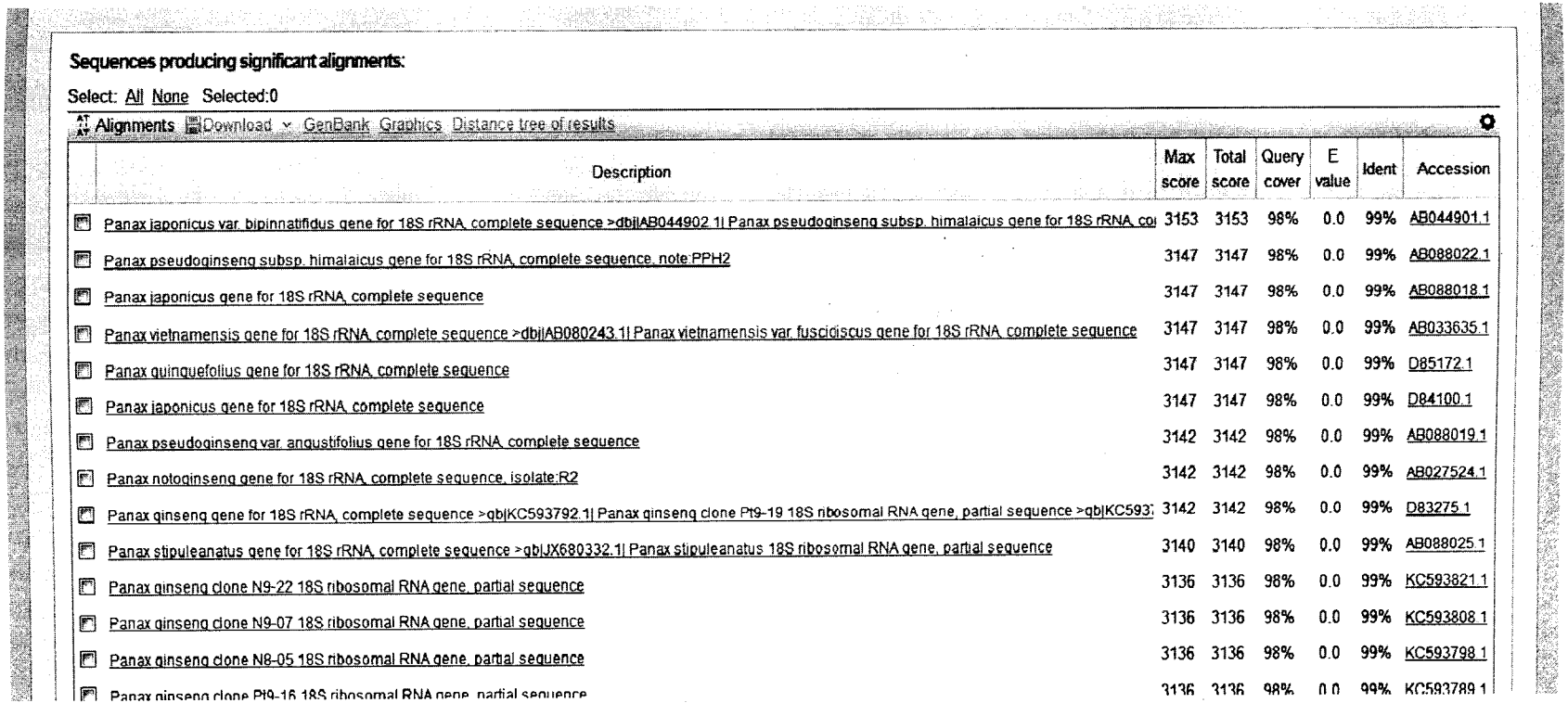
6.3.2.4. Analysis of nucleotide sequences

18S rRNA gene

Aligned sequences were used to retrieve related sequences from the GenBank using BLAST (Basic Local Alignment Search Tool) programme which was available at http://www.ncbi.nlm.nih.gov. The BLAST analysis in all the samples showed a high degree of homology (99%) with *P. ginseng, P. quinquefolius, P. notoginseng, P. japonicus, P. vietnamensis, P. stipuleanatus, P. pseudoginseng* subsp. *himalaicus, P. bipinnatifidus, P. notoginseng* (Fig. 6.8).

18S-28S rDNA ITS region

Panax assamicus collected from Meghalaya and Manipur showed a 100% identity with *P. assamicus* from GenBank. *Panax* sp. collected from Nagaland and Arunachal Pradesh showed 99% identity with *P. japonicus, P. quinquefolius, P.variablis* and *P. vietnamensis*. A 100% identity with *P. variabilis* was found for *P. variabilis* collected from Manipur. *P. bipinnatifidus* showed 99% identity with *P. bipinnatifidus* from GenBank database (Fig. 6.9-6.13).



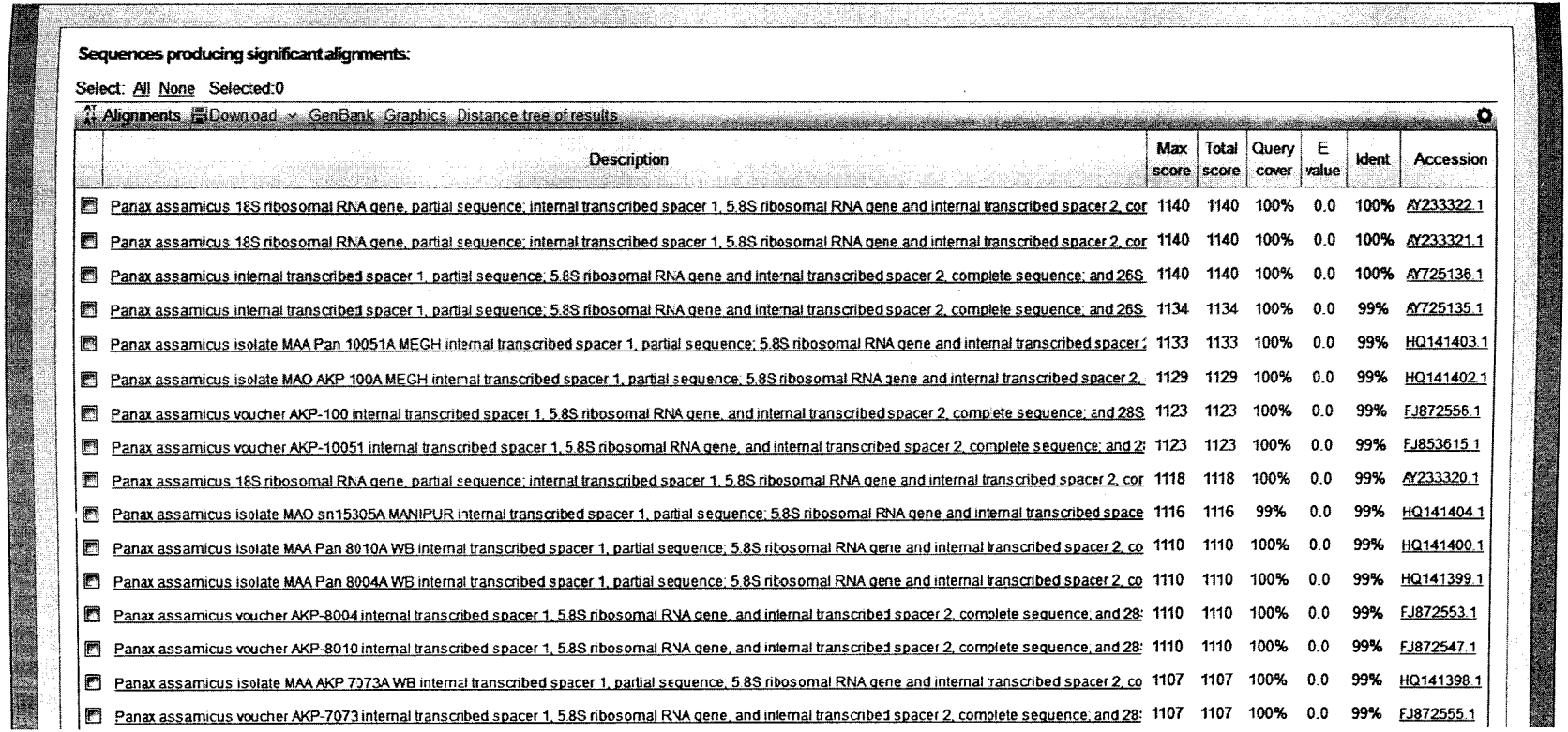
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Panax japonicus var. bipinnatifidus gene for 18S rRNA, complete sequence >dbj|AB044902.1| Panax pseudoginseng subsp. himalaicus gene for 18S rRNA, co	3153	3153	98%	0.0	99%	AB044901.1
☐	Panax pseudoginseng subsp. himalaicus gene for 18S rRNA, complete sequence, note:PPH2	3147	3147	98%	0.0	99%	AB088022.1
☐	Panax japonicus gene for 18S rRNA, complete sequence	3147	3147	98%	0.0	99%	AB088018.1
☐	Panax vietnamensis gene for 18S rRNA, complete sequence >dbj|AB080243.1| Panax vietnamensis var. fusciscus gene for 18S rRNA, complete sequence	3147	3147	98%	0.0	99%	AB033635.1
☐	Panax quinquefolius gene for 18S rRNA, complete sequence	3147	3147	98%	0.0	99%	D85172.1
☐	Panax japonicus gene for 18S rRNA, complete sequence	3147	3147	98%	0.0	99%	D84100.1
☐	Panax pseudoginseng var. angustifolius gene for 18S rRNA, complete sequence	3142	3142	98%	0.0	99%	AB088019.1
☐	Panax notoginseng gene for 18S rRNA, complete sequence, isolate:R2	3142	3142	98%	0.0	99%	AB027524.1
☐	Panax ginseng gene for 18S rRNA, complete sequence >gb|KC593792.1| Panax ginseng clone Pt9-19 18S ribosomal RNA gene, partial sequence >gb|KC593	3142	3142	98%	0.0	99%	D83275.1
☐	Panax stipuleanatus gene for 18S rRNA, complete sequence >gb|JX680332.1| Panax stipuleanatus 18S ribosomal RNA gene, partial sequence	3140	3140	98%	0.0	99%	AB088025.1
☐	Panax ginseng clone N9-22 18S ribosomal RNA gene, partial sequence	3136	3136	98%	0.0	99%	KC593821.1
☐	Panax ginseng clone N9-07 18S ribosomal RNA gene, partial sequence	3136	3136	98%	0.0	99%	KC593808.1
☐	Panax ginseng clone N8-05 18S ribosomal RNA gene, partial sequence	3136	3136	98%	0.0	99%	KC593798.1
☐	Panax ginseng clone Pt9-16 18S ribosomal RNA gene, partial sequence	3136	3136	98%	0.0	99%	KC593789.1

Fig. 6.8: BLAST analysis of the 18S rDNA fromNCBI database for all the species of *Panax*.



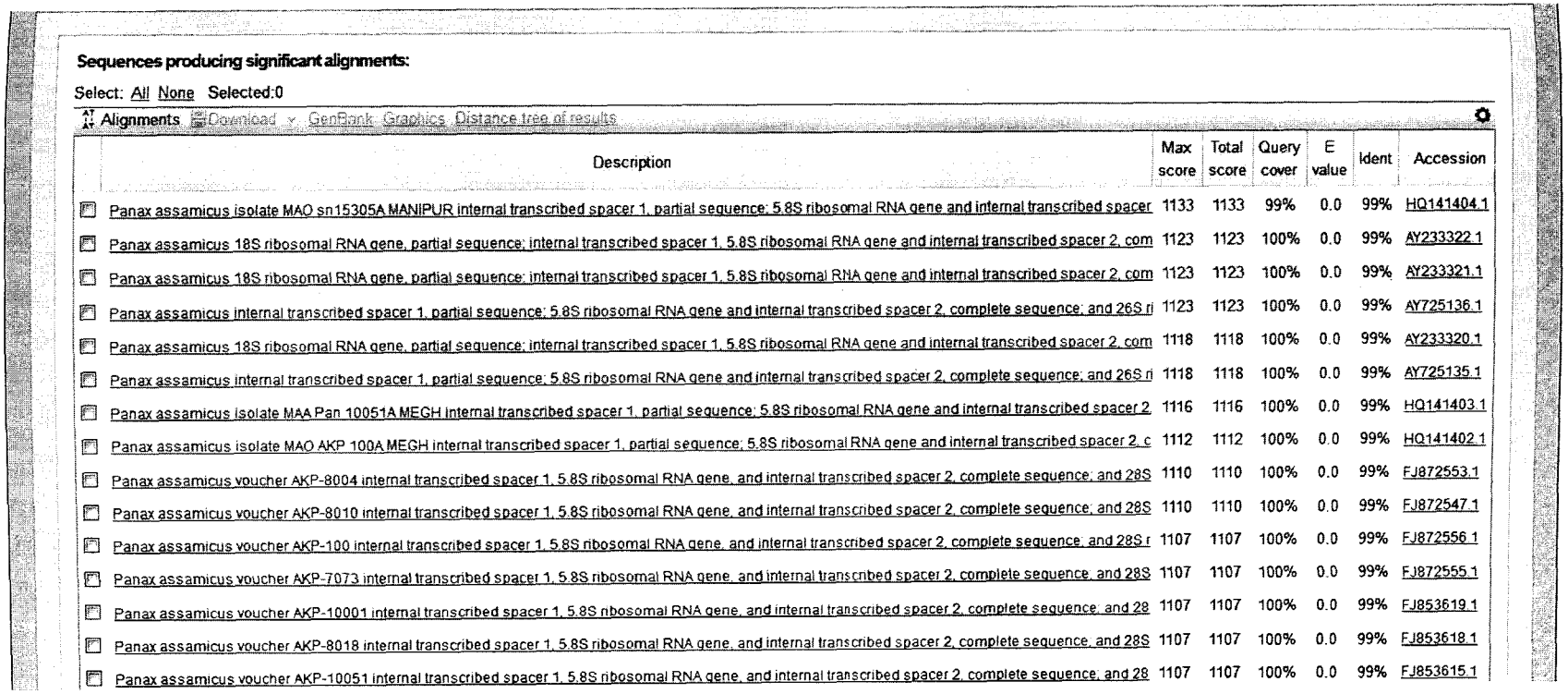
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Panax assamicus 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, cor	1140	1140	100%	0.0	100%	AY233322.1
☐	Panax assamicus 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, cor	1140	1140	100%	0.0	100%	AY233321.1
☐	Panax assamicus internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S	1140	1140	100%	0.0	100%	AY725136.1
☐	Panax assamicus internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S	1134	1134	100%	0.0	99%	AY725135.1
☐	Panax assamicus isolate MAA Pan 10051A MEGH internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2	1133	1133	100%	0.0	99%	HQ141403.1
☐	Panax assamicus isolate MAO AKP 100A MEGH internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2,	1129	1129	100%	0.0	99%	HQ141402.1
☐	Panax assamicus voucher AKP-100 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S	1123	1123	100%	0.0	99%	FJ872556.1
☐	Panax assamicus voucher AKP-10051 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 2	1123	1123	100%	0.0	99%	FJ853615.1
☐	Panax assamicus 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, cor	1118	1118	100%	0.0	99%	AY233320.1
☐	Panax assamicus isolate MAO sn15305A MANIPUR internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed space	1116	1116	99%	0.0	99%	HQ141404.1
☐	Panax assamicus isolate MAA Pan 8010A WB internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, co	1110	1110	100%	0.0	99%	HQ141400.1
☐	Panax assamicus isolate MAA Pan 8004A WB internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, co	1110	1110	100%	0.0	99%	HQ141399.1
☐	Panax assamicus voucher AKP-8004 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28	1110	1110	100%	0.0	99%	FJ872553.1
☐	Panax assamicus voucher AKP-8010 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28	1110	1110	100%	0.0	99%	FJ872547.1
☐	Panax assamicus isolate MAA AKP 7073A WB internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, co	1107	1107	100%	0.0	99%	HQ141398.1
☐	Panax assamicus voucher AKP-7073 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28	1107	1107	100%	0.0	99%	FJ872555.1

Fig. 6.9: BLAST analysis of the 18S-28S rDNA ITS region of *P. assamicus* (Meghalaya) from NCBI database.



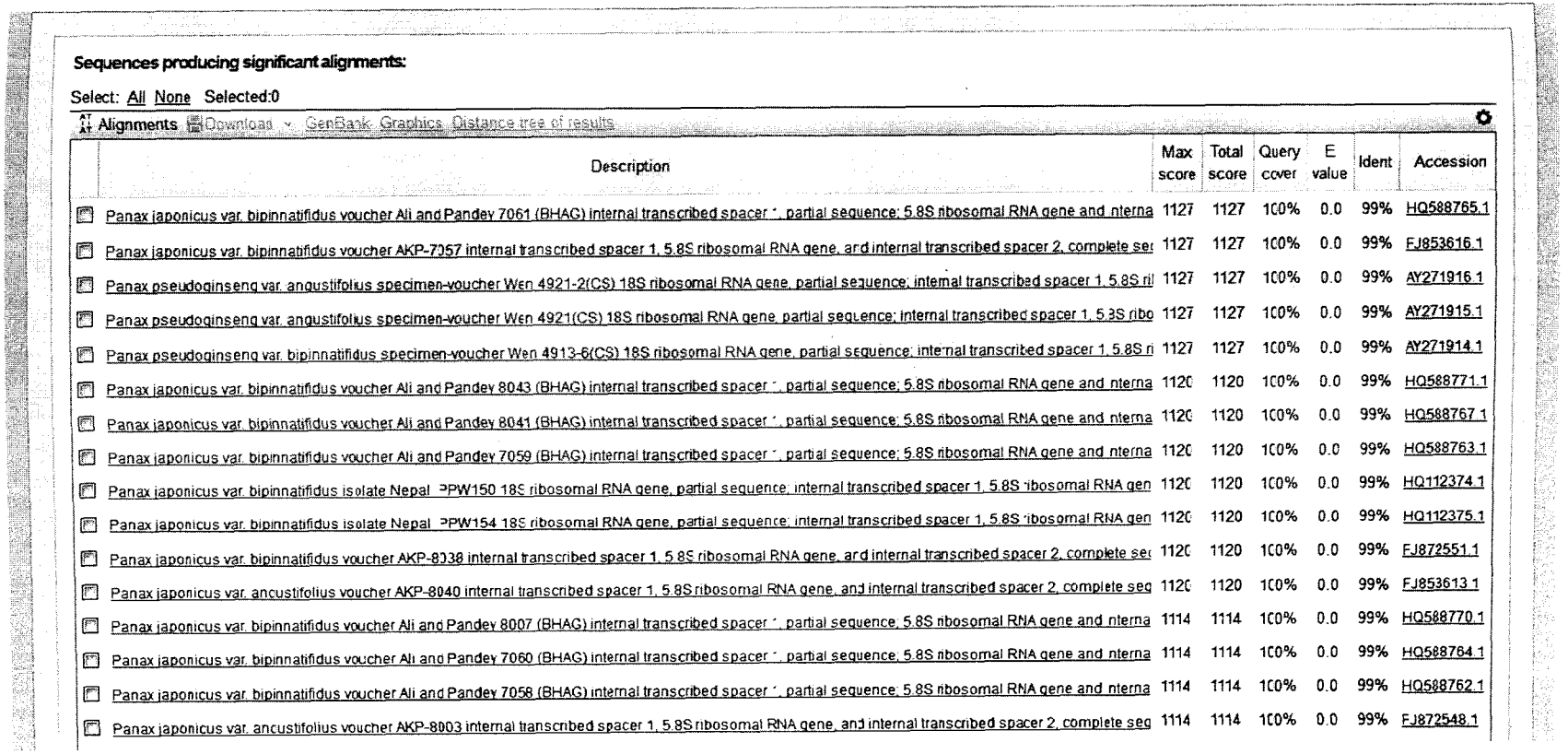
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Panax assamicus isolate MAO sn15305A MANIPUR internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer	1133	1133	99%	0.0	99%	HQ141404.1
Panax assamicus 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, com	1123	1123	100%	0.0	99%	AY233322.1
Panax assamicus 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, com	1123	1123	100%	0.0	99%	AY233321.1
Panax assamicus internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ri	1123	1123	100%	0.0	99%	AY725136.1
Panax assamicus 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, com	1118	1118	100%	0.0	99%	AY233320.1
Panax assamicus internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ri	1118	1118	100%	0.0	99%	AY725135.1
Panax assamicus isolate MAA Pan 10051A MEGH internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2	1116	1116	100%	0.0	99%	HQ141403.1
Panax assamicus isolate MAO AKP 100A MEGH internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, c	1112	1112	100%	0.0	99%	HQ141402.1
Panax assamicus voucher AKP-8004 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S	1110	1110	100%	0.0	99%	FJ872553.1
Panax assamicus voucher AKP-8010 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S	1110	1110	100%	0.0	99%	FJ872547.1
Panax assamicus voucher AKP-100 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S r	1107	1107	100%	0.0	99%	FJ872556.1
Panax assamicus voucher AKP-7073 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S	1107	1107	100%	0.0	99%	FJ872555.1
Panax assamicus voucher AKP-10001 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28	1107	1107	100%	0.0	99%	FJ853619.1
Panax assamicus voucher AKP-8018 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S	1107	1107	100%	0.0	99%	FJ853618.1
Panax assamicus voucher AKP-10051 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28	1107	1107	100%	0.0	99%	FJ853615.1

Fig. 6.10: BLAST analysis of the 18S-28S rDNA ITS region of *P. assamicus* Manipur from NCBI database.



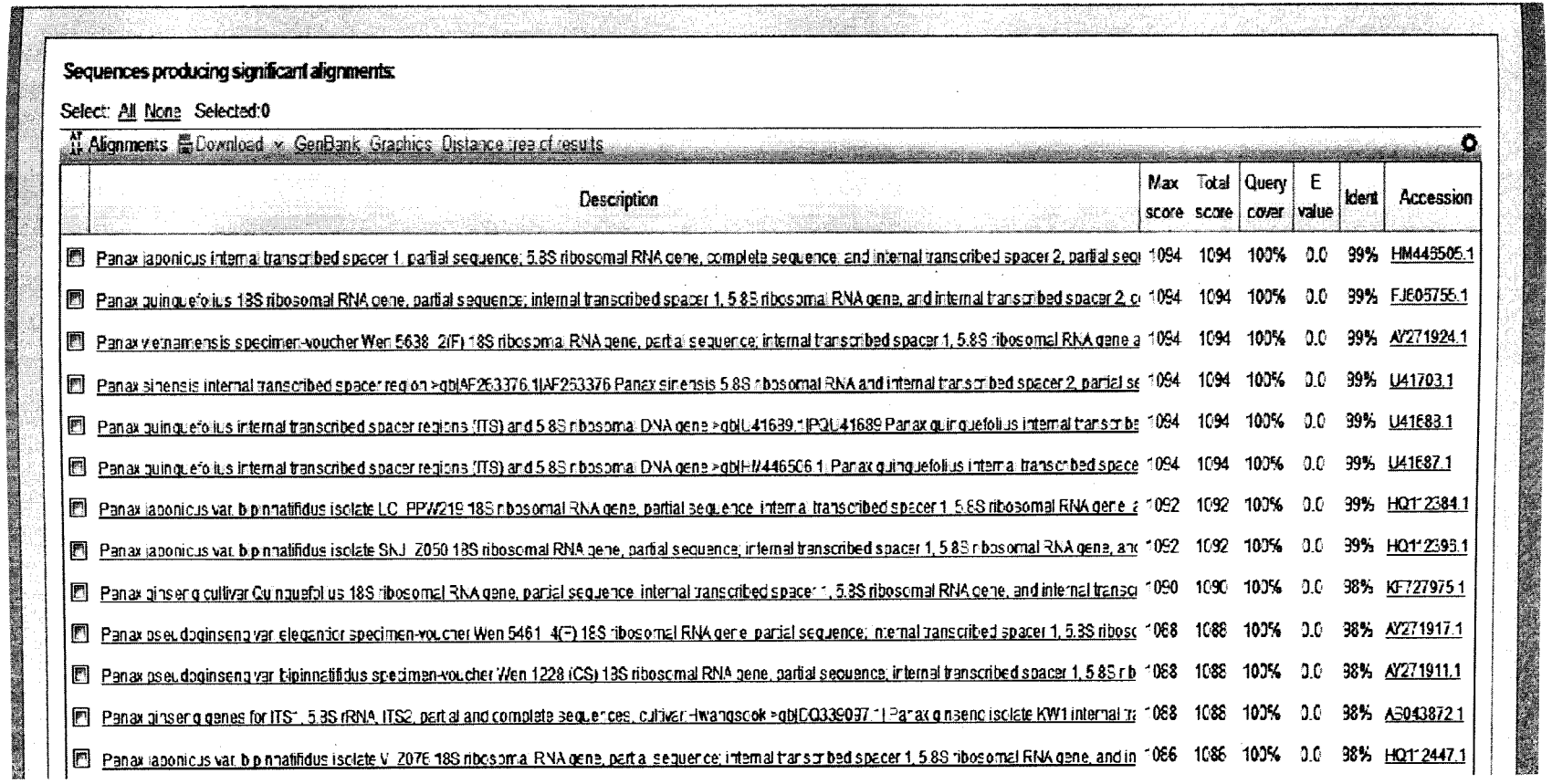
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Panax japonicus var. bipinnatifidus voucher Ali and Pandey 7061 (BHAG) internal transcribed spacer ', partial sequence; 5.8S ribosomal RNA gene and nterna	1127	1127	100%	0.0	99%	HQ588765.1
Panax japonicus var. bipinnatifidus voucher AKP-7057 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete se	1127	1127	100%	0.0	99%	FJ853616.1
Panax pseudoginseng var. angustifolius specimen-voucher Wen 4921-2(CS) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ril	1127	1127	100%	0.0	99%	AY271916.1
Panax pseudoginseng var. angustifolius specimen-voucher Wen 4921(CS) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo	1127	1127	100%	0.0	99%	AY271915.1
Panax pseudoginseng var. bipinnatifidus specimen-voucher Wen 4913-6(CS) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ri	1127	1127	100%	0.0	99%	AY271914.1
Panax japonicus var. bipinnatifidus voucher Ali and Pandey 8043 (BHAG) internal transcribed spacer ', partial sequence; 5.8S ribosomal RNA gene and nterna	1120	1120	100%	0.0	99%	HQ588771.1
Panax japonicus var. bipinnatifidus voucher Ali and Pandey 8041 (BHAG) internal transcribed spacer ', partial sequence; 5.8S ribosomal RNA gene and nterna	1120	1120	100%	0.0	99%	HQ588767.1
Panax japonicus var. bipinnatifidus voucher Ali and Pandey 7059 (BHAG) internal transcribed spacer ', partial sequence; 5.8S ribosomal RNA gene and nterna	1120	1120	100%	0.0	99%	HQ588763.1
Panax japonicus var. bipinnatifidus isolate Nepal PPW150 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gen	1120	1120	100%	0.0	99%	HQ112374.1
Panax japonicus var. bipinnatifidus isolate Nepal PPW154 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gen	1120	1120	100%	0.0	99%	HQ112375.1
Panax japonicus var. bipinnatifidus voucher AKP-8038 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete se	1120	1120	100%	0.0	99%	FJ872551.1
Panax japonicus var. ancustifolius voucher AKP-8040 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seq	1120	1120	100%	0.0	99%	FJ853613.1
Panax japonicus var. bipinnatifidus voucher Ali and Pandey 8007 (BHAG) internal transcribed spacer ', partial sequence; 5.8S ribosomal RNA gene and nterna	1114	1114	100%	0.0	99%	HQ588770.1
Panax japonicus var. bipinnatifidus voucher Ali and Pandey 7060 (BHAG) internal transcribed spacer ', partial sequence; 5.8S ribosomal RNA gene and nterna	1114	1114	100%	0.0	99%	HQ588764.1
Panax japonicus var. bipinnatifidus voucher Ali and Pandey 7058 (BHAG) internal transcribed spacer ', partial sequence; 5.8S ribosomal RNA gene and nterna	1114	1114	100%	0.0	99%	HQ588762.1
Panax japonicus var. ancustifolius voucher AKP-8003 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seq	1114	1114	100%	0.0	99%	FJ872548.1

Fig. 6.11: BLAST analysis of the 18S-28S rDNA ITS region of *P. bipinnatifidus* (Arunachal Pradesh) from NCBI database.



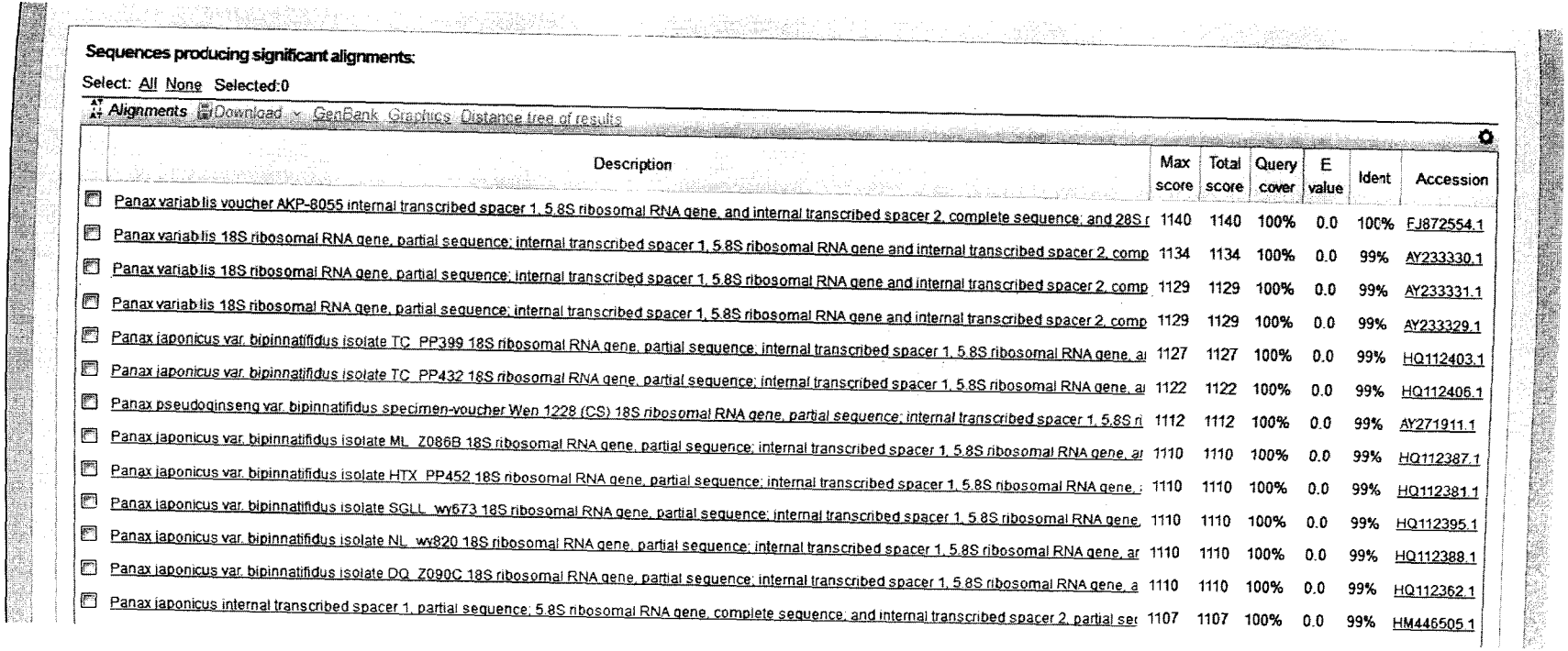
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Panax japonicus internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial seq	1094	1094	100%	0.0	99%	HM446505.1
Panax quinquefolius 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, c	1094	1094	100%	0.0	99%	FJ605756.1
Panax vietnamensis specimen-voucher Wen 5638 2(F) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene a	1094	1094	100%	0.0	99%	AY271924.1
Panax sinensis internal transcribed spacer region >gb|AF253376.1|AF253376 Panax sinensis 5.8S ribosomal RNA and internal transcribed spacer 2, partial se	1094	1094	100%	0.0	99%	U41703.1
Panax quinquefolius internal transcribed spacer regions (ITS) and 5.8S ribosomal DNA gene >gb|U41689.1|PQU41689 Panax quinquefolius internal transcribe	1094	1094	100%	0.0	99%	U41688.1
Panax quinquefolius internal transcribed spacer regions (ITS) and 5.8S ribosomal DNA gene >gb|HM446506.1| Panax quinquefolius internal transcribed space	1094	1094	100%	0.0	99%	U41687.1
Panax japonicus var. bipinnatifidus isolate LC_PPW219 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, a	1092	1092	100%	0.0	99%	HQ112384.1
Panax japonicus var. bipinnatifidus isolate SNJ_Z050 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and	1092	1092	100%	0.0	99%	HQ112395.1
Panax ginseng cultivar Quinquefolius 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcr	1090	1090	100%	0.0	98%	KF727975.1
Panax pseudoginseng var. elegantior specimen-voucher Wen 5461 4(F) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosc	1088	1088	100%	0.0	98%	AY271917.1
Panax pseudoginseng var. bipinnatifidus specimen-voucher Wen 1228 (CS) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S rib	1088	1088	100%	0.0	98%	AY271911.1
Panax ginseng genes for ITS1, 5.8S rRNA, ITS2, partial and complete sequences, cultivar:Hwangsook >gb|DQ339037.1|Panax ginseng isolate:KW1 internal tr	1088	1088	100%	0.0	98%	AB043872.1
Panax japonicus var. bipinnatifidus isolate V_Z076 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and in	1086	1086	100%	0.0	98%	HQ112447.1

Fig. 6.12: BLAST analysis of the 18S-28S rDNA ITS region of *Panax* sp. (Arunachal Pradesh and Nagaland) fromNCBI database.



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Panax variabilis voucher AKP-8055 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S r	1140	1140	100%	0.0	100%	FJ872554.1
Panax variabilis 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, comp	1134	1134	100%	0.0	99%	AY233330.1
Panax variabilis 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, comp	1129	1129	100%	0.0	99%	AY233331.1
Panax variabilis 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, comp	1129	1129	100%	0.0	99%	AY233329.1
Panax japonicus var. bipinnatifidus isolate TC_PP399 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, a	1127	1127	100%	0.0	99%	HQ112403.1
Panax japonicus var. bipinnatifidus isolate TC_PP432 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, a	1122	1122	100%	0.0	99%	HQ112406.1
Panax pseudoginseng var. bipinnatifidus specimen-voucher Wen 1228 (CS) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ri	1112	1112	100%	0.0	99%	AY271911.1
Panax japonicus var. bipinnatifidus isolate ML_Z086B 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, a	1110	1110	100%	0.0	99%	HQ112387.1
Panax japonicus var. bipinnatifidus isolate HTX_PP452 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene,	1110	1110	100%	0.0	99%	HQ112381.1
Panax japonicus var. bipinnatifidus isolate SGLL_wy673 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene,	1110	1110	100%	0.0	99%	HQ112395.1
Panax japonicus var. bipinnatifidus isolate NL_wy820 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, ar	1110	1110	100%	0.0	99%	HQ112388.1
Panax japonicus var. bipinnatifidus isolate DQ_Z090C 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, a	1110	1110	100%	0.0	99%	HQ112362.1
Panax japonicus internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial se	1107	1107	100%	0.0	99%	HM446505.1

Fig. 6.13: BLAST analysis of the 18S-28S rDNA ITS region of *P. variabilis* (Manipur) from NCBI database.

6.3.2.5. Sequence characteristics

18S rRNA gene

A total of 18 sequences of *Panax* species were used including the 8 sequences of the studied species. The outgroup *Hedera* was excluded from the data set. The sequence data set ranged from 1692-1694. The sequence characteristics of this region were calculated using Seqstate Version 1.4.1 (Müller, 2005) and DnaSP version 5.10.1(Librado and Rozas, 2009) (Table 6.4). Out of 1694 characters analysed 11 were variable/polymorphic sites, 1680 were invariable/monomorphic sites, 10 were autapomorphic sites, GC (%) content was 49.65% and transition/tranversion ratio was 0.95. Only 1 site was parsimony informative which indicates that it is more conserved and has not evolved rapidly even though it is phylogenetically informative. The total number of indel sites analysed was 3 with an average indel length of 1. Indel diversity k (i) was 0.53 which is the average number of nucleotide differences. Tajima'D test (the test is based on the differences between the number of segregating sites and the average number of nucleotide differences) was 1.09 with $p>1.0$ which was not significant.

18S-28S rDNA ITS region

A total of 41 sequences of *Panax* species were used including the 8 sequences of the studied species. The outgroup *Hedera* was excluded from the data set. The sequence data set ranged from 602-617. The sequence characteristics of this region were calculated using Seqstate Version 1.4.1 and DnaSP version 5.10.1 (Table 6.5). Out of 620 characters analysed 113 were variable/polymorphic sites, 480 were invariable/monomorphic sites, 45 were autapomorphic sites, GC (%) content was 60.42% and transition/tranversion ratio was 2.94. 68 sites were parsimony informative which indicates that it is highly variable and is phylogenetically informative. The total number of indel sites analysed were 27 with an

average indel length of 1.22. Indel diversity k (i) was 1.45 which is the average number of nucleotide differences. Tajima'D test was 2.53 with $p<0.001$ which was significant.

Table 6.5: Sequence characteristics of *18S rRNA* gene and 18S-28S rDNA ITS region in *Panax* species used for phylogenetic analyses.

Sequence Characteristics	18S RNA	ITS
Genome	Nuclear	Nuclear
No of sequence used	18	41
Characters analysed	1694	620
Total no of sites (excluding sites with gaps/missing data)	1691	593
Length range	1692-1694	602-617
Variable or polymorphic sites	11	113
Invariable or monomorphic sites	1680	480
Parsimony informative sites	1	68
Autapomorphic sites	10	45
Transition/Transversion	0.95	2.94
GC (%)	49.65	60.42
Total no of indels site analysed	3	27
Indel diversity k(i)	0.53	1.45
Average indel length	1	1.22
Tajima's D	1.09	2.53
Statistical Significance	p>0.10 not significant	p < 0.001significant

6.3.2.6. Phylogenetic analysis of *18S rRNA* gene

The aligned sequences were used for phylogenetic analyses (Table 6.6). Maximum Likelihood tree was constructed using Mega version 5 (Tamura *et al.*, 2011). The analysis involved 19 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1603 positions in the final dataset. The tree with the highest log likelihood (-2413.7750) was shown. The parameters used in the model are shown in Table 6.7. The Neighbour joining tree also yielded results more or less similar to that obtained with Maximum likelihood method.

The phylogenetic tree from NJ and ML methods (Fig. 6.14-6.15) constructed revealed three clades. The first clade included *P. assamicus, P. bipinnatifidus Panax* sp. (new species in present study), *P. ginseng, P. japonicus, P. japonicus var. bipinnatifidus, P. pseudoginseng subsp. himalaicus, P. variabilis and P. zingiberensis* supported by 100% bootstrap value. *P. pseudoginseng* and *P. stipuleanatus* formed a separate clade. *P. quinquefolius, P. notoginseng* and *P vietnamensis* formed together one clade (BS=62%).

The 50% majority-rule consensus tree resulted from Bayesian analysis applying the GTR+G model showed the support for relationships between the taxa with posterior probability values (Fig. 6.16). The tree topology was congruent with that of the Maximum Likelihood tree. The first clade was supported with maximum posterior probability value (100%). *P. pseudoginseng* and *P. stipuleanatus* were grouped together with 69% posterior probability value. The third clade which grouped together *P. quinquefolius, P. notoginseng* and *P vietnamensis* was supported by 1 % posterior probability.

Table 6.6: Accessions of *Panax* species and outgroup sampled for *18S rRNA* gene.

Taxon	Voucher	Location	Genbank Accession No.
Panax assamicus Ban.	NEHU-11969	Meghalaya	KM365074
P. assamicus Ban.	NEHU-12032	Meghalaya	KM365075
P.assamicus Ban.	NEHU-11970	Manipur	KM365076
P. variabilis J. Wen	NEHU-11971	Manipur	KM365077
Panax sp.	NEHU-11973	Nagaland	KM365078
Panax sp.	NEHU-11974	Arunachal Pradesh	KM365079
P. bipinnatifidus Seem.	NEHU-11972	Arunachal Pradesh	KM365081
P. bipinnatifidus Seem.	NEHU-12035	Arunachal Pradesh	KM365080
P. ginseng C. A. Meyer	K. Komatsu *et al.*, Y220	Yunnan, China	D83275
P. quinquefolius L.	H. Fushimi, 15	*TMPU	D85172
P. japonicus C. A. Meyer	s.leg., Baisanchi-5,6	Hubei, China	AB088018
P. bipinnatifidus (Seem.)	K. Komatsu *et al.*, S483-3,4,15,21,S484-25,26,29	Sichuan, China	AB088021
P. notoginseng (Burk.) F.H.Chen	H. Fushimi, 96002	Yunnan, China	D85171
P. pseudoginseng Wall.	T. Watanabe, s.n.	*KMPG	AB088026
P. pseudoginseng Wall. Subsp. *himalaicus* Hara	T. Watanabe, s.n	*KMPG	AB088024
P. stipuleanatus H.T. Tsai et K.M. Feng	K. Komatsu *et al.*, Y277-1	Yunnan, China	AB088025
P. vietnamensis Ha et Grushv	S.Kodata et K. Komatsu, VTM455,456,457,458	Quang Nam, Vietnam	AB033635
P. zingiberensis C.Y. Wu et Feng	K. Komatsu *et al.*, Y293	Yunnan, China	AB085764
Outgroup			
Hedera helix L.	Phylogenetic relationships of the Santalales and relativesJ1990.		X16604.1

*TMPU: Herbal garden, Toyama Medical & Pharmaceutical University, Japan.

*KMPG: Medicinal Plant Garden, Kitazato University, Nepal.

Table 6.7: Parameters of Maximum Likelihood analysis and selected model for *18S rRNA* gene.

Model	Transition/ Tranversion (*R*)	Nucleotide frequencies (%)				Substitution rate matrix				
		A	**T/U**	**G**	**C**		**A**	**T/U**	**C**	**G**
GTR+G	0.66	24.53	25.71	27.84	21.93	**A**	-	*4.65*	*7.21*	**3.70**
(G=0.05)						**T/U**	*4.44*	-	**15.36**	*5.04*
						C	*8.06*	**18.00**	-	*14.33*
						G	**3.26**	*4.65*	*11.29*	-

NOTE - Each entry is the probability of substitution (*r*) from one base (row) to another base (column). Rates of different transitional substitution are shown in **bold** and those of transversional substitutions are shown in *italics*.

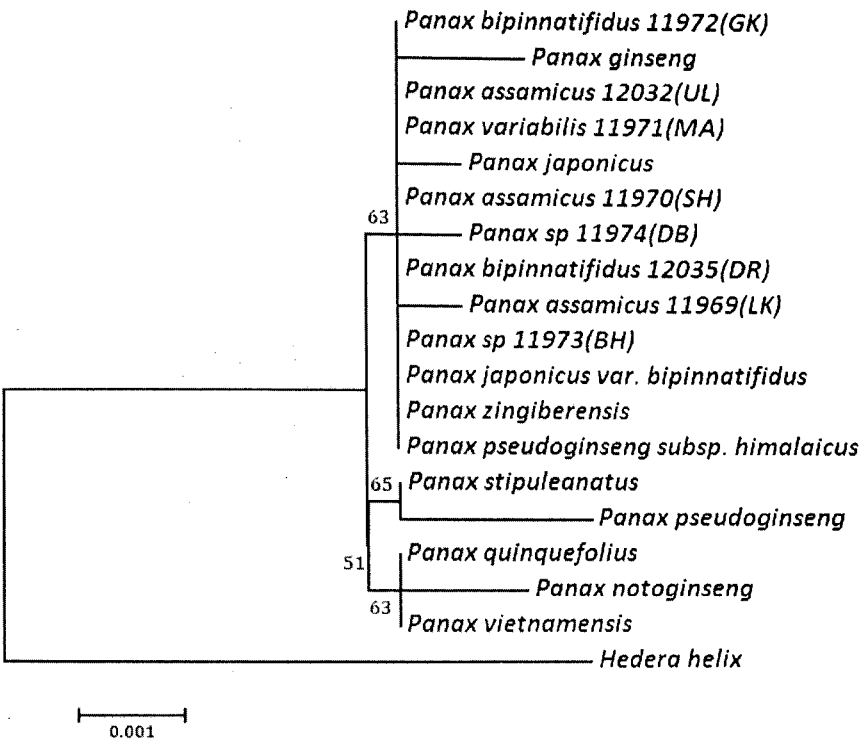


Fig. 6.14: Neighbour Joining strict consensus tree constructed using aligned sequences of *18S rRNA* gene (codes in parenthesis denotes studied samples).

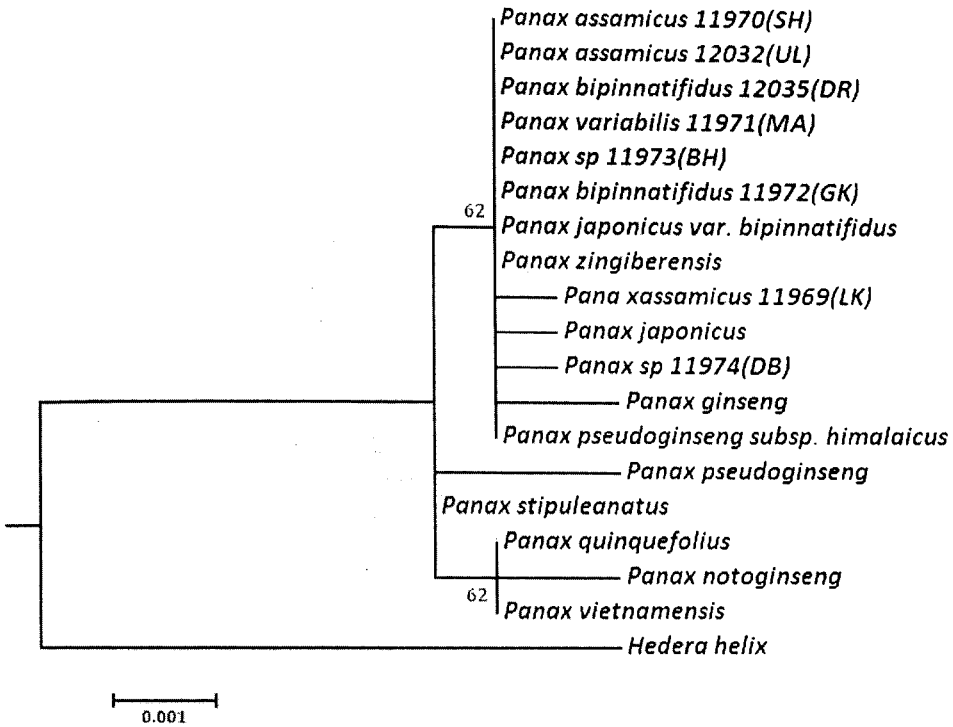


Fig. 6.15: Maximum Likelihood strict consensus tree constructed using aligned sequences of *18S rRNA* gene (codes in parenthesis denotes studied samples).

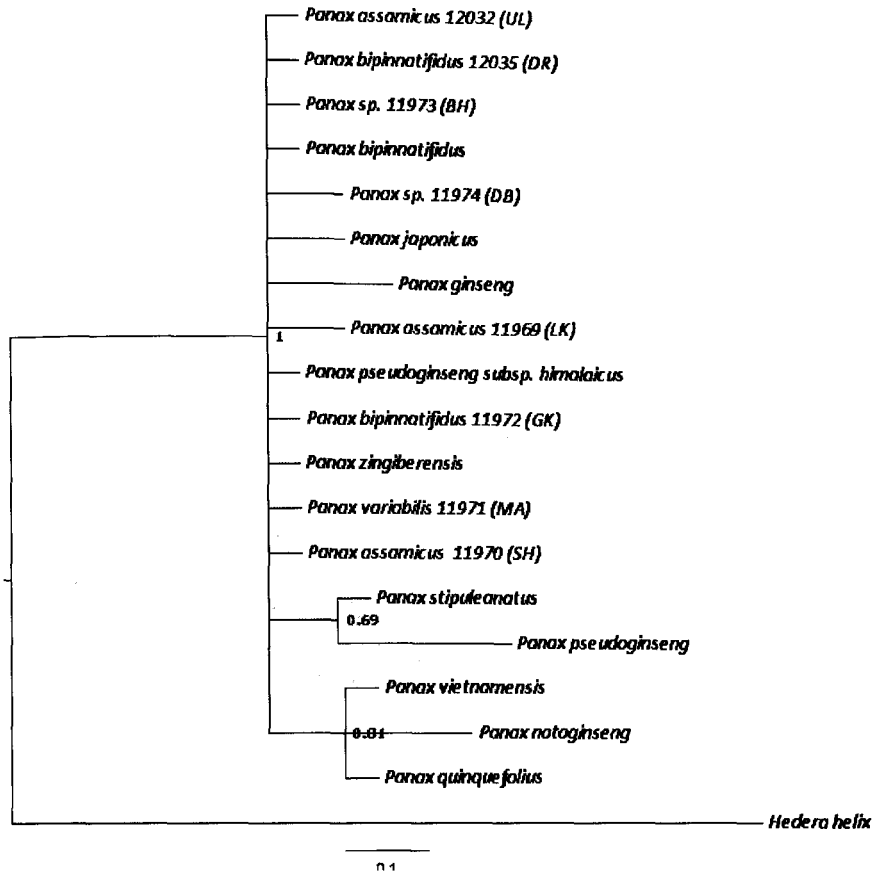


Fig. 6.16: The 50% majority rule consensus tree inferred from Bayesian analysis of the *18S rRNA* gene with posterior probability values (Red colour denotes studied samples).

6.3.2.7. Phylogenetic analysis of the 18S-28S rDNA region

Multiple sequence alignment using CLUSTAL X was carried out for the studied ITS sequences and the related sequences of *Panax* ITS region retrieved from GenBank using BLAST programme and the aligned sequences were then used for phylogenetic analyses (Table 6.8). The best model which was selected under ML method (Maximum Likelihood method) for nucleotide substitution using jModel test was GTR+G+I (general time reversible model+ gamma distribution+ invariable sites). Maximum Likelihood tree was constructed using Mega version 5. The analysis involved 42 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 592 positions in the final dataset. The tree with the highest log likelihood (-1986.9671) was shown. The parameters used for the maximum Likelihood analysis under the selected model are shown in Table 6.9. The Neighbour joining tree yielded more or less similar results as obtained with the Maximum likelihood method.

The Maximum likelihood tree revealed that the sampled populations of *P. assamicus* from Meghalaya and Manipur grouped together (BS=93%) and showed a close relationship with *P. bipinnatifidus* clade (BS=92%). The populations of *P. bipinnatifidus* from Arunachal Pradesh were grouped together in one clade with the other populations from China. *P. variabilis* from Manipur was grouped together with *P. variabilis* from China (BS=88%). *Panax* sp. populations grouped together (BS=98%) and formed a separate clade. This clade showed close relationship with the basal clade i.e., *P. notoginseng* (BS=51%) which in turn was related to the *P. pseudoginseng-P. stipuleanatus* clade (BS=99%).

The 50% majority-rule consensus tree resulted from Bayesian analysis applying the GTR+G+I model showed strong support for relationships between the taxa with posterior probability values (Fig. 6.17). The tree topology was congruent with that of the Neighbour Joining and Maximum Likelihood tree. *P. assamicus*, *P. bipinnatifdus* and *P. variabilis*

were grouped together in the first clade. *P. assamicus* occupied the top sub-clade and the Meghalaya populations were supported with 97% posterior probability with the other accessions from Meghalaya but the sample from Manipur was grouped together with the accession from Manipur with 95% posterior probability. The relationship between these populations can be seen with a posterior probability of 100%. The second sub-clade included *P. bipinnatifidus* and both the populations from Arunachal Pradesh were grouped together with a posterior probability of 98%. *P. variabilis* from Manipur was grouped together with the other accessions with 100% posterior probability. Clade I was connected with clade II which comprises of *P. ginseng, P. japonicus* and *P. quinquefolius* with posterior probability of 57%. *P. vietnamensis* forms a single clade IV and was supported with 62% posterior probability with clade III. *Panax sp.* from the two populations grouped together with 100% posterior probability and formed a separate and basal clade to the rest of the apical clade with posterior probability <50%. *P. notoginseng* formed the basal clade to the rest with 100 % posterior probability. The *P. pseudoginseng- P.stipuleanatus* clade grouped together with 54% posterior probability. *P. trifolius* formed the basal clade to the rest of the clades which was supported by earlier studies.

Table 6.8: Accessions of *Panax* and outgroup sampled for the 18S-28S rDNA region.

Taxon Name	Voucher	Geographical Origin	GenBank Accessions
Panax assamicus Ban.	NEHU-11969	Meghalaya	KM365082
P. assamicus Ban.	NEHU-12032	Meghalaya	KM365083
P.assamicus Ban.	NEHU-11970	Manipur	KM377619
P. variabilis J. Wen	NEHU-11971	Manipur	KM377620
Panax sp.	NEHU-11973	Nagaland	KM365072
Panax sp.	NEHU-11974	Arunachal Pradesh	KM365073
P. bipinnatifidus Seem.	NEHU-11972	Arunachal Pradesh	KM37762
P. bipinnatifidus Seem.	NEHU-12035	Arunachal Pradesh	KM377621
Panax assamicus Ban.	Ali ang Thongam 10001(BHAG)	Manipur, India	FJ853619
P. assamicus Ban.	MAO sn15305A (BHAG)	Manipur, India	HQ141404
P. assamicus Ban.	Pandey and Mao 100 (BHAG)	Meghalaya, India	FJ872556
P. assamicus Ban.	Pandey 5018(BHAG)	Meghalaya, India	AY233322
P. assamicus Ban.	Pandey 5000H (BHAG)	West Bengal, India	AY233320
P. assamicus Ban.	Ali and Pandey 7073 (BHAG)	West Bengal, India	FJ872555
P. bipinnatifidus Seem.	Ali and Pandey 7006 (BHAG)	Arunachal Pradesh, India	HQ588775
P. bipinnatifidus Seem.	Pathak and Bhaumik 4115 (CAL)	Arunachal Pradesh, India	AY725134
P. bipinnatifidus Seem.	Wen 1228(CS)	Yunnan, China	AY271911
P. bipinnatifidus Seem.	Wen 1166	Sichuan, China	U41678
P. bipinnatifidus Seem.	Wen 4913-6 (CS)	Eastern Nepal	AY271914
P. bipinnatifidus Seem.	Ali and Pandey 7061(BHAG)	Sikkim	HQ588765
P. bipinnatifidus Seem.	Ali and Pandey 7057 (BHAG)	Sikkim, India	FJ853616

P. bipinnatifidus Seem. var. *angustifolius*	Ali and Pandey 8040 (BHAG)	West Bengal, India	FJ853613
P. bipinnatifidus Seem. var. *angustifolius*	Ali and Pandey 8032 (BHAG)	West Bengal, India	FJ872552
P. ginseng C.A.Meyer	Wen 3127 (F)	Jilin, China	AY233326
P. ginseng C.A.Meyer	Y. D. Kim s.n. (TX)	Korea	U41682
P. japonicus C. A. Meyer	T. Kajita & T. Kurosawa 862	Japan	U41702
P. japonicus C. A. Meyer	M. Hasebe s.n.	Japan	U41701
P. notoginseng F.H.Chen ex C.Y. Wu & K.M.Feng	Wen 1200 (CS)	Yunnan, China	U41684
P. notoginseng F.H.Chen ex C.Y. Wu & K.M.Feng	Wen 1244 (F)	Guangdong, China	U41685
P. pseudoginseng Wall.	Wen 4900 (F)	Jiri, Nepal	AY233327
P. quinquefolius L.	Wen 1083 (A)	Ohio, USA	U41687
P. quinquefolius L.	Wen 1528 (CS)	Virginia, USA	U41689
P.stipuleanatus H.T.Tsai & K.M.Feng	Wen 1204 (F)	Yunnan, China	U41696
P. trifolius L.	Kramer & Kramer s.n. (CS)	Ohio, USA	U41698
P.variabilis J.Wen	Wen 5693- 4 (F)	Yunnan, China	AY233331
P.variabilis J.Wen	Ali and Pandey 8055	Nagaland, India	FJ872554
P.vietnamensis Ha & Grushv.	Wen 5638-2 (F)	Yunnan, China	AY271924
P.wangianus S.C.Sun	Wen 1174 (CS)	Sichuan, China	U41690
P.wangianus S.C.Sun	Wen 1176 (CS)	Sichuan, China	U41691
P. zingiberensis C.Y.Wu & K.M.Feng	Wen 1199	Yunnan, China	U41699
P. zingiberensis C.Y.Wu & K.M.Feng	Wen 1227 (CS)	Yunnan, China	U41700
Outgroup	Wen 2481 (CS)	Cult. in Fort Collins	AF242241
***Hedera helix* L.**			

Table 6.9: Parameters of Maximum Likelihood analysis and selected model for 18S -28S rDNA region.

Model	Transition/ Tranversion (*R*)	Nucleotide frequencies (%)				Substitution rate matrix				
		A	**T/U**	**G**	**C**		**A**	**T/U**	**C**	**G**
GTR+G	2.15	22.31	18.76	30.80	28.13	**A**	-	*4.47*	*5.13*	**12.16**
+I						**T/U**	*5.32*	-	**29.02**	*4.21*
G=0.94						**C**	*3.72*	**17.68**	-	*2.78*
I=24.94%						**G**	**9.65**	*2.80*	*3.05*	-

NOTE - Each entry is the probability of substitution (*r*) from one base (row) to another base (column). Rates of different transitional substitution are shown in bold and those of transversionsal substitutions are shown in *italics*.

Fig. 6.17: Neighbor joining strict consensus tree constructed using aligned sequences of 18S-28S Internal Transcribed Spacer (ITS) region (codes in bracket denote studied samples).

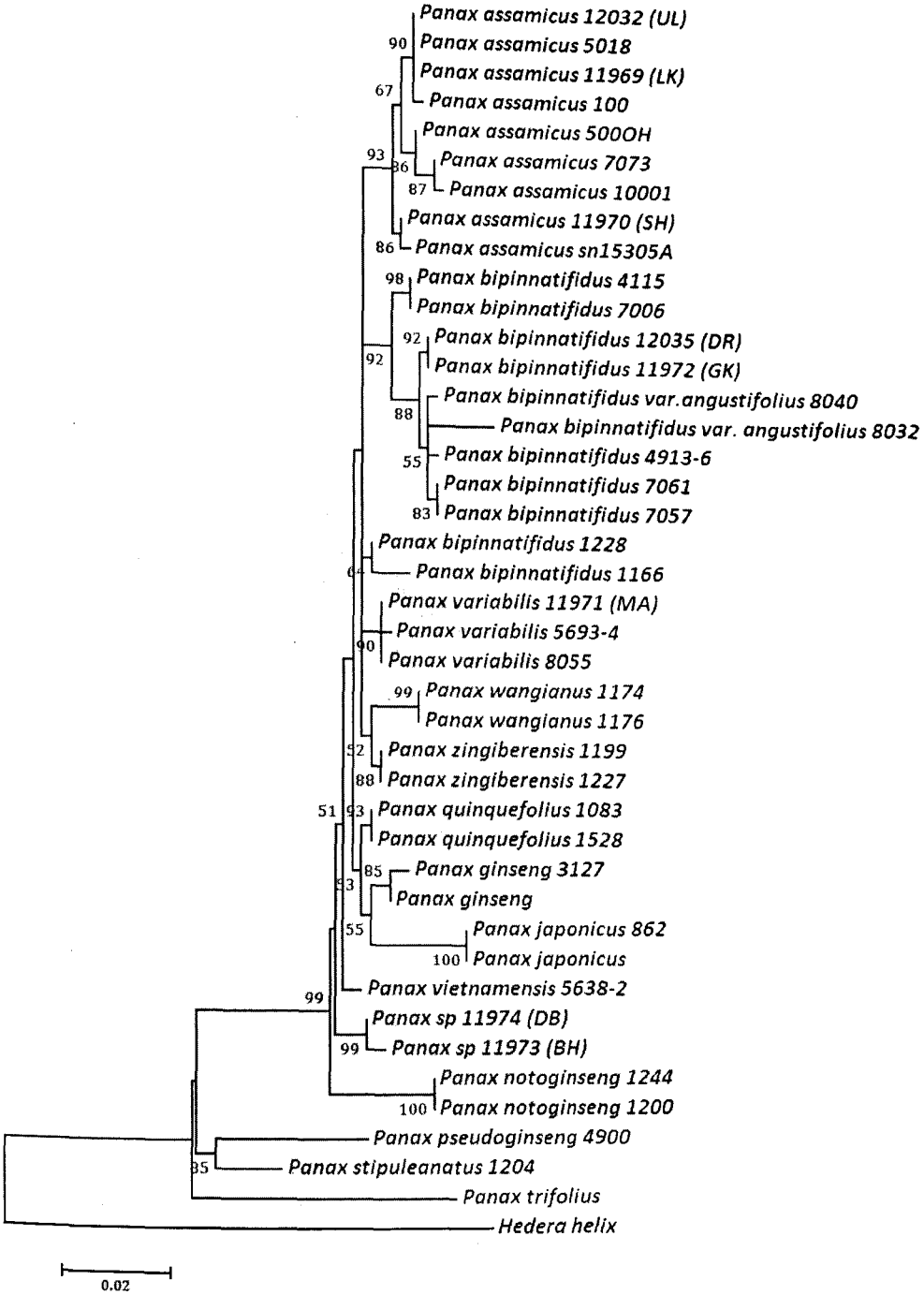


Fig. 6.18: Maximum Likelihood strict consensus tree constructed using aligned sequences of 18S-28S Internal Transcribed Spacer (ITS) region (codes in bracket denote studied samples).

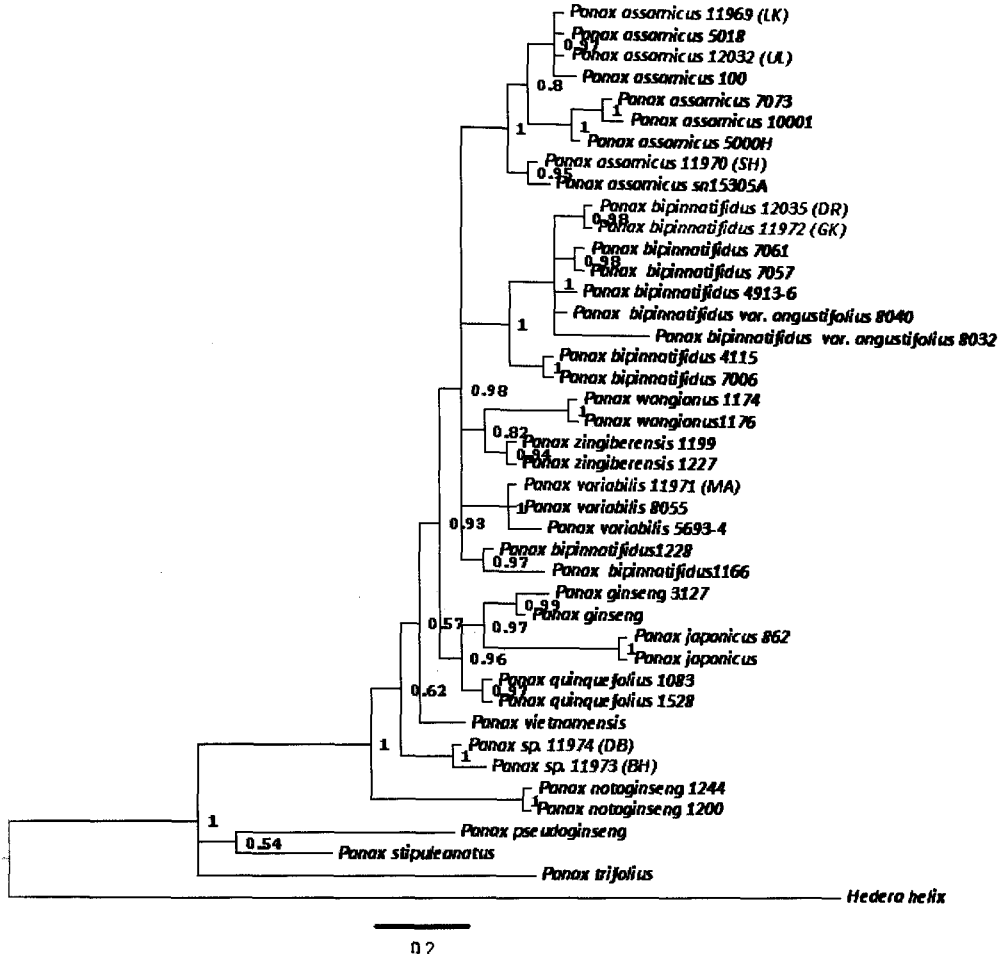


Fig. 6.19: The 50% majority rule consensus tree inferred from Bayesian analysis of 18S-28S internal Transcribed Spacer (ITS) region with posterior probability values (Red colour denotes studied samples).

6.3.3. PCR-Restriction Fragment Length Profile (PCR-RFLP)/ Amplicon Restriction Pattern (ARP)

Three restriction enzymes, *BstN*1, *Msp*I and *PspG*1 were used which produced polymorphic restriction patterns in the ITS amplicons.

6.3.3.1. PCR-restriction fragment length profile (PCR-RFLP) with *BstN*1

Restriction enzyme *BstN*1 generated different patterns for all the *Panax* species (Fig. 6.23, a-k). For *P. assamicus*, five replicates each from Nongkrem (NK), Upper Shillong (US), Manipur (SH) population were analysed. Laitkseh (UL) and Tyllang (TY) have four sub-populations and three replicates were taken from each due to lesser number of individuals and from Laitkor (LK) with three sub-populations, three replicates were taken from each. Five replicates from each population of Arunachal Pradesh viz. Dirang (DR) and Gomkang (GK) were analysed.

Two different profiles were obtained viz., PB1 and PB2 (Table 6.10). PB1 profile was obtained in all the populations of *P. assamicus* from Meghalaya and Manipur. PB2 profile was found in *P. bipinnatifidus* populations from Arunachal Pradesh, *Panax sp.* from Arunachal Pradesh and Nagaland and *P. variabilis* from Manipur. Manual check of the nucleotide sequences for all *Panax* species showed an extra recognition site for the enzyme which lies downstream the ITS region thereby producing the fragments which were generated in the gel.

Table 6.10: PCR-RFLP profile for *Panax* species with the restriction fragments using *BstN*1 (+ = band present; - = band absent).

Profiles for *BstN*1	Samples	Restriction size fragments (bp)							
		~480	~450	~360	~280	~260	~250	~120	<50
PB1	*P. assamicus* (UL,TY,LK,NK, US,SH)	-	-	+	-	+	-	+	+
PB2	*P. bipinnatifidus* (DR,GK) *Panax sp.* (DB and BH) *P. variabilis* (MA)	-	+	-	-	-	+	-	+

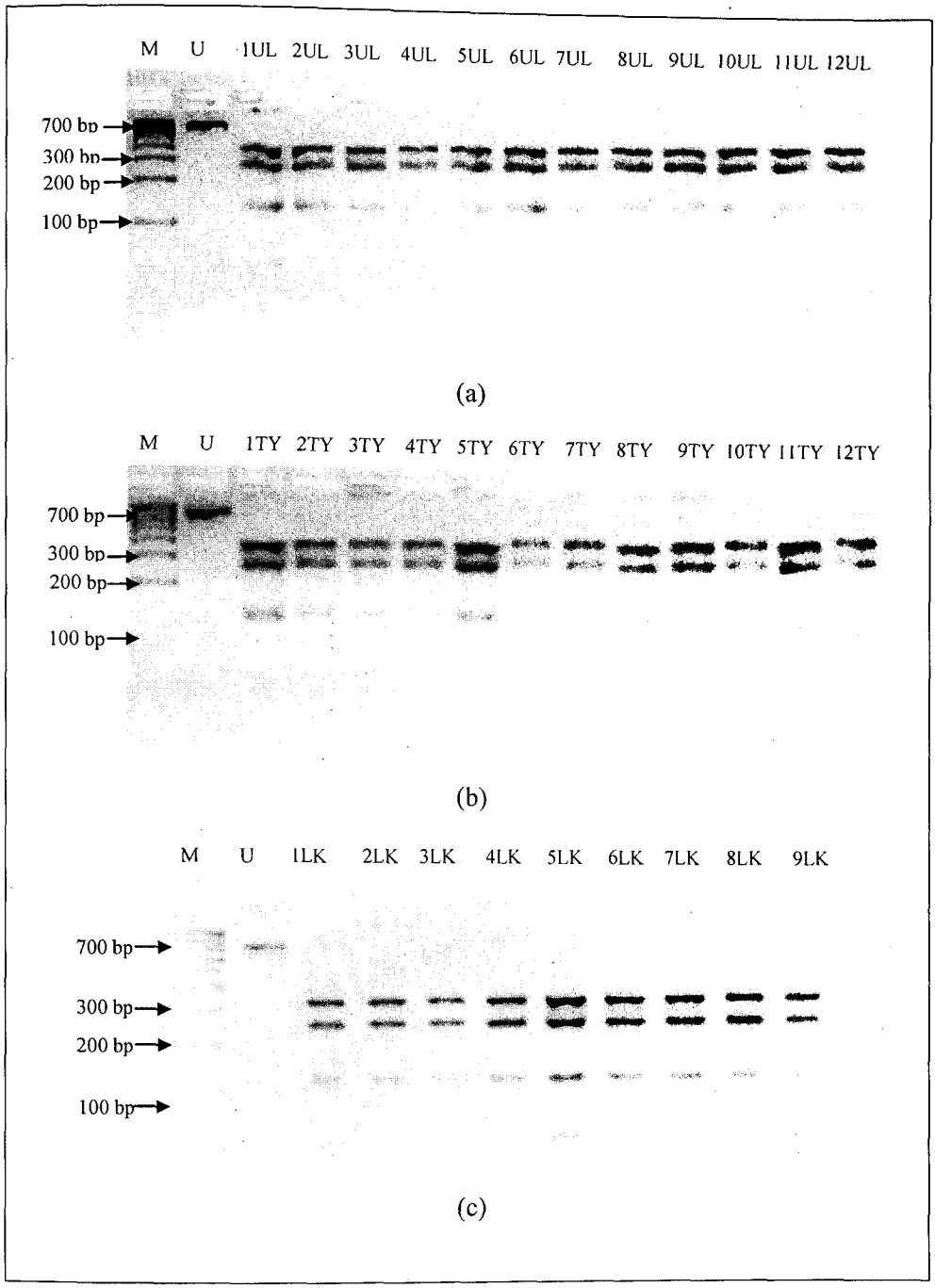


Fig. 6.20 (a-c): PCR-RFLP of *P. assamicus* from Meghalaya (UL-Laitkseh, TY- Tyllang, LK-Laitkor) digested with endonuclease enzyme *BstN*1 (M=100 bp ladder, U= undigested DNA).

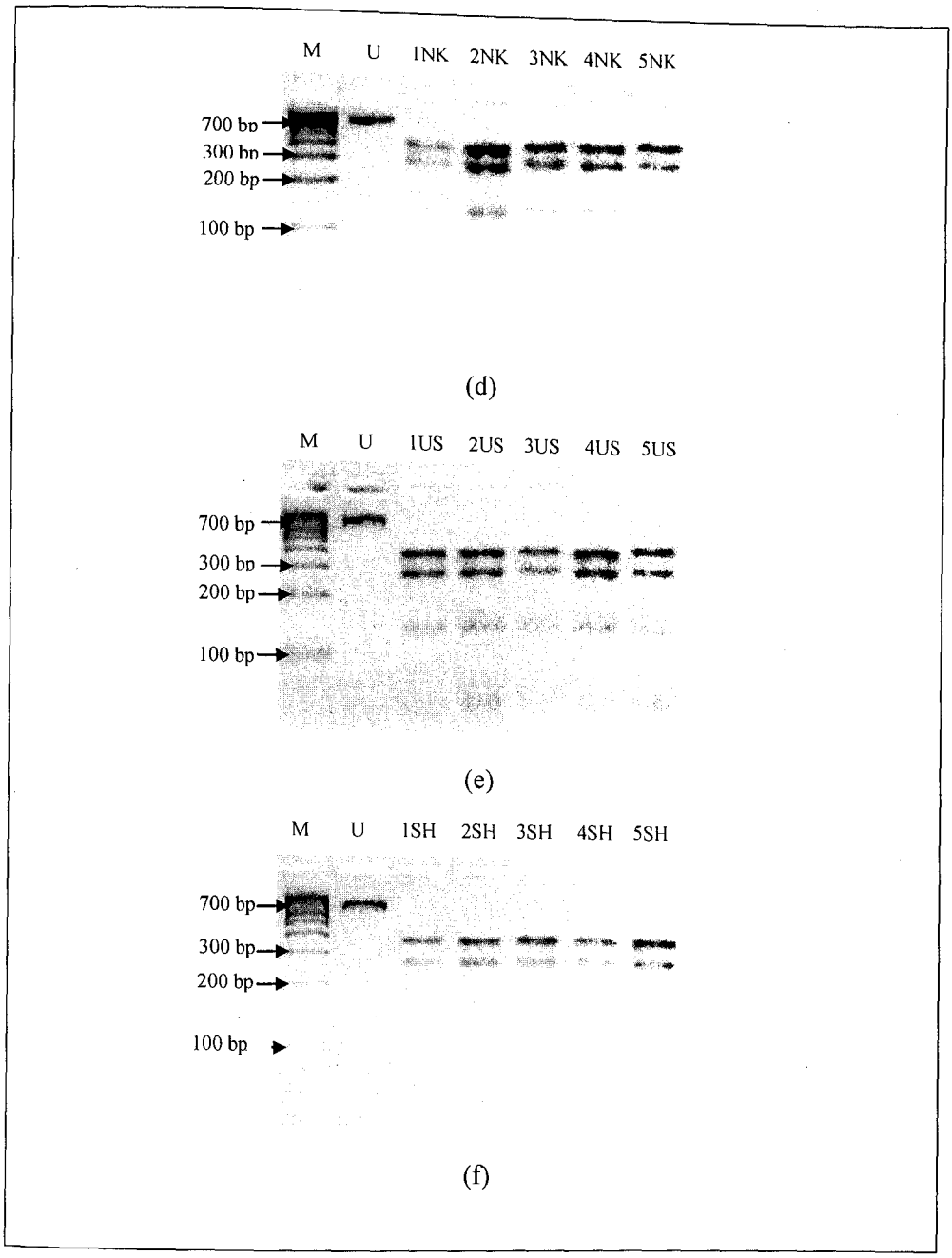


Fig. 6.20 (d-f): PCR-RFLP of *P. assamicus* from Meghalaya (NK-Nongkrem, US- Upper Shillong) and Manipur (SH) digested with endonuclease enzyme *BstN*1 (M=100 bp ladder, U= undigested DNA).

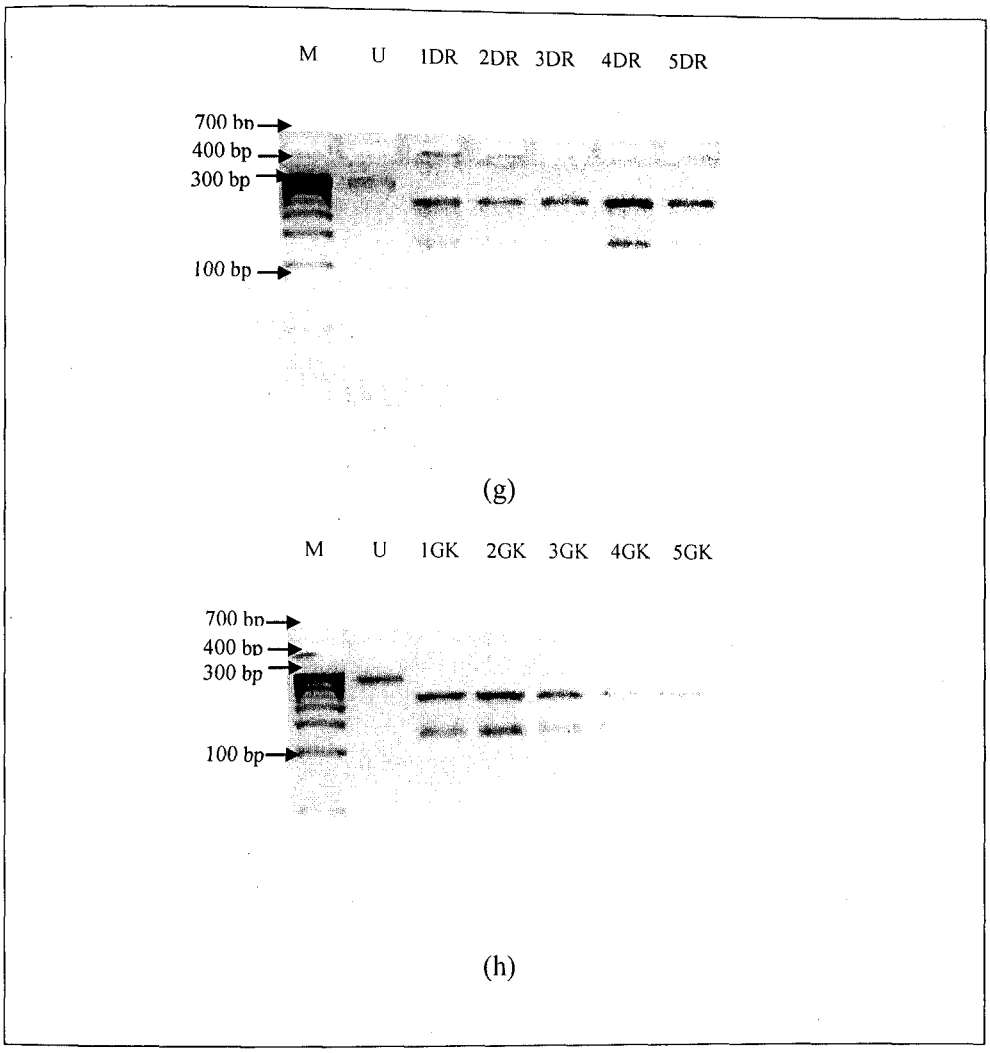


Fig. 6.20 (g-h): PCR-RFLP of *P. bipinnatifidus* from Arunachal Pradesh (DR-Dirang, GK-Gomkang) digested with endonuclease enzyme *BstN*1 (M=100 bp ladder, U= undigested DNA).

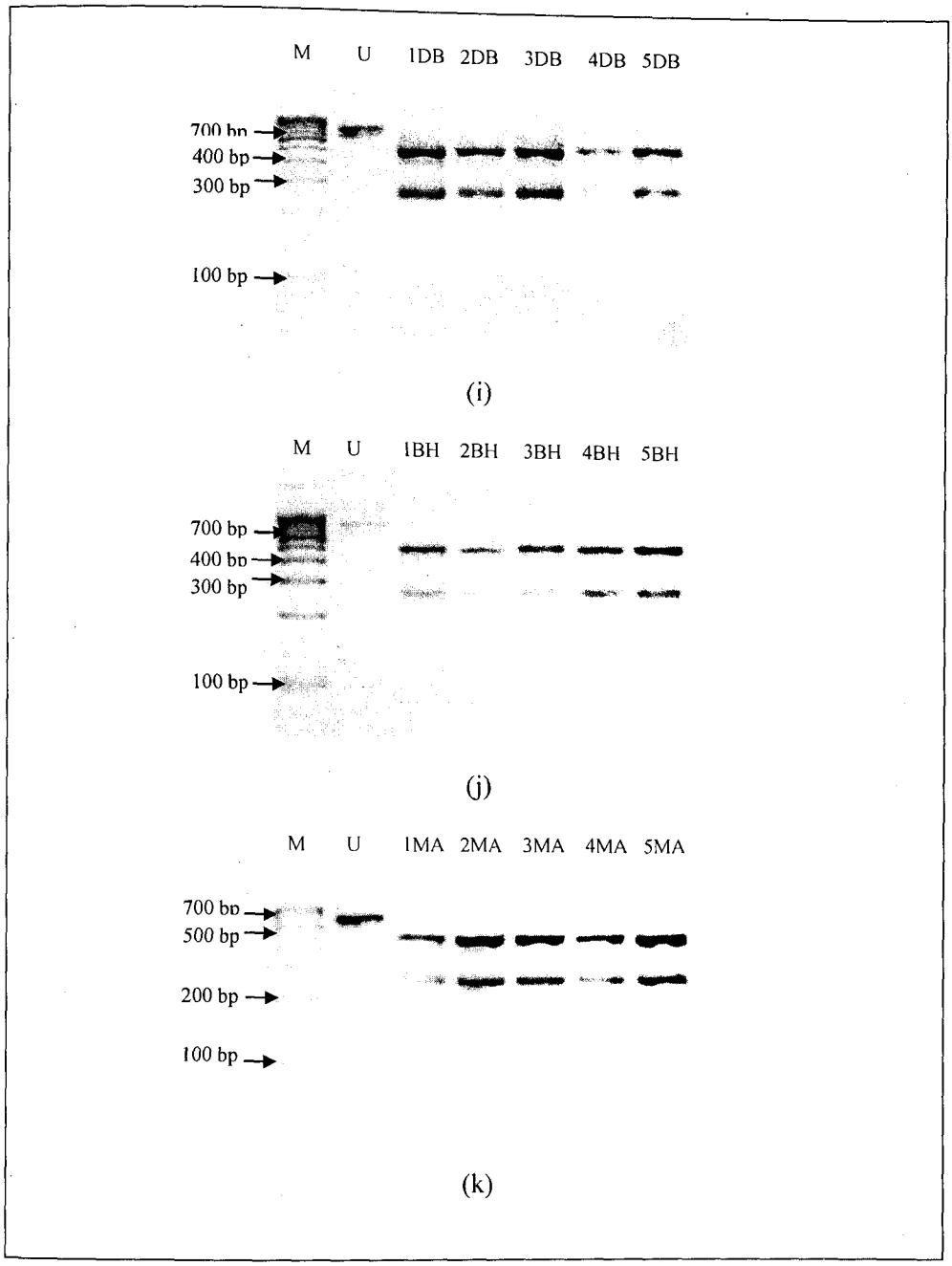


Fig. 6.20 (i-k): PCR-RFLP of *Panax sp.* from Arunachal Pradesh (DB); and Nagaland (BH) and *P. variabilis* from Manipur (MA) digested with endonuclease enzyme *BstN*1 (M=100 bp ladder, U= undigested DNA).

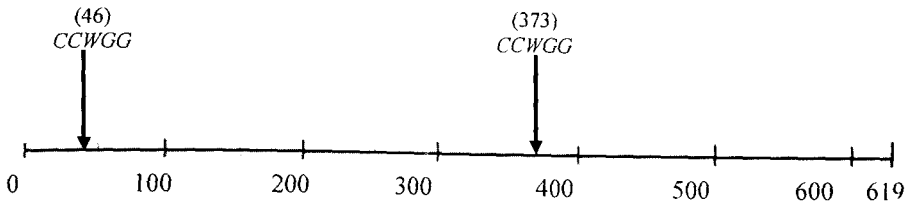


Fig. 6.21: Map of profile PB1 representing *P. assamicus* showing the restriction site using *BstN*1. (Distances not to scale).

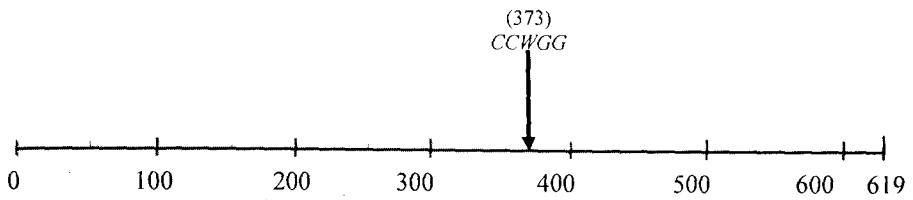


Fig. 6.22: Map of profile PB2 representing *P. bipinnatifidus, P. variabilis* and *P. haridasanii* from Nagaland showing the restriction site using *BstN*1. (Distances not to scale).

Restriction digestion analysis of profile PB1

All the samples of *P. assamicus* collected from Meghalaya and Manipur were digested with restriction enzyme *BstN*1 which showed restriction profile PB1. Computer simulated restriction digestion analysis of the ITS region identified two restriction sites at position 46 bp, and 373 bp which generated three bands of sizes 46 bp, 281 bp and 246 bp (Fig. 6.24). During digestion fragments of size ~360 bp, ~260 bp, ~120 bp and <50 bp were generated. The 46 bp appeared as ~120 bp because of the presence of extra sequence (52-60 bp) upstream the ITS region which is the 18S rDNA. The 246 bp came out as ~360 bp together with the additional sequence (67-75 bp) lying downstream the ITS region. Computer simulated analysis identified only two restriction sites but manual checking of the sequence revealed the location of another restriction site which was downstream the ITS region at position 671 bp for Meghalaya populations and 669 bp for Manipur populations thereby

producing the fourth fragment in the gel which was <50 bp. The difference in the cutting locations in these populations was due to the presence of Indels.

Restriction digestion analysis of profile PB2

Restriction profile PB2 was obtained for all the five replicates of *P. bipinnatifidus* populations from Arunachal Pradesh (DR and GK), *P. variabilis* from Manipur populations (MA) and *Panax* sp. populations from Arunachal Pradesh (DB) and Nagaland (BH) when digested with endonuclease *BstN*1. Computer simulated restriction analysis of the ITS region identified only one restriction site at position 373 bp which yields two fragments of sizes 373 bp and 251 bp respectively (Fig 6.25). Digestion results showed the presence of ~450 bp, ~250 bp and < 50 bp fragments (Table 6.9). The fragment of size 373 bp appeared as ~450 bp due to the extra sequences (50-75 bp) of the 18S rDNA region which lies upstream of ITS region. The presence of another restriction site in all the species upstream the ITS region cuts the sequence at position 668 bp in *P. bipinnatifidus*, 672 bp in *Panax* sp. and 673 bp in *P. variabilis* generating the third fragment of size < 50 bp as seen in the gel. The difference in the cutting locations of these three species was due to the presence of Indels in the sequence.

6.3.3.2. PCR-restriction fragment length profile (PCR-RFLP) with *MspI*

Restriction enzyme *Msp*I generated two different patterns viz., PM1 and PM2 for all the species of *Panax* (Fig. 6.26, a-k) (Table 6.11). PM1 profile was obtained in all the populations of *P. assamicus* from Meghalaya and Manipur and in *P. bipinnatifidus* populations from Arunachal Pradesh. Profile PM2 was obtained for *P. variabilis* populations from Manipur and *Panax* sp. populations from Arunachal Pradesh and Nagaland. Manual check of the nucleotide sequences for all *Panax* species showed some recognition sites in the sequence where substitution of a single nucleotide base could have

provided restriction sites for the enzyme thereby producing the fragments which were generated in the gel.

Table 6.11: PCR-RFLP profile for *Panax* species with the restriction fragments using *Msp*I (+ = band present; - = band absent).

Profiles for *Msp*I	Samples	Restriction size fragments (bp)				
		~530	~220	~130	~100	<50
PM1	*P. assamicus* (UL,TY,LK,NK,US,SH) *P. bipinnatifidus* (DR,GK)	+	+	-	-	-
PM2	*P. variabilis* (MA) *Panax sp.*(DB, BH)	+	-	+	+	-

provided restriction sites for the enzyme thereby producing the fragments which were generated in the gel.

Table 6.11: PCR-RFLP profile for *Panax* species with the restriction fragments using *Msp*I (+ = band present; - = band absent).

Profiles for *Msp*I	Samples	Restriction size fragments (bp)				
		~530	~220	~130	~100	<50
PM1	*P. assamicus* (UL,TY,LK,NK,US,SH) *P. bipinnatifidus* (DR,GK)	+	+	-	-	-
PM2	*P. variabilis* (MA) *Panax sp.*(DB, BH)	+	-	+	+	-

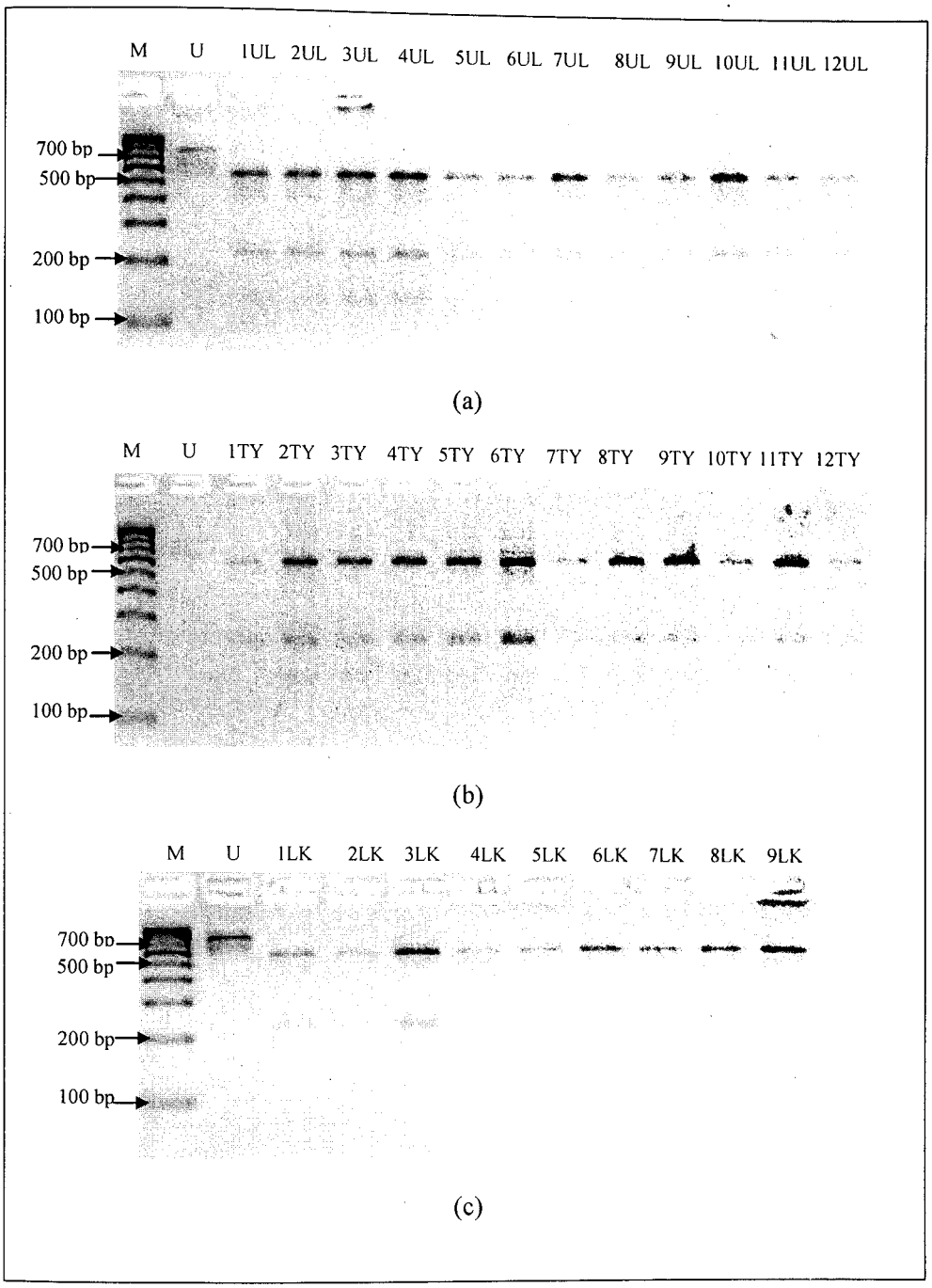


Fig. 6.23 (a-c): PCR-RFLP of *P. assamicus* from Meghalaya (UL-Laitkseh, TY- Tyllang, LK-Laitkor) digested with endonuclease enzyme *Msp*I (M=100 bp ladder, U= undigested DNA).

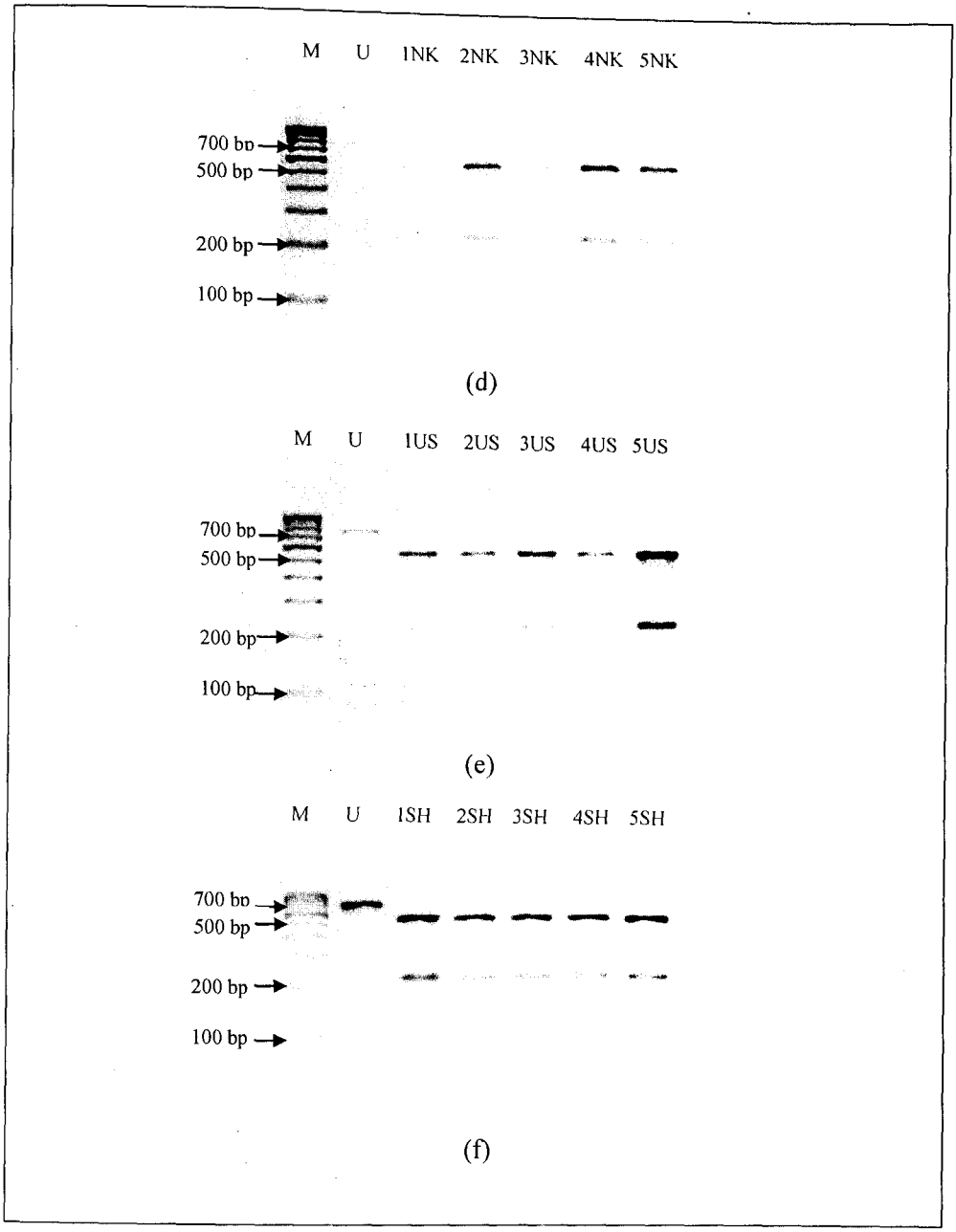


Fig. 6.23 (d-f): PCR-RFLP of *P. assamicus* from Meghalaya (NK-Nongkrem, US-Upper Shillong) and Manipur (SH) digested with endonuclease enzyme *Msp*I (M=100 bp ladder, U= undigested DNA).

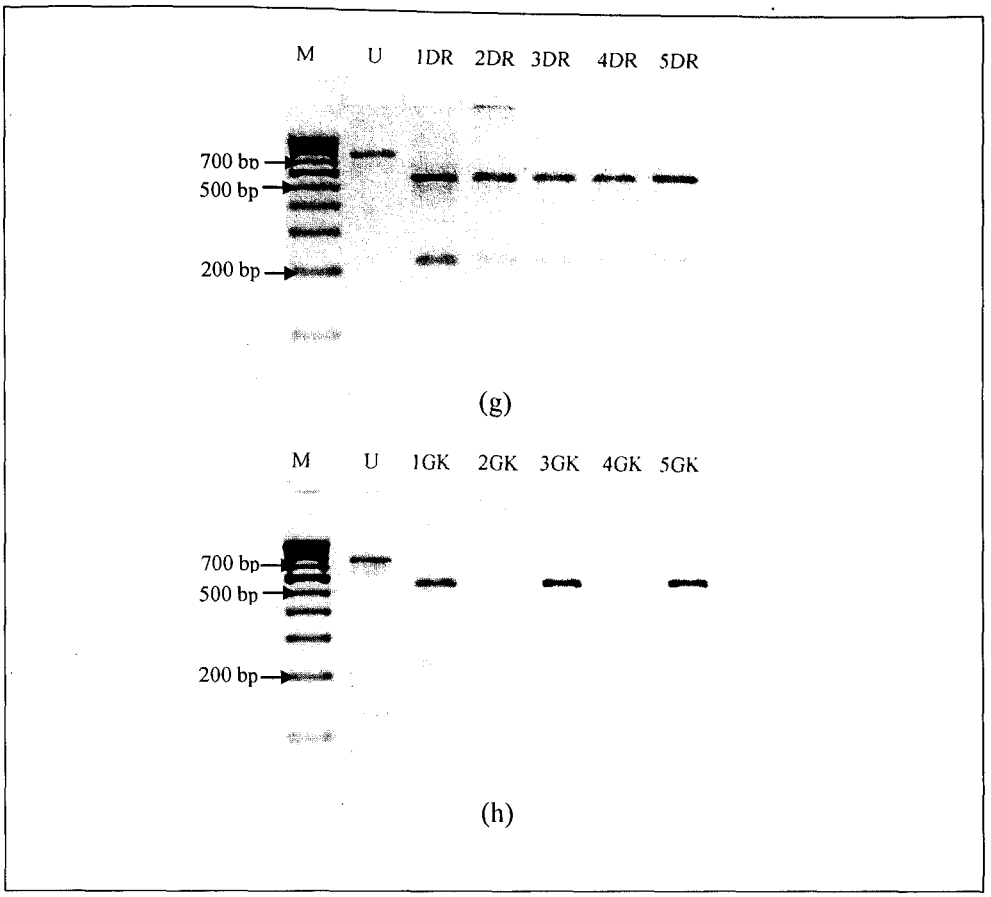


Fig. 6.23 (g-h): PCR-RFLP of *P. bipinnatifidus* from Arunachal Pradesh (DR- Dirang, GK-Gomkang) digested with endonuclease enzyme *Msp*I (M=100 bp ladder, U= undigested DNA).

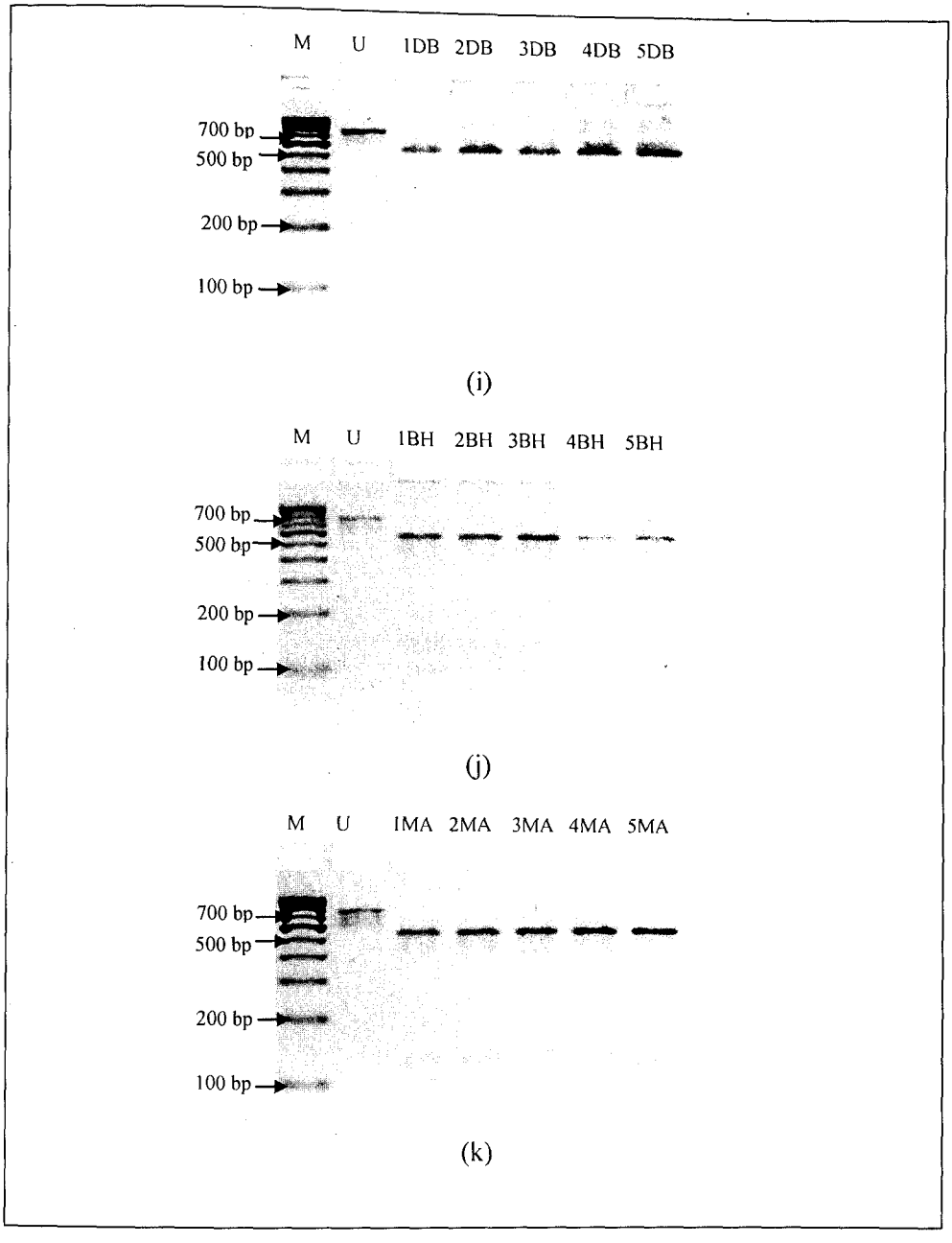


Fig. 6.23 (i-k): PCR-RFLP of *Panax* sp. from Arunachal Pradesh (DB); and Nagaland (BH) and *P. variabilis* from Manipur (MA) digested with endonuclease enzyme *Msp*I (M=100 bp ladder, U= undigested DNA).

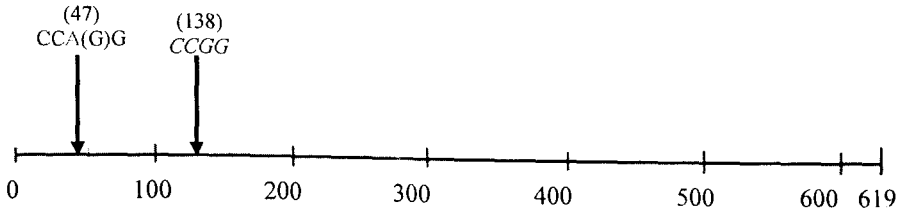


Fig. 6.24: Map of profile PM1 representing *P. assamicus* showing the restriction site using *Msp*I. (Distances not to scale).

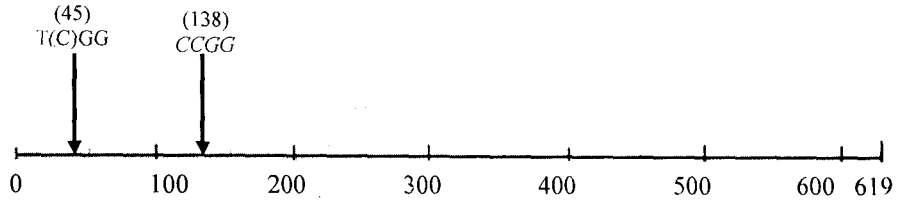


Fig. 6.25: Map of profile PM1 representing *P. bipinnatifidus* showing the restriction site using *Msp*I. (Distances not to scale).

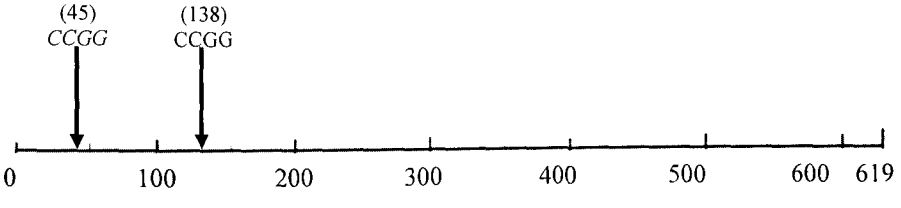


Fig. 6.26: Map of profile PM2 representing *P. variabilis and Panax* sp. showing the restriction site using *Msp*I. (Distances not to scale).

Restriction digestion analysis of profile PM1

The samples of *P. assamicus* and *P. bipinnatifidus* collected were digested with restriction enzyme *Msp*I which showed restriction profile PM1. Computer simulated restriction digestion analysis of the 18S-28S rDNA ITS region using the software DS Gene (version 1.1) identified one restriction site at position 138 bp which generated two bands of sizes 138 bp and 481 bp (Fig. 6.27). Fragments of size ~530 bp and ~220 bp were generated on actual digestion in all the samples. The fragment 138 bp appeared as ~220 bp because the primer amplified not only the ITS region but also the partial sequence of the 18S rDNA which lies upstream the ITS region and constitutes about 49-52 bp. The fragment 481 bp appeared as ~530 bp due to the presence of 65-70 additional bases downstream the ITS region which is the 28S rDNA.

However a manual check of the sequence in *P. assamicus* base by base from 1 - 619 bp revealed a site where the substitution of one nucleotide base could have produced a restriction site for the enzyme. A stretch of nucleotide bases -"CCAG" at position 45^{th}- 48^{th} bp within which if the nucleotide base 'A' is substituted by 'G' at position 47^{th}, the restriction enzyme would identify it as a recognition site and cut the sequence generating two fragments of sizes 45 bp and 574 bp fragments. Due to the transition of purine bases at one position, instead of two recognition sites (45 bp and 138 bp) which generates bands of sizes 45 bp, 93 bp and 481 bp, only one recognition site with band sizes of 138 bp and 481 bp were generated (Fig 6.27).

In *P. bipinnatifidus*, a manual check of the sequence from 1-619 bp shows a site which due to substitution, a restriction site could be produced. If the nucleotide 'T' at position 45^{th} is substituted by 'C' a restriction site would have been recognised due to transition and instead of one recognition site at 138 bp, two sites at 45 bp and 138 bp will be produced which generates bands of sizes 45 bp, 93 bp and 481 bp (Fig 6.28).

Restriction digestion analysis of profile PM2

All the five replicates of *P. variabilis* from Manipur populations (MA) and *Panax sp.*populations from Arunachal Pradesh (DB) and Nagaland (BH) were digested with endonuclease *Msp*I and showed the restriction profile PM2 (Fig. 6.29). Computer simulated restriction analysis of the ITS region recognized two restriction sites at positions 45 bp and 138 bp which generated three fragments of sizes 45 bp, 93 bp and 484 bp . Fragments of ~530 bp, ~130 bp and ~100 bp were generated on digestion in the gel. The fragment of size 45 bp appeared as ~130 bp due to the extra sequences (50-75 bp) of the 18S rDNA region which lies upstream of ITS region and the fragment 484 bp was observed as ~530 bp with the additional sequence (65-70 bp) from the 28S rDNA .

6.3.3.3. PCR-restriction fragment length profile (PCR-RFLP) with *PspG1*

The restriction enzyme *PspG*1 generated two different profiles PP1 and PP2 for all the species of *Panax* (Fig. 6.30, a-k, Table 6.12). PP1 profile was found in all the populations of *P. assamicus* from Meghalaya and Manipur. PB2 profile was found in *P. bipinnatifidus* populations from Arunachal Pradesh, *Panax* sp. from Arunachal Pradesh and Nagaland and *P. variabilis* from Manipur. Manual check of the nucleotide sequences for all *Panax* species showed an extra recognition site for the enzyme which lies downstream the ITS region thereby producing the fragments which were generated in the gel.

Table 6.12: PCR-RFLP profile for *Panax* species with the restriction fragments using *PspG1* (+ = band present; - = band absent).

Profiles for *PspG1*	Samples	Restriction size fragments (bp)						
		~430	~330	~280	~260	~250	~120	<50
PP1	*P. assamicus* (UL,TY,LK,NK,US,SH)	-	+	-	+	-	+	+
PP2	*P. bipinnatifidus* (DR andGK) *Panax sp.* (DB and BH) *P. variabilis* (MA)	+	-	-	-	+	-	+

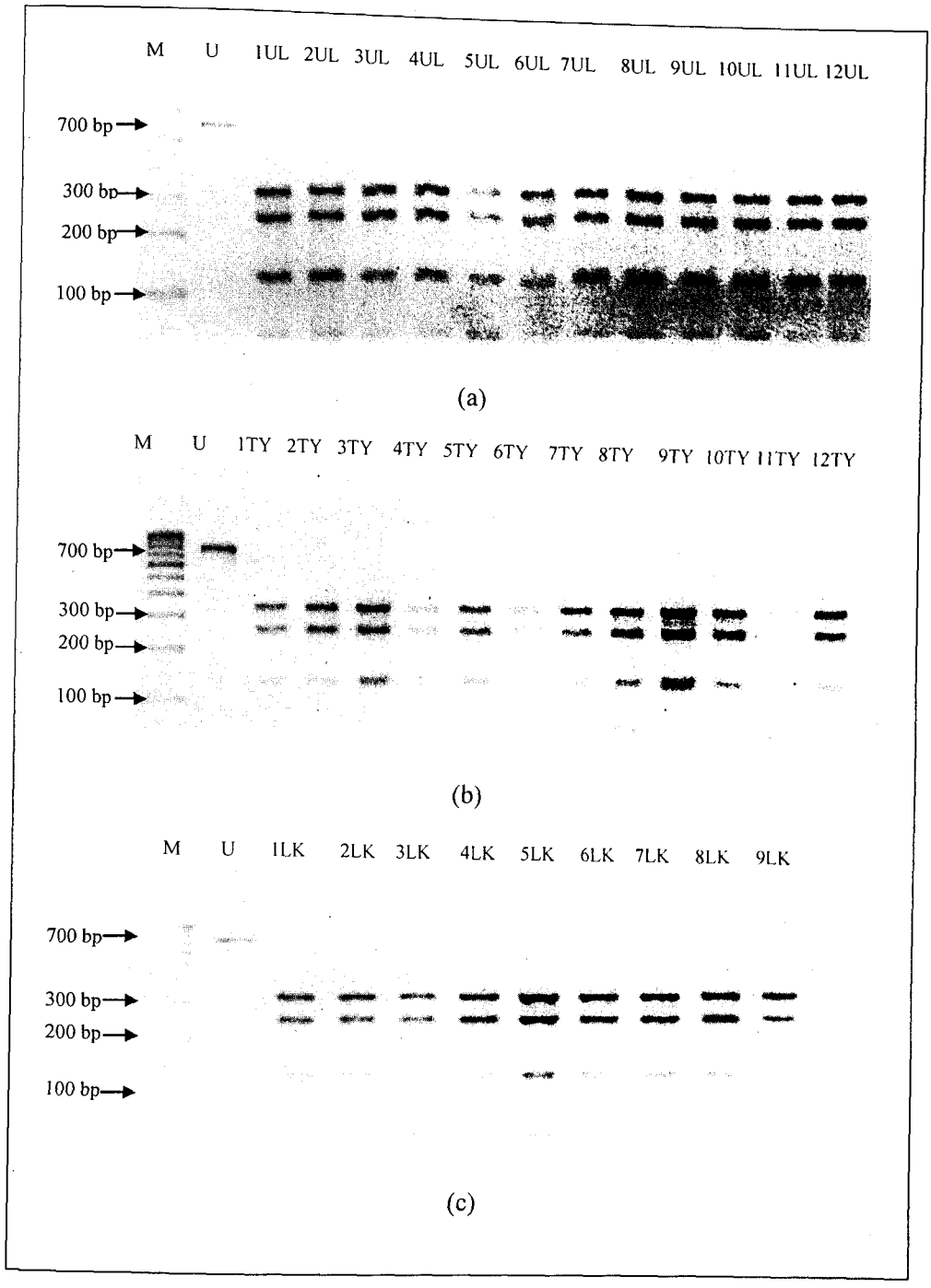


Fig. 6.27 (a-c): PCR-RFLP of *P. assamicus* from Meghalaya (UL-Laitkseh, TY-Tyllang, LK- Laitkor) digested with endonuclease enzyme *pspG*1 (M=100 bp ladder, U= undigested DNA).

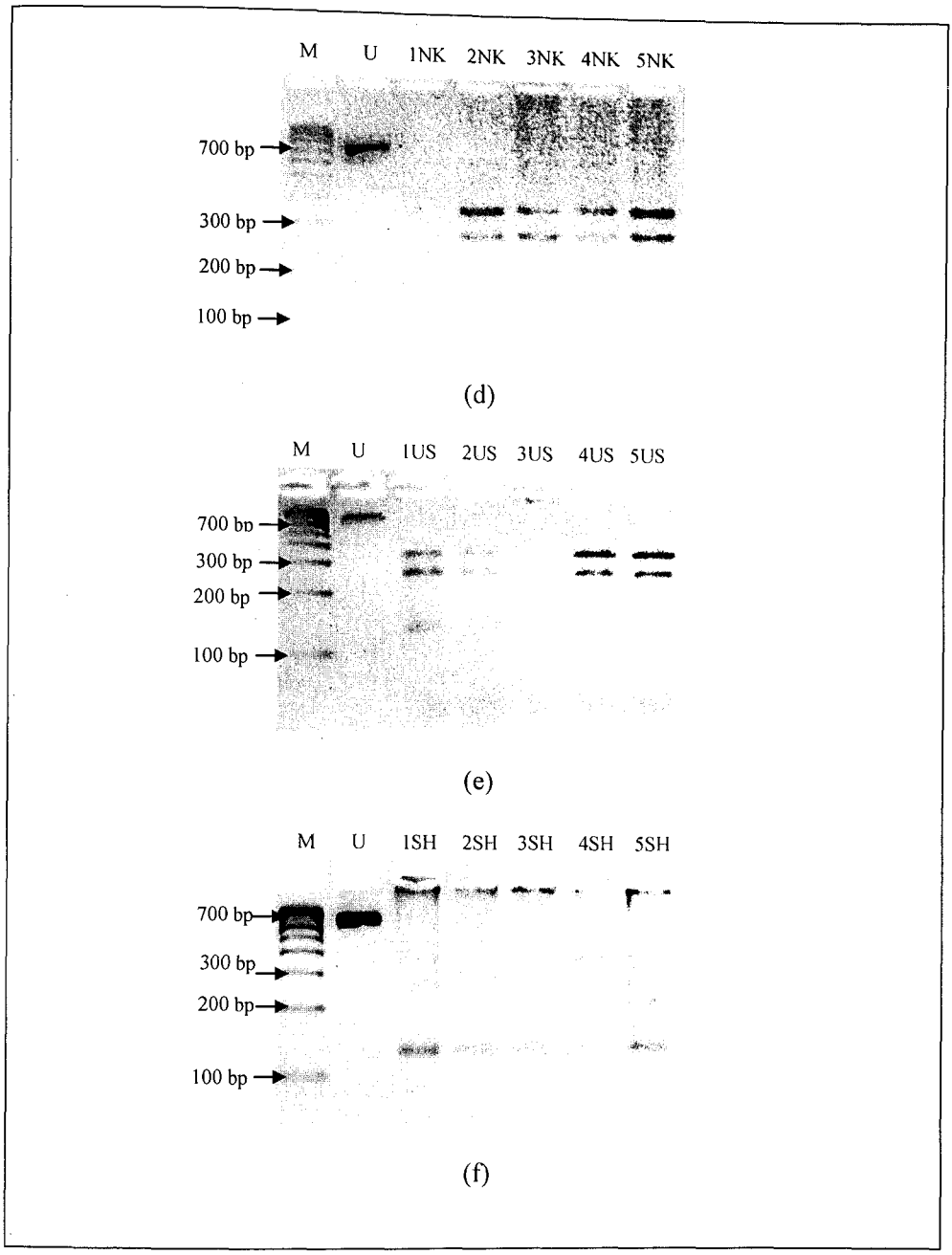


Fig. 6.27 (d-f): PCR-RFLP of *P. assamicus* from Meghalaya (NK-Nongkrem, US- Upper Shillong) and Manipur (SH) digested with endonuclease enzyme *PstI* (M=100 bp ladder, U= undigested DNA).

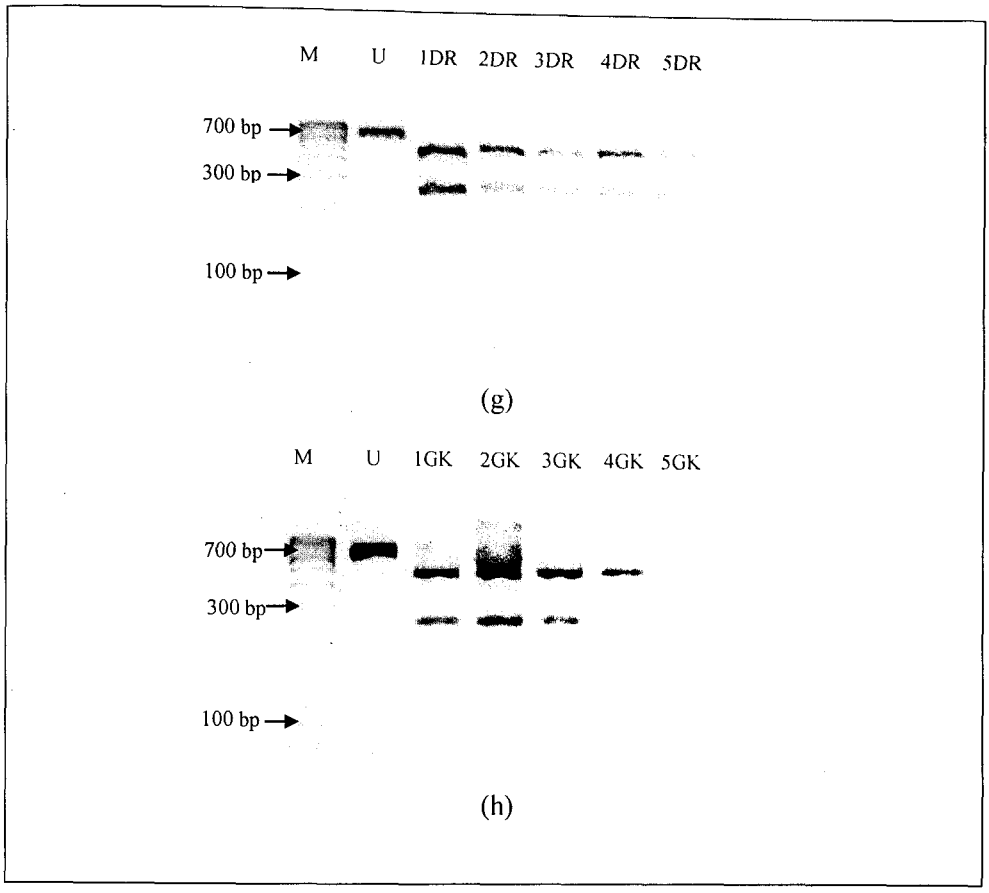


Fig. 6.27 (g-h): PCR-RFLP of *P. bipinnatifidus* from Arunachal Pradesh (DR-Dirang, GK-Gomkang) digested with endonuclease enzyme *PspG*I(M=100 bp ladder, U= undigested DNA)

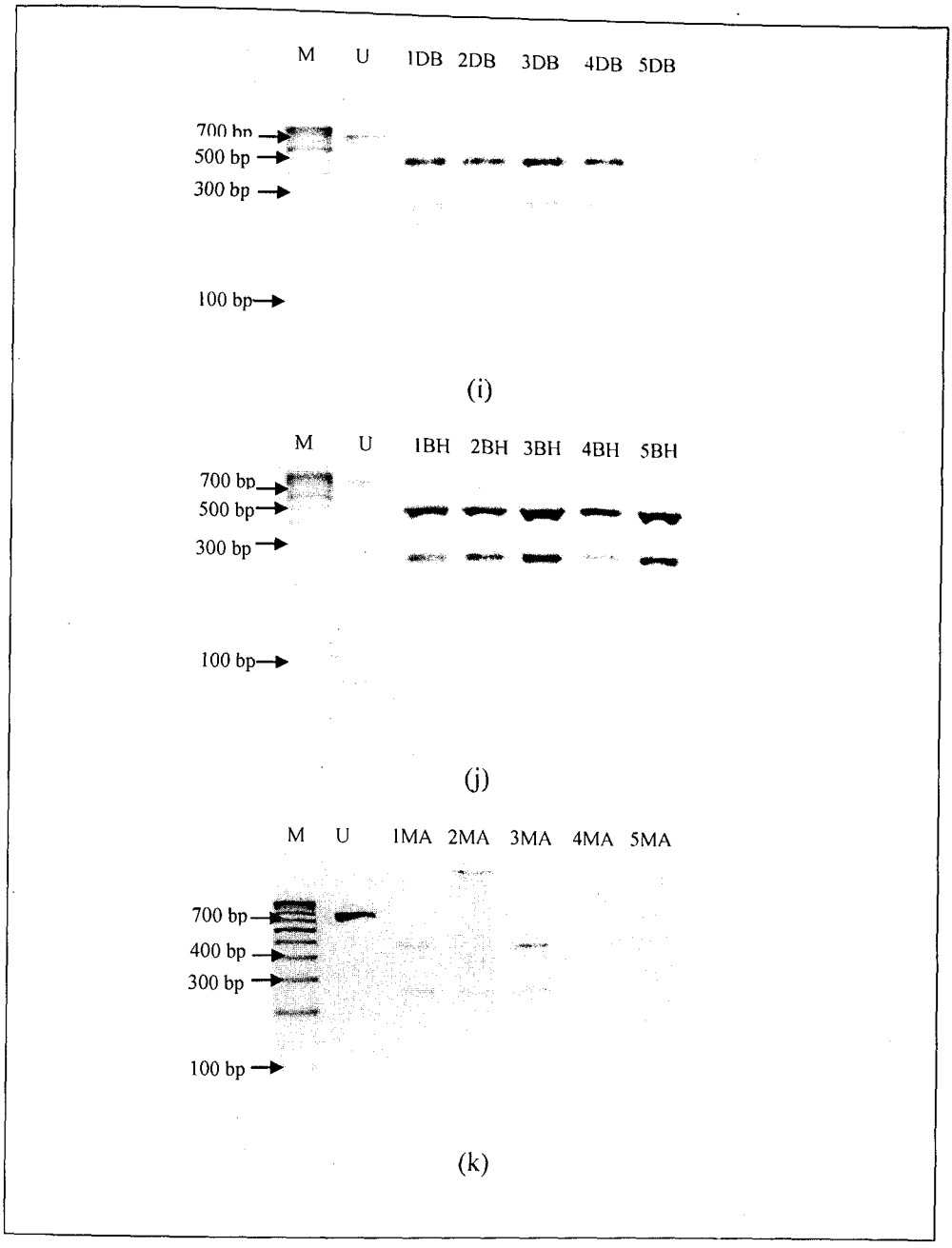


Fig. 6.27 (i-k): PCR-RFLP of *Panax* sp. from Arunachal Pradesh (DB); and Nagaland (BH) and *P. variabilis* from Manipur (MA) digested with endonuclease enzyme *pspG*1 (M=100 bp ladder, U= undigested DNA).

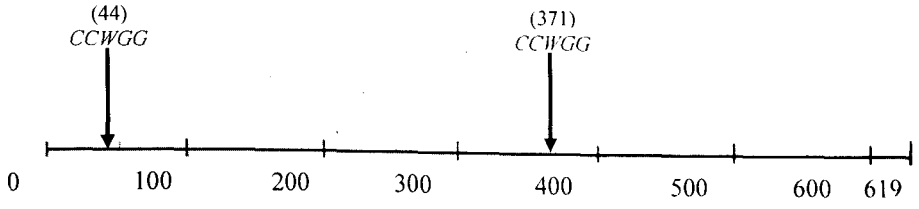


Fig. 6.28: Map of profile PP1 representing *P. assamicus* showing the restriction site using *PspG*1. (Distances not to scale)

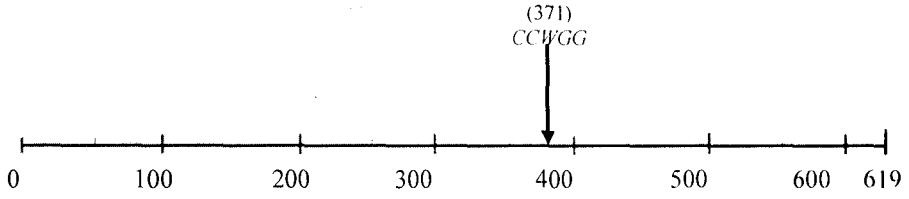


Fig. 6.29: Map of profile PP2 representing *P. bipinnatifidus, P. variabilis and Panax* sp. from Nagaland showing the restriction site using *PspG*1. (Distances not to scale).

Restriction digestion analysis of profile PP1

The amplicons of the ITS region of all the samples of *P. assamicus* collected from Meghalaya and Manipur were digested with restriction enzyme *PspG*1 which showed restriction profile PP1. The profile PP1 comprised of four bands of sizes ~330 bp, ~260 bp, ~120 bp and ~50 bp on restriction digestion with the enzyme *PspG*1 (Table 6.12). Computer simulated restriction digestion analysis via DS Gene (version 1.1) identified two restriction sites for this enzyme located at positions 44 bp and 371 bp which yields three bands of sizes 283 bp, 248 bp and 44 bp (Fig. 6.28). The 44 bp appeared as ~120 bp due to the extra sequence (52-60 bp) upstream the ITS region which comprise the 18s rDNA region. The 248 bp came out as ~330 bp together with the additional sequence (67-75 bp) lying downstream the ITS region. Computer simulated analysis identified only two restriction sites but manual checking of the sequence revealed the location of another restriction site which was present downstream of the ITS region at position 669 bp for Meghalaya populations and 667 bp for Manipur populations thereby producing the fourth fragment in the gel which was <50 bp. The difference in the cutting locations in these populations was due to the presence of Indels.

Restriction digestion analysis of profile PP2

Restriction profile PP2 was obtained for all the replicates of *P. bipinnatifidus* populations from Arunachal Pradesh (DR and GK), *P. variabilis* from Manipur populations (MA) and *Panax sp.*populations when digested with endonuclease *PspG*1. Computer simulated restriction analysis of the ITS region identified only one restriction site at position 371 bp which yields two fragments of sizes 371 bp and 248 bp respectively (Fig. 6.29). Digestion results showed the presence of ~430 bp, ~250 bp and < 50 bp fragments (Table 6.12). The fragment of size 373 bp appeared as ~430 bp due to the extra sequences (50-75 bp) of the

18S rDNA region which lies upstream of ITS region. The presence of another restriction site in all the species upstream the ITS region cuts the sequence at position 666 bp in *P. bipinnatifidus*, 670 bp in *Panax sp.*and 671 bp in *P. variabilis* generating the third fragment of size < 50 bp as seen in the gel. The difference in the cutting locations of these three species was due to the presence of Indels in the sequence but fragments of the same size were produced.

Cluster analysis

Cluster dendrogram was constructed by using the PCR-RFLP profiles of the 18S-28S rDNA ITS region of all the *Panax* species studied. The cluster dendrogram showed two major clusters, I and II (Fig 6.33). Cluster I comprises all the sub-populations of *P. assamicus* from Meghalaya and Manipur which was seen from the PCR-RFLP profiles generated by the three restriction enzymes *BstN*I and *PspG*I which showed the same profile for all the sub-populations. The same profile was obtained for *Msp*I in both the populations. Cluster II comprised of two sub-clusters, the first cluster (IIA) consists of *P. bipinnatifidus* populations from Arunachal Pradesh and the second cluster (IIB) includes *P. variabilis* from Manipur population and *Panax* sp. populations from Arunachal Pradesh and Nagaland. The enzyme *BstN*I and *PspG*I produced the same profile as in *P. variabilis* and *Panax* sp. but restriction enzyme *Msp*I generates the same profile with *P. assamicus*. However, sub-cluster IIB includes *P. variabilis* and *Panax* sp. populations. All the three enzymes generate the same profile which grouped these two species together.

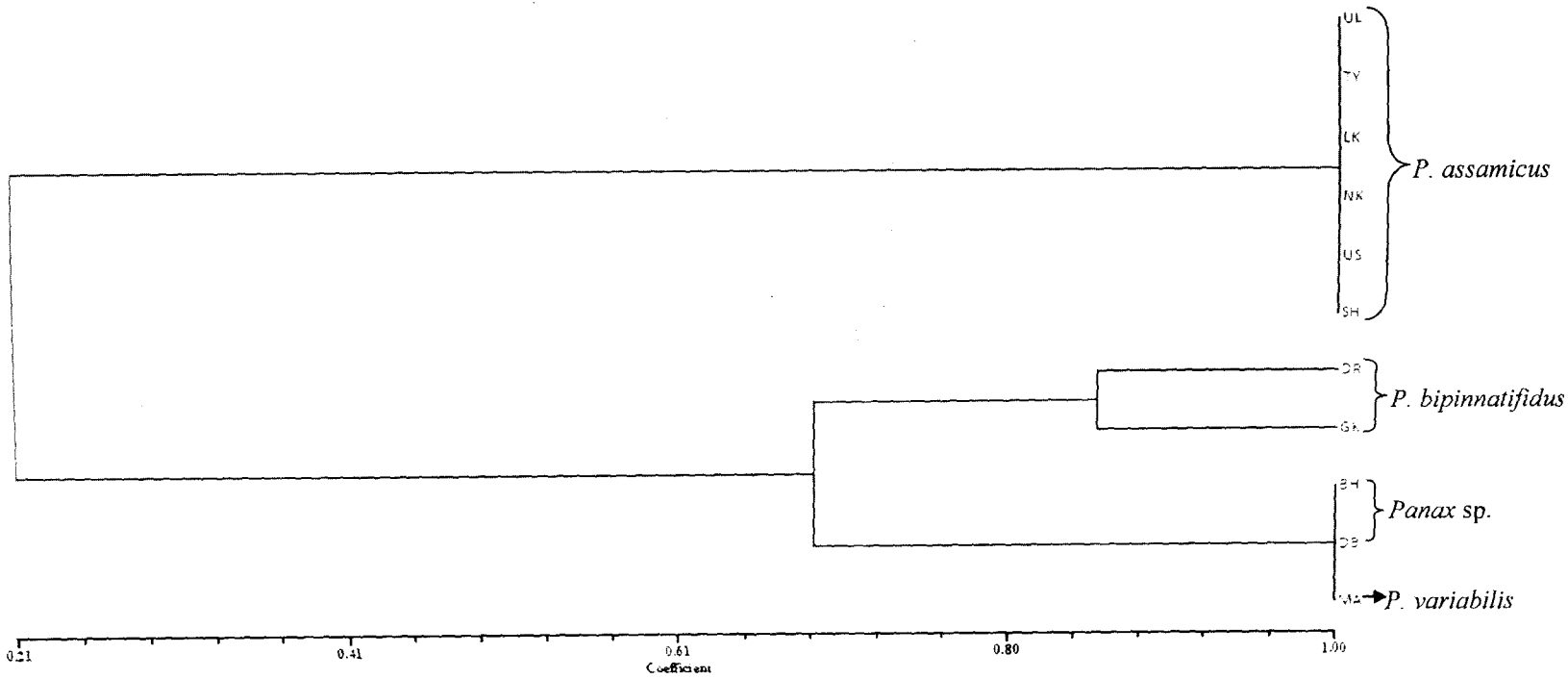


Fig. 6.30: Cluster dendrogram based on the ARP profiles using endonuclease enzyme *Msp*I, *PspG*1 and *BstN*1.

6.3.4. Secondary structure of 5.8S rRNA

The secondary structures in all the studied species of *Panax* were compared with the retrieved sequences from GenBank (Fig. 6.18-6.22). *P. assamicus* from both the populations in Meghalaya matched the secondary structure in *P. assamicus* from GenBank. *P. assamicus* from Manipur population did not match the secondary structure from Meghalaya, instead it had similar structure with another population from Manipur which was retrieved from GenBank (HQ141404). The difference in the structure was due to the transition of a purine base 'A' by purine base 'G' at one nucleotide position. The populations of *P. bipinnatifidus* from Arunachal Pradesh had the same structure with that of *P. bipinnatifidus* from Nepal. *Panax sp.* from both the populations had similar structures. Since *Panax sp.* showed 99% similarity with *P. quinquefolius* and *P. vietnamensis*, a comparison was made between them but their structures did not match. *P. variabilis* collected from Manipur population matched the secondary structure with *P. variabilis* from China.

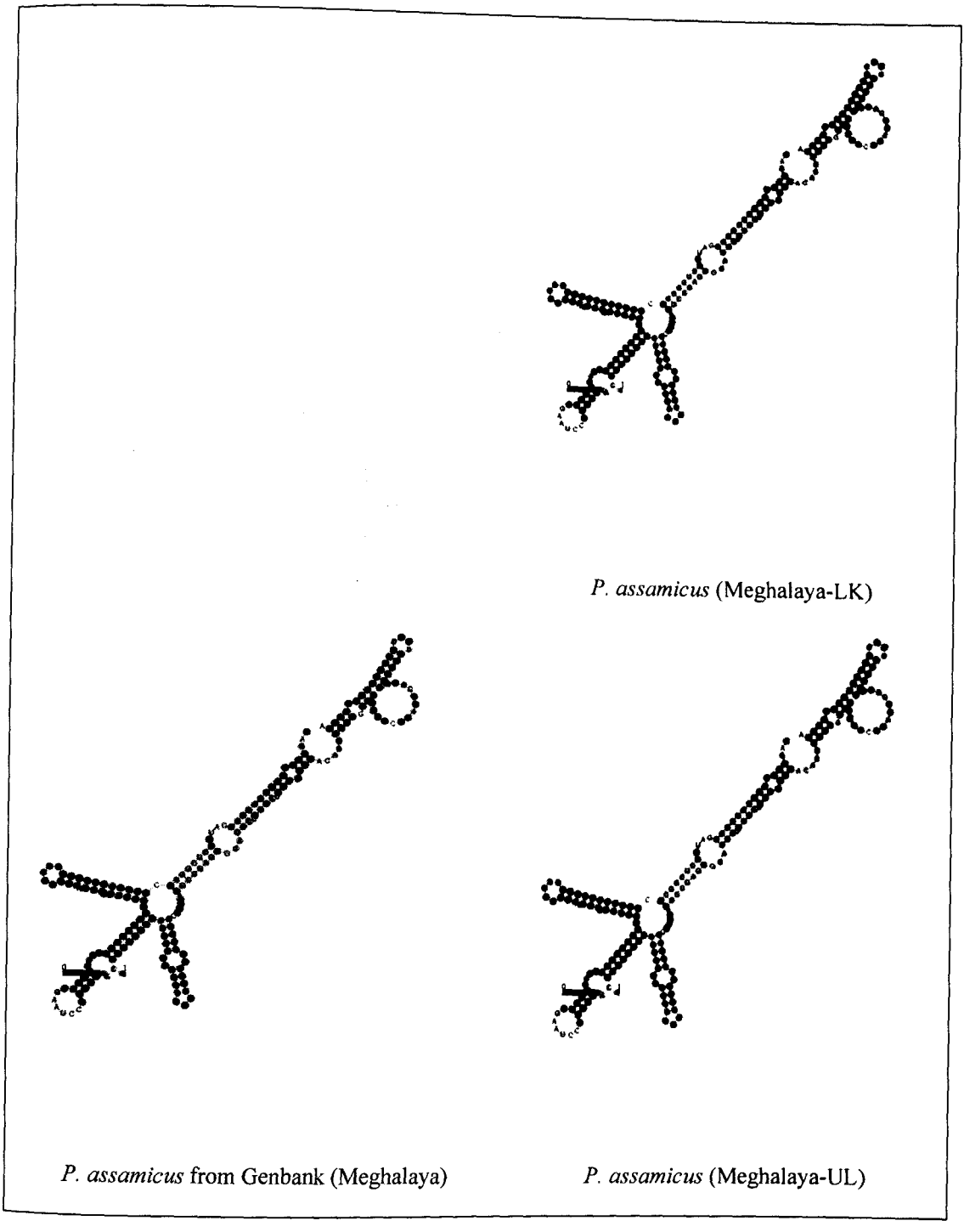


Fig. 6.31: 5.8S rRNA secondary structure using the minimum free energy (MFE). Comparison between the studied species and GenBank retrieved sequence in *P. assamicus*.

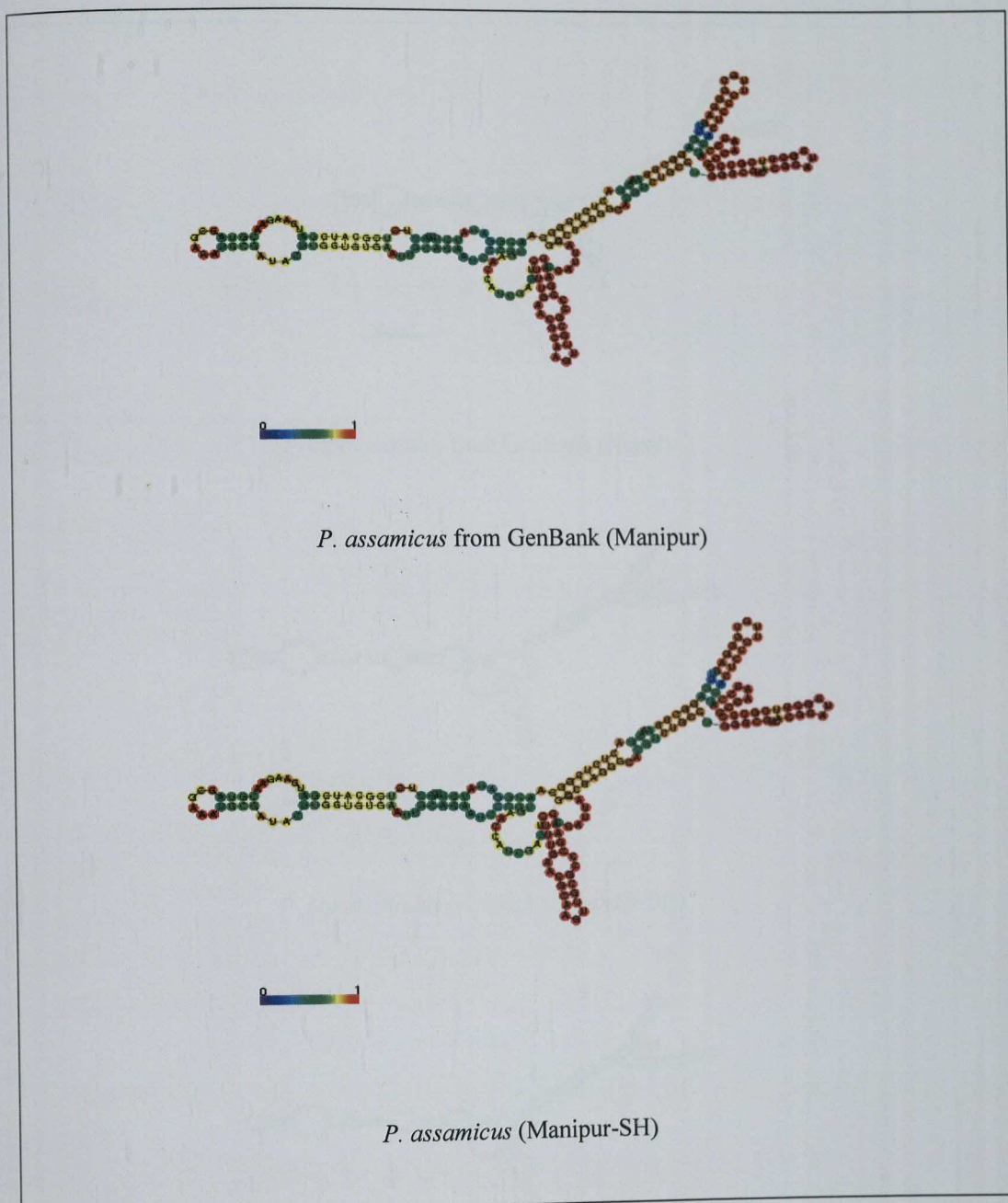


Fig. 6.32: 5.8S rRNA secondary structure using the minimum free energy (MFE). Comparison between the studied species and GenBank retrieved sequence in *P. assamicus*.

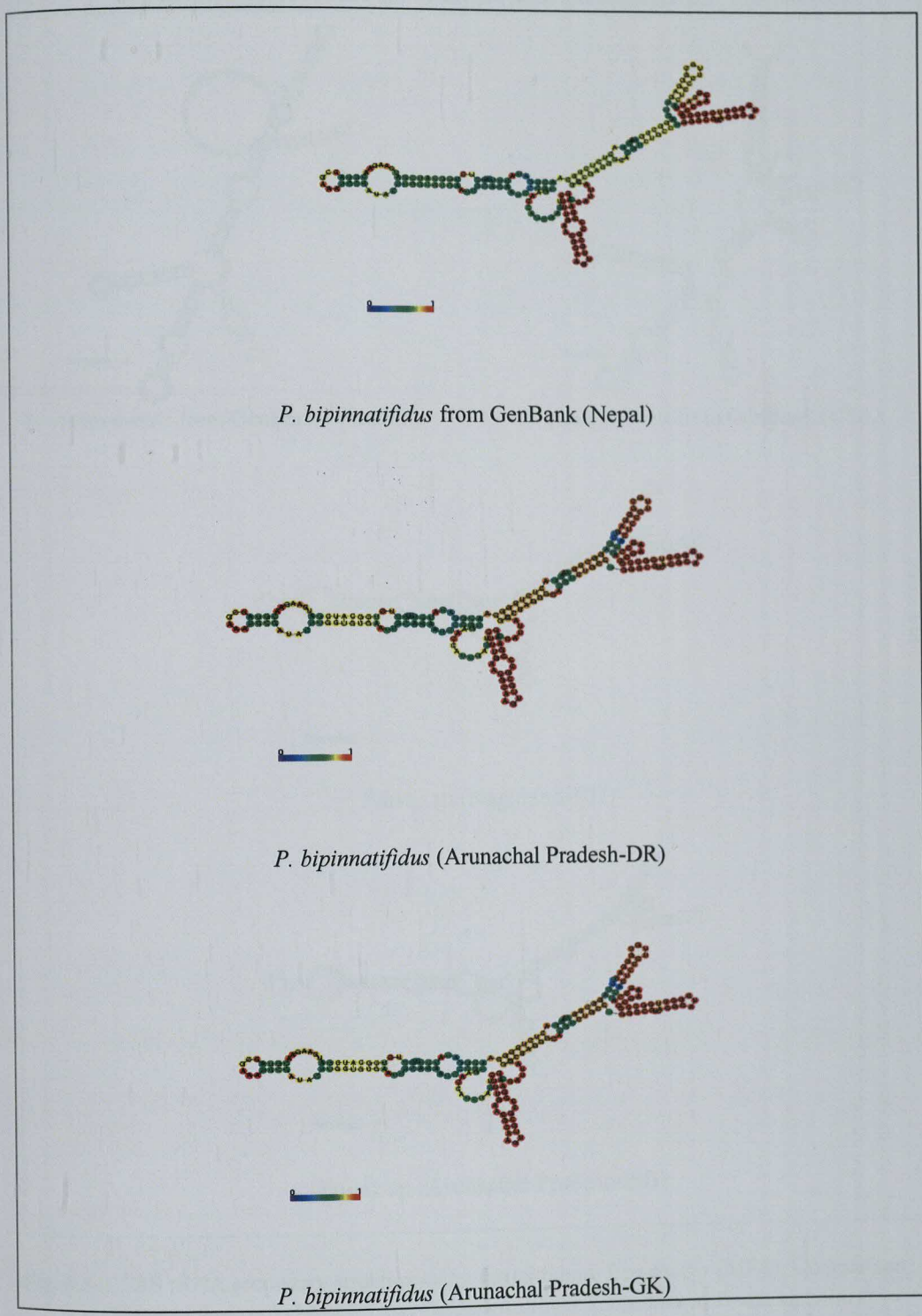


Fig. 6.33: 5.8S rRNA secondary structure using the minimum free energy (MFE). Comparison

between the studied species and GenBank retrieved sequence in*P. bipinnatifidus*

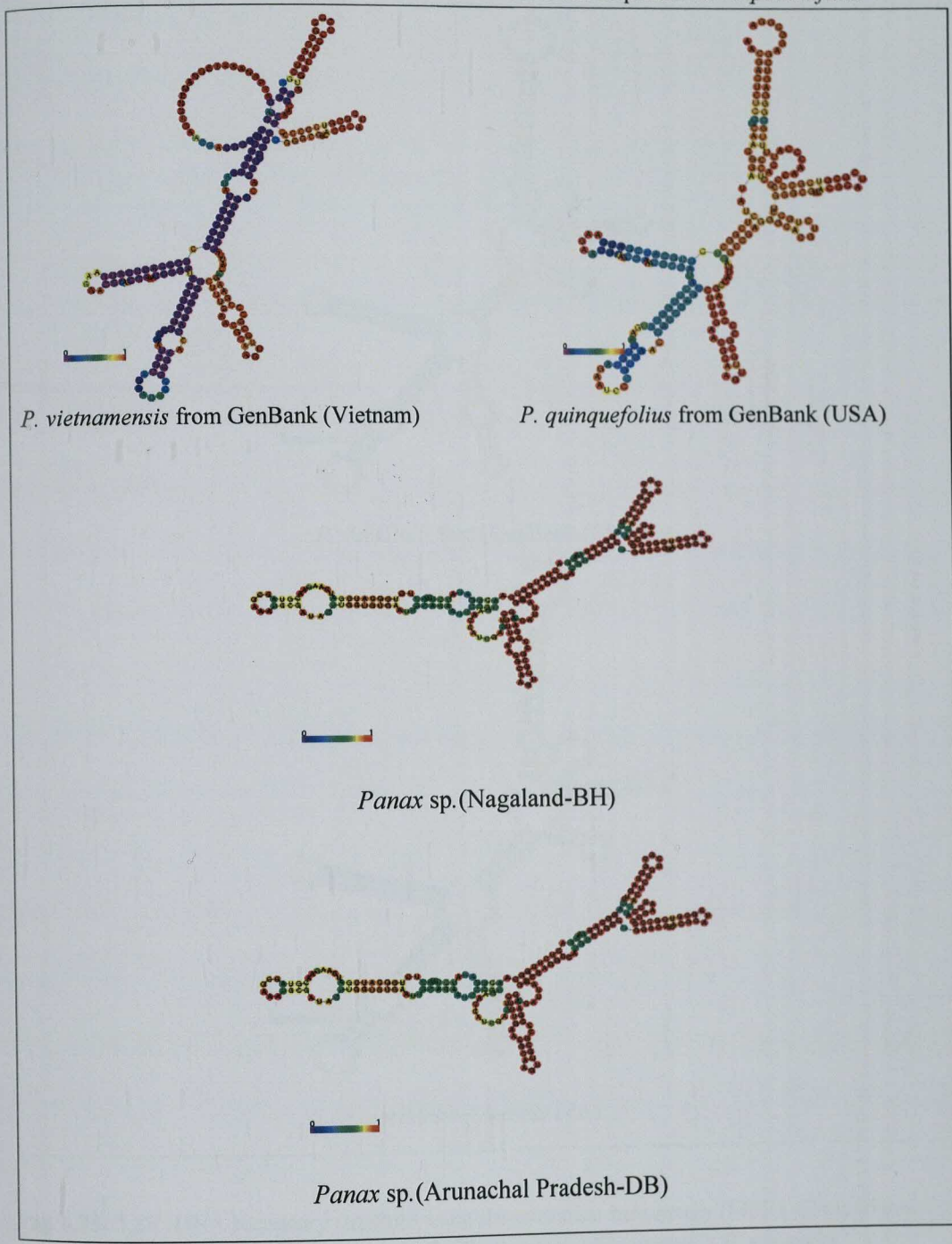


Fig. 6.34: 5.8S rRNA secondary structure using the minimum free energy (MFE). Comparison between the studied species and GenBank retrieved sequence in *Panax* sp. with *P. quinquefolius and P. vietnamensis.*

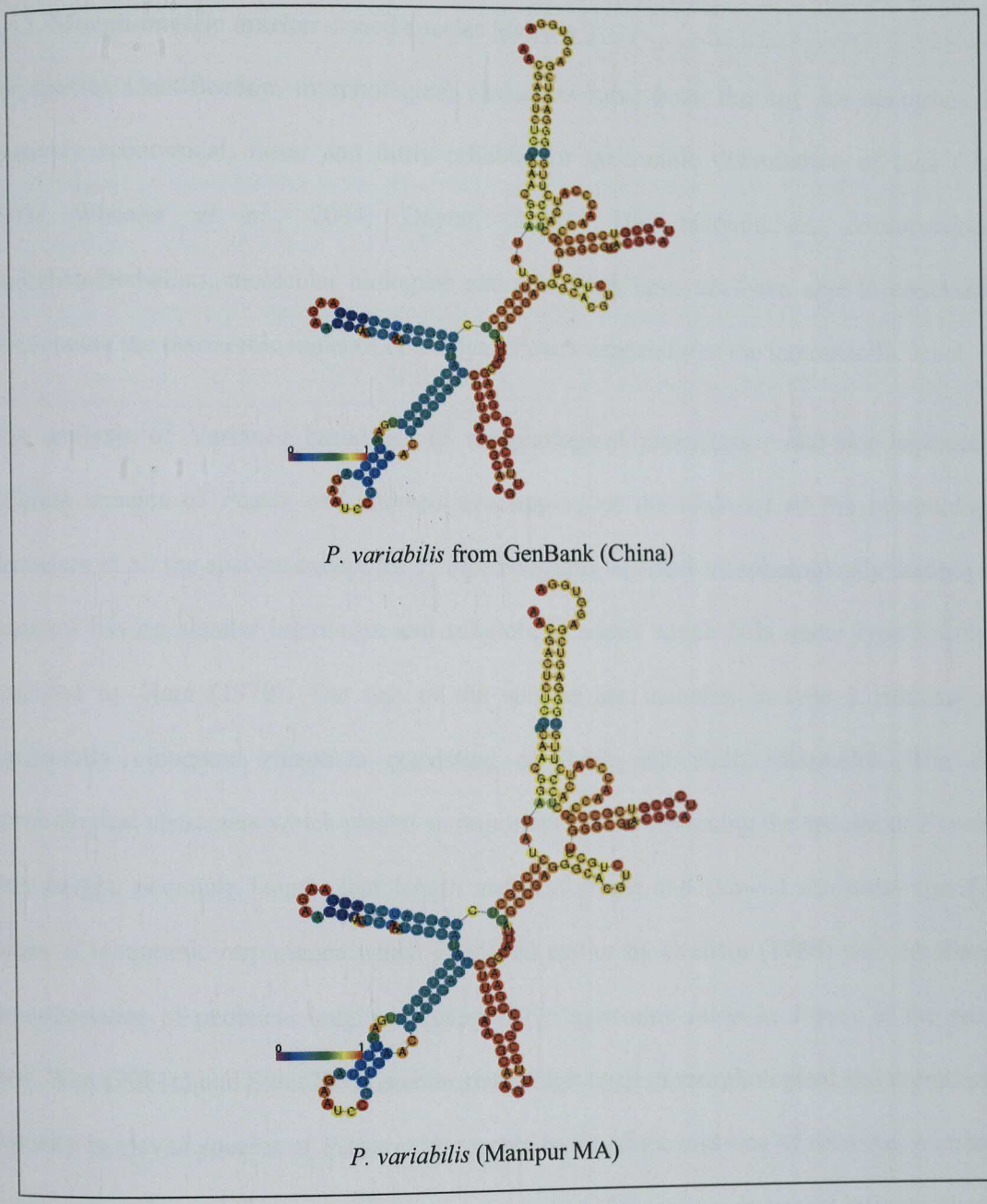


Fig. 6.35: 5.8S rDNA secondary structure using the minimum free energy (MFE). Comparison between the studied species and GenBank retrieved sequence in *P. variabilis*.

6.4. DISCUSSION

6.4.1. Morphometric marker-based species identity

For species identification, morphological characters have been the key for centuries. It is relatively economical, faster and fairly reliable for taxonomic delimitation of taxa (Dunn, 2003; Wheeler *et al.*, 2004; Dayrat, 2005). The taxonomists, conservationists, naturalists/herbalists, molecular biologists and ecologists have not been able to conclusively differentiate the taxonomic ranks of Himalayan *Panax* especially at the interspecific level.

The analysis of Variance based on 15 morphological characters could not separate the different species of *Panax* and showed overlapping of the majority of the morphological characters in all the species except for *P. bipinnatifidus* which is morphologically distinct with rhizomes having slender internodes and subglobose nodes which falls under type 3 rhizome classified by Hara (1970). The rest of the species are included in type 2 rhizome with horizontally elongated rhizomes consisting of thick and short internodes. The other morphological characters which played an important role in separating the species of *Panax* are plant height, peduncle length, leaf length and leaf shape and showed the most significant values of taxonomic importance which is proved earlier by Graham (1966) who emphasized the importance of peduncle length in recognizing taxonomic ranks in *Panax* at the species level. Wen (2001a) and Shu (2007) demonstrated high level of morphological and reproductive plasticity in eleven species of *Panax* with respect to the shape and size of rhizome, number of leaves, leaflet shape, pubescence of leaves and number of flowers in an umbel inflorescence.

Cluster analysis taking morphometric could partially seggregate the species. While *P. bipinnatifidus* had formed a clear sub-clade others exhibited intermixing of population.

The present study clearly demonstrates that ordination can offer a dependable tool to resolve the confusion and lead to correct identification of *Panax* species. The PCA revealed three distinct species viz., *P. assamicus, P. bipinnatifidus* and *P. variabilis*. Howeve there was a substantial overlap between *P. pseudoginseng* and *Panax* sp.

P. assamicus as described by Banerjee (1968) have broadly linear leaves, midribs distinct from both surfaces, apex of petals inflexed apiculate, disc flat and bracteoles which are linear-lanceolate in shape. *P. bipinnatifidus* bears no bracts, has a pinnate or bipinnate leaflet and varies from narrow to broad elliptic and a lobed double serrate margin, Seemann (1868) treated all morphotypes of Himalayan *Panax* other than pinnate or bipinnate leaflets as *P. pseudoginseng,* which is now well established as a separate/distinct species (Wen 2001; Yoo *et al.*, 2001). *P. pseudoginseng* was described by Wallich (1829) in having fascicled roots, presence of persistent stipules which are ovate in shape. *P. variabilis* was described by Jun Wen from China and Pandey *et al.* (2009) from Nagaland but no details of morphological features have been published. The species from the present study is differentiated in having obovate to broadly elliptic leaves. *Panax* sp. which was newly discovered from Arunachal Pradesh and Nagaland has been identified with rhizome 20-30 cm long, horizontal, prominent stem scars, lanceolate or oval to broadly elliptic leaves and leaflets which vary from 5-9 per petiole. The leaves are densely setose, peduncle longer than petioles, anthers oblong, fruit a berry with a black tip. All these characters which do not match with any other existing species possibly could make it a new species.

6.4.2. Molecular phylogeny-based species identity

The *18S rRNA* gene was found to be the most conserved region (conserved sites=99.17%) compared to ITS region (conserved sites=77.42%) (Table 6.4). The 18S rRNA gene with variable sites of 0.65% and parsimony informative sites of 0.06 % proves that the region being conserved is also phylogenetically informative and used for broad scale phylogenetic reconstruction which is seen in angiosperms (Nickrent and Soltis, 1995, Soltis *et al.*, 1997b) and evolves slowly. The ITS region being the most highly variable region with variable sites accounting to 18.23% across *Panax* species has the highest number of parsimony informative sites with 9.68% and is phylogenetically informative which has been proved in the phylogenetic reconstruction in angiosperms (Baldwin *et al.*, 1995).

The small-subunit sequences of rRNA such as 18S rRNA contain variable and conserved regions, including several evolutionarily conserved 'functional domains' (Gutell *et al.*, 1985), which suggests the 18S rRNA gene sequence gives more substantive information for the phylogenetic relationship. According to different taxonomists the species of *Panax* from Asia were divergent. The Asian species were identified according to the definition of Zhou *et al.* (1975), and the Indian ginseng was identified as *P. pseudoginseng* subsp. *himalaicus* following Hara's definition (1970), for it was consistent with the original description of *P. pseudoginseng* not only in terms of its morphology but also in geographic distribution. Moreover, the determination of some Asian *Panax* plants remained controversial due to the variation of morphological characters and the existence of intermediate forms which was shown by their high degree of homology (99%). The 18S rRNA gene could not distinguish all the *Panax* species which was shown by the phylogenetic tree.

The phylogeny of *Panax* has been studied using ITS sequences (Wen and Zimmer, 1996) chloroplast DNA restriction sites (Choi and Wen, 2000) and chloroplast *trn*C-*trn*D intergenic region (Lee and Wen, 2004). These studies have proved that *Panax trifolius* is sister to a clade which includes all the *Panax* species. The second diverged group constitutes the *P. pseudoginseng-P. stipuleanatus* clade. *P. notoginseng* is basal to a large clade containing *P. quinquefolius* and all the Asian *Panax* taxa. These findings have been confirmed by morphological and palynological evidences (Wen 2001b; Wen and Nowicke, 1999). The trnC-trnD phylogeny indicates that the eastern North American species *P. quinquefolius* forms a clade with the Asiatic species *P. ginseng* and *P. japonicus*. *P. assamicus* was described by Banerjee (1968) based on specimens collected from Meghalaya, India. Its species status has been questioned by Hara (1970) and Wen (2001b). Morphologically *P. assamicus* is very similar to *P. wangianus* from west central China and to *P. zhengyianus* J.Wen from south western China. All the three taxa have narrow leaflets, elongated rhizomes with thick and short internodes and fruits which are bright red in colour with a black tip. Studies on the Indian ginseng by Pandey *et al.* (2002) revealed that the Indian *P. assamicus* is quite distinct from the morphologically similar *P. wangianus* from west central China and *P. zhengyianus* from south western China. *P. bipinnatifidus* was described as being characterized by its divided leaflets (Seemann, 1868). Li (1942) and Hara (1970) realized that the degree of the division of its leaflets varied greatly. The identity of *P. bipinnatifidus* was revealed in earlier studies by Wen and Zimmer (1996), Lee and Wen (2004), Pandey *et al.* (2009), Pandey and Ali (2010). *P. variabilis* was reported by Pandey *et al.* (2009) from north-east India which was confirmed through ITS rDNA phylogeny.

In all the phylogenetic trees constructed, the present study confirmed the identity of *P. assamicus* and *P. bipinnatifidus* which were grouped accordingly in the phylogenetic tree. *P. variabilis* which was only reported in China was found to be present in North-east in the state of Manipur. However, *Panax* sp. did not form a clade with any of the other species, it was identified as a new species which bears close similarity with *P. vietnamensis* and *P. quinquefolius*. The identity of the species studied result was further confirmed through the secondary structure of the conserved 5.8S rDNA region. *P. assamicus* , *P. bipinnatifidus* and *P. variabilis* have different structures which matches the structures of the sequences retrieved from GenBank. However, *Panax* sp. has a completely different structure from the rest and did not match with the closely allied species indicating it a new species. PCR-RFLP showed a distinct profile for *P. assamicus* but *P. variabilis* and *Panax* sp. shared the same profile. However, *P. bipinnatifidus* shared a profile between *P. assamicus, Panax* sp. *and P. variabilis.*

A comparison of sequence homologies of the different genera under Araliaceae, different species of *Panax* genus and members of same species collected from different geographical locations. A comparison was also made between the unidentified species (*Panax* sp.) with the other species of *Panax* (Table 6.12). Based on this, we developed a scale for relative positioning of ITS sequences (Fig. 6.36). The ITS sequence homology of members belonging to the *Panax* genus but different species ranged from 92-98%. Species from different geographical locations exhibited homology of 98-99%. The unidentified species showed 94-99% homology with the other species of *Panax*. However, it showed 99% homology with P. *quinquefolius* and *P. vietnamensis* which indicated that despite morphological dissimilarity that this species is genetically close to these two species.

Table 6.13: Similarity percentage for nucleotide sequences retrieved from the database covering different genera, different species within a genus, and members of the same species using the internal transcribed spacer region of rDNA.

Inter-species/intra-species level	Species combination		Genera/species combination	% similarity
Different genera under Araliaceae	*Panax* species	vs	*Aralia racemosa* (U41675), *Brassaia actinophylla* (AF242245), *Cephalaralia cephalobotrys* (AF242245), *Cuphocarpus aculeatus* (AF229737), *Cussonia spicata* (AF229765), *Dendropanax hainanensis* (AF242236), *Eleutherococcus nodiflorus* (U63184), *Pentapanax plumosus* (AF242255), *Polyscias guilfoylei* (AF242246), *Pseudopanax linearis* (U63178), *Osmoxylon novo-guineense* (AF229726), *Hedera helix* (U63186), *Heteropanax fragrans* (AF242242), *Kalopanax pictus* (U63187), *Macropanax dispermus* (AF229767), *Meryta sinclairii* (U63194), *Metapanax davidii* (AF242233), *Munroidendron racemosum* (AF229738), *Schefflera arboricola* (AF242243), *Sciadodendron excelsum* (AF242231), *Tetrapanax papyriferus* (U63192), *Trevesia palmata* (AF242247)	88 - 96
Different species of genus *Panax*	*P. assamicus* (FJ872556)	vs	*P. bipinnatifidus* (AY291911), *P. ginseng* (U41682), *P. quinquefolius* (U41687), *P. variabilis* (AY233331), *P. vietnamensis* (AY271924), *P. wangianus* (U41690), *P. zingiberensis* (U41700)	98
			P. japonicus (U41701), *P. notoginseng* (U41685)	96
			P. stipuleanatus (U41696	95
			P. pseudoginseng (AY233327), *P. trifolius* (U41698)	93
	P. bipinnatifidus (AY291911)		*P. ginseng* (U41682), *P. quinquefolius* (U41687), *P. variabilis* (AY233331), *P. vietnamensis* (AY271924), *P. zingiberensis* (U41700)	99
			P. wangianus (U41690)	98
			P. japonicus (U41701), *P. notoginseng* (U41685)	97
			P. stipuleanatus (U41696)	95
			P. pseudoginseng (AY233327), *P. trifolius* (U41698)	94
	P. ginseng (U41682)		*P. quinquefolius* (U41687), *P. vietnamensis* (AY271924), *P. zingiberensis* (U41700)	99
			P. japonicus (U41701), *P. variabilis* (AY233331), *P. wangianus* (U41690)	98
			P. notoginseng (U41685)	97
			P. stipuleanatus (U41696)	96
			P. pseudoginseng (AY233327), *P. trifolius* (U41698)	94
	P. japonicus (U41701)		*P. quinquefolius* (U41687	98
			P. variabilis (AY233331), *P. zingiberensis* (U41700)	97
			P. vietnamensis (AY271924), *P. wangianus* (U41690)	96
			P. notoginseng (U41685), *P. stipuleanatus* (U41696)	95

		P. pseudoginseng (AY233327)	93
		P. trifolius (U41698)	92
	P. notoginseng (U41685)	*P. quinquefolius* (U41687), *P. variabilis* (AY233331), *P. vietnamensis* (AY271924), *P. wangianus* (U41690), *P. zingiberensis* (U41700)	97
		P. stipuleanatus (U41696)	95
		P. pseudoginseng (AY233327), *P. trifolius* (U41698)	93
	P. pseudoginseng (AY233327)	*P. stipuleanatus* (U41696)	96
		P. quinquefolius (U41687), *P. variabilis* (AY233331), *P. vietnamensis* (AY271924), *P. zingiberensis* (U41700)	94
		P. trifolius (U41698), *P. wangianus* (U41690)	93
	P. quinquefolius (U41687	*P. variabilis* (AY233331), *P. vietnamensis* (AY271924), *P. zingiberensis* (U41700)	99
		P. wangianus (U41690)	98
		P. stipuleanatus (U41696)	96
		P. trifolius (U41698)	94
	P. stipuleanatus (U41696)	*P. vietnamensis* (AY271924)	96
		P. variabilis (AY233331), *P. wangianus* (U41690), *P. zingiberensis* (U41700)	95
		P. trifolius (U41698)	94
	P. trifolius (U41698)	P. variabilis (AY233331), P. vietnamensis (AY271924), P. zingiberensis (U41700)	94
		P. wangianus (U41690)	93
	P. variabilis (AY233331)	P. zingiberensis (U41700)	99
		P. vietnamensis (AY271924), P. wangianus (U41690)	98
	P. vietnamensis (AY271924)	P. zingiberensis (U41700)	99
		P. wangianus (U41690)	98
	P. wangianus (U41690)	P. zingiberensis (U41700)	99
Different geographic locations	*P. assamicus* (FJ872556)	*P. assamicus* (HQ141404)	99
		P. assamicus (AY233320)	99
	P. assamicus (HQ141404)	*P. assamicus* (AY233320)	99
	P. bipinnatifidus (AY725134)	*P. bipinnatifidus* (AY271911)	99
		P. bipinnatifidus (AY271914)	99
		P. bipinnatifidus (HQ588765)	99
		P. bipinnatifidus (FJ853613)	99

	P. bipinnatifidus (AY271911)	*P. bipinnatifidus* (AY271914)	98
		P. bipinnatifidus (HQ588765)	98
		P. bipinnatifidus (FJ853613)	98
	P. bipinnatifidus (AY271914)	*P. bipinnatifidus* (HQ588765)	99
		P. bipinnatifidus (FJ853613)	99
	P. bipinnatifidus (HQ588765)	*P. bipinnatifidus* (FJ853613)	99
	P. variabilis (AY233331)	*P. variabilis* (FJ872554)	99
	Panax sp.(Arunachal Pradesh)	*Panax sp.*(Nagaland)	99
Unidentified species			
	Panax sp.	*P. quinquefolius* (U41687), *P. vietnamensis* (AY271924)	99
		P. assamicus (FJ872556), *P. assamicus* (HQ141404), *P. assamicus* (AY233320)	97-98
		P. bipinnatifidus (AY271911), *P. bipinnatifidus* (AY271914), *P. bipinnatifidus* (HQ588765), *P. bipinnatifidus* (FJ853613), *P. bipinnatifidus* (AY725134)	96-98
		P. ginseng (U41682), *P. variabilis* (FJ872554)	98
		P. wangianus (U41690), *P. zingiberensis* (U41700)	
		P. japonicus (U41701), *P. notoginseng* (U41685)	97
		P. stipuleanatus (U41696)	95
		P. pseudoginseng (AY233327), *P. trifolius* (U41698)	94

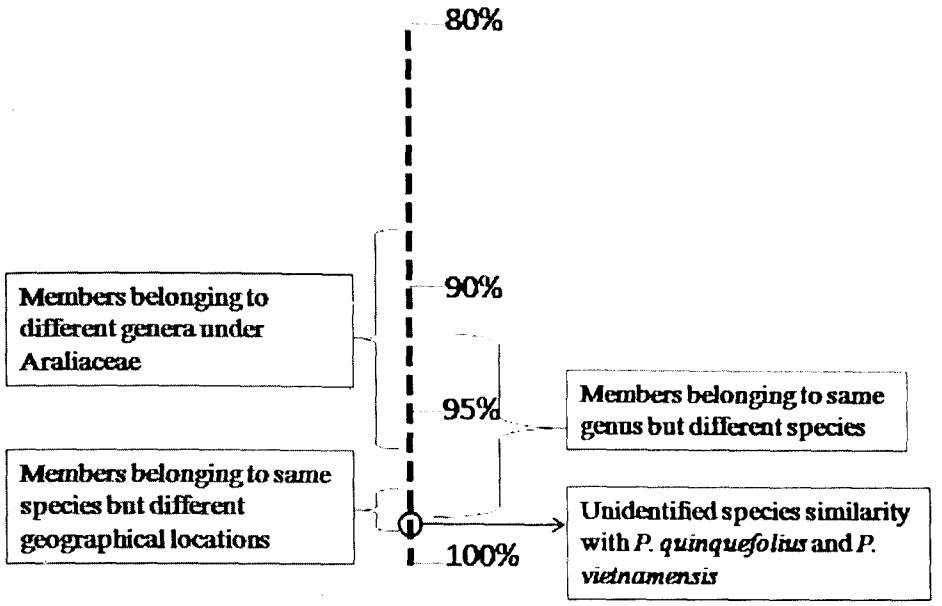


Fig. 6.36: Scale developed for comparison of ITS sequence homologies among different genera, different species, among members of same species and the unidentified specie for the data mined from GenBank. Relative positioning of the unidentified species with respect to sequence homologies for *P. quinquefolius* and *P. vietnamensis* have been shown.

In the present study, the morphometric analyses could separate clearly *P. assamicus*, *P. bipinnatifidus* and *P. variabilis*. However, there was overlapping between *P. pseudoginseng* and *Panax* sp. In contrast, a clear cut distinction among all the species was possible through molecular phylogeny i.e. 18S-28S rDNA region.

CHAPTER 7

ASSESSING THE IMPACT OF DISTURBANCE ON PANAX ASSAMICUS METAPOPULATION

7.1. INTRODUCTION

The populations of *Panax* species have been exposed to various anthropogenic disturbances including extraction of rhizomes for medicinal use/trade purpose by the local people. The impact of such disturbances on *Panax* metapopulation has not been understood. The metapopulation level understanding is essential for developing an effective conservation strategy for the species.

Population ecology has so far been addressing issues mostly related to demography and temporal dynamics of species populations at local scale. Considering the rapid and large scale changes in land-use, environmental stochasticity and external influences on natural populations during the past decade, the study of populations at a higher spatial scale has become inevitable today. For developing an effective species management strategy, particularly for those which are threatened and endemic having only a few remnant populations, it is necessary to understand the behaviour of their populations and population dynamics at landscape scale. Studies on population dynamics vis-à-vis habitat fragmentation have highlighted the necessity of the assessment of impacts at metapopulation level (Bruna and Oli, 2005).

Species occurring in specific or highly fragmented areas and those having reduced number of populations or individuals or are declining face the risk of extinction (World Conservation Union, 1994). At present, habitat loss due to anthropogenic activity is considered to be the single most important cause of loss of plant diversity (Wilcove *et al.*,

1998). Besides, human-caused changes in the environment and natural stochasticity in populations also pose additional threat to species persistence (Nakaoka, 1996; Menges, 1997; Holsinger, 2000; Vucetich *et al.*, 2000).

Patterns of birth and death that primarily determine population size vary widely among and within species (Roff, 1992). The persistence of a population depends on a numerical equilibrium over time. The simulation models based on the schedules of fecundity, recruitment and survival, and the integration of such parameters into the models allow exploration of their relative importance (Schemske *et al.*, 1994; Horvitz and Schemske, 1995; Caswell, 2001). Thus these simulation models help us in interpreting the mechanisms that govern population dynamics and in estimating extinction probabilities, particularly for threatened species (Schemske *et al.*, 1994; Menges, 2000).

In some cases, management recommendations for threatened plants are broadly created based on deterministic models (Manders, 1987), these models being static, do not take into account the random and unpredictable changes in environmental conditions, or any other factors resulting in stochastic extinction. On the other hand, stochastic computer simulation models, based on the temporal variation in observed demographic parameters, are regarded valuable for PVA investigation (Shaffer and Samson, 1985; Menges, 1992). Variations in these parameters may be obtained from long-term demographic monitoring data and are influenced by several ecological and genetic factors. Influential factors include environmental variability, intraspecific density, interspecific competition, herbivory, mutualism, pathogens, pollen limitation, dispersal, heterozygosity and allelic diversity (Schemske *et al.* 1994). The distribution and dynamics of organisms at a large spatial scale have been the focus of ecological research since early part of the twentieth century (Wright, 1931; Fisher, 1937; Skellam, 1951, 1952; MacArthur and Wilson, 1967; Levins, 1969, 1970). The theories of island biogeography and metapopulation dynamics have been

particularly powerful in this respect as they have offered a quantitative basis to analyse regional scale ecological dynamics (Hanski and Simberloff, 1997).

The archetype metapopulation theory based on source-sink relationship (Levins, 1969, 1970) has subsequently been broadened to encompass a wider class of population structure, including non equilibrium and mainland-island forms (Hastings and Harrison, 1994; Harrison and Taylor, 1997; Hanski, 1999). The inter-population migration processes, local population extinction and regional distribution of suitable habitats as discrete patches within a larger matrix of unsuitable habitat are the key elements in the metapopulation concept. Metapopulation dynamics have been defined by some authors as the product of local population dynamics and dispersal (Husband and Barrett, 1996). Antonovics *et al.* (1994) described it as a system of interconnected populations. Globally, very few studies are available on plant metapopulations (Husband and Barrett, 1996). Population dynamic sstudies on any plant species in India at a spatially large scale have not been attempted so far (Hanski, 1999). Metapopulation theory states that the scaling from local to regional dynamics may not be straightforward, and that the regional scale availability of habitat, migration and extinction play a role in determining whether a system of local populations of a species can persist.

Populations that exhibit a metapopulation structure are difficult to identify. For instance, the persistence and dynamics of metapopulations are critically dependent on the amount and regional configuration of suitable habitat (Hanski, 1997). In contrast, the dynamics of a population existing on an undisturbed continuous area of suitable habitat would be an extrapolation of local processes. Thus, determining the form of regional dynamics is not simply a matter of typology, and this links directly to demographic parameters and ecologically important aspects of population organization (Thomas and Kunin, 1999).

Species are threatened with extinction on a global scale from habitat loss, over-exploitation, introduced species, pollution, demographic, genetic and environmental fluctuations, and natural catastrophes (World Conservation Monitoring Centre, 1992). As a result of this, metapopulation models have further broadened to address the conservation needs of plants and animal species and hence Population Viability Analysis (PVA) was developed to assess extinction risk and to compare management options. PVA is a method for predicting the future fate of plant and animal populations based on demographic and environmental and genetic parameters (Shaffer, 1981; Gilpin and Soule, 1986; Boyce, 1992; Ferson and Akcakaya, 1993; Norton, 1995).

PVA process, based on computer simulation models, provides an important model for interdisciplinary discussion and synthesis, and is a widely applied management tool in conservation biology. For ranking management strategies, PVA is used according to their relative impacts on the persistence of wildlife populations (Clark *et al.*, 1991; Lindenmayer *et al.*, 1993) and through these management options, populations are built up to adequate sizes and the risk of extinction is reduced to a certain level (Given, 1994).

The impact of several management regimes on plant populations has been evaluated through PVA studies (Haig *et al.*, 1993; Lindenmayer and Possingham, 1996; Drechsler, 1998). Such applications have not been applied for threatened plant populations in India.

Several computer programs are used for evaluating PVA. The World Conservation Breeding Specialist group of the World Conservation Union (IUCN) has conducted more than 80 PVAs using VORTEX (Lacy, 1993). Other generic package such as ALEX (Possingham and Davies, 1995), SPOMSIM (Moilanen, 2004). GAPPS and INMAT are also used. RAMAS Metapop is one such package that has been used to conduct PVA for threatened taxa (Beissinger and Mccullough, 2002). It is used by ecologists, resource

managers, and population biologists worldwide who need to predict population structure and size through time and assess population and species extinction risks. The greatest strength of RAMAS Metapop is that it can be used for stage structured populations besides its ability to model age structure. This allows the use of RAMAS Metapop for PVA of plants species, as most plant PVAs are stage or size-classified (Lefkovitch matrix models). In addition, RAMAS Metapop has both option to assess the PVA under different management regime.

In this chapter the metapopulation dynamics of *Panax assamicus* was studied. The model was developed based on RAMAS Metapop version 5.0. Additionally, management interventions were simulated to explore the most effective strategy for conservation of the species. The study had the following specific objectives:

1. To study and compare survivorship and deterministic growth rate (λ) among different populations of *Panax assamicus* in Meghalaya.
2. To identify the demographic processes which contribute most to λ, thereby important in maintaining a viable population.
3. To simulate the future metapopulation trend and to assess extinction probability
4. To determine the minimum viable population (MVP) size.

7.2. METHODS

The projection matrix model was used for metapopulation analysis of *Panax assamicus* in Meghalaya. All populations were mapped and patch sizes were estimated by mapping the outermost plants of a population and the area of the convex polygon defined by these locations was determined using Geographical Information System (GIS) software ArcMap 9.3. The demographic study was conducted over a period of three years, i.e., from 2009 to 2011. Seven life history stage classes were selected viz., seedlings, 1-leaved, 2-leaved, 3-

leaved, 4-leaved, 5-leaved and 6-leaved adults. Patch isolation was measured as the mean distance of a patch to two nearest populations. The number of plants harvested/uprooted from the permanent plots were recorded and the percentage of harvested was then worked out.

Field demographic data was used to estimate the vital rates for each stage. Vital rates considered in this study were survival, and fecundity rates. Stage abundance (column vector of the matrix, n_t) was defined as the total population size in each population at each life cycle stage. This was enumerated by determining density per unit area in each population. Plant density was surveyed using 25 stratified random permanent sample plots of 10 m x 10 m each. Each of this plot was further sub-sampled through 4 random 5 m x 5 m quadrats. Thus, in total 100 quadrats of 5 m x 5 m were surveyed in each population patch over a period of 3 years. Sampled quadrats were then pooled for estimation of density/ha. Population size was then estimated by multiplying the area of each patch with plant density.

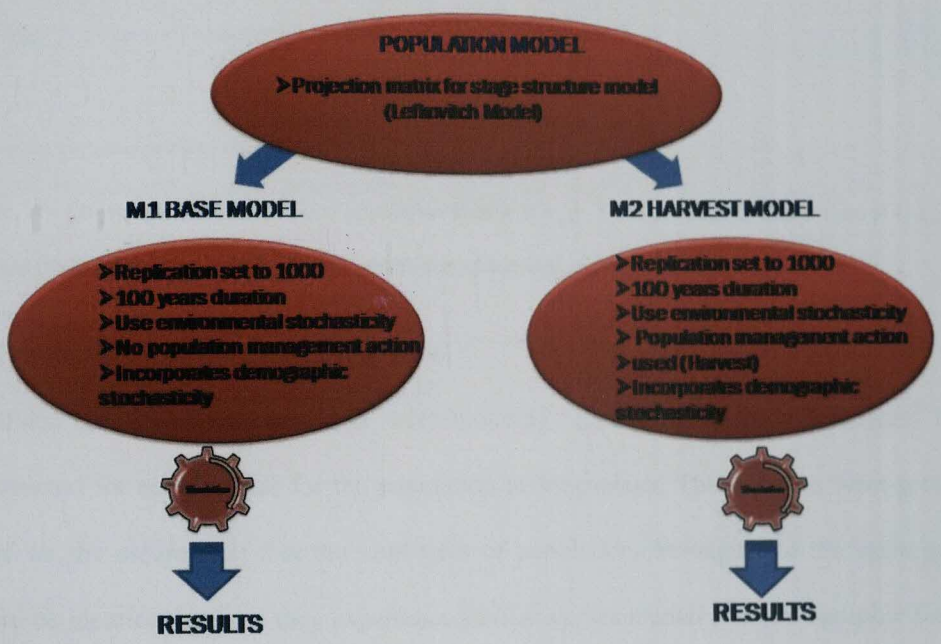


Fig 7.1: Flowchart depicting the steps used in RAMAS metapop V 5.0.

The projection matrix model takes the form: $A.n_t = n_{t+1}$ which is represented as:

$$\begin{bmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{bmatrix} \times \begin{bmatrix} n_1 \\ n_2 \\ n_3 \end{bmatrix} = n_{t+1}$$

Matrix A Column vector n_t

Where, A= Projection Matrix; n_t= Total population at time t; n_{t+1}= Total population size at time t+1. a_{11}, a_{21}, a_{31}...are the stage elements representing probabilities of survival and transition.

7.2.1. Estimation of the Matrix Elements

Separate transition matrices and corresponding standard deviation matrices were constructed for each habitat for the population in Meghalaya. The matrices were grouped based on the assumption that the vital rates of populations belonging to the same group would be identical because they experience similar environmental and demographic factors that determine their dynamics. The populations within each group were sufficiently close to justify this assumption. Several studies have taken such approach of grouping based on habitat for demographic analysis such as PVA (Akcakaya, 2005). The field demographic data obtained from the study of population dynamics were used for constructing stage-based transition matrices. Each element of the matrix representing survival rates and fecundity of plants were summarized from field data following Caswell (1989). Survival rate is the proportion of individuals that survived from a previous stage to the next. Therefore survival rate for stage 2 (S_2) is calculated as:

$$S_2 = N_3/N_2$$

Where N_2 and N_3 are the number of individuals at stage 2 and 3 respectively.

Fecundity (F) was calculated using the average number of seedling per flowering or reproductive plant in each population. All reproductive plants were assumed to produce the same average number of seedlings (Akcakaya, 1991). Fecundity is the only entry in the matrix that does not represent a probability, rather, it represents the average number of seedlings a single flowering plant produce in a given year:

$$\text{Fecundity (F)} = S_{(t+1)}/A_t$$

Where, A_t is the number of adults at time t and S_{t+1} is the total number of seedlings recruited at time t+1.

Although estimation of seed number would have been ideal for fecundity data, the fate of seeds could not be followed in the plots because of logistic constraints that need weekly monitoring given the species biology. Therefore, the stage specific fecundities (fx) by a less direct method as described above *i.e.*, considering seedling population data was followed (Damman and Cain, 1998). Stage-based transition matrices (Caswell, 2001) were built from field data collected over 3 years period *i.e.*, 2009, 2010 and 2011.

7.2.2. Deterministic Population Projection

Projection is carried out by a series of multiplication of the matrix (A) as: $n_{t+2} = A.n_{t+1}$; $n_{t+3} = A.n_{t+2} \ldots n_{t+1}$ until a stable configuration is reached. The finite rate of increase (λ) was calculated as:

$$\text{Finite rate of increase } (\lambda) = n_{t+1}/n_t$$

Sensitivity determines how much various life-history stage transitions affect the population dynamics by examining how changes in a particular stage affect the magnitude of the leading eigen value.

$$\text{Sensitivity } (S_{ij}) = w_i/W$$

Where, wi is the population vector at the i^{th} generation and W is the sum of all population vector.

One problem with this approach is that some of the variables, *i.e.*, survival rates, are intrinsically restricted in their range to values between 0.0 and 1.0, while others, *i.e.*, fecundities, may be very large.

Elasticity is a measure of proportional effect, *i.e.*, the effect that a change in a given matrix element has as a proportion to the change in that element:

$$\text{Elasticity } (Eij) = (aij/\lambda)\ Sij$$

Where, Eij is the elasticity value and represents the proportion of λ due to transition a_{ij} (de Kroon *et al.*, 1986).

7.2.3. Matrix Model Formulation

Demographic data was used to construct a stage structured projection matrix model (base-model) (Caswell, 2001; Lefkovitch, 1978) for each group. We also made use of data from biotic interference to create an alternate model. Therefore, two different matrices designated as M1 (base-model) and M2 matrices (alternate model) were used. M1 contained the vital rate elements that was estimated from actual survivals in the demographic plots, while M2 matrix contained vital rate elements that was estimated by adding actual survivals including those plants that would have survived if there was not apparent disturbance in the sites. During estimation of M2 matrix, care was taken to include those individuals only whose mortality was evidently caused by unnatural processes such as disturbance. The method of using the two model matrices was followed to compare the population performance in two contrasting scenarios *i.e.*, with disturbance in natural conditions (M1) and without disturbance (M2). Disturbances from all sources and forms were included such as grazing, landslide, cultivation, uprooted plants, wild animal

disturbance etc. All demographic groups of the species had a separate M1 and M2 model. We modelled the stochastic population dynamics using Monte Carlo based software RAMAS Metapop version 5.0 (Akçakaya, 1998). Parameters that were included in the Model are vital rates, demographic and environmental stochasticity, and initial population structure/stage abundance. Constraints were imposed within RAMAS Metapop to ensure that all simulated survival rates remain within the bounds of 0.0 and 1.0 with minimal truncation of the distribution. Environmental stochasticity was modelled through introducing random fluctuations in stage specific fecundities and survivals. For this, the program assigns during each time step to each transition rate a random value drawn from a specified log-normal distribution whose mean and standard deviation are given by the empirical matrices. A log-normal distribution for stochastic simulations was chosen because several matrix elements had small mean values, but large standard deviations (Akçakaya and Root, 2003; Regan, 2004). Since no catastrophic event disturbed the populations during the 3 years of study period, changes in population size most likely reflected true environmental and demographic stochasticity. Demographic stochasticity was modelled using binomial distribution (Akçakaya, 1991, 2002). Demographic stochasticity is the variation in the average chances of survival that occurs because a population is made up of a finite integer of individuals. For demographic stochasticity can be represented as:

$$\text{Demographic stochasticity} = S_i.N_i(t)$$

The number of survivors for ith stage was drawn from a binomial distribution with two parameters *viz.*, Survival rate 'S' and Ni(t) (as sample size).

In the first set, all simulations were run with 1000 replications until time to extinction is achieved. In the second set, a threshold population size (N_e) was set for quantification of quasi-extinction (Ginzburg *et al.*, 1982). The quasi-extinction time was quantified because extinction of a species is usually achieved at much longer time in which all the individuals

die. Given the time constraint, the present study could not afford that long time and achieve 100% extinction. The species studied is listed as endangered due to heavy extraction from the wild. Besides, population falling below critical size may not be viable due to demographic and genetic effects. Therefore, it was pertinent to evaluate risk at an early time (Bretagnolle and Inchausti, 2005). A threshold size of Ne= 100 was set for *Panax assamicus* since the plant is a long-lived perennial and is likely to persist for a long duration with more than 100 individuals.

7.3. RESULTS

7.3.1. Demographic stages

Panax assamicus could be distributed into 8 stages i.e., seedling stages and seven other leaf-stages from 1-leaved to 7-leaved stage. 3 and 4-leaved stage are reproductive individuals (Fig 8.2). 7-leaved stage is difficult to locate and occurred in just one patch with a single individual.

Fig. 7.2: Leaf stages of *Panax assamicus*

7.3.2. Spatial Characteristics

Panax assamicus metapopulation had only 5 populations. The mean patch size was 89.6 ± 42.1 ha. Total plant density was 27 ±11.61/ha. The mean patch isolation was 12.28 ± 4.7 (Table 7.1).

Table 7.1: Spatial characteristics of populations of *Panax assamicus*.

Population	Patch size (ha)	Population density/ha	Population size	Population isolation (km)*	
				Individual	Mean
Tyllang	153	5	765	24.7	
Nongkrem	21	44	924	5.9	
Upper Shillong	13	63	819	4.5	12.28 (4.7)
Laitkor	224	3	672	3.6	
Laitkseh	37	20	740	22.7	

* (mean distance from three nearest population). Value in parentheses is the corresponding standard error of the mean.

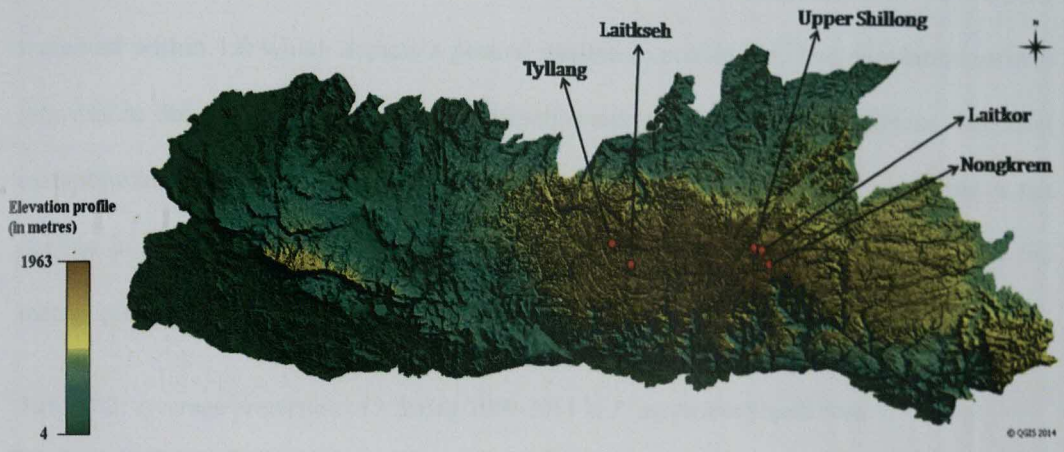


Fig. 7.3: Locations of the 5 populations of *Panax assamicus* in Meghalaya

7.3.3. Population viability analysis

7.3.3.1. Deterministic analysis

1. Finite Rate of Increase (λ)

Deterministic analysis yielded the finite rate of increase (λ) which is the determining factor in the future growth of the metapopulation. All the populations of *Panax assamicus* exhibited discrete population growth pattern and the growth rate declined (λ <1) over 3 years of study period i.e. 2009-2011. The growth rate (λ) in all the five populations remained within 1.0 which depicts a general decline in population. The population growth rate was in the order Upper Shillong>Laitkseh>Laitkor>Tyllang and Nongkrem. The total metapopulation level growth rate (λ) also remained negative (λ=0.898) indicating a net decline in the total population size of the species (Table 7.2). However, no patch in the metapopulation disappeared during the three years of study period.

Table 7.2: Average projection of λ during 2009-2011 in *P. assamicus* populations

Population	Growth rate (λ)
Tyllang	0.860
Nongkrem	0.852
Upper Shillong	0.970
Laitkor	0.872
Laitkseh	0.875
Metapopulation	**0.886**

2. Elasticity

Three demographic processes contribute to the finite rate of increase of *Panax assamicus* *i.e.*, fecundity (F) growth (G) and survival (L) during the three year study period. The analysis of stage matrices for different populations revealed that for Laitkseh population,

there was no vital rate element for growth (G) and fecundity (F), whereas in Nongkrem population fecundity (F) element was absent (Table 7.3). The contribution of all these demographic processes to overall growth of the population in the life cycle of *P. assamicus* was represented by the relative sensitivity of survival, growth and fecundity to λ. Survival growth contributes the maximum towards the growh rate. Contribution of growth to elasticity was highest in Upper Shillong which is near to 0.1 followed by Tyllang population. However, in Laitkor and Nongkrem populations, the elasticity values fall below 0.001. There was no contribution of growth in Laitkseh population. Fecundity had the least elasticity which shows it had relatively less contribution to λ. In Laitkseh and Nongkrem populations, fecundity did not have any contribution to *P. assamicus* and demonstrated the actual behaviour of a typical iteroparous forest herb where survival had the highest elasticity compared to growth and fecundity (Fig.7.4).

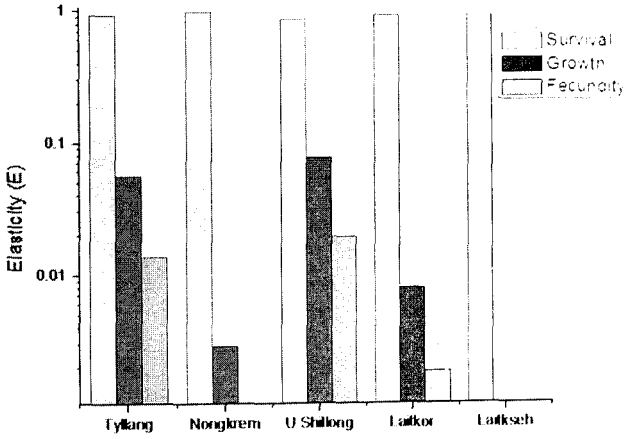


Fig. 7.4: Elasticity of *P. assamicus*

Table 7.3: Stage matrix of *P. assamicus* in Tyllang sub-population

Tyllang	Stage matrix						
Seedling	**Seedlings**	**1-leaved**	**2-leaved**	**3-leaved**	**4-leaved**	**5-leaved**	**6-leaved**
Seedling	0.535	0.0	0.0	0.0	0.006	0.015	0.35
1-leaved	0.084	0.832	0.0	0.0	0.0	0.0	0.0
2-leaved	0.0	0.07	0.814	0.0	0.0	0.0	0.0
3-leaved	0.0	0.0	0.058	0.946	0.0	0.0	0.0
4-leaved	0.0	0.0	0.0	0.041	0.794	0.0	0.0
5-leaved	0.0	0.0	0.0	0.0	0.0	0.565	0.0
6-leaved	0.0	0.0	0.0	0.0	0.0	0.017	0.5
	Standard deviation matrix						
Seedling	**Seedling**	**1-leaved**	**2-leaved**	**3-leaved**	**4-leaved**	**5-leaved**	**6-leaved**
Seedling	0.38	0.0	0.0	0.0	0.006	0.015	0.01
1-leaved	0.077	0.006	0.0	0.0	0.0	0.0	0.0
2-leaved	0.0	0.074	0.23	0.0	0.0	0.0	0.0
3-leaved	0.0	0.0	0.049	0.0	0.0	0.0	0.0
4-leaved	0.0	0.0	0.0	0.019	0.129	0.0	0.0
5-leaved	0.0	0.0	0.0	0.0	0.0	0.262	0.0
6-leaved	0.0	0.0	0.0	0.0	0.0	0.024	0.001

Table 7.4: Stage matrix of *P. assamicus* in Nongkrem sub-population

Nongkrem	Stage matrix						
Seedling	**Seedlings**	**1-leaved**	**2-leaved**	**3-leaved**	**4-leaved**	**5-leaved**	**6-leaved**
Seedling	0.798	0.0	0.0	0.00	0.002	0.018	0.35
1-leaved	0.07	0.591	0.0	0.0	0.0	0.0	0.0
2-leaved	0.0	0.025	0.538	0.0	0.0	0.0	0.0
3-leaved	0.0	0.0	0.052	0.656	0.0	0.0	0.0
4-leaved	0.0	0.0	0.0	0.114	0.839	0.0	0.0
5-leaved	0.0	0.0	0.0	0.0	0.011	0.762	0.0
6-leaved	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Standard deviation matrix						
Seedling	**Seedling**	**1-leaved**	**2-leaved**	**3-leaved**	**4-leaved**	**5-leaved**	**6-leaved**
Seedling	0.09	0.0	0.0	0.0	0.003	0.002	0
1-leaved	0.01	0.43	0.0	0.0	0.0	0.0	0.0
2-leaved	0	0.03	0.16	0.0	0.0	0.0	0.0
3-leaved	0.0	0.0	0.07	0.26	0.0	0.0	0.0
4-leaved	0.0	0.0	0.0	0.02	0.17	0.0	0.0
5-leaved	0.0	0.0	0.0	0.0	0.02	0.13	0.0
6-leaved	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 7.5: Stage matrix of *P. assamicus* in Upper Shillong sub-population.

Upper Shillong	Stage matrix						
Seedling	**Seedlings**	**1-leaved**	**2-leaved**	**3-leaved**	**4-leaved**	**5-leaved**	**6-leaved**
Seedling	0.839	0.0	0.0	0.0	0.006	0.015	0.35
1-leaved	0.0	0.782	0.0	0.0	0.0	0.0	0.0
2-leaved	0.0	0.03	0.97	0.0	0.0	0.0	0.0
3-leaved	0.0	0.0	0.017	0.948	0.0	0.0	0.0
4-leaved	0.0	0.0	0.0	0.052	0.85	0.0	0.0
5-leaved	0.0	0.0	0.0	0.0	0.025	0.83	0.0
6-leaved	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Standard deviation matrix						
Seedling	**Seedling**	**1-leaved**	**2-leaved**	**3-leaved**	**4-leaved**	**5-leaved**	**6-leaved**
Seedling	0.22	0.0	0.0	0.0	0.001	0.001	0.0
1-leaved	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2-leaved	0.0	0.03	0.03	0.0	0.0	0.0	0.0
3-leaved	0.0	0.0	0.02	0.01	0.0	0.0	0.0
4-leaved	0.0	0.0	0.0	0.07	0.07	0.0	0.0
5-leaved	0.0	0.0	0.0	0.0	0.03	0.02	0.0
6-leaved	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 7.6: Stage matrix for *Panax assamicus* in Laitkor sub-population.

Laitkor	Stage matrix						
Seedling	**Seedlings**	**1-leaved**	**2-leaved**	**3-leaved**	**4-leaved**	**5-leaved**	**6-leaved**
Seedling	0.738	0.0	0.0	0.0	0.003	0.009	0.35
1-leaved	0.01	0.73	0.0	0.0	0.0	0.0	0.0
2-leaved	0.0	0.032	0.768	0.0	0.0	0.0	0.0
3-leaved	0.0	0.0	0.059	0.911	0.0	0.0	0.0
4-leaved	0.0	0.0	0.0	0.036	0.873	0.0	0.0
5-leaved	0.0	0.0	0.0	0.0	0.025	0.67	0.0
6-leaved	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Standard deviation matrix						
Seedling	**Seedling**	**1-leaved**	**2-leaved**	**3-leaved**	**4-leaved**	**5-leaved**	**6-leaved**
Seedling	0.12	0.0	0.0	0.0	0.002	0.001	0.0
1-leaved	0.01	0.11	0.0	0.0	0.0	0.0	0.0
2-leaved	0.0	0.02	0.08	0.0	0.0	0.0	0.0
3-leaved	0.0	0.0	0.01	0.06	0.0	0.0	0.0
4-leaved	0.0	0.0	0.0	0.02	0.09	0.0	0.0
5-leaved	0.0	0.0	0.0	0.0	0.01	0.52	0.0
6-leaved	0.0	0.0	0.0	0.0	0.0	0.0	0.1

Table 7.7: Stage matrix of *P. assamicus* in Laitkseh sub-population.

Laitkseh	Stage matrix						
Seedling	**Seedlings**	**1-leaved**	**2-leaved**	**3-leaved**	**4-leaved**	**5-leaved**	**6-leaved**
Seedling	0.87	0.0	0.0	0.0	0.016	0.1402	0.35
1-leaved	0.006	0.766	0.0	0.0	0.0	0.0	0.0
2-leaved	0.0	0.009	0.84	0.0	0.0	0.0	0.0
3-leaved	0.0	0.0	0.03	0.824	0.0	0.0	0.0
4-leaved	0.0	0.0	0.0	0.034	0.688	0.0	0.0
5-leaved	0.0	0.0	0.0	0.0	0.0	0.875	0.0
6-leaved	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Standard deviation matrix						
Seedling	**Seedling**	**1-leaved**	**2-leaved**	**3-leaved**	**4-leaved**	**5-leaved**	**6-leaved**
Seedling	0.03	0.0	0.0	0.0	0.005	0.001	0.0
1-leaved	0.01	0.22	0.0	0.0	0.0	0.0	0.0
2-leaved	0.0	0.0	0.14	0.0	0.0	0.0	0.0
3-leaved	0.0	0.0	0.03	0.24	0.0	0.0	0.0
4-leaved	0.0	0.0	0.0	0.02	0.01	0.0	0.0
5-leaved	0.0	0.0	0.0	0.0	0.0	0.17	0.0
6-leaved	0.0	0.0	0.0	0.0	0.0	0.0	0.0

7.3.3.2. Stochastic Risk Analyses

Trajectory Summary

A summary of the abundance of the metapopulation (and each of its populations) as it changes through time (Fig. 7.5. a,b) The average, ±1 standard deviation, minimum and maximum abundances are the output of the model. The ±1 standard deviations are

displayed symmetrically around the mean, regardless of actual distribution. A continuous decline with time was observed in the habitat patches and also in the metapopulation size (Fig. 7.6).

A large decline was expected in all the populations of *P. assamicus* within 40 years except for Upper Shillong population where the decline was expected after 60 years in both the M1 and M2 scenarios. After a sharp decline the trajectories stabilized in both the scenarios which indicated an almost deterministic decline in the future. However, the summary on the meatpopulation size declines in both M1 and M2 scenario which leads to an extrememely low abundance. In M1 scenario, the metapopulation size after a sharp decline becomes stable after 65 years and in M2 scenario the population becomes stable after 40 years.

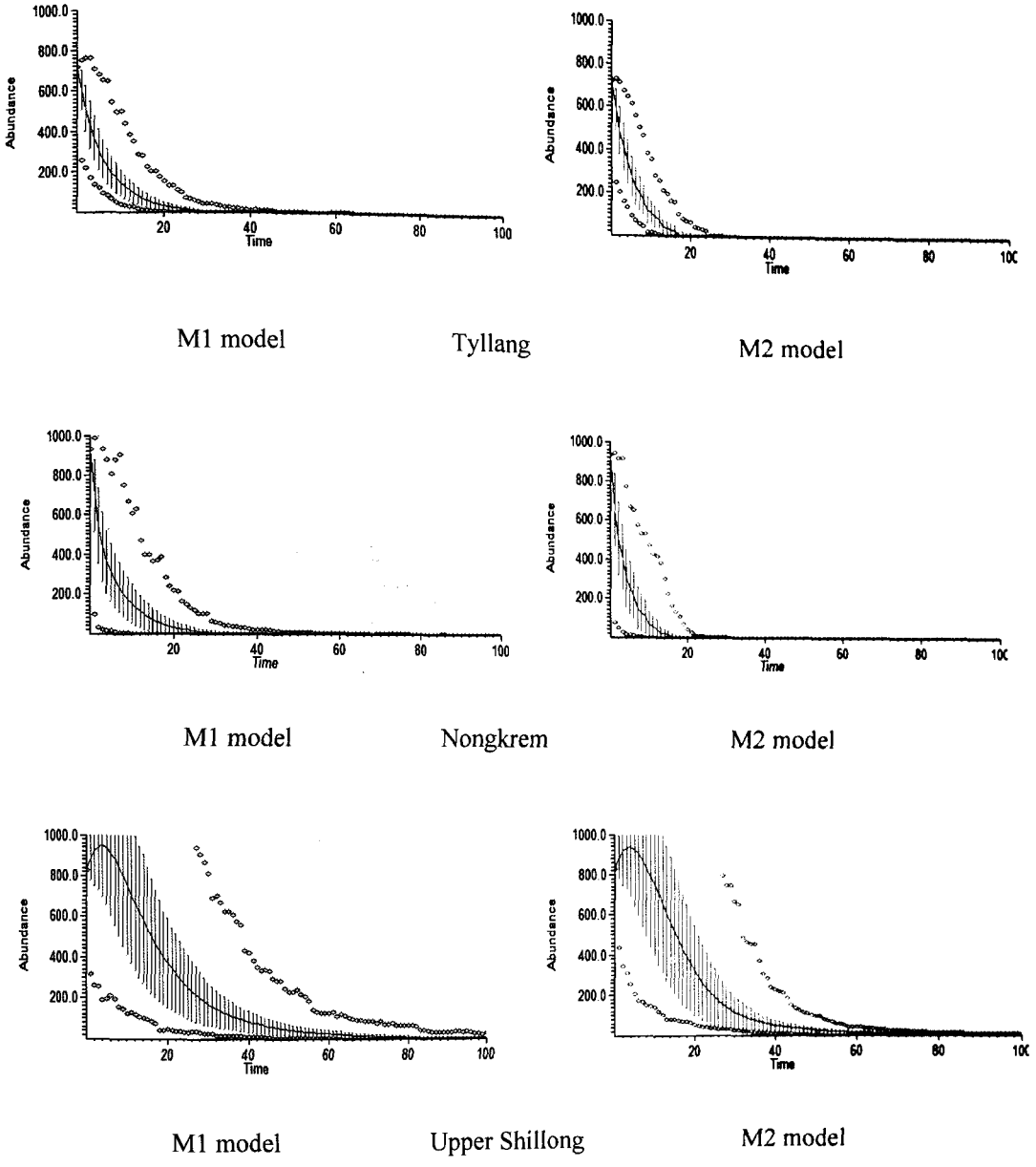


Fig. 7.5 (a): Trajectories summaries over 100 years of *Panax assamicus*. The mean and 95% confidence intervals after 1000 replications are presented. Minimum and maximum abundance are represented by red arrows. Some of the maximum confidence intervals and range are not shown to confine the scale of the curve for better representation.

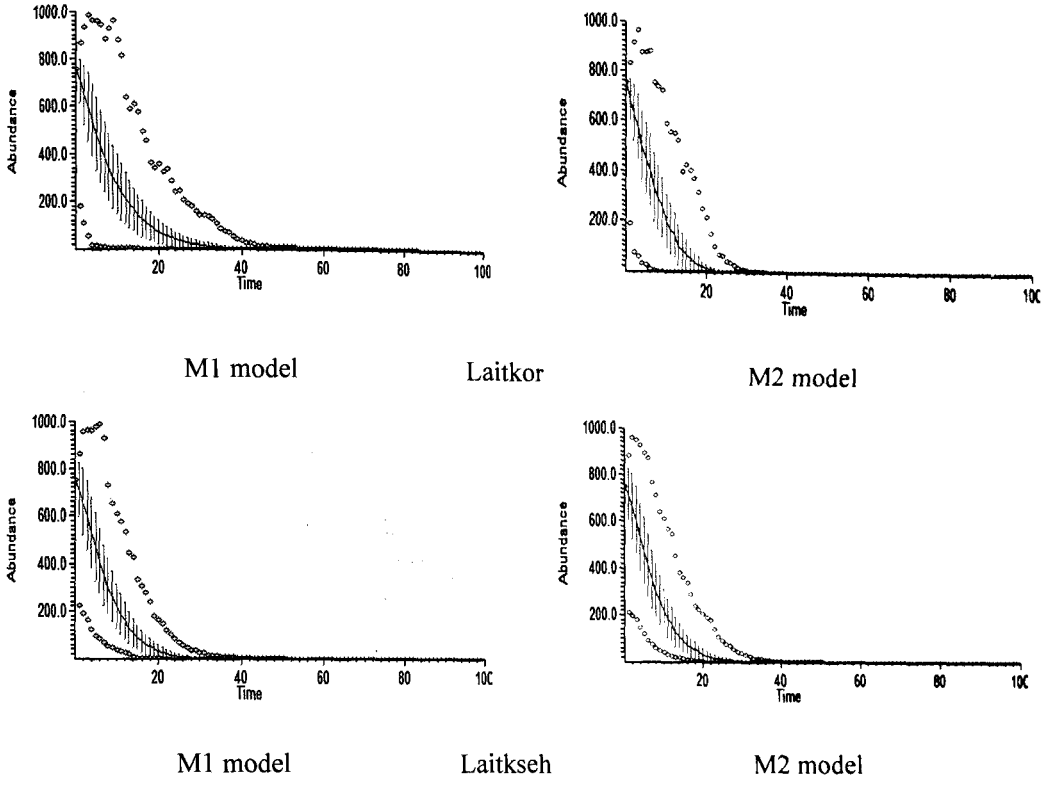
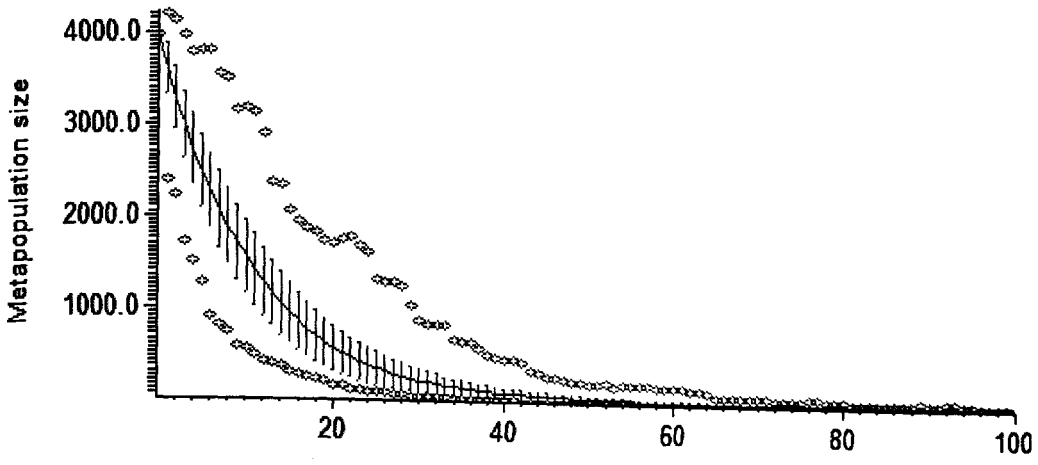
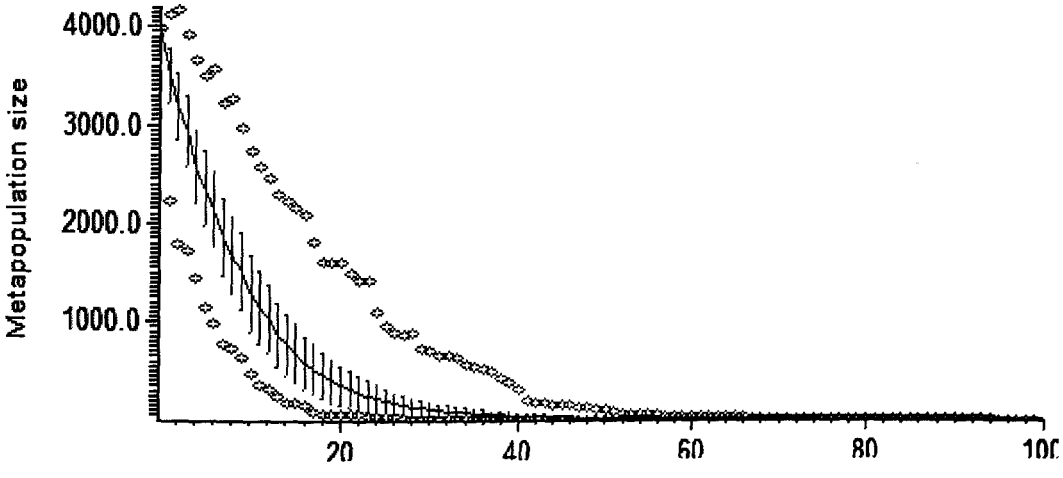


Fig. 7.5 (b): Trajectories summaries over 100 years of *Panax assamicus*. The mean and 95% confidence intervals after 1000 replications are presented. Minimum and maximum abundance are represented by red arrows. Some of the maximum confidence intervals and range are not shown to confine the scale of the curve for better representation.



M1 model

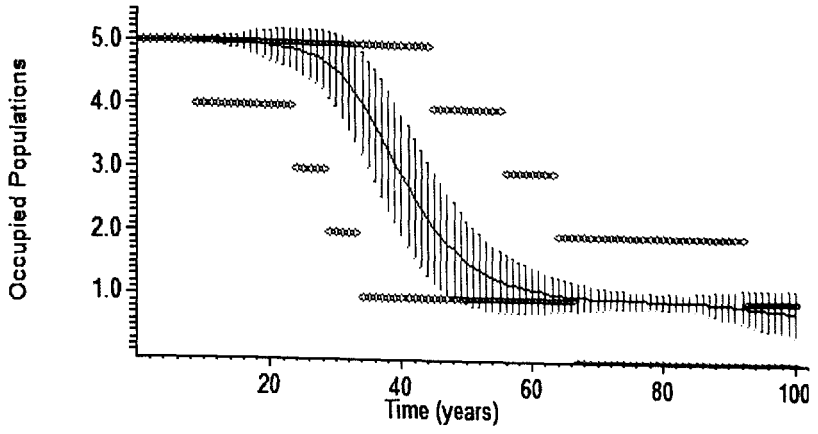


M2 model

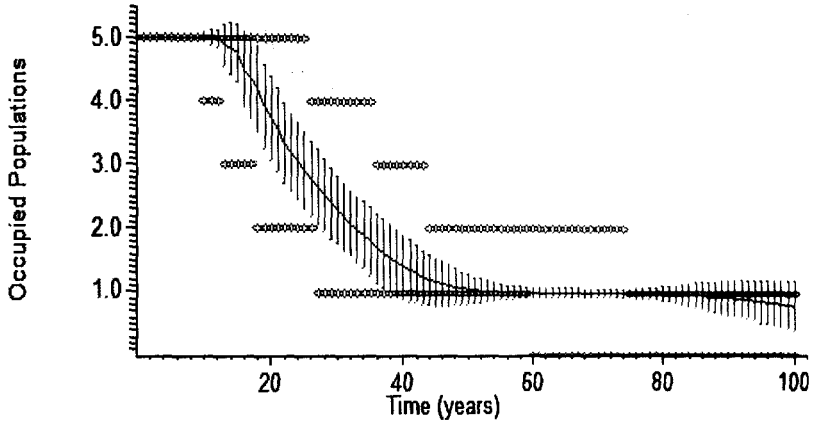
Fig. 7.6: Trajectory summary of *P. assamicus* in M1 and M2 scenario of all the populations.

Metapopulation viability

The change in the number of occupied populations of *P. assamicus* through time depicted a sharp decline in the number of patches and only 50% of which would remain extant within 24 years (Fig. 7.7). Most of the remaining sub-populations are from Upper Shillong under M1 scenario where maximum time steps during the simulations were occupied by the populations. This was evident from the local occupancy of populations (Fig. 7.8). The metapopulation occupancy in M2 scenario showed a similar trend and approximately 50% of the population would be extant by 16th year. The statistical summary of the occupancy rate revealed that the average time the sub-population would persist through time was predicted at 34 (Nongkrem), 38 (Tyllang), 42 (Laitkseh), 45 (Laitkor) and 98 (Upper Shillong) years under M1 scenario. The average time for persistence under M2 scenario was predicted at 18 (Tyllang), 20 (Nongkrem), 26 (Laitkor), 38 (Laitkseh) and 98 (Upper Shillong) years. The populations at Upper Shillong did not show any change in both M1 and M2 scenarios.

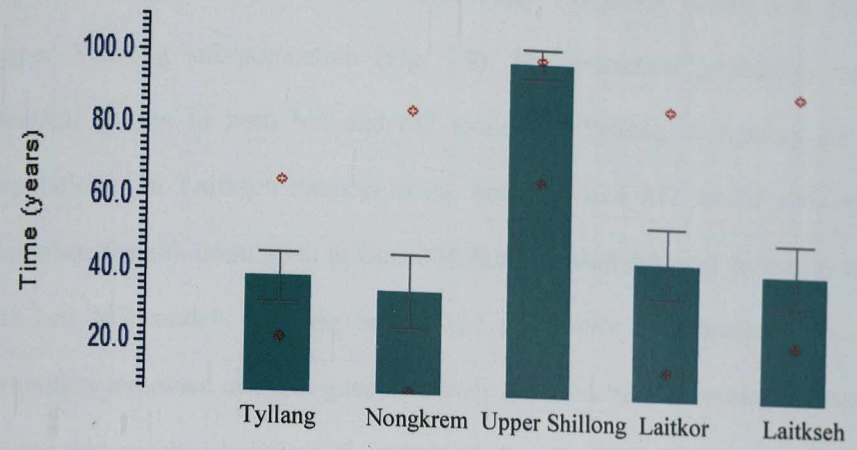


M1 model

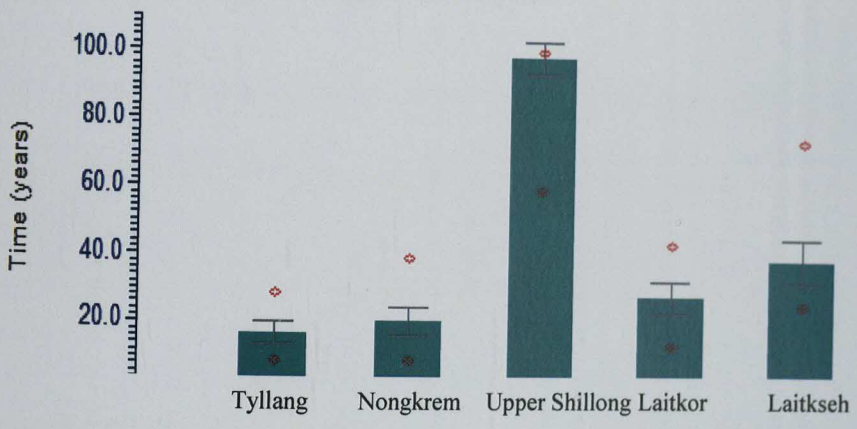


M2 model

Fig. 7.7: Metapopulation occupancy of *P. assamicus* (line represents mean value of the 1000 replications; bars show standard deviation, 95% confidence interval), (one time step= 1 year).



M1 model

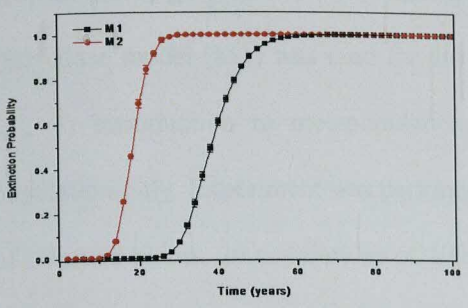


M2 model

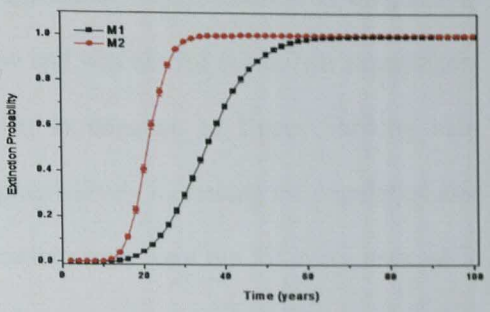
Fig. 7.8: Local occupancy of *P. assamicus* in the five sub-populations

Probability of extinction

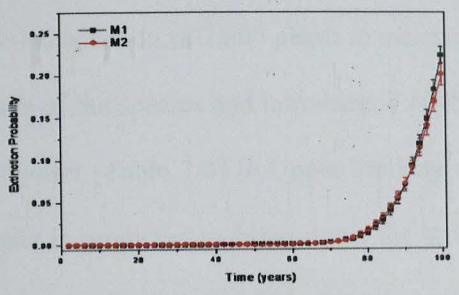
Time to extinction refers to the complete extinction of the populations while extinction risk is the probability of extinction in 100 years. The extinction curves for all the sub-populations approached extinction probability 1.0 much before 100 years except for Upper Shillong sub-population (Fig. 7.9). The extinction probability curves revealed identical shapes in both M1 and M2 models in Tyllang, Nongkrem and Laitkor sub-populations. In Laitkseh subpopulation, both M1 and M2 model showed overlapping. However, the sub-population in Upper Shillong showed different extinction curves for both M1 and M2 models reaching below 0.25 probability of extinction. The probability of extinction remained at 0 but goes up slowly after reaching 75 years, M1 model curve was higher and reaches below 0.23 probability in 100 years. The metapopulation extinction curve shows a similar trend with the Upper Shillong sub-population in which the M1 and M2 model showed overlapping but after 75 years deviates from each other. The M2 model showed 0.20 probability of extinction in 100 years.



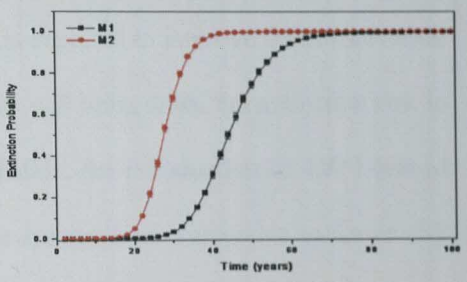
(a)



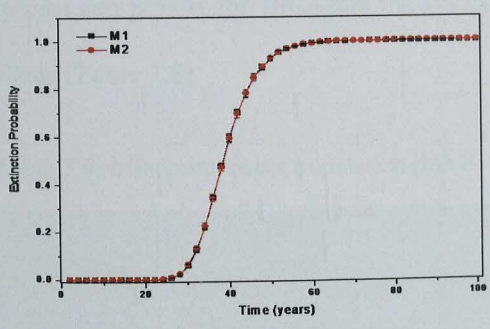
(b)



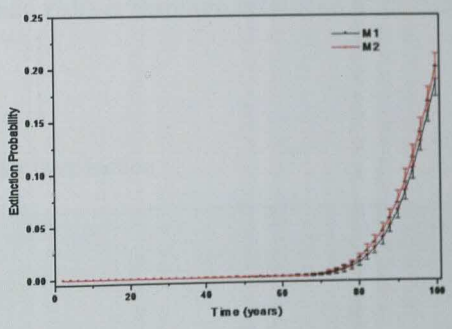
(c)



(d)



(e)



(f)

Fig. 7.9: Extinction probability in 100 years (a-Tyllang, b-Nongkrem, c-Upper Shillong, d- Laitkor, e-Laitkseh, f-metapopulation).

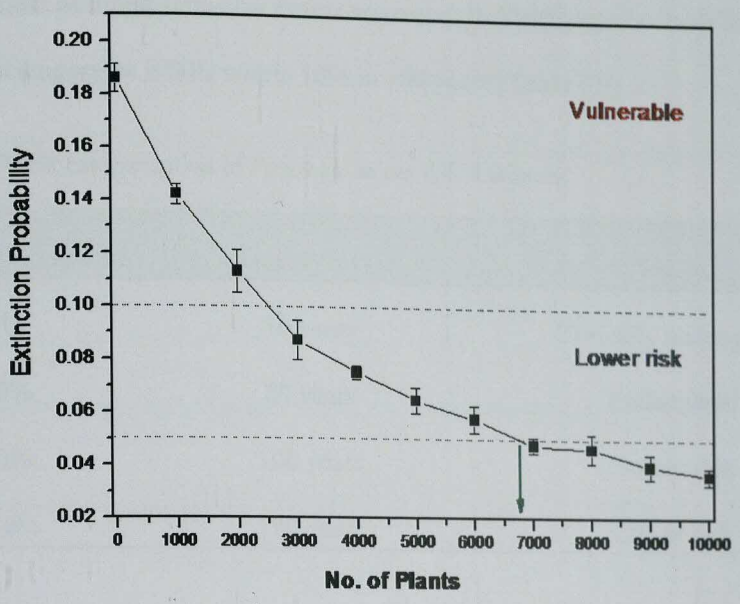
7.3.3.3. Management intervention (Introduction of plants to populations)

Simulation experiment was performed to analyze the effect of management by introduction of 1-leaved stage plants on the viability of *P. assamicus* metapopulation in Meghalaya. Only Base model (M1) was used for this purpose and was carried out in two experiments i.e., (i) Introduction to metapopulation, and (ii) introduction to Upper Shillong sub-population only. Experiment was performed by progressively increasing the population size of 1-leaved plants. 20 simulations of 1000 replications each were run for every increase in population size. The extinction risk projected under each set was noted and plotted against number of plants introduced.

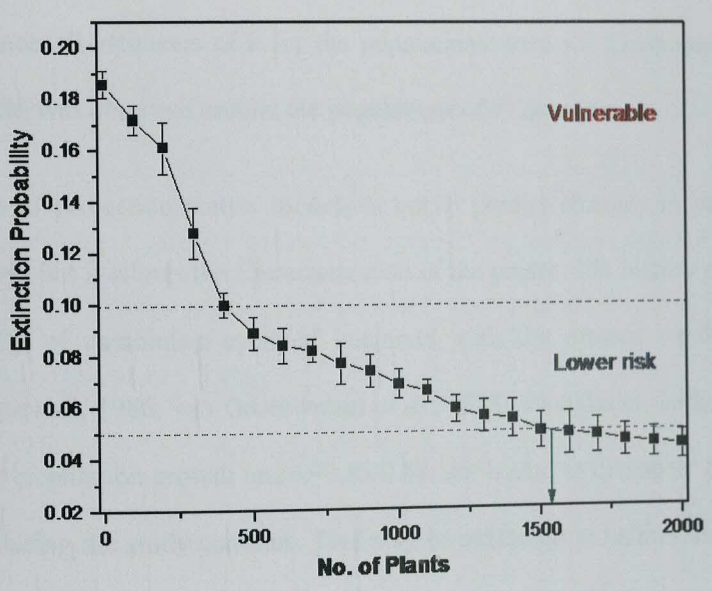
The introduction of 2,800 plants to metapopulation is expected to improve the conservation status of the species and introduction of 6,700 plants will bring down the extinction risk in 100 years (Table 7.8) In Upper Shillong sub-population, the introduction of 400 1-leaved plants is predicted to bring down the status of the species from vulnerable to lower risk while introduction of 1,600 plants is predicted to bring down the extinction risk to 5% in 100 years. The Minimum Viable Population size which is a minimum threshold size that a species can persist for 100 years (at 5% extinction risk) is therefore estimated to be 5,520 plants (Table 7.8).

Table 7.8: Minimum viable population (MVP) size for introduction

	Introduction to	
	All 5 populations	Only US population
No. of plants introduce that is effective to reach Minimum Viable Population (MVP) size	6700 (1340 each)	1600



(a)



(b)

Fig. 7.10: Management intervention (a) Introduction to metapopulation (b) Introduction to Upper Shillong sub-population.

7.3.3.4. Assigning threat status

The assessment of threat status for *Panax assamicus* in Meghalaya is classified under Vulnerable category as it falls within 10% in 100 years (Table 7.9).

Table 7.9: Threat categorization of *Panax sp.* as per IUCN criteria

Extinction risk	Time (years)	Status
5%	10 years	Critically endangered
20%	20 years	Endangered
10%	100 years	Vulnerable
<10%	100 years	Lower risk

7.4. DISCUSSION

Deterministic analysis yielded the finite rate of increase (λ) which is the determining factor in the future growth of the metapopulation. In the present study, the populations were declining, since all estimates of λ for the populations were <1. Difference in population growth rate (λ) was observed around the populations of *P. assamicus*.

The purpose of projection matrix models is not to predict changes in population in the future scenario but it allows the characterization of the present life history of a species, and the possibility of examining eventual outcomes with the present condition remaining constant (Casewell, 1986; van Groenendael *et al.*, 1988). Nongkrem, laitkor, Laitkseh and Tyllang with population growth rate λ=0.85-0.88, are bound to disappear if the conditions that persist during the study continue. This may be attributed to habitat disturbance which deteriorates habitat quality resulting from change in micro-climatic conditions. This is also in conformity with the order of percentage of harvested plants which is highest in Nongkrem (5.44%) followed by Tyllang (3.32%) and laitkor (3.0%). Laitkseh population is relatively undisturbed population and had second highest λ, while Upper Shillong

population had the highest λ, probably because of its protected location. The λ value obtained in the present study was lower when compared to *P. quinquefolium* (Charron and Gagnon, 1991; Nantel *et al.*, 1996) that varied from 0.88-1.06. The low growth rate in *P. assamicus* may be due to its population density, small size of the population, greater demographic and environmental stochasticity. Low seedling recruitment and establishment rates, the presence of a relatively long pre-reproductive period, slow individual growth rate, greater longevity of established individuals, relatively stable population growth rate close to 1.0 indicate that *P. quinquefolium* is a species of stable habitat.

Elasticity values of rare plants indicate which life history stages are most critical to population growth (Schemske *et al.*, 1994). This has implications in conservation intervention. Therefore, while conserving the whole population, special protection to these sub-sets of population will make the conservation effort a success. In, elasticity analysis, survival contributed the most to the population growth and fecundity the least. The relatively less importance of fecundity in *P. assamicus* may be explained based on the argument of Silvertown *et al.* (1992), who stated that there is often a trade-off between the different processes. Trade-offs between growth and seed reproduction, and survival and fecundity have been frequently observed in plant demographic studies (Silvertown, 1987). However, the relationship between survival and growth and their trade-offs against each other is not very common in nature.

The use of M1 and M2 models in the PVA brings to light the impact of harvesting on the overall metapopulation health of *P. assamicus* which has an impact on the population growth rate (λ). Nantel *et al.* (1996) reported that λ declined with increasing harvest of the plants. Inclusion of alternate matrix model is important since change in λ has significant implication to the persistency of species (Morris and Doak, 2005). At the metapopulation level the impact is less with a marginal difference of extinction risk i.e. approx. 2%. The

impact is however more prominent at the population level as evident from the disparity of M1 and M2 extinction curves in three populations i.e. Nongkrem, Laitkor and Tyllang population, which is most affected. Apparent from M1 and M2 models, the persistency of Upper Shillong population is least affected by harvesting and the similarity of its extinction curves with that of metapopulation curves implies that it plays an important role in the persistency of the metapopulation as a whole. Such a trend would lead the metapopulation to extremely low abundance. This trend for rare species has also been reported by earlier workers viz., Schtickzelle *et al.*, 2005 in their PVA models. In most cases, especially in models of exponentially growing populations, the simulated population sizes at a given time step will have a skewed distribution. The time to extinction curves had a skewed distribution in the species which is in conformity with the conclusion of Levinton and Ginzburg (1984), who concluded that skewed distribution and high variance are typical of time to extinction curves for threatened and rare plants.

Results from simulation experiment suggested that the most effective, convenient and cost effective strategy is the introduction of plants to Upper Shillong population only instead of individuals to the entire metapopulation. The MVP size for *P. assamicus* suggests that appropriate actions need to be planned before it goes extinct. Though extinction and recolonization is a natural process, these species however need immediate attention since whole metapopulation is at stake. Although from MVP estimation it is evident that the populations have not yet reached the threshold minimum population size (or effective population size), it is necessary to ensure its persistence by initiating effective conservation efforts. Therefore, the application of population viability analysis in this study has provided an insight into the population study that helped in describing the potential management actions.

CHAPTER 8

INTERPOPULATION VARIATION IN GINSENOSIDE CONTENTS IN PANAX SPECIES COMPLEX

8.1. INTRODUCTION

Medicinal plants are the basis of traditional herbal medicine and in Western medicine they serve as the source of many kinds of drugs (Sucher and Carles, 2008). They can be important source of previously unknown chemical substances with potential therapeutic effects. The world health organization has estimated that over 75% of the world's population still relies on plant derived medicines, usually obtained from traditional healers, for its basic health care needs (Herrera *et al.*, 2008). Herbal medicines are in great demand in both developed and developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs.

The active principles or constituents (phytochemicals) in medicinal plants are chemical compounds known as secondary plant products. Secondary metabolites are low-molecular weight compounds occurring within the plant kingdom. More than 100,000 structures have already been described (Buckingham, 1994). The estimates of the total number in plants alone exceed 500,000 (Mendelsohn and Balick, 1995) and many more are yet to be discovered. The major pharmacologically active components in ginseng are a class of triterpene saponins, commonly known as ginsenosides (Yahara *et al.*, 1979; Kasai *et al.*, 1983; Ma *et al.*, 1999). Ginsenosides were first isolated in 1963 (Shibata *et al.*, 1963, 1965) and are composed of a dammarane skeleton (17 carbons in a four-ring structure) with various sugar moieties attached to the C-3 and C-20 positions (Matsuura *et al.*, 1984; De Smet, 2002). Ginsenosides have been classified into two main groups: the glycosides of 20(S)-protopanaxadiol which consists of Rb1, Rb2, Rc, Rd Rg3 and Rh2, and those of 20 (S) protopanaxatriol which includes Rg1, Rg2, Re, Rf, Rh1 and R1(Attele *et al.*, 1999;

Shibata, 2001) (Fig. 8.1). More than 40 of these saponins have been isolated and identified (Teng *et al.*, 2003) and the pharmacological activities of the ginsenosides Rb1 and Rg1 have been clearly established (Table 8.1).

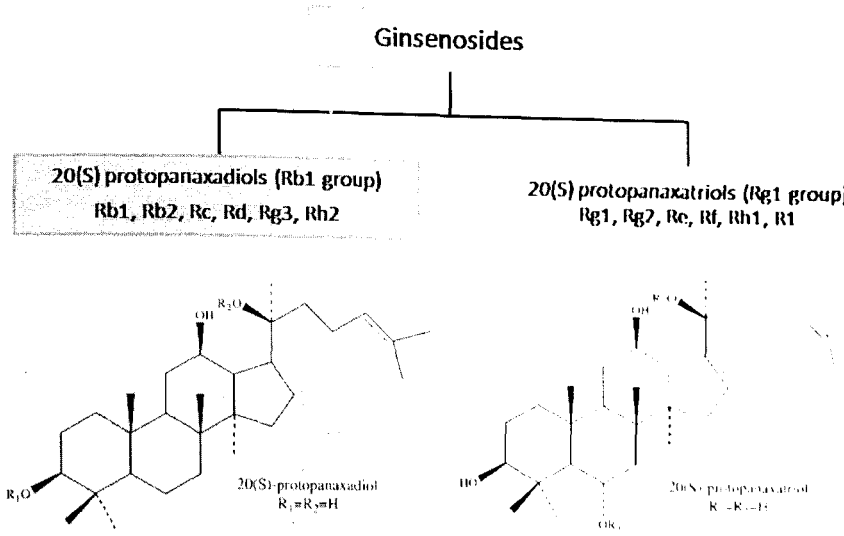


Fig. 8.1: Classification of ginsenosides.

Table: 8.1: Pharmacological activities of ginsenosides.

Rb1 group	Rg1 group
Exhibits sedative	Stimulating and anti-fatigue effect
A depressant of the central nervous system	A weak stimulant of the central nervous system
Anticonvulsive	Enhances the motoric activity
Analgesic	
Antipyretic	
Anti-inflammatory	
Antipsychotic activities	
Improves gastro-intestinal mobility	

Ginseng is well known for its use in traditional medicine (Zhang *et al.*, 2006) such as in modulating blood pressure, metabolism and immune functions (Liu and Xiao, 1992; Attele *et al.*, 1999; Spelman *et al.*, 2006; Xiang *et al.*, 2008). Ginsengs have been not only used as therapeutic agents but also marketed as dietary supplements and raw materials of health food (Shen *et al.*, 2003; Wang *et al.*, 2008). *Panax ginseng* commonly known as Asian ginseng is the most familiar herbal medicine which has been used as a tonic, sedative, anti-fatigue, or anti-gastric ulcer drug, and also has antidiabetic and antitumor activities (Lee *et al.*, 1997a; Shin *et al.*, 2006). *P. quinquefolius* or American ginseng is used to reduce stress, lower high blood sugar and adjust immunity (Vuksan *et al.*, 2001a). Modern pharmacological studies have shown that *P. notoginseng* has anticarcinogenic and hepatoprotective activities, as well as protective effects on cardiovascular and cerebrovascular systems (Konoshima *et al.*, 1999).

The utility of each of the *Panax* species differs from one another (Matsuura *et al.*, 1984; Shibata *et al.*, 1985).The relative amounts of ginsenosides have been used to differentiate the *Panax* species. *P. quinquefolius* has little or no ginsenoside Rf, has a lower ratio of ginsenoside Rg1 to Rb1 compared to *P. ginseng* (van Breemen *et al.*, 1995; Li *et al.*, 1996) and is consistent with the pharmacology of Rg1 and Rb1 (Shibata *et al.*, 1985). *P. quinquefolius* is also considered to be balanced and less stimulating than *P. ginseng* (Hobbs, 1996).

The most commonly used *Panax* species are *P. ginseng, P. quinquefolius, P. notoginseng, P. vietnamensis* and *P. japonicus*. Till today most of the research has focused on Korean ginseng which has been used in Asia for more than 5000 years as a tonic and a panacea that can promote longevity (Dharmananda 2002). Little information is available on ginsenoside variation in the wild populations of the Himalayan ginseng. Establishing the nature of

phytochemical variation would be of interest both for conservation as well as commercial utilization.

The main objectives of the study are:

1. To study the ginsenoside content in all the *Panax* species.
2. To screen out the factors responsible for the higher concentration of ginsenoside in plants.
3. Inter and intrapopulation variation in the ginsenosides content.
4. To identify the superior populations for medicinal uses.

8.2. METHODS

8.2.1. Processing of samples

The rhizomes of 15-20 cm were collected from Arunachal Pradesh, Meghalaya, Manipur and Nagaland and were washed with water and air dried. The air dried rhizomes were kept in dehydrator at 40°C for further drying. The moisture content (%) was determined by Infra Red Moisture meter which should be less than 10°C. The dried rhizomes were sliced into pieces and grounded in a mixer and sieved through mesh No.10. The sieved powder were then packed in zip lock polythene bags and labeled for further analysis.

8.2.2. Sample preparation for ginsenoside quantification

The finely powdered sample (1 g) was extracted three times with 20 ml 80% methanol using a sonicator for 60 minutes at 40°C. The samples were then centrifuged and the supernatant was concentrated under reduced pressure. The residue was dissolved in 80% methanol and adjusted to a final volume of 10 ml. The samples before injection were then filtered through a 0.2 μm membrane nylon filter.

8.2.3. Standard solutions

Ginsenoside Rb1 (1 mg/ml), and Rg2 (1 mg/ml) standard stock solution was prepared in methanol. A series of standard operating solutions of different concentrations were obtained by diluting the mixed standard stock solution.

8.2.4. HPLC conditions for ginsenoside quantification

A HPLC method was developed using a reversed-phase C18 column (Perkin Elmer, 5 µm) with a gradient mobile phase of 0.5% Phosphoric acid/Acetonitrile (30:70). The flow rate was 1.0 ml/min with column temperature at 40°C and injection volume was 20 µl. Detection was accomplished by monitoring at 203 nm and solvent used was HPLC grade methanol.

8.2.5. Statistical Analyses

Statistica version 6 available at www.statsoft.com, Origin Version 7 usable at www.OriginLab.com were used to analyse the data. ANOVA was carried out to test the variation in ginsenoside content among species and between the populations. Tukey's HSD test was performed to specify which groups in the sample differ significantly. Multiple stepwise forward regression analyses was carried out to identify the most statistically significant variables that contribute to the concentration of ginsenoside content.

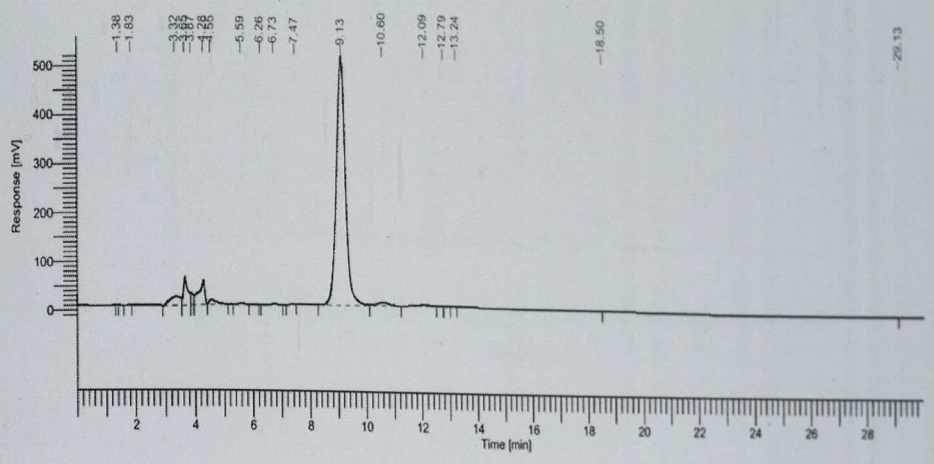
8.3. RESULTS

Ginsenoside Rb1 and Rg2 in extract from samples were identified by comparison of the retention times with the ginsenoside standards obtained from the chromatogram (Fig. 8.2). The retention time was 9.13 mins for Rb1 and 8.68 mins for Rg2. The profiles of the samples collected from some of the populations for *P. assamicus*, *P. bipinnatifidus*, *Panax* sp., *P. pseudoginseng* and *P. variabilis* revealed that the retention time for Rb1 in all the samples ranged from 8.93-9.17 minutes (Fig. 8.3, a-k). Rb1 content (%) was highest in *P.*

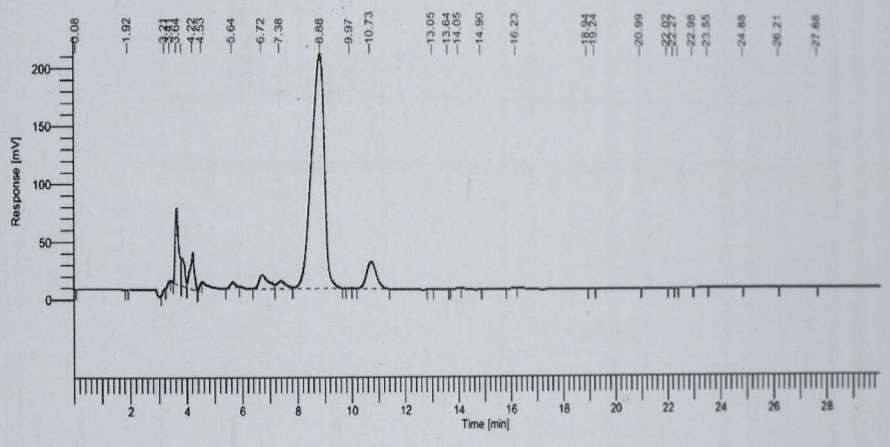
assamicus. The trend was *P. assamicus* > *P. bipinnatifidus* > *P. pseudoginseng* > *Panax* sp. > *P. variabilis*. The highest content was recorded for *P. assamicus* population at Laitkseh (=3.05) and lowest in *P. variabilis* population (0.45). ANOVA followed by Tukey's multiple-range test revealed that there was significant variation in Rb1 ginsenoside content in all the populations and even among the species (Tukey's HSD test, $p<0.05$; ANOVA, $p<0.001$) (Table 8.2, 8.3).

The retention time for Rg2 in all the samples ranged from 8.4-8.8 minutes (Fig. 8.3, a-k). Rg2 content (%) was highest in *P. assamicus*. The trend was *P. assamicus* > *P. pseudoginseng* > *P. bipinnatifidus*, *P. variabilis* > *Panax* sp. The highest content was recorded for *P. assamicus* population at Laitkseh (= 0.55) and lowest in *Panax* sp. population (0.006). ANOVA followed by Tukey's multiple-range test revealed that there was significant variation in Rg2 ginsenoside content in all the populations and even among the species (Tukey's HSD test, $p<0.05$; ANOVA, $p<0.01$ and $p<0.001$) (Table 8.2, 8.3). The highest and lowest total ginsenoside content ranged from 3.60 to 0.19 % per gm.

The Rg2/Rb1 ratio for *P. assamicus* population ranged from 0.05-0.18, for *P. bipinnatifidus* it was 0.05. The lowest Rg2/Rb1 ratio was lowest in *Panax* sp. with 0.03, *P. pseudoginseng* had the highest range of 0.11-0.37 and for *P. variabilis* it was reported at 0.16.

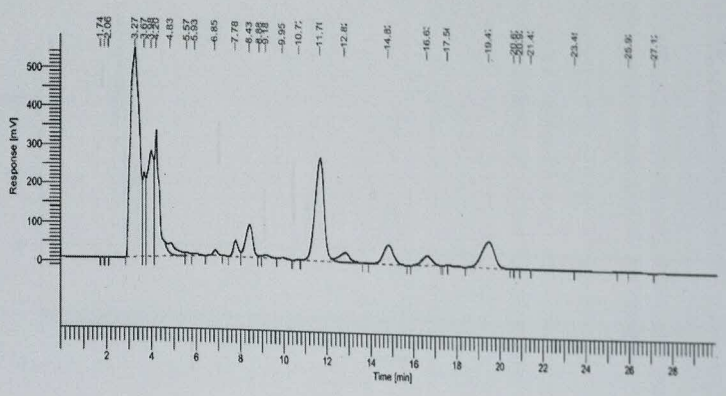


Rb1 standard (Rb1 group)

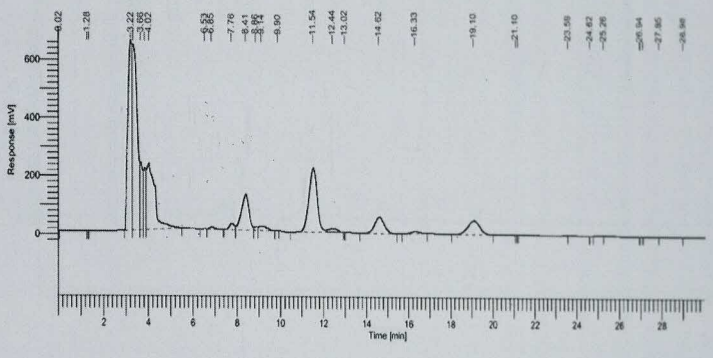


Rg2 standard (Rg1 group)

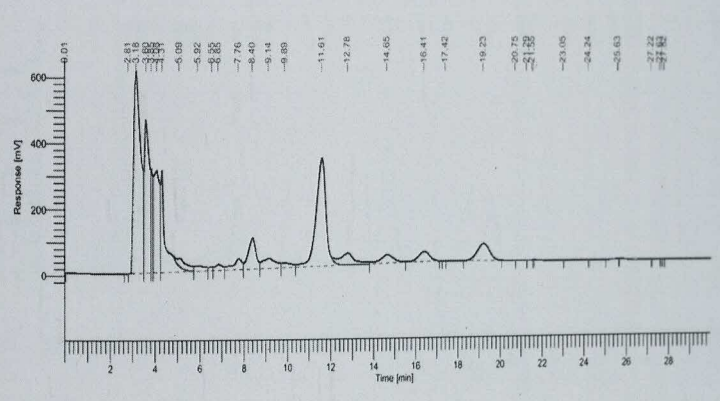
Fig. 8.2: HPLC chromatograms of standards.



(a)

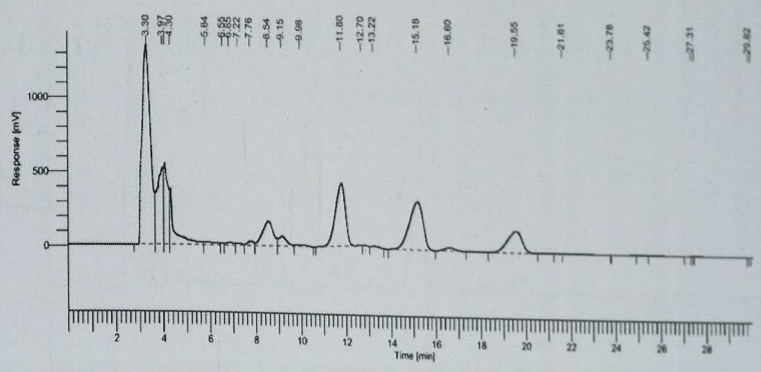


(b)

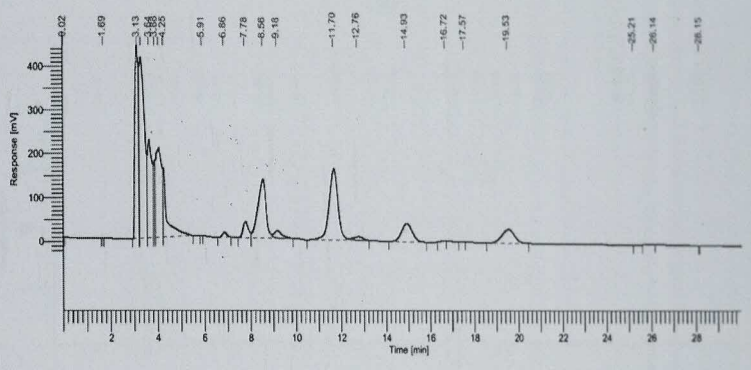


(c)

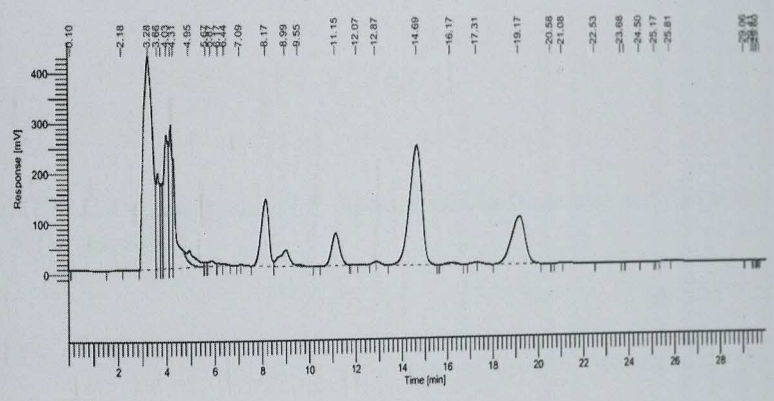
Fig. 8.3 (a-c): HPLC chromatograms of *P. assamicus* (a-Nongkrem, b-Laitkor, c-Upper Shillong).



(d)

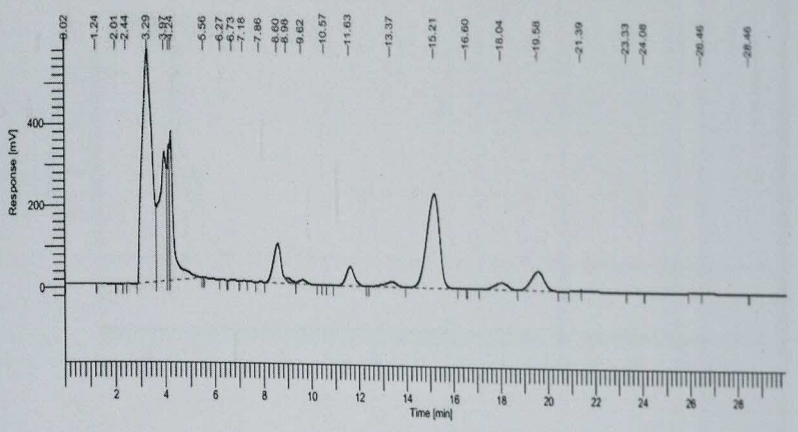


(e)

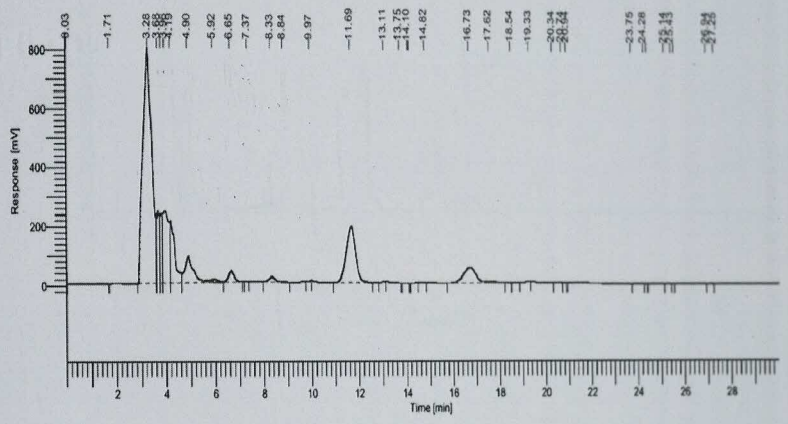


(f)

Fig. 8.3 (d-f): HPLC chromatograms of *P. assamicus* (d-Laitkseh, e-Tyllangr, f-Manipur).

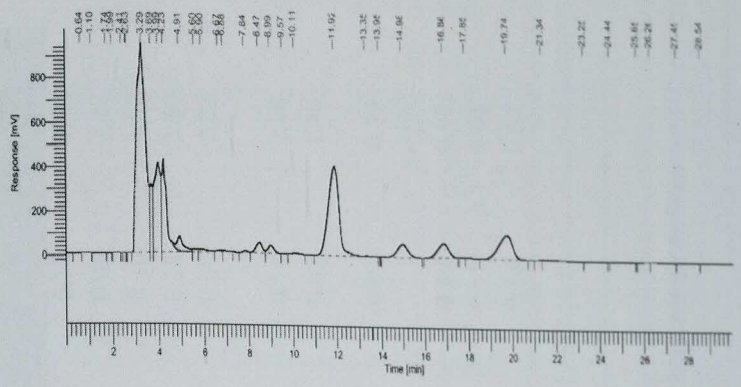


(g)

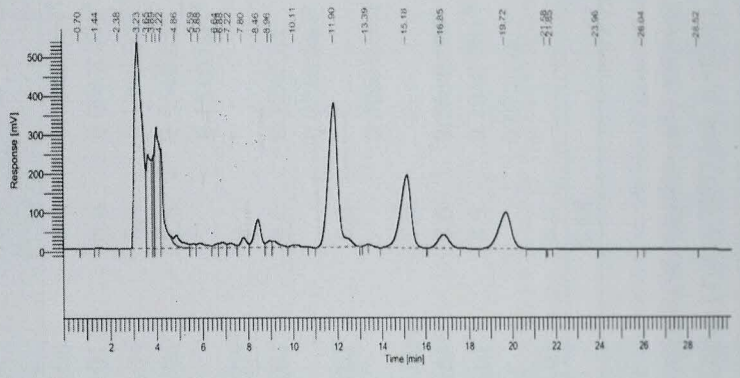


(h)

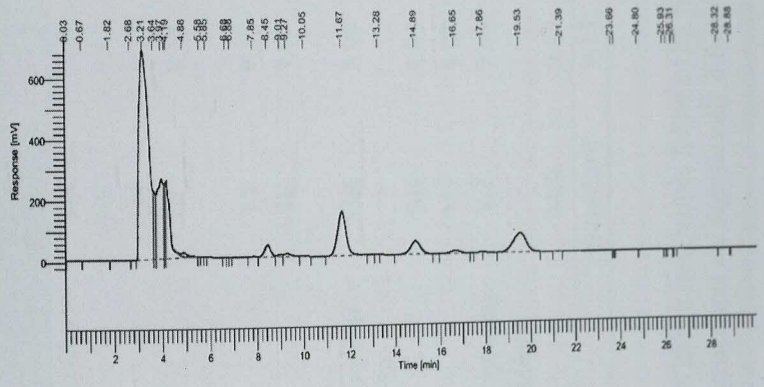
Fig. 8.3 (g-h): HPLC chromatograms of *P. bipinnatifidus* (g-Gomkang) and *Panax* sp. (h- Nagaland).



(i)



(j)



(k)

Fig. 8.3 (i-k): HPLC chromatograms of *P. pseudoginseng* (i-Mtsugho, j-khezha) and *P. variabilis* (k-Manipur).

Table 8.2: Populations showing Ginsenoside content (%).

Species	Populations	Rb_1		Rg_2		Rb1/Rg2 ratio	Rg2/Rb1 ratio	Total Ginsenoside content (%)
		RT (mins)	(%/gm)	RT (mins)	(%)			
P. assamicus	LK	9.14	1.54±0.08 ^{d}	8.6	0.18±0.002^{d}	8.84	0.12	1.72
	NK	9.17	1.16±0.01 ^{e}	8.6	0.06±0.001^{h}	20.95	0.05	1.22
	US	9.14	1.24±0.04^{e}	8.6	0.13±0.002^{ef}	9.83	0.10	1.37
	UL	9.14	3.05±0.06^{a}	8.8	0.55±0.01^{a}	5.60	0.18	3.60
	TY	9.13	2.07±0.02^{b}	8.7	0.15±0.002^{d} ^{e}	14.10	0.07	2.22
	SH	9.00	1.78±0.03^{c}	8.6	0.32±0.02^{b}	5.60	0.18	2.10
P. bipinnatifidus	GK	8.98	1.33±0.02^{e}	8.6	0.07±0.004^{g} ^{h}	19.53	0.05	1.40
Panax sp.	BH	8.93	0.18±0.004^{i}	8.4	0.006±0.001 ^{i}	34.58	0.03	0.19
P. pseudoginseng	MT	9.02	0.71±0.04^{g}	8.6	0.26±0.009^{c}	2.77	0.37	0.97
	KZ	9.00	0.92±0.01^{f}	8.5	0.10±0.001^{fg}	9.6	0.11	1.02
P. variabilis	MA	9.14	0.45±0.01^{h}	8.5	0.07±0.003^{g} ^{h}	6.57	0.16	0.52
	Standard	**9.13**		**8.68**				

Note: Within a row, values followed by same superscripts are not significantly different while those followed by different superscripts are significantly different from each other (Tukey HSD test, $p < 0.05$).

Table 8.3: One way ANOVA showing effect of species and sites on Rb1 and Rg2 ginsenosides (*p<0.01, **p<0.001).

Parameters	Variation due to	df	F	p value
Rb1	Species	4	19.7394**	0.000
	Sites	10	456.00**	0.000
Rg2	Species	4	4.869*	0.002
	Sites	10	516.742**	0.000

8.3.1. Factors influencing the presence of ginsenosides in North-east India

The ginsenoside Rb1 was positively correlated with soil organic carbon ($p<0.0001$), calcium, ($p<0.05$), zinc ($p<0.01$), air temperature ($p<0.05$), light intensity ($p<0.01$) and iron ($p<0.05$) and negatively correlated with available phosphorus ($p<0.0001$), altitude ($p<0.0001$) and copper ($p<0.05$) (Table 8.4). The ginsenoside Rg2 was positively correlated with soil organic carbon ($p<0.0001$), soil temperature ($p<0.0001$) and magnesium ($p<0.001$) and negatively correlated with altitude ($p<0.0001$), available phosphorus ($p<0.001$) and copper ($p<0.05$).

Table 8.4: Forward stepwise multiple regression equations showing the factors influencing ginsenoside Rb1 and Rg2. Coefficient represent B values for prediction (N=60).

Dependent variable (Y)	Regression equation	Adjusted R2	p-value
Ginsenoside Rb1	$Y= 3.726 - 0.014(P_{av}) - 0.004$ (Alt) + 0.436 (SOC) + 0.455 (Ca) - 0.017 (Cu) + 0.012 (Zn) + 0.064 (AT) + 0.0003 (LI) + 0.016 (Fe)	0.915	<0.000
Ginsenoside Rg2	$Y= -0.076 + 0.099$ (SOC) - 0.0007 (Alt) + 0.03 (ST) – $0.002 (P_{av}) + 0.05$ (Mg) – 0.006 (Cu)	0.751	<0.000

8.4. DISCUSSION

Individual ginsenosides have divergent and important effects on health. Therefore, discovering significant variation in ginsenoside contents and composition in *Panax* species would have significant implications for future research, development, and regulation of ginseng products which is in high demand.

In the present study, the variation in the ginsenoside content from all the populations ranges from 0.19 to 3.60 %. The variation observed in ginsenoside concentration in the different populations may be of both environmental and genetic origin (Baldwin and Peterson, 1999; Bazzaz *et al.*, 1987). The total ginsenoside contents as well as individual ginsenoside contents varied among the species. This has also been shown in *P. ginseng* and *P. quinquefolius* (Lewis, 1988). In this study, the highest contents of Rb1 and Rg2 were recorded for *P. assamicus* population at Laitkseh. The lowest Rb1 value was recorded for *P. variabilis* and for Rg2 it was *Panax* sp. Both the ginsenosides showed significant difference when comapared among the species and among populations.

The ginsenoside Rb1 in *P. assamicus, P. bipinnatifidus, Panax sp., P. pseudiginseng* and *P. variabilis* was positively correlated with soil organic carbon, calcium, zinc, air temperature, light intensity, and iron. The ginsenoside Rg2 was found to be positively correlated with soil organic carbon, soil temperature and magnesium. Influences from the environment, such as soil nutrients and texture, humidity, soil moisture, air, and light conditions, can affect the chemical profile of populations. Rb1, Rc, and Rd have been shown to be most susceptible to varying environmental conditions (Li *et al.*, 1996).

The quality and composition of ginsenosides in the ginseng plants are influenced by a range of factors such as species, age, part of the plant, cultivation method, harvesting season and preservation method (Lim *et al.*, 2005; Shlag and McIntosh, 2006). The ginsenoside Rf is unique to *P. ginseng* while F11 is found exclusively in *P. quinquefolius*. Thus the Rf/F11 ratio is used as a phytochemical marker to distinguish American ginseng from Asian ginseng (Li *et al.*, 2000; Assinewe *et al.*, 2003). Traditionally the ratio Rg1/Rb1 is linked to the ethnopharmacology properties of ginseng preparations (Dharmananda, 2002). The ratio less than 0.4 is indicative of *P. quinquefolius*, while a high ratio is characteristic of *P. ginseng* (Nakamura *et al.*, 2007). Indeed, Rb1 acts as weak CNS depressant while Rg1 stimulates the CNS. The low ratio of Rg1/Rb1 is linked to the so-called "cool" or calming properties of American ginseng, while the high ratio Rg1/Rb1 could be the reason of the "warm" or stimulating characteristics of Asian ginseng (Chang *et al.*, 2003). The Rg1 (Rg2)/Rb1 ratio for *P. pseudoginseng* which had the highest value ranged from 0.11-0.37, *P. assamicus* population ranged from 0.05-0.18. The ratio for *P. bipinnatifidus* was 0.05 and for *P. variabilis* it was 0.16. The lowest Rg2/Rb1 ratio was lowest in *Panax* sp. with 0.03.

The Rg1/Rb1 value calculated for all the species also proved to be a biochemical marker for establishing the identity of the species. However, the ratios had to be confirmed by including more populations under each species. The data on ginsenoside content confirmed that *P. assamicus* had the highest concentration of both ginsenoside Rb1 and Rg2 and the populations from West Khasi Hills and Manipur showed the highest concentration which might be due to various factors like habitat, no anthropogenic disturbance and other environmental conditions.

CHAPTER 9
GENERAL DISCUSSION

At least 80% of the traditional medicines used for primary health care are derived from plants (Farnsworth and Soejarto, 1991; Pei Shengji, 2001). In developing countries of Africa and Asia, more than 80% of the rural population relies on medicinal plants for their primary health care needs. India is one among the leading countries in Asia in terms of richness of traditional knowledge related to the use of plant species. North-eastern India has been in focus for its rich diversity of medicinal plants and associated folk/traditional medicinal knowledge including Siddha, Ayurveda, Amchi, Unani and Homeopathy system of medicines. The use of herbal products for medicine, food supplements and cosmetics is gaining ground globally and the plant products are being preferred over synthetic compounds world-wide as they are cost-effective and have negligible side effects. Because of this, the demand for herbal products has been increasing steadily, and the growth of international trade in plants of medicinal importance is phenomenal during the past two decades. This in turn is leading to over-extraction of medicinal plants from the wild, thus deteriorating the size of natural populations and their habitats (Bhutani, 2008; Mao *et al.*, 2009, De *et al.*, 2010). Due to overharvesting of medicinal plants, and other disturbances caused by deforestation, timber logging and several other anthropogenic factors most of the medicinal plant species are on the brink of extinction. The threat status of these medicinal plants has not been evaluated properly because of deficient data. For effective conservation strategy, the correct threat assessment of the species is a pre-requisite.

Panax spp. in north-eastern India has been heavily extracted due to its high medicinal uses. The herbal practitioners in the region use the rhizome as a tonic and vitalizer. The rhizome is being extensively collected by the local people from the wild. In Nagaland and Manipur, the rhizome is being sold at Rs 700 to 1,000/- per kg. The rhizome is being sold also as powder in these two states. The rhizomes of *Panax* are also extracted in Arunachal Pradesh and are sold at Rs 2,000-3000/- per kg. According to some sources, many of these extracted rhizomes are sold to the neighbouring countries. *Panax*, commonly known as Ginseng is threatened in their natural habitats in north-eastern India (Nayar and Sastry, 1990). Therefore, it is imperative that appropriate conservation measures need to be developed to recover the species and replenish its depleted populations and ensuring their perpetuation in nature. However, our understanding on Himalayan *Panax* species biology is extremely poor, which is so critical for taking any effective conservation measure for the species. Even, the taxonomy of the species remained unresolved and the population data are not available due to extremely low population size and sparse distribution of the species in the difficult mountain terrain.

Therefore, the present study was conducted in five states of north-eastern India, where *Panax* species occur i.e. Arunachal Pradesh, Manipur, Meghalaya, Nagaland and Sikkim to study the distribution pattern, niche and population characteristics of *Panax* species complex. As mentioned above, the taxonomy of *Panax* is highly controversial due to the appearance of intermediates in most of the morphological features (Hara, 1970; Zhou *et al.*, 1975; Hoo and Tseng 1978; Yang, 1981; Wen and Zimmer, 1996). The taxonomists have not been able to differentiate the taxonomic ranks of Himalayan *Panax* especially at the species level. Therefore, it was essential to resolve the taxonomic ambiguity of Himalayan *Panax* species complex. Since the use of only molecular phylogeny approach is not always successful in

establishing species level identity, a combined approach encompassing morphometric, molecular, ecological niche and biochemical marker was tried to resolve these closely related species within *Panax* species complex. Since ginsenosides constitute the pharmacologically active constituents in *Panax* species, the ginsenoside contents were quantified in different *Panax* species, related to the prevailing environmental factors, and the relative contents of various ginsenosides was used as a possible marker to differentiate the species.

In north-eastern India, six species of *Panax* were reported by earlier workers viz., *P. assamicus*, *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *P. sokpayensis* and *P. sikkimensis* which have restricted distributions with the exception of *P. pseudoginseng* that extends from north-eastern India up to Nepal. The species of *Panax* have an altitudinal range of 1490 m in Meghalaya up to 3,300 m asl in Sikkim. During the study, in addition to *P. assamicus*, *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis*, *P. sokpayensis* and *P. sikkimensis*, a new species (*Panax* sp.) that was morphologically distinct from all the existing species was discovered. An effort was made to distinguish this species through the combined approach of molecular phylogeny, cluster classification using morphometric traits, ecological niche modeling and the ratio of two major ginsenoside groups.

The potential distribution area of each *Panax* species was modeled using Ecological Niche Modelling. The most important factor which determined the distribution of the potential habitats of *P. assamicus* (Manipur), *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *Panax* sp. was Normalized Difference Vegetation Index (NDVI) but for *P. assamicus* from Meghalaya, Digital Elevation Model (DEM) was the key factor. The contribution of NDVI for the former set of species ranged between 88.4 and 94.6%. *P. bipinnatifidus* had the maximum value with a contribution of 94.6%. For *P. assamicus* from Meghalaya, DEM contributed about

64% to the model. The greater contribution of NDVI than the other factors to the potential distribution models of most species revealed the importance of NDVI layer in defining habitat suitability which is related to the phenological cycle of the vegetation of that particular area. The relatively high importance of vegetation parameters such as herbaceous cover, NDVI and EVI in predicting distribution of species was in conformity with the distribution models developed by several earlier workers (Stohlgren *et al.*, 2001; Osborne *et al.*, 2001; RouraPascual *et al.*, 2006; Bino *et al.*, 2008).

The distribution models developed for different species were quite accurate as reflected in the high AUC values (AUC>0.9) for all the species of *Panax*. The values ranged between 0.97 and 0.99 for *P. assamicus*, *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *Panax* sp. The models with AUC>0.95 but <1.0 are considered very good (Thuiller *et al.*, 2005). The high AUC values for different species revealed that the habitat distribution models generated by MaXent have the ability to differentiate between suitable and unsuitable habitats of *Panax* species. With the AUC values very close to 1.0, the models exhibit their capability for strong prediction. The niche overlap according to Schoener's D statistic (Schoener, 1968) in all the *Panax* species ranged from limited to moderate overlap. This indicates that although these species are different, they do share many environmental variables.

The importance of each environmental predictor variable was assessed using jackknife operation (Yost *et al.*, 2008) and showed the environmental variable with highest gain when used in isolation but when omitted, decreases the gain the most was NDVI values of January, March, July and December, and elevation. These variables contributed the most to the Maxent models in *Panax* species.

The potential distribution maps generated through ENM indicates that *P. assamicus* is distributed in Meghalaya and Manipur. The distribution of the species was also predicted in Tuensang district of Nagaland bordering Myanmar. However, it could not be located in Tuensang. *P. bipinnatifidus* was predicted with continuous distribution from Sikkim extending to Bhutan and Arunachal Pradesh, Myanmar, West Bengal and Sikkim. *P. pseudoginseng* was predicted in Nagaland, Manipur, Bhutan and Myanmar. *P. variabilis* showed a restricted distribution confined only to the northern part of Senapati district of Manipur and areas in southern part of Kohima district of Nagaland bordering Senapati. A continuous distribution pattern of *Panax* sp. was predicted in Arunachal Pradesh spreading to Tuensang district of Nagaland, and also in southern part of Bhutan, Tibet, Myanmar, Sikkim, West Bengal and Nepal.

The niche breadth of a species describes the range of environmental factors or resources that it can use (Gaston *et al.*, 1997). In the present study, *P. assamicus* with niche breadth of 2.2 had the highest niche breadth, followed by *P. pseudoginseng* (2.0), *Panax* sp. (1.7) and the lowest being in *P. bipinnatifidus* (1.0) and *P. variabilis* (1.0). PCA using various environmental factors confirmed that *P. assamicus, p. pseudoginseng* and *Panax* sp. have broad ecological niches. Such species are geographically widespread and also abundant locally (Brown, 1984). In contrast, *P. bipinnatifidus* and *P. variabilis* had very narrow niches, and therefore, had very restricted distribution. Species with broad niche can persist in a wide range of habitat types while species with narrow niche are restricted to a few places where its niche requirements are met (Gaston 1993; Kunin and Gaston 1997). Widespread species are more likely to have access to a more diverse resource base utilization (Gaston *et al.*, 1997). Therefore, the niche breadth of the widespread species should be broader than those of geographically restricted

species. There is an alternative hypothesis predicting that species with small geographical ranges should have elevated extinction risk, because small ranges make them highly vulnerable to the effects of environmental stochasticity and localized catastrophes (Simberloff, 1998; Lawton, 1995).

Generalists are not sensitive to any of the environmental changes but specialists are prone to such changes (Lawton *et al.*, 1994; Johnson, 1998). Therefore, the extinction risk due to small geographical range is more in specialists, but lesser in generalists and with increase in geographical range, the risk of extinction declines.

In the present study, the phylogeny of *Panax* using nuclear 18S rRNA gene could not separate the species as they exhibited high degree of homology (99%). Earlier, the phylogeny of *Panax* was studied using ITS sequences (Wen and Zimmer, 1996), chloroplast DNA restriction sites (Choi and Wen, 2000) and chloroplast *trn*C-*trn*D intergenic region (Lee and Wen, 2004). These studies have been able to resolve the systematic position of some of the species of *Panax*. In their analyses, *Panax trifolius* formed the basal clade with a sister clade that includes all the *Panax* species. *P. pseudoginseng* and *P. stipuleanatus* were the second diverged group followed by *P. notoginseng* which is basal to a large clade containing *P. quinquefolius* and all the Asian *Panax* taxa. The trnC-trnD phylogeny supports the above relationship and is consistent with the morphological and palynological evidences (Wen 2001b; Wen and Nowicke, 1999).

P. assamicus described by Banerjee (1968) is very similar to *P. wangianus* Sun. from west central China and to *P. zhengyianus* J.Wen from south western China in the morphological features viz., narrow leaflets, elongated rhizomes with thick and short internodes and fruits

which are bright red in colour with a black tip. The identity of *P. assamicus* was resolved through the use of ITS nuclear ribosomal DNA (Pandey *et al.*, 2002) that separates the species from *P. wangianus* and *P. zhengyianus*.

P. bipinnatifidus with divided leaflets was described by Seemann (1868) and the degree of division varied greatly. The identity of *P. bipinnatifidus* was established in earlier studies by Wen and Zimmer (1996), Lee and Wen (2004) and recently by Pandey *et al.* (2009), Pandey and Ali (2010). *P. variabilis* was described by Jun Wen from China but a detailed description of the plant was not available. However, it was reported by Pandey *et al.* (2009) from north-east India which was confirmed through ITS rDNA phylogeny.

The unidentified species collected from Arunachal Pradesh and Nagaland showed resemblance with *P. vietnamensis* and *P. quinquefolius* in the morphological features as well as in ITS rDNA phylogeny. To confirm the identity of this species, perhaps as a new species, need further investigation.

In order to further confirm the identity of all the six *Panax* species collected in the present study, secondary structure of the highly conserved 5.8S rRNA region was analyzed. The secondary structures of *P. assamicus, P. bipinnatifidus* and *P. variabilis* added to the identity confirmation. However, in *Panax* sp. the secondary structure did not match with any of the species of *Panax* not even to the closely related *P. quinquefolius* and *P. vietnamensis*. Analyses through PCR-RFLP showed a distinct profile for *P. assamicus*. *P. variabilis* and *Panax* sp. shared the same profile. However, *P. bipinnatifidus* shared a profile between the above species. Therefore, PCR-RFLP using the three restriction enzymes viz., *Bst*N1, *Msp*I and *Psp*G1 could be used as a marker for *P. assamicus*.

Due to high percentage of homology in the ITS sequence datasets, all the *Panax* species fall in the range of 92-98%. However, the unidentified species of *Panax* in the present study which showed clear differences from *P. assamicus*, *P. pseudoginseng*, *P. bipinnatifidus* and *P. variabilis* could be a new species that had high percentage of homology in the nucleotide sequence with *P. vietnamensis* (99%) and *P. quinquefolius* (99%).

The high level of ITS sequence divergence observed between *P. trifolius* from eastern North America and the Asian relatives suggested an early divergence compared to *P. quinquefolius* with the Asian species that had occurred much later (Wen and Zimmer, 1996). The hypothesis proposed by Tiffney (1985) argued on the multiple origins of eastern Asian and eastern north American disjunct pattern of evolution of plants. It was based on fossil evidences and the geological history of the north temperate region, which has been subsequently supported by molecular data for several species of *Panax* (Wen and Zimmer, 1996; Wen and Jansen, 1992, 1995; Xiang *et al.*, 1994; Wen *et al.*, 1996). The areas of the Himalayas and central and western China are regarded as the center of diversity of *Panax*. With majority of the species present, the evolutionary radiation of *Panax* may have occurred in this region. The relatively low ITS sequence divergence among most Asiatic species suggests that these species are relatively recently diverged (Wen and Zimmer, 1996). Most species of *Panax* species occur in the mountainous regions of central and western China and the Himalayas. The rise of the Himalayas and the formation of many mountain ranges in central and western China due to the impact from the collision of the Indian plate with Asia since the late Tertiary and Quaternary may have created several isolated habitats ideal for speciation of *Panax* (Axelrod *et al.*, 1998, Wen, 1999). This is consistent with the observed pattern of close relationships among many Asiatic species and in the present study at both morphological and molecular levels. However,

P. quinquefolius and *P. trifolius* which occur in eastern North America are very distinct, representing different phylogenetic lines within *Panax*. The comparative phylogenetic study on Araliaceae based on the ITS sequences of nuclear ribosomal DNA suggested that *Panax* is monophyletic and the monophyly is supported by several synapomorphies (Hoo, 1961; Atuyokova *et al.*, 2005).

Population viability analysis is a critical step for species conservation. It was analysed for *P. assamicus* using two model scenario (M1 and M2 model) to simulate the future of the species based on certain ecological and demographic features of the species populations following Akcakaya (2000). The PVA presented were demographically structured models. The PVA for *P. assamicus* yielded four important outputs *viz*., (i) finite rate of increase (ii) elasticity analysis (iii) metapopulation occupancy and, (iv) extinction risk analysis. Additionally, threat status of the three species was assessed using IUCN criteria and management options were explored to suggest conservation measure of the species.

All the populations of *Panax assamicus* exhibited discrete population growth pattern and the growth rate declined (λ <1) over 3 years of study period i.e. 2009-2011.The growth rate (λ) in all the five populations ranged from 0.86-0.97 which depicts a general decline in population. This indicates that there was a constant reduction in the populations at each time step. The λ value for Upper Shillong population (0.97) suggested that the population will remain if the prevailing conditions are not disturbed. However, other populations with λ=0.85-0.88 are more or less not stable and will disappear in the near future if the present conditions persist.

The life history stages are most critical to population growth. Metapopulation modeling could identify the life history stage that is most vulnerable through elasticity analysis (Schemske *et al.* 1994). As reported by Caswell (1986), *P. quinquefolium* has size dependent mortality and the most vulnerable stage of the life cycle appears to be the period of seedling establishment. Once the seedlings are established, *P. quinquefolium* individuals had high life expectancy (>20 years) (Charron, 1989). In *P. assamicus* too, the most vulnerable stage of the life cycle was the period of seedling establishment. In elasticity analysis, survival which contributes the most to the population growth rate played an important factor and fecundity the least. In *Panax* species, even though the seedling mortality rate is high the stability of the population is more sensitive to a decrease in the survival rate of large individuals than to a reduction in the production of seeds or in the establishment of seedlings. Such elasticity values have high conservation implications *i.e.,* special protection to these sub-sets of population instead of the whole population, will boost conservation efforts of such species.

Population viability analysis has been applied to compare potential management actions. The long-term and reliable set of demographic monitoring data which showed temporal variation in population dynamics coupled with ecological factors affecting the population and the detailed autecological information on the species are important factors for such analysis (Burgman *et al.* 1988). The simulation results from the introduction of 1-leaved stage plants to the metapopulation suggest that the introduction of 6,700 plants to the metapopulation will improve the conservation status of the species and the introduction of 6, 700 individuals to Upper Shillong population is predicted bring down the extinction risk to 5% in 100 years. The Minimum viable population (MVP) is important from conservation point of view. The Minimum Viable Population size which is a minimum threshold size that a species can persist

for 100 years (at 5% extinction risk) is therefore estimated to be 5,520 plants. Therefore, for *P. assamicus* due to the long dormancy and low seed production, conservation strategy will be to introduce the 1-leaved stage into the metapopulation.

Ginseng with a long history of medicinal use are amongst the most popular and best-selling herbal medicines worldwide (Ernst, 2002). To date most of the research has focused on Korean ginseng which has been used in Asia for more than 5000 years as a tonic and a panacea that can promote longevity. Ginsenosides which are the main active compounds derived from the roots and rhizomes of different *Panax* species are used as markers for identification and also for the quality control of ginseng drugs and commercial products. The different ginsenosides fall under two groups viz., Rb1 and Rg1 group. The Rb1/Rg1 ratio has been used for the standardization of ginseng products. In particular, the ratio differs among different species: Rb1/Rg1 values usually between 1and 3 are characteristic of *P. ginseng*, while Rb1/Rg1 values around 10 or greater are indicative of *P. quinquefolius*. Also, the presence or absence of marker compounds is used for species differentiation.

The total as well as individual ginsenosides vary depending on the species which is seen in *P. ginseng* and *P. quinquefolius* (Lewis, 1988). In the present study, the total ginsenoside for *P. assamicus* ranged from 1.22-3.60, for *P. pseudoginseng* it ranged from 0.97-1.02. In *P. bipinnatifidus*, the content was 1.40, in *P. variabilis* it was 0.52 and the lowest was in *Panax* sp. The Rg2/Rb1 ratio for *P. assamicus* population ranged from 0.05-0.18, for *P. bipinnatifidus* it was 0.05. *P. pseudoginseng* had the highest range of 0.11-0.37 and for *P. variabilis* it was reported at 0.16 and the lowest ratio was reported in *Panax* sp. with 0.03. The variation in the total ginsenoside content among the populations of the same species could be due to varied environmental conditions such as wild and cultivated (Betz *et al.*,1984), soil and fertility

conditions (Konsler *et al.*, 1990), age of the roots/rhizomes (Soldati and Tanaka, 1984), and extraction methods (Schulten and Soldati, 1981). From regression analysis, it was concluded that these two major ginsenosides were related to certain microclimatic and edaphic factors. For Rb1 the correlated variables were: soil organic carbon, calcium, zinc, air temperature, light intensity, iron, available phosphorus and copper. The variation in the altitude influenced the concentration of Rb1. However, for Rg2 the favourable factors include, soil organic carbon, soil temperature and magnesium. Li *et al.* (1996) reported the sensitivity of the ginsenoside to varying environmental conditions which was also confirmed in the present study. Variation among and within individual ginsenosides may be pharmacologically important because individual ginsenosides differ in their effects on human physiology (Ki *et al.*, 1998). The ratios between the abundant ginsenosides of the Rb1 and Rg1 group have been used as a marker to identify the species in *Panax*, use in quality control and for identification of ginseng samples. However, most studies have been carried out for *P. ginseng*, *P. quinquefolius*, *P. japonicus*, *P. notoginseng* and *P. vietnamensis*. No study on these ratios was available for Himalayan ginseng.

Notoginsenoside R1, pseudo-ginsenoside F11, Rg1, Rb1 and Re were used as markers for separating *P. notoginseng*, *P. quinquefolius* and *P. ginseng* (Wan *et al.*, 2007). The ratio of Rb1 to Rg1 and Rg1 to Rb1 is often used as a basis for separating *P. ginseng* which had a higher ratio of Rg1 to Rb1 compared to *P. quinquefolius* (Chen *et al.*, 2008) but the ratio of Rb1/Rg1 was higher for *P. quinquefolius* compared to *P. ginseng* (Soldati and Stitcher, 1980). The main constituent in *P. vietnamensis* was majonoside R2 , have attracted much attention due to the high pharmacological potentials (Duc *et al.*, 1993; Hong *et al.*, 1998; Konoshima *et al.*, 1998). The ratio Rg2 to Rb1 could be used as a marker in the present study for

differentiating the species. However, ratio needs to be confirmed from more populations of these species and studies on more phytochemicals from ginseng will provide a better understanding on their applicability to drug formulation.

Morphometric analyses could separate *P. assamicus*, *P. bipinnatifidus*, *P. variabilis* and *Panax* sp. into distinct species. Ecological niche characterization revealed that *P. bipinnatifidus* and *P. variabilis* have two distinct niches. However, *P. assamicus* and *Panax* sp. showed overlapping niches. Ecological niche modeling could segregate all the species distinctly. Molecular analysis through phylogeny of the 18S-26S rDNA ITS region, could separate all the species clearly. Biochemical marker could also separate the species based on the Rg2/Rb1 ratio. It was clear for *Panax* species with a value of 0.03. However, for other species, it was overlapping, hence needs further investigation with more populations.

Thus, the present study on *Panax* species complex could reaffirm the identity of most of the species of Himalayas. The extensive field survey in the entire north-eastern region provided a holistic distribution pattern of different *Panax* species of Himalayas. During the process, the study could locate a morphologically divergent species that might be a new species. The study has also proved the efficiency of ENM in predicting the potential distribution areas of a species. The study has demonstrated that based on niche breadth and fundamental niche of the species, it is possible to segregate the species. The study concludes that a combined approach encompassing morphometric, ecological niche characterization, biochemical marker and molecular phylogeny would yield better result in establishing species identity than following any of these approaches individually.

SUMMARY

Ginseng, the members of the genus *Panax* are known as 'King of Herbs'. It has been highly valued for its mystical properties and has a 5000-year long history as a traditional herbal medicine originating from ancient China. The ginseng genus, belonging to the family Araliaceae

is one of the approximately 120 genera of flowering plants with an eastern Asian and eastern North American disjunct distribution. *Panax* consists of approximately 18 species, of which 16 are from eastern Asia and two from eastern North America. In India, Araliaceae is represented by 15 genera distributed mostly in north and northeastern region. The genus *Panax* in India comprises of *Panax assamicus* Ban., *P. bipinnatifidus* Seem., *P. pseudoginseng* Wall., *P. sokpayensis* Sharma and Pandit., and *P. sikkimensis* Ban. In India, *Panax* species are found only in north-eastern region and Darjeeling hills of West Bengal. Among the north-eastern states, the species have been reported from Arunachal Pradesh, Meghalaya, Manipur, Nagaland and Sikkim.

Several Himalayan species of *Panax* have been debatable due to sympatry of morphologically distinct taxa and the existence of occasional morphological intermediates. The Himalayas, central and Western China are considered as the centre of diversity of *Panax*. High medicinal value of *Panax* is responsible for their extraction from the wild, which has reduced the sizes of most of their natural populations. The illegal trade in wild ginseng has caused a drastic decline in their population sizes in the wild during the recent years. This has made at least two *Panax* species as threatened. The Himalayan *Panax* species are already enlisted as vulnerable in the

Red Data Book of India. In CAMP (2003), *P. wangianus* (present day identified as *P. assamicus*) was classified as an endangered species. Because of deficient data, other species of Himalayan *Panax* are yet to be classified under IUCN criteria (IUCN, 2010).

The study was conducted in Arunachal Pradesh, Manipur, Meghalaya, Nagaland and Sikkim. In Arunachal Pradesh, *Panax* species are found in the temperate forest of Tawang district and subtropical broadleaved forest of West Kameng district. In Manipur, *Panax* species are found in temperate forests of Senapati and Ukhrul districts at an altitude of 1,800 to 2,400 m asl where Oaks are the dominant trees. In Meghalaya, *Panax* populations are found in subtropical broadleaved forest which occurs above 1,200 m asl. In Nagaland, the species are found mainly in the wet temperate forests classified under Naga hills wet temperate forest which is distributed along the Assam/Burma border from 1,800 m asl. In Sikkim, *Panax* species are found in the temperate forest and subtropical or lower montane forests. Thus, all *Panax* species were at an altitudinal range of 1,490-3,306 m asl in north-east India.

Panax species recorded in the present study are, *P. assamicus, P. bipinnatifidus, P. pseudoginseng, P. variabilis* and one unidentified species (referred as *Panax* sp.). The *Panax* species in the present study consists of: (i) horizontally elongated rhizomes with thick and short internodes which includes *P. assamicus, P. variabilis, P. pseudoginseng*, and *Panax* sp. (unidentified), and (ii) horizontally elongated rhizomes with slender and elongated internodes and subglobose nodes that includes *P. bipinnatifidus*.

The present study was undertaken with the following objectives:

1. Study the distribution pattern, niche and population characteristics of *Panax* species complex in north-eastern India;

2. Resolve the taxonomic ambiguity of Himalayan *Panax* species complex using molecular phylogeny approach; and
3. Quantify the ginsenoside contents for Himalayan *Panax* species complex and correlate it with the ecological conditions of the species.

Ecological niche characterization

Each species had a different range of occurrence with respect to different edaphic and climatic variables. The range of air temperature where *Panax assamicus* grows was 18-28°C. The corresponding ranges for *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *Panax* sp. were 13.1-14°C, 20- 24.9°C, 18-18.6°C and 23.2-26.6°C, respectively. The range of relative humidity for *Panax assamicus* was 58.5-94.9% while that for *P. bipinnatifidus* it was 52-60%. The respective ranges for *P. pseudoginseng*, *P. variabilis* and *Panax* sp. were 76-99 %, 86-96% and 62-79.4%, respectively. The range of light intensity for *P. assamicus* was 800-2,500 lux while that for *P. bipinnatifidus* and *P. variabilis* it was 700-1,300 lux. *P. pseudoginseng* occurred in the range of 650-2,900 lux and *Panax* sp. grew in the range of 600-1,640 lux. The range of soil temperature for *P. assamicus* was 14.8-21.1°C while that for *P. bipinnatifidus* it was 10.2-11.4°C. *P. pseudoginseng* had a range of 15.9-18.4°C, *P. variabilis* 14.8-15.6°C and *Panax* sp. grew in the soil temperature range of 16.7-19.2°C. The texture of the soils for *Panax assamicus*, *P. pseudoginseng* and *Panax* sp. showed a range of textural class from loamy sand to sandy . In *P. variabilis* and *P. bipinnatifidus* the soil texture was sandy. The range of water holding capacity for *P. assamicus* was 31-76.8% while that for *P. bipinnatifidus* it was 46.8-53.8%. *P. pseudoginseng* had a range of 31.3-51.9%, *P. variabilis* 43.3-51.2% and *Panax* sp. grew in the range of 28.6-51.7%. The soil bulk density ranges for *P. assamicus*, *P. bipinnatifidus*, *P. pseudoginseng*, and *P. variabilis* and *Panax sp.*, were 0.4-0.8 g cm^{-3}, 0.7-

1.2 g cm^{-3} 0.3-1 g cm^{-3}, 0.5-1g cm^{-3} and 0.5-0.6 g cm^{-3}. The range for soil porosity in *P. assamicus* was 69.1-86.2%, while that for *P. bipinnatifidus* was 55.3-75.6%. The range for *P. pseudoginseng*, *P. variabilis* and *Panax* sp. was 62.4-87.5%, was76.3-81.3% and 62.9-81% respectively. The soil moisture content range for soils of *P. assamicus* growing areas was 22.1-47.7%. The corresponding range for *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *Panax sp.*, were 29.5-31.45%, 31.1-46%, 44.1-46.7% and 30.6-47.5%. The range of soil pH for *P. assamicus* was 4.4-6.0 while that for *P. bipinnatifidus* was 4.3 to 4.8. *P. pseudoginseng*, *P. variabilis* and *Panax* sp. grows in the range of 4.9-7.1, 4.8-5.9 and 5.2-7.3. The range of total kjeldahl nitrogen for *P. assamicus* was 0.4-1.0% while that for *P. bipinnatifidus* was 0.4-0.5%. *P. pseudoginseng* had a range of 0.4-1.0%, *P. variabilis* with 0.6-0.7% and *Panax* sp. with 0.5-1.25%. The range of soil organic carbon for *P. assamicus*, *P. bipinnatifidus P. pseudoginseng P. variabilis* and *Panax* sp. was 1-5.8% , 4.1-4.2%, 3.9-5.5%, 5.2-6.2% and 2.5-4.4%. The range of available phosphorus for *P. assamicus* was 16-25.9 µg g^{-1} while that for *P. bipinnatifidus* was 17.3-18 µg g^{-1}. *P. pseudoginseng P. variabilis* and *Panax* sp. grow in the range of 23.8-78 µg g^{-1}, 59-65.2 µg g^{-1} and 22.8-143.3 µg g^{-1}. The range for exchangeable potassium for *P. assamicus* was 78.9-362.5 µg g^{-1} while that for *P. bipinnatifidus* was 165.5-178 µg g^{-1}. The respective ranges for *P. pseudoginseng*, *P. variabilis* and *Panax* sp. was 192.5-360 µg g^{-1}, 135-275 µg g^{-1} was 138-440 µg g^{-1} respectively. The range of total magnesium for *P. assamicus* was 1.7-11 mg g^{-1} while that for *P. bipinnatifidus* was 3.2-3.6 mg g^{-1}. *P. pseudoginseng* had a range of 2.9-4.9 mg g^{-1}, *P. variabilis* was 2.7-3.6 mg g^{-1} and *Panax* sp. grow in the range of 3.1-5.7 mg g^{-1}. The range of total calcium for *P. assamicus* was 0.03-11.09 mg g^{-1}. The corresponding ranges for *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *Panax* sp. was 0.1-0.2 mg g^{-1}, 0.01-0.6 mg g^{-1}, 0.1-0.2 mg g^{-1} was 0.05-1 mg g^{-1}. The range for total iron in *P. assamicus* was 27.3-83.6 mg g^{-1} while that for *P. bipinnatifidus* was

27.7-28.6 mg g^{-1}. *P. pseudoginseng* grows in the range of 24.5-37 mg g^{-1}, *P. variabilis* with 23.5-31.6 mg g^{-1} and *Panax* sp. with 29.5-59.2 mg g^{-1}. The range for total manganese in *P. assamicus* was 0.1-1.4 mg g^{-1}. The corresponding ranges for *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *Panax* sp. was 0.1-0.3 mg g^{-1}, 0.2-0.3 mg g^{-1} and 0.2-0.5 mg g^{-1}. The corresponding ranges for total zinc in *P. assamicus*, *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *Panax* sp. was 40.4-101 µg g^{-1}, 70.7-73.8 µg g^{-1}, 55.8-93.6 µg g^{-1}, 42.8-53.8 µg g^{-1} and 53.9-69.5 µg g^{-1}. The range for total copper in *P. assamicus* was 23.53-48.05 µg g^{-1} while that for *P. bipinnatifidus* was 29.2-32.4 µg g^{-1}. *P. pseudoginseng*, *P. variabilis* and *Panax* sp. was 16.9-47.2 µg g^{-1}, 27.8-28.2 µg g^{-1} was 32.3-52.4 µg g^{-1}.

Niche breadth and Niche differentiation

The mean Levin's niche breadth for all the environmental parameters clearly showed that *P. assamicus* had the broadest niche breadth of 2.2 followed by *P. pseudoginseng* with a niche breadth of 2.0. *Panax* sp. had an intermediate niche breadth of 1.7 and both *P. bipinnatifidus* and *P. variabilis* showed the same niche breadth of 1.0.

The scatter plot, an output of PCA with the species and the corresponding environmental parameters revealed the distinct niches (environmental requirements) of each species, although three species viz., *P. assamicus, P. pseudoginseng* and *Panax* sp. showed overlapping but stretched out group indicating the wide range of species. However, *P. variabilis* and *P. bipinnatifidus* were clumped which shows that these species have a narrow range of variation.

Although the distribution of different *Panax* species was correlated with different environmental factors, available phosphorus, soil moisture content, total Kjeldahl nitrogen, copper and manganese, porosity, relative humidity, air temperature, exchangeable potassium,

soil organic carbon, soil pH and iron were important factors responsible for their distribution/occurrence.

Ecological niche modeling

The potential distribution area of each *Panax* species was modeled using Ecological Niche Modelling. The most important factor which determined the distribution of the potential habitats of *P. assamicus* (Manipur), *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *Panax* sp. was Normalized Difference Vegetation Index (NDVI) but for *P. assamicus* from Meghalaya, Digital Elevation Model (DEM) was the key factor. The contribution of NDVI for the former set of species ranged between 88.4 and 94.6%. *P. bipinnatifidus* had the maximum value with a contribution of 94.6%. For *P. assamicus* from Meghalaya, DEM contributed about 64% to the model. The greater contribution of NDVI than the other factors to the potential distribution models of most species revealed the importance of NDVI layer in defining habitat suitability which is related to the phenological cycle of the vegetation of that particular area. The relatively high importance of vegetation parameters such as herbaceous cover, NDVI and EVI in predicting distribution of species was in conformity with the distribution models developed by several earlier workers (Stohlgren *et al.*, 2001; Osborne *et al.*, 2001; RouraPascual *et al.*, 2006; Bino *et al.*, 2008).

The distribution models developed for different species were quite accurate as reflected in the high AUC values (AUC>0.9) for all the species of *Panax*. The values ranged between 0.97 and 0.99 for *P. assamicus*, *P. bipinnatifidus*, *P. pseudoginseng*, *P. variabilis* and *Panax* sp. The models with AUC>0.95 but <1.0 are considered very good (Thuiller *et al.*, 2005). The high AUC values for different species revealed that the habitat distribution models generated by

MaXent have the ability to differentiate between suitable and unsuitable habitats of *Panax* species. With the AUC values very close to 1.0, the models exhibit their capability for strong prediction. The niche overlap according to Schoener's D statistic (Schoener, 1968) in all the *Panax* species ranged from limited to moderate overlap. This indicates that although these species are different, they do share many environmental variables.

The importance of each environmental predictor variable was assessed using jackknife operation (Yost *et al.*, 2008) and showed the environmental variable with highest gain when used in isolation but when omitted, decreases the gain the most was NDVI values of January, March, July and December, and elevation. These variables contributed the most to the Maxent models in *Panax* species.

The potential distribution maps generated through ENM indicates that *P. assamicus* is distributed in Meghalaya and Manipur. The distribution of the species was also predicted in Tuensang district of Nagaland bordering Myanmar. However, it could not be located in Tuensang. *P. bipinnatifidus* was predicted with continuous distribution from Sikkim extending to Bhutan and Arunachal Pradesh, Myanmar, West Bengal and Sikkim. *P. pseudoginseng* was predicted in Nagaland, Manipur, Bhutan and Myanmar. *P. variabilis* showed a restricted distribution confined only to the northern part of Senapati district of Manipur and areas in southern part of Kohima district of Nagaland bordering Senapati. A continuous distribution pattern of *Panax* sp. was predicted in Arunachal Pradesh spreading to Tuensang district of Nagaland, and also in southern part of Bhutan, Tibet, Myanmar, Sikkim, West Bengal and Nepal.

Establishing taxonomic identity of *Panax* species from north-eastern India

Morphometric analysis

Cluster Analysis using the 15 morphological characters of 56 specimens produced two major clusters in the dendrogram. The first cluster comprised of *P. assamicus* with a few individuals at lower distance measure. The second cluster was formed at greater distance and divided into sub-clusters which included, *P. assamicus, P. bipinnatifidus, P. variabilis, P. pseudoginseng* and *Panax* sp. *P. bipinnatifidus* formed a subcluster of its own but all the other specimens were intermixed with each other and did not give a clear distinction in all the species. However, there was significant difference among the species and also among the populations in the morphological characters.

PCA scatter plot showed that *Panax* in north-east formed five distinct groups i.e. *P. assamicus, P. pseudoginseng, Panax sp., P. variabilis and P. bipinnatifidus.* The samples of *P. bipinnatifidus* and *Panax sp.* showed overlapping and more condensed indicating less variation. *P. variabilis* and *P. pseudoginseng* also showed overlapping but with a broad ellipse indicating a wide range of variation. *P. assamicus* on the other hand showed overlapping with *P. variabilis* and *P. pseudoginseng* and is broadly stretched out indicating a wide range of variation. *P. bipinnatifidus* is narrowly stretched .

Molecular phylogeny

Approximately 21 kb genomic DNA bands were observed for all *Panax* species. The best amplification result was obtained at annealing temperature of 67°C with a total band size of 1.8Kb for 18S rRNA gene. For 18S-28S rDNA ITS region, the best amplification result was

obtained at annealing temperature of 57.5°C where a single band of approximately 700 bp was amplified.

Nucleotide sequence analysis: The BLAST for the *18S rRNA* gene showed a high degree of homology (99%) with *P. ginseng, P. quinquefolius, P. notoginseng, P. japonicus, P. vietnamensis, P. stipuleanatus, P. pseudoginseng* subsp. *himalaicus, P. bipinnatifidus, P. notoginseng.* For 18S-28S rDNA ITS region, BLAST analysis showed 100% identity of *P. assamicus, P. bipinnatifidus* and *P. variabilis. Panax sp.* collected from Nagaland and Arunachal Pradesh showed 99% identity with *P. japonicus, P. quinquefolius, P.variablis* and *P. vietnamensis.*

Sequence characteristics: A total of 18 sequences of *Panax* species were used including the 8 sequences of the studied species for the 18S rRNA gene. The sequence data set ranged from 1692-1694. Out of 1694 characters analysed, 11 were variable/polymorphic sites, 1680 were invariable/monomorphic sites, 10 were autapomorphic sites, 1 site was parsimony informative, GC (%) content was 49.65% and transition/tranversion ratio was 0.95.The total number of indel sites analysed was 3 with an average indel length of 1. Indel diversity k (i) was 0.53 Tajima'D test was 1.09 with p> 1.0 which was not significant.

A total of 41 sequences of *Panax* species were used including the 8 sequences of the studied species for the 18S-28S rDNA ITS region. The sequence data set ranged from 602-617. Out of 620 characters analysed 113 were variable/polymorphic sites, 480 were invariable/monomorphic sites, 68 sites were parsimony informative, 45 were autapomorphic sites, GC (%) content was 60.42% and transition/tranversion ratio was 2.94. The total number

of indel sites analysed were 27 with an average indel length of 1.22. Indel diversity k (i) was 1.45 and Tajima'D test was 2.53 with $p<0.001$ which was significant.

Phylogenetic analysis of 18S rRNA gene: The phylogenetic tree constructed through NJ and ML methods revealed three clades. The first clade included *P. assamicus, P. bipinnatifidus Panax sp.*(new species in present study), *P. ginseng, P. japonicus, P. japonicus var. bipinnatifidus, P. pseudoginseng subsp. himalaicus, P. variabilis and P. zingiberensis* supported by 100% bootstrap value. *P. pseudoginseng* and *P. stipuleanatus* formed a separate clade. *P. quinquefolius, P. notoginseng* and *P vietnamensis* formed together one clade (BS=62%). The 50% majority-rule consensus tree resulted from Bayesian analysis applying the GTR+G model showed the support for relationships between the taxa with posterior probability values.The tree topology was congruent with that of the Maximum Likelihood tree. The first clade was supported with maximum posterior probability value (100%). *P. pseudoginseng* and *P. stipuleanatus* were grouped together with 69% posterior probability value. The third clade which grouped together *P. quinquefolius, P. notoginseng* and *P vietnamensis* was supported by 1 % posterior probability.

Phylogenetic analysis of the 18S-28S rDNA region: The Neighbour joining tree yielded more or less similar results as obtained with the Maximum likelihood method. The Maximum likelihood tree revealed that the sampled populations of *P. assamicus* from Meghalaya and Manipur grouped together (BS=93%) and showed a close relationship with *P. bipinnatifidus* clade (BS=92%). The populations of *P. bipinnatifidus* from Arunachal Pradesh were grouped together in one clade with the other populations from China. *P. variabilis* from Manipur was grouped together with *P. variabilis* from China (BS=88%). *Panax sp.* populations grouped together (BS=98%) and formed a separate clade. This clade showed close relationship with the

basal clade i.e., *P. notoginseng* (BS=51%) which in turn was related to the *P. pseudoginseng-P. stipuleanatus* clade (BS=99%). The 50% majority-rule consensus tree resulted from Bayesian analysis applying the GTR+G+I model showed strong support for relationships between the taxa with posterior probability values (Fig 6.17). The tree topology was congruent with that of the Neighbour Joining and Maximum Likelihood tree.

***Secondary structure of 5.8S rRNA*:** The secondary structures of *P. assamicus* from both the populations in Meghalaya matched the secondary structure in *P. assamicus* from GenBank. *P. assamicus* from Manipur population had similar structure with another population from Manipur which was retrieved from GenBank (HQ141404). The populations of *P. bipinnatifidus* from Arunachal Pradesh had the same structure with that of *P. bipinnatifidus* from Nepal. *Panax sp.* from both the populations i.e. Arunachal Pradesh and Nagaland had similar structures but did not match with any of the structures in GenBank.

PCR –restriction fragment length profile (pcr-rflp)/ amplicon restriction pattern (arp): The PCR-RFLP using the endonuclease *BstN*I and *Psp*GI produced two different profiles The first profile was obtained in all the populations of *P. assamicus* from Meghalaya and Manipur and the second profile was obtained for *P. bipinnatifidus*, *Panax sp.* and *P. variabilis*. Restriction enzyme *Msp*I generated two profiles, PM1 profile was obtained for *P. assamicus* populations and *P. bipinnatifidus* populations. Profile PM2 was obtained for *P. variabilis* population and *Panax sp.* populations from Arunachal Pradesh and Nagaland.

Cluster dendrogram constructed by using the combined PCR-RFLP profiles of the 18S-28S rDNA ITS region of all the *Panax* species revealed two major clusters, I and II. Cluster I comprises all the sub-populations of *P. assamicus* from Meghalaya and Manipur. Cluster II

comprised of two sub-clusters, the first cluster (IIA) consists of *P. bipinnatifidus* populations from Arunachal Pradesh and the second cluster (IIB) includes *P. variabilis* from Manipur population and *Panax sp.* populations from Arunachal Pradesh and Nagaland.

Scale developed for identification of Panax species: The comparison of sequence homologies of the different genera under Araliaceae, different species of *Panax* genus and members of same species collected from different geographical locations revealed that the 18S-28S rDNA ITS sequence homology of members belonging to the *Panax* genus but different species ranged from 92-98%. Species from different geographical locations exhibited homology of 98-99%. The unidentified species showed 94-99% homology with the other species of *Panax*. However, it showed 99% homology with *P. quinquefolius* and *P. vietnamensis* which indicated that despite morphological dissimilarity that this species is genetically close to these two species.

Assessing the impact of disturbance on *Panax assamicus* metapopulation

Demographic stages: *Panax assamicus* have 8 stages i.e., seedling stage and seven other leaf-stages from 1-leaved to 7-leaved stage. 3 and 4-leaved stage are reproductive individuals.

Spatial Characteristics: *Panax assamicus* metapopulation had only 5 populations. The mean patch size was 89.6 ± 42.1 ha. Total plant density was 27 ±11.61/ha and the mean patch isolation was 12.28 ± 4.7.

Finite Rate of Increase (λ): Deterministic analysis yielded the finite rate of increase (λ) which revealed that all the populations of *Panax assamicus* exhibited discrete population growth pattern and the growth rate declined (λ <1) over 3 years of study period i.e. 2009-2011. The growth rate (λ) in all the five populations remained within 1.0 which depicts a general

decline in population. The population growth rate was in the order Upper Shillong>Laitkseh>Laitkor >Tyllang and Nongkrem. The total metapopulation level growth rate (λ) also remained negative (λ=0.898) indicating a net decline in the total population size of the species.

Elasticity: The contribution of all these demographic processes to overall growth of the population in the life cycle of *P. assamicus* was represented by the relative sensitivity of survival, growth and fecundity to λ. Survival growth contributes the maximum towards the growh rate. Contribution of growth to elasticity was highest in Upper Shillong which is near to 0.1 followed by Tyllang population. However, in Laitkor and Nongkrem populations, the elasticity values fall below 0.001. There was no contribution of growth in Laitkseh population. Fecundity had the least elasticity which shows it had relatively less contribution to λ. In Laitkseh and Nongkrem populations, fecundity did not have any contribution to *P. assamicus* and demonstrated the actual behaviour of a typical iteroparous forest herb where survival had the highest elasticity compared to growth and fecundity.

Stochastic Risk Analyses: A large decline was expected in all the populations of *P. assamicus* within 40 years except for Upper Shillong population where the decline was expected after 60 years in both the M1 and M2 scenarios. After a sharp decline the trajectories stabilized in both the scenarios which indicated an almost deterministic decline in the future. However, the summary on the meatpopulation size declines in both M1 and M2 scenario which leads to an extremely low abundance. In M1 scenario, the metapopulation size after a sharp decline becomes stable after 65 years and in M2 scenario the population becomes stable after 40 years.

Metapopulation viability: The occupied populations in *P. assamicus* depicted a sharp decline through time and only 50% of these would still remain within 24 years. The remaining sub-populations are from Upper Shillong under M1 scenario. The metapopulation occupancy in M2 scenario showed a similar trend and approximately 50% of the population would be extant by 16th year and the populations which will remain are from Upper Shillong.

Probability of extinction: The extinction curves for all the sub-populations approached extinction probability 1.0 much before 100 years except for Upper Shillong sub-population. The extinction probability curves revealed identical curves in 3 of the subpopulations. Laitkseh population showed overlapping in both the scenarios but Upper Shillong sub-population showed different extinction curve in M1 and M2 models.

Management intervention: Threat status of the study species was determined and *Panax assamicus* was categorized as vulnerable. Management options was analysed and introduction of approximately 400 1-leaved stage plants is required to bring down the risk of *Panax assamicus* from vulnerable category. To lower the risk, an estimate of 5,520 plants is required to be introduced to reach its Minimum Viable Population (MVP) size.

Inter-population variation in ginsenoside contents

The retention time for ginsenoside Rb1 was 9.13 mins and for ginsenoside Rg2, it was 8.68 mins. The retention time for Rb1 in all the samples ranged from 8.93-9.17 minutes. Rb1 content (%) was highest in *P. assamicus* and the trend was *P. assamicus* > *P. bipinnatifidus* >*P. pseudoginseng* >*Panax* sp. > *P. variabilis*. The highest content was recorded for *P. assamicus* population at Laitkseh (3.05%) and lowest in *P. variabilis* population (0.45). There was significant variation in Rb1 ginsenoside content in all the populations and even among the

species. The retention time for Rg2 in all the samples ranged from 8.4-8.8 minutes. Rg2 content (%) was highest in *P. assamicus* and the trend was *P. assamicus* > *P. pseudoginseng* > *P. bipinnatifidus*, *P. variabilis* > *Panax* sp. The highest content was recorded for *P. assamicus* population at Laitkseh (= 0.55) and lowest in *Panax* sp. population (0.006). There was significant variation in Rg2 ginsenoside content in all the populations and even among the species. The total ginsenoside content ranged from 3.60 to 0.19 %. The Rg2/Rb1 ratio for *P. assamicus* population ranged from 0.05-0.18, for *P. bipinnatifidus* it was 0.05. The lowest Rg2/Rb1 ratio was lowest in *Panax sp.* with 0.03, *P. pseudoginseng* had the highest range of 0.11-0.37 and for *P. variabilis* it was reported at 0.16.

The ginsenoside Rb1 was positively correlated with soil organic carbon, calcium, zinc, air temperature, light intensity, and iron and negatively correlated with available phosphorus, altitude and copper. The ginsenoside Rg2 was positively correlated with soil organic carbon, soil temperature and magnesium and negatively correlated with altitude, available phosphorus and copper.

Morphometric analyses could separate *P. assamicus*, *P. bipinnatifidus*, *P. variabilis* and *Panax* sp. into distinct species. Ecological niche characterization revealed that *P. bipinnatifidus* and *P. variabilis* have two distinct niches. However, *P. assamicus* and *Panax* sp. showed overlapping niches. Ecological niche modeling could segregate all the species distinctly. Molecular analysis through phylogeny of the 18S-26S rDNA ITS region, could separate all the species clearly. Biochemical marker could also separate the species based on the Rg2/Rb1 ratio. It was clear for *Panax* species with a value of 0.03. However, for other species, it was overlapping, hence needs further investigation with more populations.

Thus, the present study on *Panax* species complex could reaffirm the identity of most of the species of Himalayas. The extensive field survey in the entire north-eastern region provided a holistic distribution pattern of different *Panax* species of Himalayas. During the process, the study could locate a morphologically divergent species that might be a new species. The study has also proved the efficiency of ENM in predicting the potential distribution areas of a species. The study has demonstrated that based on niche breadth and fundamental niche of the species, it is possible to segregate the species. Thus, the study concludes that a combined approach encompassing morphometric, ecological niche characterization, biochemical marker and molecular phylogeny would yield better result in establishing species identity than following any of these approaches individually.

APPENDICES

Clustal 2.1 Multiple Sequence Alignment for *18S rRNA* gene

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CLUSTAL 2.1 multiple sequence alignment

P.assamicusSH       CATGCATGTGTAAGTATGAACTAATTCAGACTGTGAAACTGCGAATGGCTCATTAAATCA 60
P.variabilisMA      CATGCATGTGTAAGTATGAACTAATTCAGACTGTGAAACTGCGAATGGCTCATTAAATCA 60
P.assamicusUL       CATGCATGTGTAAGTATGAACTAATTCAGACTGTGAAACTGCGAATGGCTCATTAAATCA 60
P.bipinnatifidusDR  CATGCATGTGTAAGTATGAACTAATTCAGACTGTGAAACTGCGAATGGCTCATTAAATCA 60
P.bipinnatifidusGK  CATGCATGTGTAAGTATGAACTAATTCAGACTGTGAAACTGCGAATGGCTCATTAAATCA 60
  panax sp. BH      CATGCATGTGTAAGTATGAACTAATTCAGACTGTGAAACTGCGAATGGCTCATTAAATCA 60
  panax sp. DB      CATGCATGTGTAAGTATGAACTAATTCAGACTGTGAAACTGCGAATGGCTCATTAAATCA 60
P.assamicusLK       CATGCATGTGTAAGTATGAACTAATTCAGACTGTGAAACTGCGAATGGCTCATTAAATCA 60
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P.variabilisMA      GTTATAGTTTGTTTGATGGTATCTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATA 120
P.assamicusUL       GTTATAGTTTGTTTGATGGTATCTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATA 120
P.bipinnatifidusDR  GTTATAGTTTGTTTGATGGTATCTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATA 120
P.bipinnatifidusGK  GTTATAGTTTGTTTGATGGTATCTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATA 120
  panax sp. BH      GTTATAGTTTGTTTGATGGTATCTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATA 120
  panax sp. DB      GTTATAGTTTGTTTGATGGTATCTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATA 120
P.assamicusLK       GTTATAGTTTGTTTGATGGTATCTGCTACTCGGATAACCGTAGTAATTCTAGAGCTAATA 120
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P.variabilisMA      CGTGCAACAAACCCCGACTTCTGGAAGGGATGCATTTATTAGATAAAAGGTCGACGCGGG 180
P.assamicusUL       CGTGCAACAAACCCCGACTTCTGGAAGGGATGCATTTATTAGATAAAAGGTCGACGCGGG 180
P.bipinnatifidusDR  CGTGCAACAAACCCCGACTTCTGGAAGGGATGCATTTATTAGATAAAAGGTCGACGCGGG 180
P.bipinnatifidusGK  CGTGCAACAAACCCCGACTTCTGGAAGGGATGCATTTATTAGATAAAAGGTCGACGCGGG 180
  panax sp. BH      CGTGCAACAAACCCCGACTTCTGGAAGGGATGCATTTATTAGATAAAAGGTCGACGCGGG 180
  panax sp. DB      CGTGCAACAAACCCCGACTTCTGGAAGGGATGCATTTATTAGATAAAAGGTCGACGCGGG 180
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P.variabilisMA      CTTCTGCCCGTTGCTGCGATGATTCATGATAACTCGACGGATCGCACGGCCCTCGTGCCG 240
P.assamicusUL       CTTCTGCCCGTTGCTGCGATGATTCATGATAACTCGACGGATCGCACGGCCCTCGTGCCG 240
P.bipinnatifidusDR  CTTCTGCCCGTTGCTGCGATGATTCATGATAACTCGACGGATCGCACGGCCCTCGTGCCG 240
P.bipinnatifidusGK  CTTCTGCCCGTTGCTGCGATGATTCATGATAACTCGACGGATCGCACGGCCCTCGTGCCG 240
  panax sp. BH      CTTCTGCCCGTTGCTGCGATGATTCATGATAACTCGACGGATCGCACGGCCCTCGTGCCG 240
  panax sp. DB      CTTCTGCCCGTTGCTGCGATGATTCATGATAACTCGACGGATCGCACGGCCCTCGTGCCG 240
P.assamicusLK       CTTCTGCCCGTTGCTGCGATGATTCATGATAACTCGACGGATCGCACGGCCCTCGTGCCG 240
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P.variabilisMA      GCGACGCATCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGTGGCCTACTA 300
P.assamicusUL       GCGACGCATCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGTGGCCTACTA 300
P.bipinnatifidusDR  GCGACGCATCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGTGGCCTACTA 300
P.bipinnatifidusGK  GCGACGCATCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGTGGCCTACTA 300
  panax sp. BH      GCGACGCATCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGTGGCCTACTA 300
  panax sp. DB      GCGACGCATCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGTGGCCTACTA 300
P.assamicusLK       GCGACGCATCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGTGGCCTACTA 300
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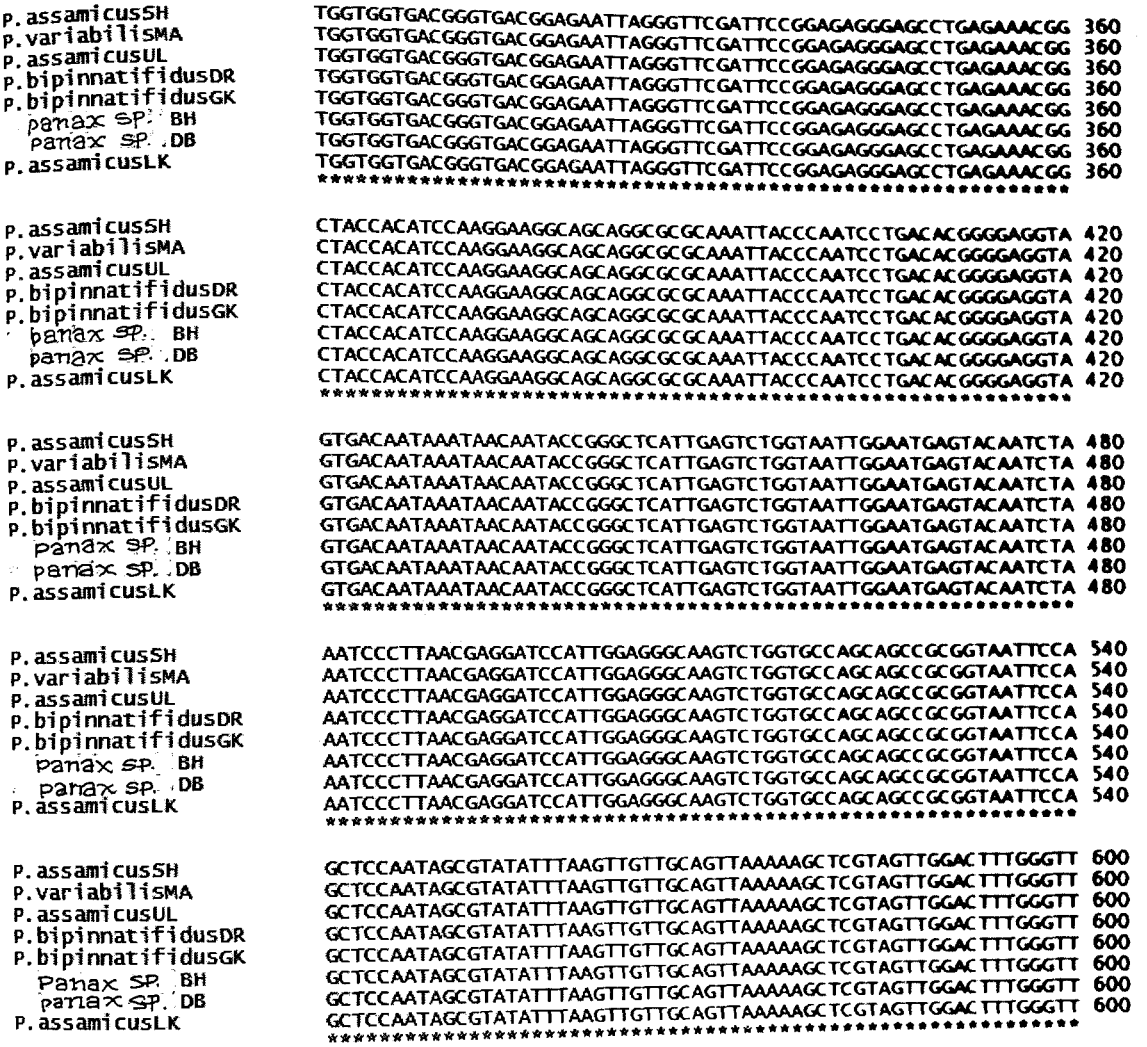
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P.variabilisMA TGGTGGTGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGG 360
P.assamicusUL TGGTGGTGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGG 360
P.bipinnatifidusDR TGGTGGTGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGG 360
P.bipinnatifidusGK TGGTGGTGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGG 360
panax sp. BH TGGTGGTGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGG 360
panax sp. DB TGGTGGTGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGG 360
P.assamicusLK TGGTGGTGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGG 360
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P.variabilisMA CTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTA 420
P.assamicusUL CTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTA 420
P.bipinnatifidusDR CTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTA 420
P.bipinnatifidusGK CTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTA 420
panax sp. BH CTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTA 420
panax sp. DB CTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTA 420
P.assamicusLK CTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTA 420
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P.variabilisMA GTGACAATAAATAACAATACCGGGCTCATTGAGTCTGGTAATTGGAATGAGTACAATCTA 480
P.assamicusUL GTGACAATAAATAACAATACCGGGCTCATTGAGTCTGGTAATTGGAATGAGTACAATCTA 480
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panax sp. BH AATCCCTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCA 540
panax sp. DB AATCCCTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCA 540
P.assamicusLK AATCCCTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCA 540
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P.variabilisMA GCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGACTTTGGGTT 600
P.assamicusUL GCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGACTTTGGGTT 600
P.bipinnatifidusDR GCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGACTTTGGGTT 600
P.bipinnatifidusGK GCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGACTTTGGGTT 600
Panax SP. BH GCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGACTTTGGGTT 600
panax SP. DB GCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGACTTTGGGTT 600
P.assamicusLK GCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGACTTTGGGTT
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P.variabilisMA     GGGTCGGCCGGTCCGCCTCTCGGTGTGCACCGATCGTCTCGTCCCTTCTGCCGGCGATGC 660
P.assamicusUL      GGGTCGGCCGGTCCGCCTCTCGGTGTGCACCGATCGTCTCGTCCCTTCTGCCGGCGATGC 660
P.bipinnatifidusDR GGGTCGGCCGGTCCGCCTCTCGGTGTGCACCGATCGTCTCGTCCCTTCTGCCGGCGATGC 660
P.bipinnatifidusGK GGGTCGGCCGGTCCGCCTCTCGGTGTGCACCGATCGTCTCGTCCCTTCTGCCGGCGATGC 660
panax sp. BH       GGGTCGGCCGGTCCGCCTCTCGGTGTGCACCGATCGTCTCGTCCCTTCTGCCGGCGATGC 660
panax sp. DB       GGGTCGGCCGGTCCGCCTCTCGGTGTGCACCGATCGTCTCGTCCCTTCTGCCGGCGATGC 660
P.assamicusLK      GGGTCGGCCGGTCCGCCTCTCGGTGTGCACCGATCGTCTCGTCCCTTCTGCCGGCGATGC 660
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P.assamicusSH      GCTCCTGTCCTTAACTGGCCGGGTCGTGCCTCCGGCGCTGTTACTTTGAAGAAATTAGAG 720
P.variabilisMA     GCTCCTGTCCTTAACTGGCCGGGTCGTGCCTCCGGCGCTGTTACTTTGAAGAAATTAGAG 720
P.assamicusUL      GCTCCTGTCCTTAACTGGCCGGGTCGTGCCTCCGGCGCTGTTACTTTGAAGAAATTAGAG 720
P.bipinnatifidusDR GCTCCTGTCCTTAACTGGCCGGGTCGTGCCTCCGGCGCTGTTACTTTGAAGAAATTAGAG 720
P.bipinnatifidusGK GCTCCTGTCCTTAACTGGCCGGGTCGTGCCTCCGGCGCTGTTACTTTGAAGAAATTAGAG 720
panax sp. BH       GCTCCTGTCCTTAACTGGCCGGGTCGTGCCTCCGGCGCTGTTACTTTGAAGAAATTAGAG 720
panax sp. DB       GCTCCTGTCCTTAACTGGCCGGGTCGTGCCTCCGGCGCTGTTACTTTGAAGAAATTAGAG 720
P.assamicusLK      GCTCCTGTCCTTAACTGGCCGGGTCGTGCCTCCGGCGCTGTTACTTTGAAGAAATTAGAG 720
                   ************************************************************

P.assamicusSH      TGCTCAAAGCAAGCCTACGCTCTGGATACATTAGCATGGGATAACATCATAGGATTTCGG 780
P.variabilisMA     TGCTCAAAGCAAGCCTACGCTCTGGATACATTAGCATGGGATAACATCATAGGATTTCGG 780
P.assamicusUL      TGCTCAAAGCAAGCCTACGCTCTGGATACATTAGCATGGGATAACATCATAGGATTTCGG 780
P.bipinnatifidusDR TGCTCAAAGCAAGCCTACGCTCTGGATACATTAGCATGGGATAACATCATAGGATTTCGG 780
P.bipinnatifidusGK TGCTCAAAGCAAGCCTACGCTCTGGATACATTAGCATGGGATAACATCATAGGATTTCGG 780
panax sp. BH       TGCTCAAAGCAAGCCTACGCTCTGGATACATTAGCATGGGATAACATCATAGGATTTCGG 780
panax sp. DB       TGCTCAAAGCAAGCCTACGCTCTGGATACATTAGCATGGGATAACATCATAGGATTTCGG 780
P.assamicusLK      TGCTCAAAGCAAGCCTACGCTCTGGATACATTAGCATGGGATAACATCATAGGATTTCGG 780
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P.assamicusSH      TCCTATTACGTTGGCCTTCGGGA-CGGAGTA-TGATTAACAGGGACAGTCGGGGGCATTC 838
P.variabilisMA     TCCTATTACGTTGGCCTTCGGGATCGGAGTA-TGATTAACAGGGACAGTCGGGGGCATTC 839
P.assamicusUL      TCCTATTACGTTGGCCTTCGGGATCGGAGTAATGATTAACAGGGACAGTCGGGGGCATTC 840
P.bipinnatifidusDR TCCTATTACGTTGGCCTTCGGGATCGGAGTAATGATTAACAGGGACAGTCGGGGGCATTC 840
P.bipinnatifidusGK TCCTATTACGTTGGCCTTCGGGATCGGAGTAATGATTAACAGGGACAGTCGGGGGCATTC 840
panax sp. BH       TCCTATTACGTTGGCCTTCGGGATCGGAGTAATGATTAACAGGGACAGTCGGGGGCATTC 840
panax sp. DB       TCCTATTACGTTGGCCTTCGGGATCGGAGTAATGATTAACAGGGACAGTCGGGGGCATTC 840
P.assamicusLK      TCCTATTACGTTGGCCTTCGGGATCGGAGTAATGATTAACAGGGACAGTCGGGGGCATTC 840
                   ************************ ******* ****************************

P.assamicusSH      GTATTTCATAGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACAACTGCGAAAGCA 898
P.variabilisMA     GTATTTCATAGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACAACTGCGAAAGCA 899
P.assamicusUL      GTATTTCATAGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACAACTGCGAAAGCA 900
P.bipinnatifidusDR GTATTTCATAGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACAACTGCGAAAGCA 900
P.bipinnatifidusGK GTATTTCATAGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACAACTGCGAAAGCA 900
panax sp. BH       GTATTTCATAGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACAACTGCGAAAGCA 900
panax sp. DB       GTATTTCATAGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACAACTGCGAAAGCA 900
P.assamicusLK      GTATTTCATAGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACAACTGCGAAAGCA 900
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P.assamicusSH      TTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGAT 958
P.variabilisMA     TTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGAT 959
P.assamicusUL      TTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGAT 960
P.bipinnatifidusDR TTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGAT 960
P.bipinnatifidusGK TTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGAT 960
  panax sp. BH     TTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGAT 960
  panax sp. DB     TTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGAT 960
P.assamicusLK      TTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGAT 960
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P.assamicusSH      ACCGTCCTAGTCTCAACCATAAACGATGCCGACCAGGGATCAGTGGATGTTGCTTTTAGG 1018
P.variabilisMA     ACCGTCCTAGTCTCAACCATAAACGATGCCGACCAGGGATCAGTGGATGTTGCTTTTAGG 1019
P.assamicusUL      ACCGTCCTAGTCTCAACCATAAACGATGCCGACCAGGGATCAGTGGATGTTGCTTTTAGG 1020
P.bipinnatifidusDR ACCGTCCTAGTCTCAACCATAAACGATGCCGACCAGGGATCAGTGGATGTTGCTTTTAGG 1020
P.bipinnatifidusGK ACCGTCCTAGTCTCAACCATAAACGATGCCGACCAGGGATCAGTGGATGTTGCTTTTAGG 1020
  panax sp. BH     ACCGTCCTAGTCTCAACCATAAACGATGCCGACCAGGGATCAGTGGATGTTGCTTTTAGG 1020
  panax sp. DB     ACCGTCCTAGTCACAACCATAAACGATGCCGACCAGGGATCAGTGGATGTTGCTTTTAGG 1020
P.assamicusLK      ACCGTCCTAGTCTCAACCATAAACGATGCCGACCAGGGATCAGTGGATGTTGCTTTTAGG 1020
                   ********** ************************************************

P.assamicusSH      ACTCCACTGGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCA 1078
P.variabilisMA     ACTCCACTGGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCA 1079
P.assamicusUL      ACTCCACTGGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCA 1080
P.bipinnatifidusDR ACTCCACTGGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCA 1080
P.bipinnatifidusGK ACTCCACTGGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCA 1080
  panax sp. BH     ACTCCACTGGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCA 1080
  panax sp. DB     ACTCCACTGGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCA 1080
P.assamicusLK      ACTCCACTGGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGTATGGTCGCA 1080
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P.assamicusSH      AGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAA 1138
P.variabilisMA     AGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAA 1139
P.assamicusUL      AGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAA 1140
P.bipinnatifidusDR AGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAA 1140
P.bipinnatifidusGK AGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAA 1140
  panax sp. BH     AGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAA 1140
  panax sp. DB     AGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAA 1140
P.assamicusLK      AGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAA 1140
                   ************************************************************

P.assamicusSH      TTTGACTCAACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGACTGAGA 1198
P.variabilisMA     TTTGACTCAACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGACTGAGA 1199
P.assamicusUL      TTTGACTCAACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGACTGAGA 1200
P.bipinnatifidusDR TTTGACTCAACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGACTGAGA 1200
P.bipinnatifidusGK TTTGACTCAACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGACTGAGA 1200
  panax sp. BH     TTTGACTCAACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGACTGAGA 1200
  panax sp. DB     TTTGACTCAACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGACTGAGA 1200
P.assamicusLK      TTTGACTCAACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGACTGAGA 1200
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P.assamicusSH      GCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTG 1258
P.variabilisMA     GCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTG 1259
P.assamicusUL      GCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTG 1260
P.bipinnatifidusDR GCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTG 1260
P.bipinnatifidusGK GCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTG 1260
  panax sp. BH     GCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTG 1260
  panax sp. DB     GCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTG 1260
P.assamicusLK      GCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTG 1260
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P.assamicusSH      TCTGGTTAATTCCGTTAACGAACGAGACCTCAGCCTGCTAAATAGCTATGTGGAGGTATA 1318
P.variabilisMA     TCTGGTTAATTCCGTTAACGAACGAGACCTCAGCCTGCTAAATAGCTATGTGGAGGTATA 1319
P.assamicusUL      TCTGGTTAATTCCGTTAACGAACGAGACCTCAGCCTGCTAAATAGCTATGTGGAGGTATA 1320
P.bipinnatifidusDR TCTGGTTAATTCCGTTAACGAACGAGACCTCAGCCTGCTAAATAGCTATGTGGAGGTATA 1320
P.bipinnatifidusGK TCTGGTTAATTCCGTTAACGAACGAGACCTCAGCCTGCTAAATAGCTATGTGGAGGTATA 1320
  panax sp. BH     TCTGGTTAATTCCGTTAACGAACGAGACCTCAGCCTGCTAAATAGCTATGTGGAGGTATA 1320
  panax sp. DB     TCTGGTTAATTCCGTTAACGAACGAGACCTCAGCCTGCTAAATAGCTATGTGGAGGTATA 1320
P.assamicusLK      TCTGGTTAATTCCGTTAACGAACGAGACCTCAGCCTGCTAAATAGCTATGTGGAGGTATA 1320
                   ************************************************************

P.assamicusSH      CCTCCACGGCCAGCTTCTTAGAGGGACTATGGCCTTTTAGGCCACGGAAGTTTGAGGCAA 1378
P.variabilisMA     CCTCCACGGCCAGCTTCTTAGAGGGACTATGGCCTTTTAGGCCACGGAAGTTTGAGGCAA 1379
P.assamicusUL      CCTCCACGGCCAGCTTCTTAGAGGGACTATGGCCTTTTAGGCCACGGAAGTTTGAGGCAA 1380
P.bipinnatifidusDR CCTCCACGGCCAGCTTCTTAGAGGGACTATGGCCTTTTAGGCCACGGAAGTTTGAGGCAA 1380
P.bipinnatifidusGK CCTCCACGGCCAGCTTCTTAGAGGGACTATGGCCTTTTAGGCCACGGAAGTTTGAGGCAA 1380
  panax sp. BH     CCTCCACGGCCAGCTTCTTAGAGGGACTATGGCCTTTTAGGCCACGGAAGTTTGAGGCAA 1380
  panax sp. DB     CCTCCACGGCCAGCTTCTTAGAGGGACTATGGCCTTTTAGGCCACGGAAGTTTGAGGCAA 1380
P.assamicusLK      CCTCCACGGCCAGCTTCTTAGAGGGACTATGGCCTTTTAGGCCACGGAAGTTTGAGGCAA 1380
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P.assamicusSH      TAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGTATTC 1438
P.variabilisMA     TAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGTATTC 1439
P.assamicusUL      TAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGTATTC 1440
P.bipinnatifidusDR TAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGTATTC 1440
P.bipinnatifidusGK TAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGTATTC 1440
  panax sp. BH     TAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGTATTC 1440
  panax sp. DB     TAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGTATTC 1440
P.assamicusLK      TAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGTATTC 1440
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P.assamicusSH      AACGAGTCTATAACCTTGGCCGACAGGCCCGGGTAATCTTTGAAATTTCATCGTGATGGG 1498
P.variabilisMA     AACGAGTCTATAACCTTGGCCGACAGGCCCGGGTAATCTTTGAAATTTCATCGTGATGGG 1499
P.assamicusUL      AACGAGTCTATAACCTTGGCCGACAGGCCCGGGTAATCTTTGAAATTTCATCGTGATGGG 1500
P.bipinnatifidusDR AACGAGTCTATAACCTTGGCCGACAGGCCCGGGTAATCTTTGAAATTTCATCGTGATGGG 1500
P.bipinnatifidusGK AACGAGTCTATAACCTTGGCCGACAGGCCCGGGTAATCTTTGAAATTTCATCGTGATGGG 1500
  panax sp. BH     AACGAGTCTATAACCTTGGCCGACAGGCCCGGGTAATCTTTGAAATTTCATCGTGATGGG 1500
  panax sp. DB     AACGAGTCTATAACCTTGGCCGACAGGCCCGGGTAATCTTTGAAATTTCATCGTGATGGG 1500
P.assamicusLK      AACGAGTCTATAACCTTGGTCGACAGGCCCGGGTAATCTTTGAAATTTCATCGTGATGGG 1500
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P.assamicusSH       GATAGATCATTGCAATTGTTGGTCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAG 1558
P.variabilisMA      GATAGATCATTGCAATTGTTGGTCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAG 1559
P.assamicusUL       GATAGATCATTGCAATTGTTGGTCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAG 1560
P.bipinnatifidusDR  GATAGATCATTGCAATTGTTGGTCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAG 1560
P.bipinnatifidusGK  GATAGATCATTGCAATTGTTGGTCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAG 1560
  panax sp. BH      GATAGATCATTGCAATTGTTGGTCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAG 1560
  panax sp. DB      GATAGATCATTGCAATTGTTGGTCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAG 1560
P.assamicusLK       GATAGATCATTGCAATTGTTGGTCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAG 1560
                    ************************************************************ 1560

P.assamicusSH       CTCGCGTTGACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAAT 1618
P.variabilisMA      CTCGCGTTGACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAAT 1619
P.assamicusUL       CTCGCGTTGACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAAT 1620
P.bipinnatifidusDR  CTCGCGTTGACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAAT 1620
P.bipinnatifidusGK  CTCGCGTTGACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAAT 1620
  panax sp. BH      CTCGCGTTGACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAAT 1620
  panax sp. DB      CTCGCGTTGACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAAT 1620
P.assamicusLK       CTCGCGTTGACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAAT 1620
                    ************************************************************ 1620

P.assamicusSH       GGTCCGGTGAAGTGTTCGGATTGCGGCGACGTGGGCGGTTCGCTGCCCGCGACGTCGAAA 1678
P.variabilisMA      GGTCCGGTGAAGTGTTCGGATTGCGGCGACGTGGGCGGTTCGCTGCCCGCGACGTCGAAA 1679
P.assamicusUL       GGTCCGGTGAAGTGTTCGGATTGCGGCGACGTGGGCGGTTCGCTGCCCGCGACGTCGAAA 1680
P.bipinnatifidusDR  GGTCCGGTGAAGTGTTCGGATTGCGGCGACGTGGGCGGTTCGCTGCCCGCGACGTCGAAA 1680
P.bipinnatifidusGK  GGTCCGGTGAAGTGTTCGGATTGCGGCGACGTGGGCGGTTCGCTGCCCGCGACGTCGAAA 1680
  panax sp. BH      GGTCCGGTGAAGTGTTCGGATTGCGGCGACGTGGGCGGTTCGCTGCCCGCGACGTCGAAA 1680
  panax sp. DB      GGTCCGGTGAAGTGTTCGGATTGCGGCGACGTGGGCGGTTCGCTGCCCGCGACGTCGAAA 1680
P.assamicusLK       GGTCCGGTGAAGTGTTCGGATTGCGGCGACGTGGGCGGTTCGCTGCCCGCGACGTCGAAA 1680
                    ************************************************************ 1680

P.assamicusSH       GAAGTCCACTGAAC 1692
P.variabilisMA      GAAGTCCACTGAAC 1693
P.assamicusUL       GAAGTCCACTGAAC 1694
P.bipinnatifidusDR  GAAGTCCACTGAAC 1694
P.bipinnatifidusGK  GAAGTCCACTGAAC 1694
  panax sp. BH      GAAGTCCACTGAAC 1694
  panax sp. DB      GAAGTCCACTGAAC 1694
P.assamicusLK       GAAGTCCACTGAAC 1694
                    ************** 1694
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Clustal 2.1 Multiple Sequence Alignment for 18S-28S rDNA ITS region

CLUSTAL 2.1 multiple sequence alignment

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P.assamicusLK           GTCGAAACCTGCATAGCAGAACGACCCGCGAACACGTTACACTACCAGGTGAGGGACGAG 60
P.assamicusUL           GTCGAAACCTGCATAGCAGAACGACCCGCGAACACGTTACACTACCAGGTGAGGGACGAG 60
P.assamicusSH           GTCGAAACCTGCATAGCAGAACGACCCGCGAACACGTTACACTACCAGGTGAGGGACGAG 60
P.bipinnatifidusDR      GTCGAAACCTGCATAGCAGAACGACCCGCGAACACGTTACACTACCAGGTGAGGGACGAG 60
P.bipinnatifidusGK      GTCGAAACCTGCATAGCAGAACGACCCGCGAACACGTTACACTATCGGGTGAGGGACGAG 60
P.variabilisMA          GTCGAAACCTGCATAGCAGAACGACCCGCGAACACGTTACACTATCGGGTGAGGGACGAG 60
  panax sp. DB          GTCGAAACCTGCATAGCAGAACGACCCGCGAACACGTTACACTACCGGGTGAGGGACGAG 60
  panax sp. BH          GTCGAAACCTGCATAGCAGAACGACCCGCGAACACGTTACAATACCGGGTGAGGGACGAG 60
                        GTCGAAACCTGCATAGCAGAACGACCCGCGAACACGTCACAATACCGGGTGAGGGACGAG 60
                        ************************************ *** ** * ************

P.assamicusLK           GGGTGCGCAAGCTCCCCAAGTTTCAAACCCATGGTCGGGGACCACCCTTGGGTGGCTCTC 120
P.assamicusUL           GGGTGCGCAAGCTCCCCAAGTTTCAAACCCATGGTCGGGGACCACCCTTGGGTGGCTCTC 120
P.assamicusSH           GGGTGCGTAAGCTCCCCAAGTTTCAAACCCATGGTCGGGGACCACCCTTGGGTGGCTCTC 120
P.bipinnatifidusDR      GGGTGCGCAAGCTCCCCAAGTTGCAAACCCATGGTCGGGGACCACCCTTGGGTGGCTCTC 120
P.bipinnatifidusGK      GGGTGCGCAAGCTCCCCAAGTTGCAAACCCATGGTCGGGGACCACCCTTGGGTGGCTCTC 120
P.variabilisMA          GGGTGCGCAAGCTCCCCAAGTTGCAAACCCATGGTCGGGGACCACCCTTGGGTGGCCCTC 120
  panax sp. DB          GGATGCGCAAGCTCCCCGAGTTGCAAACCCATGGTCGGGGACCGCCCTTGGGTGGCTCTC 120
  panax sp. BH          GGATGCGCAAGCTCCCCGAGTTGCAAACCCATGGTCGGGGACCGCCCTTGGGTGGCTCTC 120
                        ** **** ********* **** ******************** ************ ***

P.assamicusLK           GTCCGAACAACGACCCCCCGGCGCGGAATGCGCCAAGGAAATCAAACTGAACTGCACGCG 180
P.assamicusUL           GTCCGAACAACGACCCCCCGGCGCGGAATGCGCCAAGGAAATCAAACTGAACTGCACGCG 180
P.assamicusSH           GTCCGAACAACGACCCCCCGGCGCGGAATGCGCCAAGGAAATCAAACTGAACTGCACGCG 180
P.bipinnatifidusDR      GTCCGAACAACGACCCCCCGGCGCGGAATGCGCCAAGGAAATCAAACTGAACTGCACGCG 180
P.bipinnatifidusGK      GTCCGAACAACGACCCCCCGGCGCGGAATGCGCCAAGGAAATCAAACTGAACTGCACGCG 180
P.variabilisMA          GTCCGAACAACGACCCCCCGGCGCGGAATGCGCCAAGGAAATCAAACTGAACTGCACGCG 180
  panax sp. DB          GTCCGAACAACGACCCCCCGGCGCGGAATGCGCCAAGGAAATCAAACTGAACTGCACGCG 180
  panax sp. BH          GTCCGAACAACGACCCCCCGGCGCGGAATGCGCCAAGGAAATCAAACTGAACTGCACGCG 180
                        ************************************************************

P.assamicusLK           TCCCCCCCCGTTTGCGGGCGGCGGAAGCGTCTTTCTAAAACACAAACGACTCTCGGCAACG 240
P.assamicusUL           TCCCCCCCCGTTTGCGGGCGGCGGAAGCGTCTTTCTAAAACACAAACGACTCTCGGCAACG 240
P.assamicusSH           TCCCCCCCCGTTTGCGGGCGGCGGAAGCGTCTTTCTAAAACACAAACGACTCTCGGCAACG 240
P.bipinnatifidusDR      TCCCCCCCCGTTTGCGGGCGGCGGAAGCGTCTTTTTAAAACACAAACGACTCTCGGCAACG 240
P.bipinnatifidusGK      TCCCCCCCCGTTTGCGGGCGGCGGAAGCGTCTTTTTAAAACACAAACGACTCTCGGCAACG 240
P.variabilisMA          TCCCCCCCCGTTTGCGGGCGGCGGAAGCGTCTTTCTAAAACACAAACGACTCTCGGCAACG 240
  panax sp. DB          TCCCCCCCCGTTTGCGGGCGGCGGAAGCGTCTTTCTAAAACACAAACGACTCTCGGCAACG 240
  panax sp. BH          TCCCCCCCCGTTTGCGGGCGGCGGAAGCGTCTTTCTAAAACACAAACGACTCTCGGCAACG 240
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P.assamicusLK           GATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTG 300
P.assamicusUL           GATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTG 300
P.assamicusSH           GATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTG 300
P.bipinnatifidusDR      GATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTG 300
P.bipinnatifidusGK      GATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTG 300
P.variabilisMA          GATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTG 300
  panax sp. DB          GATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTG 300
  panax sp. BH          GATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTG 300
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P.assamicusLK           CAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGA 360
P.assamicusUL           CAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGA 360
P.assamicusSH           CAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGA 360
P.bipinnatifidusDR      CAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGA 360
P.bipinnatifidusGK      CAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGA 360
P.variabilisMA          CAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGA 360
  panax sp. DB          CAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGA 360
  panax sp. BH          CAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGA 360
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P.assamicusLK          GGGCACGTCTGCCTGGGCGTCACACATCGCGTCGCCCCCCCAACCCATCACTCCCTTGCGG 420
P.assamicusUL          GGGCACGTCTGCCTGGGCGTCACACATCGCGTCGCCCCCCCAACCCATCACTCCCTTGCGG 420
P.assamicusSH          GGGCACGTCTGCCTGGGCGTCACACATCGCGTCGCCCCCCCAACCCATCACTCCCTTGCGG 420
P.bipinnatifidusDR     GGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCAACCCATCACTCCCTTGCGG 420
P.bipinnatifidusGK     GGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCAACCCATCACTCCCTTGCGG 420
P.variabilisMA         GGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCAACCCATCACTCCCTTGCGG 420
  panax sp. DB         GGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCAACCCATCACTCCCTTGCGG 420
  panax sp. BH         GGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCCAACCCATCACTCCCTCGCGG 420
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P.assamicusLK          GAGTTGAGGCGGAGGGGCGGATAATGGCCTCCCGTGTCTCACCGCGCGGTTGGCCCAAAT 480
P.assamicusUL          GAGTTGAGGCGGAGGGGCGGATAATGGCCTCCCGTGTCTCACCGCGCGGTTGGCCCAAAT 480
P.assamicusSH          GAGTTGAGGCGGAGGGGCGGATAATGGCCTCCCGTGTCTCACCGCGCGGTTGGCCCAAAT 480
P.bipinnatifidusDR     GAGTTGAGGCGGAGGGGCGGATAATGGCCTCCCGTGTTTCATTGCGCGGTTGGCCCAAAT 480
P.bipinnatifidusGK     GAGTTGAGGCGGAGGGGCGGATAATGGCCTCCCGTGTTTCATTGCGCGGTTGGCCCAAAT 480
P.variabilisMA         GAGTCGAGGTGGAGGGGCGGATAATGGCCTCCCGTGTCTCACCGCGCGGTTGGCCCAAAT 480
  panax sp. DB         GAGTCGAGGCAGAGGGGCGGATAATGGCCTCCCGTGTCTCACCGCGCGGTTGGCCCAAAT 480
  panax sp. BH         GAGTCGAGGCAGAGGGGCGGATAATGGCCTCCCGTGTCTCACCGCGCGGTTGGCCCAAAT 480
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P.assamicusLK          GCGAGTCCTTGGCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATG 540
P.assamicusUL          GCGAGTCCTTGGCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATG 540
P.assamicusSH          GCGAGTCCTTGGCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATG 540
P.bipinnatifidusDR     GCGAGTCCTTGGCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATG 540
P.bipinnatifidusGK     GCGAGTCCTTGGCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATG 540
P.variabilisMA         ACGAGTCCTTGGCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATG 540
  panax sp. DB         GCGAGTCCTTGGCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATG 540
  panax sp. BH         GCGAGTCCTTGGCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATG 540
                        ************************************************************

P.assamicusLK          TCGTGCGGTGACCCGTCGCCAGCAAAATCTCTCATGACCCTGTTGCGCCGTCCTCGACGC 600
P.assamicusUL          TCGTGCGGTGACCCGTCGCCAGCAAAATCTCTCATGACCCTGTTGCGCCGTCCTCGACGC 600
P.assamicusSH          TCGTGCGGTGACCCGTCGCCAGCAAAAGCTCTCATGACCCTGTTGCGCCGTCCTCGACGC 600
P.bipinnatifidusDR     TCGTGCGGTGACCCGTCGCCAGCAAAAGCTCTCATGACCCTGTTGCGCCGTCCTCGACGC 600
P.bipinnatifidusGK     TCGTGCGGTGACCCGTCGCCAGCAAAAGCTCTCATGACCCTGTTGCGCCGTCCTCGACGC 600
P.variabilisMA         TCGTGCGGTGACCCGTCGCCAGCAAAAGCTCTCATGACCCTGTTGCGCCGTCCTCGACGC 600
  panax sp. DB         TCGTGCGGTGACCCGTCGCCAGCAAAAGCTCTCATGACCCTGTTGCGCCGTCCTCGACGC 600
  panax sp. BH         TCGTGCGGTGACCCGTCGCCAGCAAAAGCTCTCATGACCCTGTTGCGCCGTCCTCGACGC 600
                       ************************** ********************************

P.assamicusLK          GCGCTCCGACCGCGACC-- 617
P.assamicusUL          GCGCTCCGACCGCGACC-- 617
P.assamicusSH          GCGCTCCGACCGCGACC-- 617
P.bipinnatifidusDR     ACGCTCCGACCGCGACCCC 619
P.bipinnatifidusGK     ACGCTCCGACCGCGACCCC 619
P.variabilisMA         GCGCTCCGACCGCGACC-- 617
  panax sp. DB         GCGCTCCGACC-------- 611
  panax sp. BH         GTGCTCCGACCGCGACCCC 619
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BIO-DATA

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Educational Qualifications:

Sl. No.	Examination Passed	Name of Board/ University	Year of Passing		
1.	H.S.L.C	M.B.O.S.E.	1999	60.36	I
2.	H.S.S.L.C	M.B.O.S.E.	2001	56.2	II
3.	B.Sc. (Bot. Hons.)	N.E.H.U.	2004	70.13	I
4.	M.Sc. (Botany)	N.E.H.U.	2006	67.40	I

Seminars and Symposiums Attended

1. National Symposium on "*Frontiers in Biocomplexity and Biodiversity of plants*" organized by Centre for Advanced Studies in Botany, North-Eastern Hill University, Shillong-793022 on 14th -15th March, 2008.

2. The 96th Session of the "*Indian Science Congress*", held at North–Eastern Hill University, Shillong from 3rd –7th January, 2009.

Workshops and Training Courses

1. Training programme on "*Capacity Building training in Plant Taxonomy*" organized at Botanical survey of India, Eastern Circle, Shillong from 3rd -15th November, 2008.

2. Completed one month training on "*Analysis of Ginsenosides using HPLC*" from 2nd - 30th November, 2009 at FRLHT, Bangalore.

3. Attended a four day workshop on "*High performance computing applications in physical, environmental, and life sciences and bioinformatics*", held at North–Eastern Hill University, Shillong from 3rd –6th March, 2009. Jointly organized by North–Eastern Hill University, Shillong and funded by the Centre for Development of Advanced Computing (CDAC), Pune.

4. Completed one month "*Short term training course in data analysis using excel*" from August, 2010 till September, 2010 conducted by Computer Centre, North–Eastern Hill University, Shillong, Meghalaya.

5. Attended a workshop on "*Preventing extinction and improving conservation status of threatened plants through application of biotechnological tools*", held at North–Eastern Hill University, Shillong from 17th – 18th March, 2011. Organized by Centre For Advanced Studies in Botany, NEHU, Shillong and Department of Biotechnology, Government of India, New Delhi.

6. Participated in the 18th DBT-Sponsored Training Course on "*Bioinformatics: a practical Approach in Genomics and Proteomics*" conducted by the Bioinformatics Centre, North-Eastern Hill University, Shillong from 26th -29th September, 2011.

7. Training programme on "*Ecological Niche Modelling*" organised by Department of Botany, Shillong, from 21st-23rd May, 2014 under DBT's All India Coordinated Research Project.

Technical Reports

Co-authored Final Technical Report of DBT project on "Conservation of *Panax* species in north-east India: Characterization of niche and genetic variability, mapping of potential distributional areas, establishment of field gene bank and micropropagation", submitted to DBT.

DECLARATION

I Ms. Lucy Badaplin Nongbri, do hereby declare that the information furnished above is true and correct to the best of my knowledge and belief.

Date: 8.09.2014
Place: SHILLONG

Nongbri
Signature