FINAL TECHNICAL REPORT
OF
THE PROJECT

DEVELOPMENT OF SUSTAINABLE CROPPING OF GINSENG AND THALICTRUM IN MANIPUR.

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CHAPTER – I

INTRODUCTION

1.1 Plant Material

Panax pseudoginseng Wallich commonly known as Ginseng belongs to the family Araliaceae and *Thalictrum foliolosum* D.C.(Mao- *Ivei Koraio*, Manipuri- *Umang peruk*) of the family Ranunculaceae are the two plants we have been engaged with the project. Their distribution is restricted only in Senapati and Ukhrul districts of Manipur (Deb, 1961). The two plants are highly prized in these areas because its dried root is tonic, adaptogenic, aphrodisdiac, stimulant, expectorant and antipyretic used in dyspepsia, vomiting, etc. In medical field, Ginseng is well known for its activities associated with neurology and aging with poor scientific explanation. Shiva and Shreshtra (1986) worked on medicinal plants for ecodnomic exploitation in Eastern Himalayas and reported that there are four *Panax* species (*P. pseudoginseng, P. sikkimensis, P. assamanica* and *P. quinkuifolius*) which occur in Eastern Himalayas and are substitute of European and American Ginseng and *P. ginseng* of China.

Deori (1986) reported the distribution of important medicinal plants in the temperate regions of Arunachal Pradesh. He added that *Panax pseudoginseng* is distributed naturally from 2000 to 2700 metres above the sea level in the places like Tale Valley in Subansiri district, Tawang in Kameng district, Mellinja-Hot spring area in Lohit district.

Ginseng is very much familiar in all the countries of our globe. Some plants with more or less similarities with Ginseng are also named as Ginseng e.g. *Trichopus zeylanicus* sub species (Ssp), *travancoricus*-Indian Ginseng, *Eleutherococcus cissifolius* (Griff. Ex Seem); Nakai-belong to the family Araliaceae, local name *Kosamot siio* (Mao) a small tree, leaves are used for stomach disorder. It is also known as Siberian Ginseng and possesses similar adaptogenic properties of Ginseng (Sinha, 1996)

P. pseudoginseng Wall. Synonymous to *Aralia pseodoginseng* Benth was first reported by Mukherjee from Siroi Hill, Ukhrul. The plant was also reported by Deb (1961) from the same place.

As local people are uprooting the very Ginseng plants and unproperly utilized it elsewhere, it is now become a threat to its survivability to their natural habitat. Keeping in views the above mentioned facts conservation of these plants is important and one of the most effective measure would be its sustainable farming through mass propagation with the help of micro propagation (meristem culture techniques).

1.2 History of Ginseng Plant

Ginseng is one of the best known Chinese herbs. Its common name comes from the Chinese word renshen - meaning man root, after the shape of its thick taproot. The almost magical properties ascribed to ginseng in the west, together with high the price of the best quality herb, have led to much poor quality and diluted ginseng being sold to the public. Korean ginseng is said to be stronger than the Chinese variety. American ginseng, *P. quinquefolium*, lacks some of the key properties of the Oriental species, while *Eleutherococcus senticosus*, or Siberian ginseng, which grows in Russia, has been shown to have similar properties to the Oriental types although it is not a ginseng itself.

In some country like China, the best known herb is Ginseng and it has been featured as ancient tonic herb for over 3000 years. It was known is Europe from the 9th century onwards, notably when presented to Luis XIV (1638-1715) by the King of Siam, but only became widely used in the West as a result of Soviet research into "adaptogens" the 1950s. *Panax ginseng*, a slow growing woodland perennial with alimited natural distribution, was near extinct in the wild by the 19th century. Other two related plants American ginseng (*Panax quinquefolius*) and Siberian ginseng (*Eleutherococcus sentiocossus*) were discovered in the 18th century and 1950s respectively. Surprisingly, *Eleutherococcus* was found similar in its constituents with ginseng plants.

It is an ancient Taoist tonic herb, which has been used as a vital essence. In Chinese word, *qi* means a tonic in Chinese medicine. It was introduced in Europe several times from the ninth century onward but assumed no importance in eastern medicine until studies by Soviet scientists in the early 1950s established it as an "adaptogen". To increase availability of the drug, they also searched for similar properties in related native species and discovered *Eleutherococcus senticossus* (Siberian ginseng). There is report for the use of this plant as a healing herb by the Vietcong during the Vietnam War to improve recovery from gunshot wounds. In medical literature, confusion exists between *P. pseudoginseng* and *P. noltoginseng*, and whether or not they do differ notably in constituents is not clear. The main medicinal species are now rare in the wild, and are cultivated commercially in Korea, China, Russia, and the USA. Some ginseng like Brazilian ginseng which contain up to 11 per cent saponins, including pfaffic acid and its derivatives had been patented as anti-tumour drugs. The ground root is taken as a tonic, especially during convalescence and the menopause. Yet, its ability to improve stamina and resistance to stress become common knowledge and has always been valued das a tonic in old age.

1.3 Medicinal properties of ginseng plant and its related species

Used internally for convalescence, menopausal complaints, geriatric debility, physical and mental stress, and insomnia caused by prolonged anxiety. Used in the background treatment of cancer And exposure to tonic chemicals and radiation, and to improve resistance to infection. Not given to children, or taken for longer than three weeks at a time. Contraindicated with caffeine. Several related species have been used for rheumatic complaints, low vitality, and weak liver and kidney energy for d2000 years in Chinese medicine. *Eleutherococcus senticosus* has less or milder action than *P. ginseng*.

Used internally for debility associated with old age or illness, lack of appetite, insomnia, stress, shock, and chronic illness. An ingredient in many important Chinese formulae, also taken as a simple (i.e. not mixed with other herbs), often as a yang tonic before winter or a period of great stress. Also used for coronary heart disease and angina (roots), dizziness, and vertigo (flowers). Internally and externally for nose bleed, and haemorrhage from lungs, digestive tract, uterus, or injuries (root). Not usually prescribed for pregnant woman or patient under 40 years old, or with depression, anxiety, or acute inflammatory disease. Use is normally restricted to three weeks. Excess may cause headaches, restlessness if taken with caffeine, alcohol, turnips, and bitter or spicy foods. The Chinese used Ginseng for lack of appetite, forgetfulness, worry, palpitations, insomnia, sweating and general weakness. **Caution**:- should not be used for more than three weeks and should not be taken when there is acute inflammatory disease, or for depression and anxiety.

A sweet (slightly bitter), tonic herb that both relaxes and stimulates the nervous system, adrenaline like improved muscle stamina, heart tonic, controls bleeding, improve circulation, relieve pain, anti bacterial effect, encourages secretion of hormones, improves stamina, increases resistant to disease (stimulate immune), regulate blood pressure, lowers blood sugar, and reduces inflammation. It is adaptogenic, having a toxic effect on almost all organs. In short, Ginseng is used as a remedy for improving health after illness, in old age, for general weakness, and for stress. It is also considered useful for improving stamina over short periods.

Ginseng has remarkable adaptogenic properties or quality i.e., helping the body adapt to stress, fatigue and cold. Help people to adapt either by supporting the nervous system and easing nervous and emotional tension, or by maintaining health. Ginseng also has an effective remedy at times of great mental or physical stress, but in certain cases can also be taken when a relaxing effect is required, for example to relieve headaches, or to ensure a good night's sleep. Trials show that ginseng significantly improves the body's capacity to cope with hunger, extremes of temperature, and mental and emotional stress. Furthermore, ginseng produces a sedative effect

when the body requires sleep. The ginsenosides which are responsible for this action are similar in structure to the body's own stress hormones. Ginseng also found to increases immune function i.e., resistance to infection and improved liver function.

1.4 Therapeutic actions

As an adaptogenic, the action of ginseng varies. It has a stimulating effect on young people with *qi* (a Chinese word denotes the vital force or human vitality for keeping good health and longevity). It is tonic, restorative and even sedative for those weakened by illness or old age.

In China, ginseng is best known as a stimulant, tonic herb for athletes and those subject to physical stress, and as a male aphrodisiac. It is also a tonic for old age, and is traditionally taken by people in northern and central China from late middle age onwards, helping them endure the long hard winters.

In Western countries ginseng is viewed not so much as a medicine, but as a life-enhancing tonic. It is useful for those coping with stressful events, such as taking exams, ginseng is often abused in the West and should not be taken for more than 6 weeks.

Root is harvested after 4 years (the plant takes at least four years to mature), when the active constituents are most concentrated. In China, dried root is chewed to provide an energy boost. In the form of capsule, for nervous exhaustion, take a 500mg capsule one a day (do not exceed the dose, which can cause insomnia and high blood pressure) and do not take for more than 6 weeks. Avoid caffeine while taking ginseng. Do not take if pregnant. Soup is a common way of taking ginseng in China. Add 1g dried root per portion of vegetable soup and take daily.

1.5 Approved Objectives of the project:

a) Collection tours to Chandel, Ukhrul and Senapati District.
b) Determination of agrotechniques for pot culturing of the mericlones.
c) Meristem culturing of the plant species for mass production of plantlets.
d) Establishement of mericlones in pots and further mericloning of export potential plants for mass production of plantlets.
e) One week training programme of 100 scheduled tribe unemployed youths for production of plantlets.

1.6 Additional Works

1) Soil analysis of both the natural soil of the plant growing area and the artificial soil in which pot culture experiments carried out.
2) Biochemical analysis of the Ginseng and Thalictrum plant (leaves & root).
3) Identification of active compounds and its characterisation.

CHAPTER – II

SURVEY AND COLLECTION OF PLANTS

2.1 District wise survey of Plants

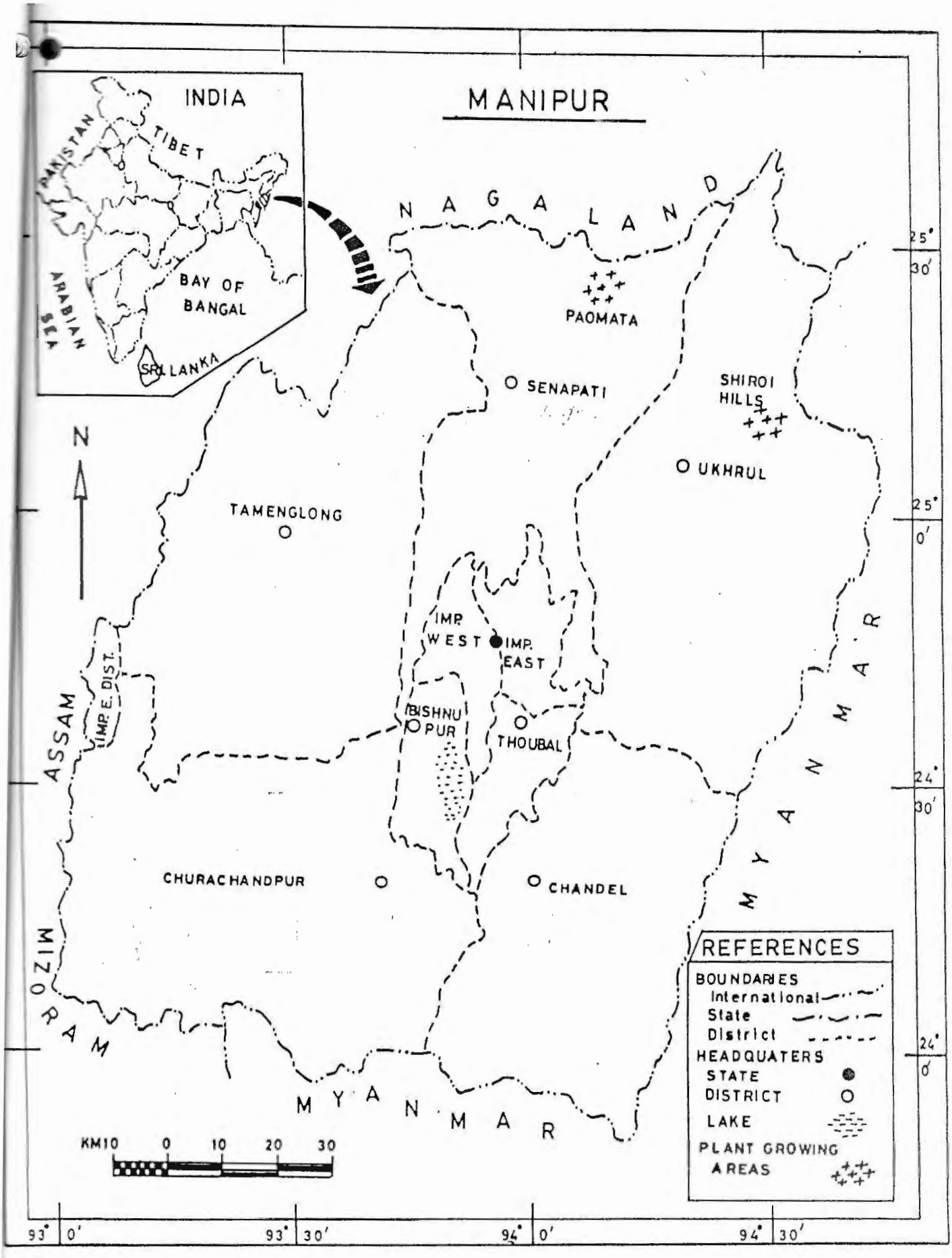
2.1.1 Senapati District

Montane wet temperate forests found in Mao and Koubru areas of Senapati District are favourable for the growth of Ginseng and Thalictrum plants in natural condition. The altitude ranges from about 1800-2400 m above the mean sea level and contain evergreen species like oak and pine trees. Two surveys had been made at Senapati Dist. (Fig 1&2). The first survey was in the 1st week of April '99 at Mao (Senapati Dist.) and we have collected ***Thalictrum*** plants along with seeds and about one kg. Weight Ginseng roots. In the second trip during the month of August 1999, a detail survey was made at the place where Ginseng grows naturally and collected about 50 small plants from their natural habitat. Some spot analysis were done at Senapati District namely: a) plant morphology, b) Phytosociology, c) environmental aspects, d) soil analysis, e site survey and mapping of the area etc.

2.1.2 First survey at Mao (4th April 1999)

Survey team comprising of 7 (seven) people including Principal Investigator, Coordinator and project staff proceeded to Mao, Senapati District for plant collection on 4th April 1999. Mao which is having 108 km. from Imphal is also one of the most thickly populated areas in the district. With the help of one porter, we went inside the forests at Okhro Ikhro where *Thalictrum* grows wildly. Few plants along with nearby sample soils were collected. After that, we went straight to Paomata, a place that is about 10 km. from Mao for collecting Ginseng and ***Thalictrum*** plants. Another porter from the area guided us to the place where Ginseng grows naturally. As Ginseng generally starts appearing from the month of April-May and it continues growing till September-October, we could not locate any Ginseng plant in the area. However, we collected Ginseng roots that were earthen artificially by the villagers in order to prevent from drying. We took if for tissue/pot culture purposes. Out of the collected specimens, some are transferred to laboratory pots and some are preserved in 5% formalene.

The local villagers have been aware about Ginseng since last year. The story is like this "local students studying at Kohima found people selling Ginseng roots in the market for medicinal purposes, then they identified it and start collecting the same Ginseng growing wildly from the local area and send it to Kohima for Rs. 50 to 130 per kg. At present, there is a high demand so the price hiked up to Rs. 1000 for One Kg. Since then, local villagers started collecting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UKHRUL
TAMENGLONG
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LAKE
PLANT GROWING AREAS

Fig. 1.

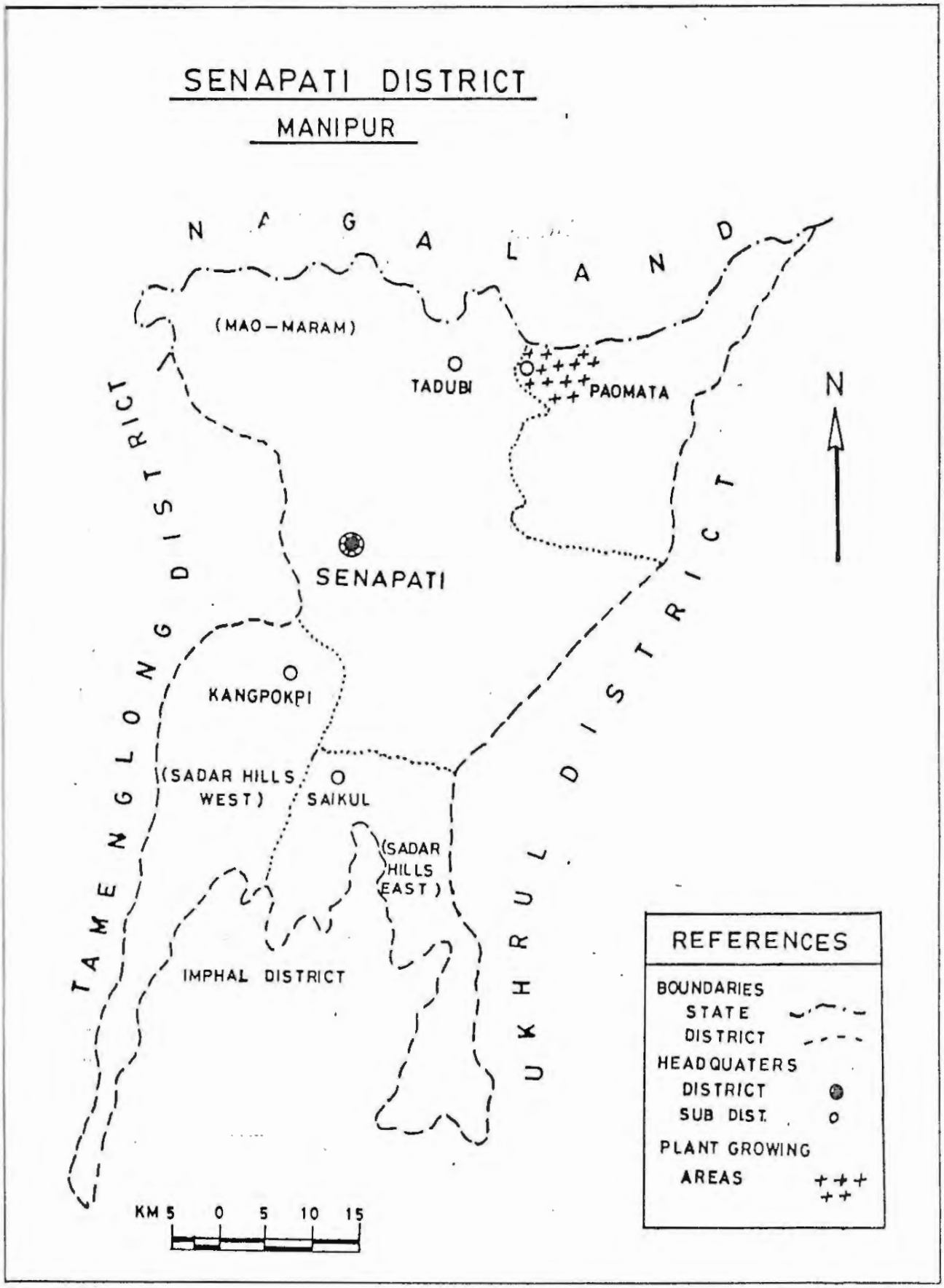
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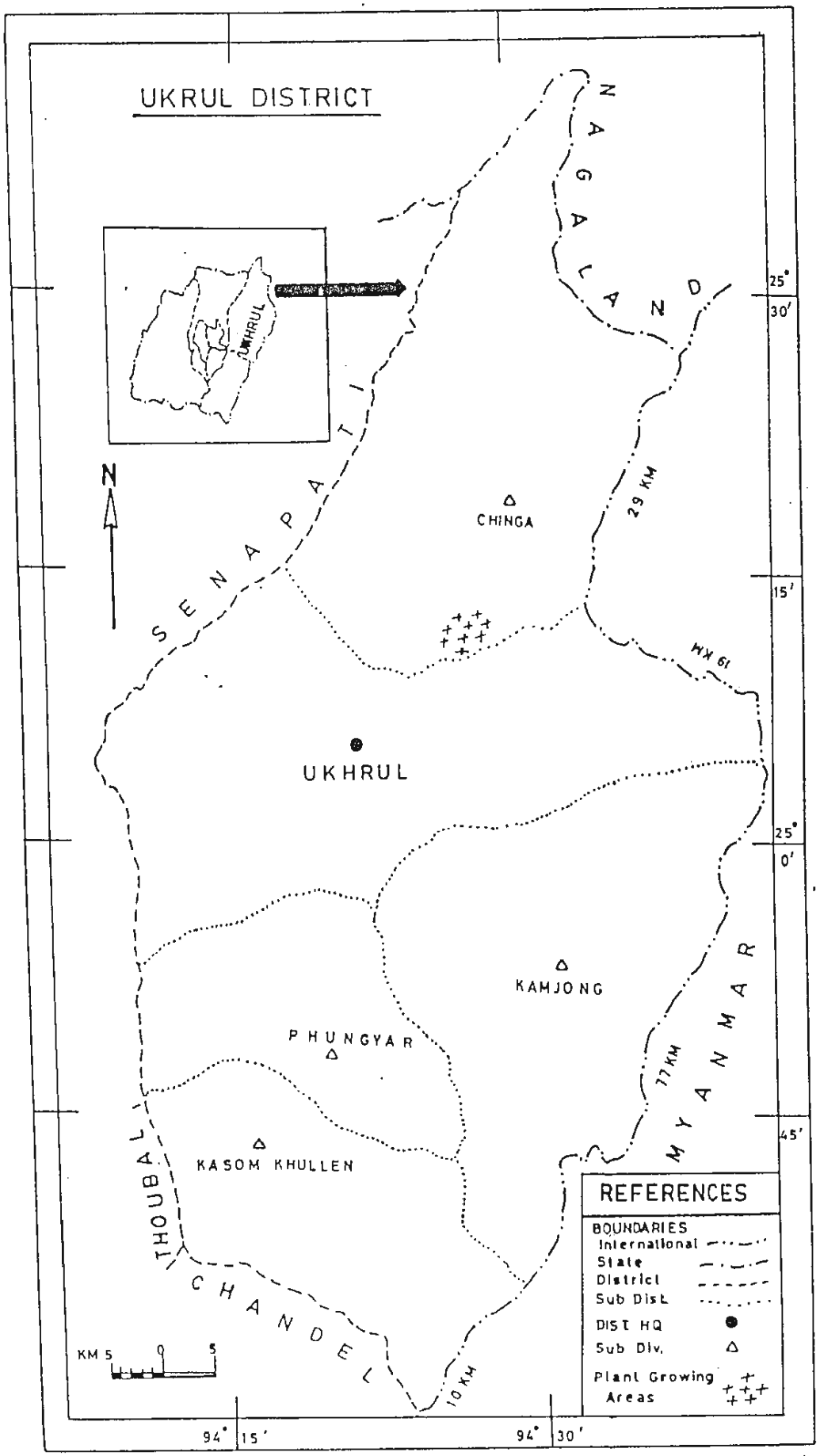
Fig. 2.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KAMJONG
PHUNGYAR
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REFERENCES
BOUNDARIES
International
State
District
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Plant Growing Areas

Fig. 3.

wild Ginseng roots and kept it very safely even by digging earth to avoid drying. They start hunting for Ginseng from nearby hilly areas. Khunjao, Oinam, Purum, Thenba Khulen and Koide are the some other areas where the plant grows naturally.

Villagers believe that Ginseng has got medicinal value for about 39 diseases and the root extract of ***Thalictrum*** is also use as tonic, opthelmia (eye problem), diuretic and purgative. ***Eleutherococcus*** leaves are used for stomach disorder and the same property as that of Ginseng.

2.1.3 Seconds survey at Mao (August 1999)

A team of seven people including two project staffs a field assistant and other research scholars of Life Sciences Department, Manipur University went to Mao, Senapati District (Fig. 1) for plant collection on 3rd August, 1999. Mao which is 108 km north from Imphal is having moderately cold climatic conditions. Due to the favourable climatic conditions, a large number of medicinal plants grows naturally (a list is given in the Table No. 9).

Survey at Okhro Ikhro (Mao) for Thalicdtrum plant was able to collect enough plants for experiments. Spot study at the site revealed that other medicinally reported plants grows around thalictrum plants. Those plants along with soil sample were also collected for further study. Mostly Thalictrum plants are found din the plain area, even in the Datuley hill, the plants are found in the areas where there are slightly plain. The soil where Thalictrum grows is of loamy type, slightly dry than that of Ginseng. So the relative abundance for Thalictrum plant is about one plant is every three-diameter area. The plant size ranges from a few centimetres up to four feet tall. Unlike that of Ginseng Thalictrum plant grows sparsely in the area. It may be due to the dispersal of seed by wind for propagation. Mostly these plants are found in shady areas under thick shrubs. Sometimes at the side of large rocks these plants are found. Fortunately, we got four feet tall Thalictrum plant in the Datuley hill.

Next day, we visited Paomata (Fig 1&2) for collection of Ginseng. At the Paomata Hill, we could not locate any ginseng plant. We proceeded up to Datuley Hill that is about 1 Km. from Paomata Bazar. About 200-metre diameter area we hunted for Ginseng plant and collected dearly 50 small plants uprooted from their mother soil along with the rhizome. Approximate abundance of Ginseng plant is about one in every four metre diameter area. The biggest plant we got has 30 cm. Height from the ground and having 10 gram rhizome weight.

The abundance of Ginseng is very peculiar in the sense that once the plant is found in the specific side others are also found around the plant. And that might be because of vegetative propagation of the plant by the rhizome. The soil is also of loamy type and moist having very high

water content (Table –8). The place was also shady and deeply under the trees. Ginseng plant is always abundant in the western side of the hillock where there is lack of sunlight.

All the plants we have collected from Mao were kept overnight in a moist condition in order to maintain the fresh viability of the plant. Next day early morning we prepared two wooden culture beds of dimensions 3'x2'x ½' and an artificial soil having composition of sandy soil and leaf mould in the ratio (1: 1). We then transferred all the collected plants in the culture bed as well as in ten different culture pots.

Table No.1- To determine the abundance and density of Ginseng plant (Quadrate Size = 10 x 10 m2)

Sl. No.	No. of quadrate	No. of individual plant/quardrate	No. of quadrate in which Sps. occurred	Total No. of quadrate studied	Density[1]	Abundance[2]
1.	1st	2				
2.	2nd	Nil				
3.	3rd	6				
4.	4th	5				
5.	5th	Nil				
6.	6th	5				
7.	7th	6				
8.	8th	Nil				
9.	9th	10				
10.	10th	5	11	15	50/15=4.45	50/11=4.7
11.	11th	2				
12.	12th	5				
13.	13th	2				
14.	14th	Nil				
15.	15th	2				

1. Density = $\frac{\text{Total No. of individuals of a species}}{\text{Total No. of quadrate studied}}$

2. Abundance = $\frac{\text{Total No. of individual of a species}}{\text{Total No. of quadrate of occurrence}}$

Table No. 2- To determine the abundance and density of *Thalictrum* plant

Sl. No	No.of quadrate studies	No.of individual plant/quadrate	No.of quadrate in which Sps. occurred	Total No. of quadrate studied	Density [1]	Abundance [2]
1.	1st	2				
2.	2nd	1				
3.	3rd	2				
4.	4th	1				
5.	5th	2				
6.	6th	2				
7.	7th	2				
8.	8th	2				
9.	9th	1				
10	10th	Nil	14	15	20/14 = 1.43	20/15 = 1.3
11.	11th	1				
12.	12th	1				
13.	13th	1				
14	14th	1				
15	15th	1				

1. Density = $\frac{\text{Total No. of individuals of a species}}{\text{Total No. Of quadrate studied}}$

2. Abundance = $\frac{\text{Total No. Of individual of a species}}{\text{Total No. Of quadrate of occurrence}}$

Table: 3 - Plant Distribution

Sl.No.	Place	Ginseng Plant	Thalictrum Plant
1.	Okhro Ikhro	Nil	17
2.	Datuley hill	50	3
3.	Paomata hill	Nil	Nil

Table: 4 - Plant height of Ginseng

Sl.No.	Height (cm)	Number of Plants
1.	10	5
2.	12	6
3.	14	4
4.	16	3
5.	18	2
6.	20	Nil
7.	22	12
8.	24	8
9.	26	8
10	28	Nil
11.	30	2

Table: 5- Plant Height of Thalictrum

Sl.No.	Height (cm)	No of Plants
1.	20	3
2.	40	2
3.	60	8
4.	80	3
5.	100	2
6.	120	2

Table: 6 - Length of Ginseng Rhizome

Sl. No.	Length (cm)	No of Rhizome
1.	0 - 2	2
2.	2 - 4	5
3.	4 - 6	8
4.	6 - 8	21
5.	8 - 10	10
6.	10 - 12	4

Table: 7- Length of Thalictrum root

Sl. No.	Plant Number	Total No. Of Roots	Length (2 - 6) cm	Length (6 - 10) cm	Length (10 - 14) cm
1.	1st	7	2	5	0
2.	2nd	6	2	2	2
3.	3rd	4	1	2	1
4.	4th	4	2	0	2
5.	5th	6	4	2	0
6.	6th	5	2	2	1
7.	7th	4	0	2	2
8	8th	7	2	4	1
9	9th	2	2	0	0
10	10th	5	3	2	0
11.	11th	2	2	0	0
12.	12th	7	2	3	2
13.	13th	10	4	2	4
14.	14th	2	2	0	0
15.	15th	5	4	1	0
16.	16th	3	2	1	0
17.	17th	2	1	0	1
18.	18th	5	2	3	0
19.	19th	3	0	2	1
20.	20th	8	6	1	1

Table : 8- List of some common plants growing intermixed with Ginseng and Thalictrum.

Sl. No.	Name of Plant	Family
1.	*Pratia nummularia*	Lobeliaceae
2.	*Fragaria* sp.	Rosaceae
3.	*Polygonum* sp.	Polygonaceae
4.	*Ploygonum* sp. (*Wakhei yendem*)	-do-
5.	*Xnthoxylum* sp.	Rutaceae
6.	*Heracleum* sp.	Apiaceae
7.	*Eupatorium adenophorum*	Compositae
8.	*Artemisia* sp.	-do-
9.	*Commelina* sp.	Commelinaceae
10.	*Oxalis corniculata*	Oxalidaceae
11.	*Curcuma* sp.	Zingiberaceae
12.	*Vanda* sp.	Orchidaceae
13.	*Smilax* sp.	Liliaceae
14.	*Selaginella* sp.	Selaginellaceae
15.	*Houttuynia cordata*	Saururaceae
16.	*Paederia foetida*	Rubiaceae
17.	*Impatiens* sp.	Balsaminaceae
18.	*Leucas* sp.	Lamiaceae
19.	*Begonia* sp.	Begoniaceae
20.	*Drymaria cordata*	Caryophyllaceae
21.	*Gynura nepalensis*	Compositae
22.	*Centella asiatica*	Apiaceae
23.	*Butea monosperma*	Papilionaceae

2.2.1 Survey at Ukhrul

Montane wet temperate forests are found in pockets of Shiroi Hills of Ukhrul, which is faviorable for Ginseng and Thalictrum plants. In Ukhrul District, two surveys were made (Fig. 3) at Shiroi Hill. In the first trip, Ginseng and Thalictrum plants were found growing in the mature stage. However, they were not found in the flowering stage. Some spot analysis were done at Shiroi Hill namely: a) plant morphology, b) phytosociology, c) environmental aspects, d) soil analysis, e) site survey and mapping of the area etc.

Flowering of Ginseng starts from the last week of May and continues upto August end. Hence, the second trip was made on June 2000 for studying the floral morphology and anatomy of the plant parts for confirmation of the plants. Two Ginseng plant species from the same site were collected. Morphologically these two plants are different. One is confirmed to be *Panax pseudoginseng* which was reported by Deb in the year 1961 from the same spot. Another Ginseng species which has got different character with that of the first may be another species of *Panax*, having the characters – tuberous root stalk, which is rarely branched, but the majority of the members are unbranched one. Leaf is palmately digitate, whose shapes of leaflets are lanceolate, oblanceolate. Sometimes the two halves of the leaflets are unequal in area. Colour of the leaves is dark green. Flower – petals are greenish in colour, regular, provided with indistinct disc below the ovary, presence of semi-inferior ovary, with two, more or less cone-shaped green and distinct styles, aestivation of the petals is imbricate. One allied species of Ginseng which is known as Siberian Ginseng, *Eleutherococcus senticosus* is also available in the site. In the Shiroi hill environment two *Thalictrum* species are also available, however they are not in the flowering stage.

Shiroi hill (Fig. 3) is having sub-tropical forest ecosystem in the Ukhrul district of Manipur at 25°13′ N latitude and 94°25′ E longitude which is 105 km away from Imphal city. It has an altitude ranging from 1730 to 1900 m from the mean sea level contain evergreen species with predominance of oak and pine trees, which widely vary in size due to altitudinal differences. The eastern part of Shiroi hill is surrounded by Myanmar (Burma) whereas, the Manipur Valley lies on the west and south-western side. The northern side of the hills is bordering Nagaland.

The soil type found in Shiroi hill is of red loamy. The sand constituted the major portion of the soil with 57.05% with 6.05% and 10% clay and silt respectively. Soil temperature is roughly about 22°C (during June) and soil moisture is about 90% (during June). pH of the soil is slightly acidic i.e., about 6.8. The flowering season i.e., August has recorded maximum humidity (945%), which fall in rainy season (June & September). This season is the wettest period of the year with mean minimum temperature of about 27°C and mean minimum temperature of about

15°C. Monthly rainfall ranged from 180 mm (September) to 300 mm (June). The mean maximum humidity ranged from 88.2% (September) to 94% (August).

2.2.2 Survey at Chandel District

Tropical dry deciduous forest along the Myanmar border are continuation of teak forest of Kabaw Valley. Comparatively, this district has lesser altitude (1200 – 1500 m above the mean sea level) and temperature is also warmer than those of Ukhrul and Senapati districts. Dori (1986) reported that *Panax* and *Thalictrum* are favourable in the montane wet temperate climate within the altitude range of about 2000 m above the mean sea level. Both the conditions are peculiar of Ukhrul and Senapati District. Hence, both the plants are available in the two districts. In the Chandel District two survey programmes were preformed during the growth season of Ginseng and Thalictrum plants. Intensive study was made during the survey work in different places of Chandel District. However, the plants were not recorded from this district. It may be because of the environmental factors that are not favourable for the growth of these plants.

2.2.3 Systematic study of *P. pseudoginseng* plant (Fig. 4)

Systematic study was performed from the plant materials collected from the Shiroi hill, Ukhrul. The characters assessed from ten plants were observed and compared with the actual characters of the plant and the characters are listed below:

Habitat : Wild, shady and moist places with loose loamy soils.

Habit : Perennial, herb, erect with green and hard stem, with a single inflorescence along with 5-6 leaves at its tip.

Root : Tuberous, perennial, more or less spreading horizontally bearing persistent fleshy scales at the base i.e., at the junction of the root and the stem which are shed during growth. The root may be branched and the branching is absent. The root are generally white. Root surfaces rough. The size of the root ranges from to cm in diameter and from to cm in length.

Stem : Simple, erect, solid, annual, unbranched, pithy, greenish in colour, cylindrical at the base but somewhat triangularly angular upwards, glabrous, cylindrical, annual, hard bearing whorl of 507 palmately compound leaves. Height of the stem ranges from 10 to 50 cm (Max. height obtained from Ukhrul survey).

Leaf : Whorl, digitate, compound with a long petiole bearing 4-5 or sometimes 6 leaflets in each leaf. The leaflets are also arranged palmately with distinct petioles. The texture of the leaflet is membranous which is finely serrated, gradually accumulate, bristle on both the surfaces but distinct at the veins and veinlets. The leaflets are ranging

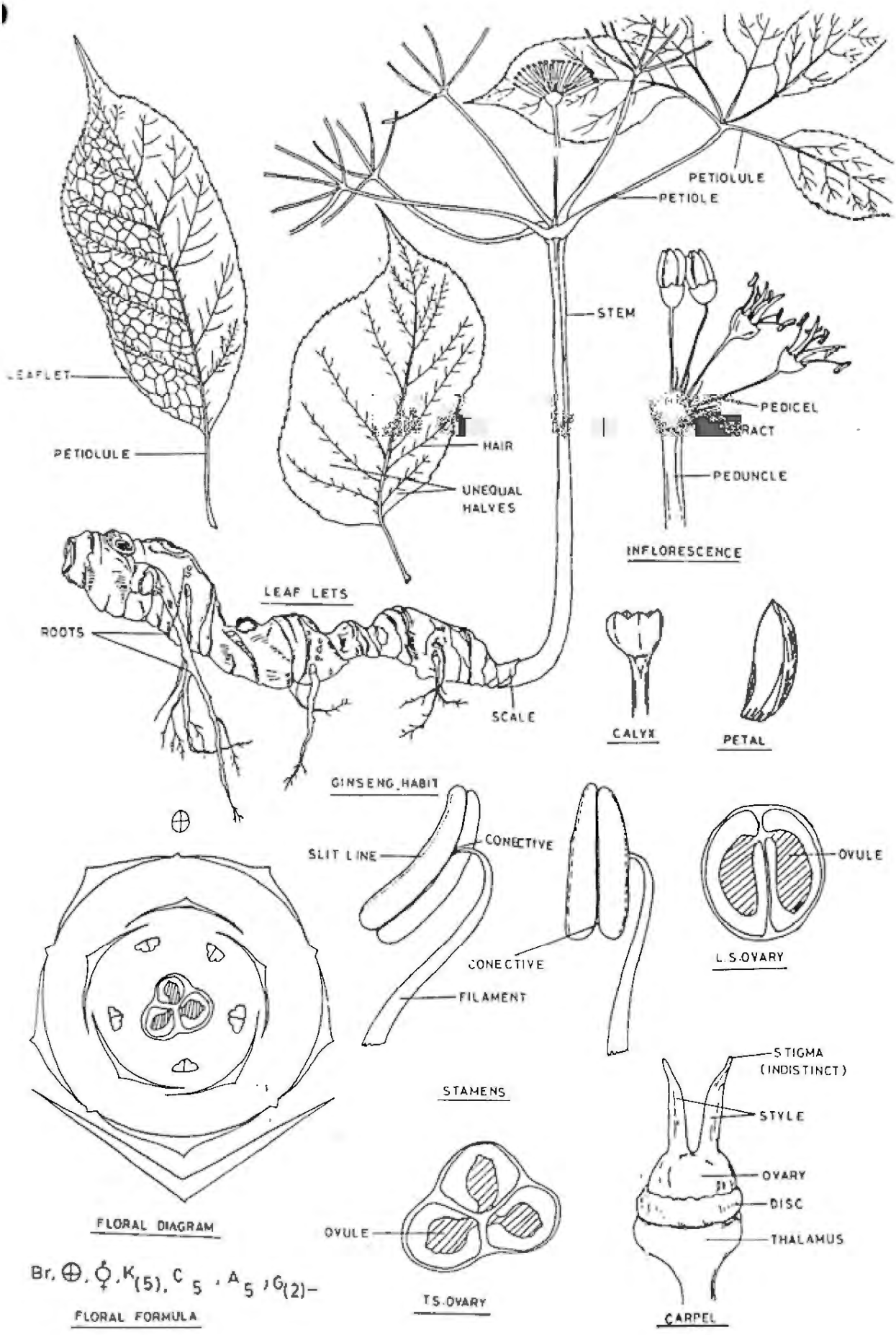
PETIOLULE
PETIOLE
STEM
LEAFLET
PETIOLULE
HAIR
UNEQUAL HALVES
PEDICEL
BRACT
PEDUNCLE
INFLORESCENCE
LEAF LETS
ROOTS
SCALE
GINSENG HABIT
CALYX
PETAL
SLIT LINE
CONECTIVE
CONECTIVE
FILAMENT
STAMENS
OVULE
L.S.OVARY
FLORAL DIAGRAM
Br, ⊕, ♀, K(5), C5, A5, G(2)–
FLORAL FORMULA
OVULE
T.S.OVARY
STIGMA (INDISTINCT)
STYLE
OVARY
DISC
THALAMUS
CARPEL

Fig. 4.

from lanceolate, oblanceolate, ovate to obviate in shape. The leaf bare of the leaflet is obtuse or sometimes reounded. Sometimes the two halves of the leaflet are unequal in shape but generally equal in shape, deep green in colour. Reticulate, unicostate, divergent, exstipulate. The mean size of leaf is 10.4 cm in length, 4.36 cm in width and petiole length is 14.09 cm.

Inflorescence : Simple umbel with a long pendulate ranting from 6.2-27 cm in length, glabours, green in colour.

Flower : Minutely bracteate, pentamerous, pedicellate, complete, hermaphrodite, actinomorphic, small, greenish, pedicel hairy with 1.1 to 1.5 cm in length. The flower is perigynous, perianth biseriate.

Bract : Sickle shaped with a concave towards centre of the bract, green, minute, 1-2 mm in length.

Calyx : Inconspicuous or cupuliform, adnate to ovary, 5 minute teeth, gamasepalous, green valvate.

Corolla : 5 petal, polypetalous, ovate, arising from the disc, caduceus, imbricate in bud condition, pale green outside, white inside.

Androecium : 5 stamen, polyandrous, alternate with the petals, opposite to sepals, distinct, arising from the disc, anther white, filament white, the anther 2-celled, dorsifixed, about two third towards the anther tips, anther slightly curved, dehiscing longitudinally, introse while young and introse when mature enough. Connective is extended upto the tip of the anther but very indistinct. The filament is glabrous.

Gynoecium : 2 carpels, syncarpous, styles 2, free, distinct, connate at the base, style fleshy, more or less cone-shaped, green in colour and glabrous. Stigma indistinct, capitate, greenish white. The ovary is half or semi inferior, terminal, light green trilocular with one ovule in each locule, axile placentation, pendulous.

Disc : : Epigynous disc present covering ovary upto half usually confluent with the style bases, curved, circular, curved face with some grooves.

Fruit : Berry or Drupe.

Floral Formula : $Br, \oplus, \male, K_{(5)}, C_5, A_5, G_{(2)}$

2.2.4 Systematic study of *Panax* sps. (Fig. 5)

Systematic study was performed from the plant materials collected from the Shiroi hill, Ukhrul. The characters were assessed from ten plants, so as to observe the correct characters of the plant and the characters are listed below:

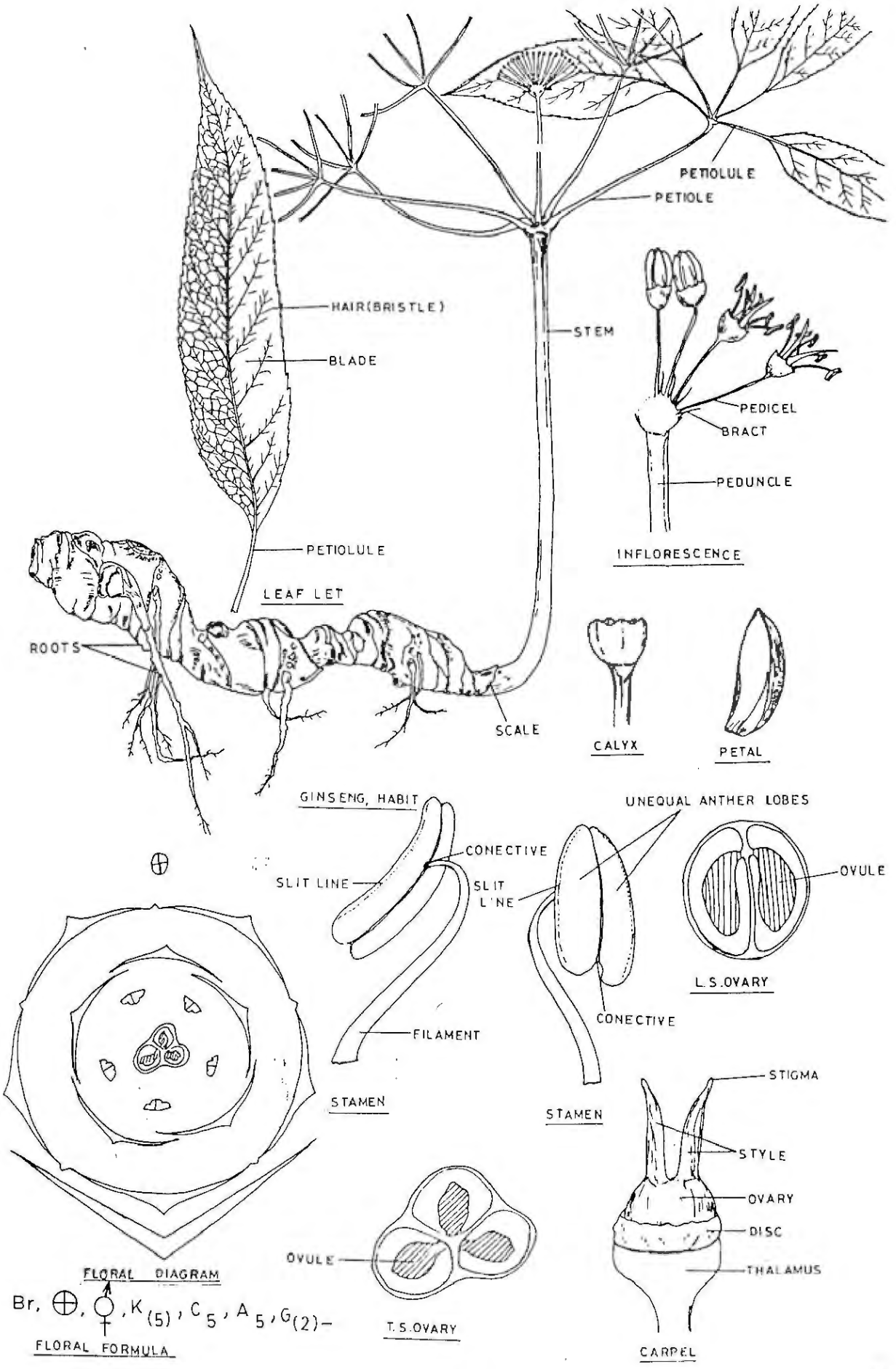
PETIOLULE
PETIOLE
HAIR(BRISTLE)
STEM
BLADE
PEDICEL
BRACT
PEDUNCLE
INFLORESCENCE
PETIOLULE
LEAF LET
ROOTS
SCALE
GINSENG, HABIT
CALYX
PETAL
UNEQUAL ANTHER LOBES
CONECTIVE
SLIT LINE
SLIT LINE
OVULE
L.S.OVARY
FILAMENT
CONECTIVE
STAMEN
STAMEN
STIGMA
STYLE
OVARY
DISC
THALAMUS
OVULE
T.S.OVARY
CARPEL
FLORAL DIAGRAM
Br, ⊕, ♀, K(5), C5, A5, G(2)–
FLORAL FORMULA

Fig. 5

Habitat : Wild, shady and moist places with loose reddish loamy soils.

Habit : Tuberous, perennial, more or less spreading horizontally bearing persistent fleshy scales at the base i.e., at the junction of the root and the stem which are shed during growth. The root stock may be branched and the branching is absent. The root stocks are generally white. Root surfaces rough.

Stem : Simple, erect, solid, unbranched, pithy, greenish in colour, glabrous, cylindrical, annual, hard bearing whorl of 5 palmately compound leaves. Height of the stem ranges from 10 to 65 cm (Max. height obtained from Ukhrul survey).

Leaf : Whorl, difitate, compound with a long petiole bearing 5-6 leaflets in each leaf. The leaflets are also arranged palmately with distinct petiolcs. The texture of the leaflet is membranous which is finely serrated, gradually acuminate, but acute apexes are also reported. Bristle on both the surfaces but distinct at the veins and veinlets. The leaflets are lanceolate in shape which bases of the blade are acute in shape. Two halves of the leaflets are always equal in area. The colour of the leaflets are dark green but the dorsal surface is somewhat silvery in colour. Vanation is reticulate, unicosted and divergent, exstipulate, whorled phylotaxy. The mean size of leaf is 10.46 cm in length, 4.36 cm in width and petiole length is 14.09 cm.

Inflorescence : Simple umbel with a long pendulate, glabrous, green in colour.

Flower : Minutely bracteate, pentamerous, pedicellate, complete, hermophrodite, actinomorphic, small, greenish, pedicel hairy with 1.1 to 1.5 cm in length. The flower is perigynous, perianth biseriate.

Bract : Sickle shaped with a concave towards centre of the bract, green, minute, 1-2 mm in length.

Calyx : Inconspicuous or cupuliform, adnate to ovary, 5 minute teeth, gamosepalous, green valvate.

Corolla : 5 petal, polypetalous, ovate, arising from the disc, caduceus, quincuncial in bud condition, pale green outside, white inside.

Androecium : 5 stamen, polyandrous, alternate with the petals, opposite to sepals, distinct, arising from the disc, anther white, filament white, the anther 2-celled, dorsifixed about two third towards the anther tip, anther slightly curved, dehiscing longitudinally, introse while young and inrose when mature enough. Connective is extended upto the tip of the anther but very indistinct. Sometimes anther lobes are unequal in size. The filament is glabrous.

Gynoecium : 2 carpels, syncarpous, styles 2, free, distinct, connate at the base, style fleshy, more or less cone-shaped, green in colour and glabrous. Stigma indistinct, capitate, greenish white. The ovary is half inferior, terminal, light green trilocular with one ovule in each locule, exile placentation, pendulous.

Disc : Epigynous disc present covering ovary upto half usually confluent with the style bases, curved, circular, curved face with some grooves.

Fruit : Berry or Drupe.

Floral Formula : Br, ⊕, ♂, $K_{(5)}$, C_5, A_5, $G_{(2)}$.

Panax sp. is taller than the *Panax pseudoginseng*. The leaflets of the *Panax* sp. is lanceolate in shape, ventral surface is dark green and dorsal surface is somewhat silvery in colour whereas in *Panax pseudoginseng* the shape of the leaflets are ranging from ovate, obovate to lanceolate which colour is light green. The most important distinguishing point in between the two is in aestivation of the corolla, there is quincuncial in *Panax* sp. and imbricate in *Panax pseudoginseng*.

2.2.5 Soil analysis

Analysis of three different types of soils for different parameters (Table No. 9) were done. The soil studied were:

- Natural soil of Ginseng,
- Natural soil of Thalictrum,
- Pot cultured artificial soil.

(a) Organic carbon estimation: Walkley and Black's method (1947) was adopted for this estimation. It is a titrimetric quantitative estimation where soil containing 1N $K_2Cr_2O_7$, conc. H_2SO_4, ortho-phosphoric acide and DPA indicator was titrated against normal ferrous ammonium sulphate solution.

(b) Carbonate detection: By using conc. HCl to the soil sample and monitored the rapid effervescence that indicates the presence of carbonate in the soil. The experiment is not a quantitative still we are able to compare the three different soil samples for their carbonate content.

(c) Nitrate detection: The soil suspension in the water is taken (not the filtrate) and allowed to react with 0.5% DPA in conc. H_2SO_4 and blue colour developed are compared accordingly.

(d) Moisture content of the soil: Specific weight of fresh soul is oven dried for 2-3 days at 60°C until a constant weight is maintained and their moisture content are calculated and compared.

(e) pH of the soil: All the soils are made suspended in water (1 g : 40 ml H_2O) and filtered. Then, the pH is measured for the filtrate of all types of soil samples.

Table:9 – Soil Chemical parameters of Natural and Artificial soil samples studied

Sl. No.	Parameter	Thalilctrum (Okhro Ikho)	Ginseng (Datuley Hill)	Artificial (Pot cultured)
1.	Moisture (%)	11.75	49.57	132.6
2.	pH7	7.6	7.8	9.2
3.	Org. carbon (%)	3.15	3.43	3.81
4.	Nitrate	***	**	*
5.	Carbonate	*	**	***

* Slightly present, ** Moderate and *** Highest.

2.2.6 Phytosociology

In nature, plants cannot grow independently in a particular area. They grow along with some plants which have mutual benefit among themselves. In the survey study also Ginseng and *Thalictrum* plants are found growing along with some plants in the Senapati and Ukhrul districts. A list of the dominant intermixing plants are given in Table No. 8 and are of 23 in number.

CHAPTER – III

LABORATORY CULTURE (POT AND TISSUE)

3.1 Plant Material

The collected plant specimens in the form of roots from the collection sites of Senapati and Ukhrul districts are brought to the laboratory of Ginseng Project at Life Sciences Department, Manipur Unviersity for experiment. The soils for the pot culture experiment were prepared in a proportion of sandy soil : charcoal : leaf mold (6:1:3). Three Ginseng roots per pot have been transferred in 16 different pond water per pot every alternate day. After a week of planting, small planlets started appearing from the buds. The growth rate is about 1.3 cm per day in the first week. On 20th April 1999 the height of the plant was recorded as 19.5 cm. In case of *Thalictrum* the growth rate is 2.5 cm per day and attains a height of 22.5 cm within a week. The plants developed from the pot culture experiment was used as a mother plant to get explants for tissue culture for micropropagation.

3.2 Laboratory Experiments

3.2.1 (A) Pot Culture Experiment

The collected plant specimens in the form of roots from the collected sites of the Senapati Dist. are brought to the laboratory of Ginseng Project, Life Sciences Department, Manipur University for experiments. The soils for the pot culture experiment were prepared in a proportion of *sandy soil : charcoal : leaf mold (6:1:3)* and pot culture in 16 different pots. Using this condition the maximum height is attained within two weeks and growth ceases. The plant developed from the pot's experiments will be used as a mother plant to get explants for tissue culture experiments for micro-propagation.

For pot culture, the appropriate soil composition was designed in such a easy manner that it excluded most of chemical composition (Enclosed table).

Table 10. Soil composition for pot culture

Sl. No.	Soil Name	Composition (%)	pH
1.	Sandy soil	85%	8.0
2.	Leaf mold	10%	
3.	Charcoal	5%	

3.2.2 (B) Biochemical Analysis

Biochemical constituents of the root of Ginseng and Thalictrum were compared in four different parameters. The percentage constituents of Phenol, Carbohydrates, Protein and Phytosterols in these two plants were studies. The Phenol and carbohydrate content is little higher in Ginseng than Thalictrum about 0.04 mg/g and 14.3 mg/g respectively. Other metabolites, like protein and Phytosterol are higher (10.08 mg/g and 1.5 mg/g respectively) in Thalictrum than that of Ginseng. The comparative data is also enclosed herewith. It is only the preliminary biochemical studies, further work is need to be done.

Table – 11. Comparative data for biochemical studies

Sl. No.	Parameter's name	Ginseng (mg/g) Mean ± S.D.	Thalictrum (mg/g) Mean ± S.D.
1.	Carbohydrate	43.5 ± 0.08	29.2 ± 0.45
2.	Phenol	0.245 ± 0.11	0.204 ± 0.13
3.	Phytosterol	3.6 ± 0.443	5.1 ± 0.24
4.	Protein	2.4 ± 0.02	12.48 ± 0.30
5.	Sugar (Total)	32.8 ± 0.40	9.8 ± 0.56

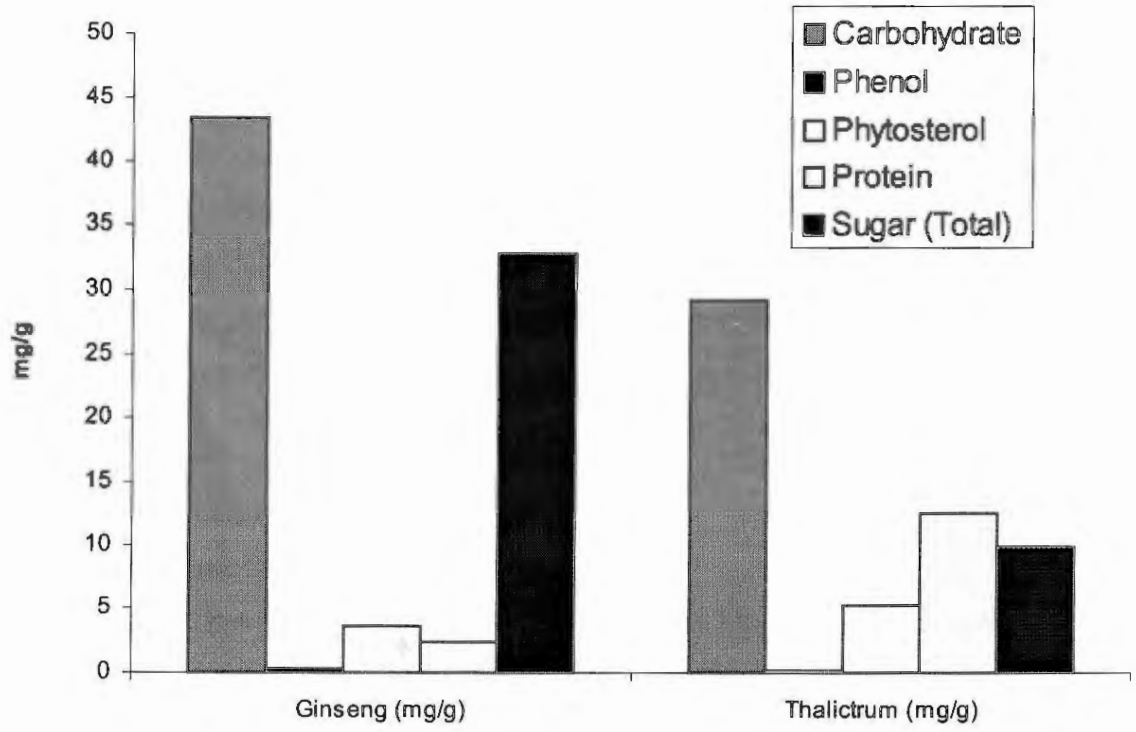

Figure-6: Comparative bar chart for biochemical analysis of Ginseng and Thalictrum.

3.2.3 Plant Tissue Culture

Literature in the micropropagation of *Panax pseudoginseng* is vast and can collect a lot within this few months but that of *Thalictrum foliolosum* is extremely limited and work in this area has not been done to a considerable extent. Sterilization of the explant for Ginseng and Thalictrum were standardised by developing a protocol for surface sterilisation (using Sodium-Hypochloride, Mercuric chloride, Tween 20 and 70% alcohol).

We have done inoculation experiments with convincing achievement of culture. Yet we able to achieve the very initial stages of micro propagated plants with trial methods. So far we have used 100 conical flask for the inoculation by using MS media with different hormonal composition of IAA, IBA, Kinetin, BA & NAA etc. For convenience, modification in MS media and hormonal composition were made in the first stage. MS media is used for tissue culture experiments as this media has highest number of nutrients and MS media is used particularly for Ginseng. In the current month, explants are in the incubation stages. We concentrate on one very much specific technique for micro propagation experiments that is by using MS media.

All the culture were placed in a culture room maintained at 23 ± 1°C with 16 h light/8h dark photoperiod at 15 μ mol photons/m^2/sec.

Plant tissue culture plays an important role in micropropagation and biotechnological breeding techniques in gensing and thalictrum plants. In this report, we study the establishment of tissue culture of ginseng and thalictrum from the rhizome and nodal shoots respectively. It is important to establish a reproducible tissue for micropropagation of ginseng and thalictrum. Successful tissue cultures have been reported for many members of ginseng, but those of Thalictrum is not yet known. On the other hand, micropropagation of the genus Thalictrum and ginseng by tissue culture are economically important because propagation of these plants in the laboratory do not demand large amount of tissues. From the collected specimens the rhizomes with buds of ginseng and the nodal shoots of Thalictrum were used for taking explants in culture media.

3.2.4 Material and Methods:

3.2.4.1 Plant materials and culture conditions

Field mother plants of *Panax pseudoginseng* and *Thalictrum foliolosum* collected from the Mao and Ukhrul areas which were successful in pot culture were taken as the explant. For each genotype, plants of Thalictrum were propagated in pot culture within the duration as its propagation requires rhizomes.

3.2.4.2 Culture media

Most of the culture media were based on the mineral salts and organic constituents of Murashige and Skoog (1962), designated MS. All the media contained 30 g sucrose in every one liter and 8 g agar. The medium was adjusted to pH 5.8 before autoclaving at 120 ± 3°C for 30 minutes, many trial methods were made by using different pH range i.e., (5.2 upto 5.8).

Culture establishment: Shoot tips of the Thalictrum and rhizome of ginseng from rapidly extending shoots were surface disinfected by immersion in solution containing 10% Sodium hypochloride and a drop of Tween 20 for 15 minutes. After washing in sterile double distilled water, the shoot tips and rhizomes were dipped in 0.1% mercuric chloride solution for 10 minutes followed by thorough rinsing in distilled water. Finally the plant materials of ginseng were dipped into 70% ethanol for 3 minutes and rinsed in sterile double distilled water 3 to 5 times. In Thalictrum plants materials ethanol dip was skipped as it affected the plant material badly. Sterilisation process ended in mercuric chloride and rinsed in double distilled water. Surface sterilised plants materials were cut into 5 mm^3 pieces and used as explants. Cultures were subcultured at monthly intervals by transferring the materials to various fresh shoot culture media.

3.2.4.3 Axillary bud proliferation

In preliminary experiments, axillary shoots produced from axillary buds of Thalictrum were obtained by using MS media supplemented with IAA, IBA, BA, kinetin or in combination of kinetin with IAA & IBA with BA. Axillary budding was scored 8 weeks after cuture initiation. In the equal concentration of hormone auxin and kinetin, callus formation of Thalictrum can be seen. In the same hormonal combinations of the above axillary shoots of ginseng cannot be seen, instead structures like callus can be seen in the auxin-kinetin combination.

Rooting essay were preformed under the same conditions on MS media supplemented with different concentrations of IBA, BA and in combination of IAA and kinetin. Higher concentration of IAA with kinetin was also tested for root initiation (Introduction of Plant Physiology, Hopkins 1995). Upto 5 weeks, no root initiation was observed and the whole phenomenon is still under observation.

The influence of growth regulators on the induction of Thalictrum shoots is presented in Table 12. For IAA and kinetin, all the concentrations of IAA tested positive in shoo induction while in kinetin the number of shoots formed were almost nil. When IAA is used along with kinetin it gives better result in the induction of callus.

The influence of growth regulators on the induction of ginseng callus and shoots is presented in table 13. In the individual uses of IAA, it induces callus in ginseng explant.

Table-12. Effect of Growth Regulators (IAA and Kinetin) and additives on Shoot and Callus Induction from Nodal Expants of Thallictrum after four weeks of Culture.

Sl. No.	Treatment	Growth Regulator (μM) IAA	KIN	Axillary Shoot Induction (%)*	Callus (%)	Shoot Length (c.m.) Mean ± S.D.
1	Control	Nil		20	nd	1.5± 0.16
2.	T_1	2	-	20	nd	1.5± 0.14
3.	T_2	4	-	40	nd	1.9± 0.30
4.	T_3	6	-	40	nd	2.1± 0.14
5.	T_4	8	-	40	nd	1.6± 0.14
6.	T_5	10	-	60	nd	2.3± 0.08
7.	T_6	12	-	80	nd	1.9± 0.22
8.	T_7	14	-	40	nd	1.9± 0.16
9.	T_8	-	4	20	nd	1.5± 0.16
10.	T_9	-	8	20	nd	2.0± 0.14
11.	T_{10}	-	12	40	nd	1.7± 0.20
12.	T_{11}	-	16	nd	nd	nd
13.	T_{12}	-	20	nd	nd	nd
14.	T_{13}	4	4	nd	60	nd
15.	T_{14}	4	8	nd	40	nd
16.	T_{15}	8	4	nd	20	nd

* Average of Five Explants by taking three replications.
nd - Not Determined

Table-13. Effect of Growth Regulators (IAA and Kinetin) and additives on Shoot and Callus Induction from Rhizomatous Explants of Ginseng after four weeks of Culture.

Sl. No.	Treatment	Growth Regulator (μM) IAA	KIN	Axillary Shoot Induction (%)*	Callus (%)	Shoot Length (c.m.) Mean ± S.D.
1	Control	Nil		20	nd	1.5± 0.14
2.	T_1	2	-	20	nd	2.0± 0.21
3.	T_2	4	-	20	nd	2.5± 0.17
4.	T_3	6	-	nd	nd	nd
5.	T_4	8	-	nd	20	nd
6.	T_5	10	-	nd	40	nd
7.	T_6	12	-	nd	20	nd
8.	T_7	14	-	nd	nd	nd
9.	T_8	-	4	nd	nd	nd
10.	T_9	-	8	20	nd	2.0± 0.14
11.	T_{10}	-	12	nd	nd	nd
12.	T_{11}	-	14	nd	nd	nd
13.	T_{12}	-	20	nd	nd	nd
14.	T_{13}	4	4	nd	20	nd
15.	T_{14}	4	8	nd	80	nd
16.	T_{15}	8	4	nd	60	nd

* Average of Five Explants by taking three replications.
nd - Not Determined

Table-14. Effect of Growth Regulators (IBA and BA) and additives on Shoot and Callus Induction from Nodal Explants of Thallictrum after four weeks of Culture.

Sl. No.	Treatment	Growth Regulator (μM) IBA	BA	Axillary Shoot Induction (%)*	Callus (%)	Shoot Length (c.m.) Mean ± S.D.
1	Control	Nil		20	nd	2.5± 0.05
2.	T_1	2	-	nd	nd	nd
3.	T_2	4	-	nd	nd	nd
4.	T_3	6	-	20	nd	2.0± 0.17
5.	T_4	8	-	40	nd	2.2± 0.37
6.	T_5	-	2	40	nd	2.2± 0.14
7.	T_6	-	4	40	nd	2.0± 0.14
8.	T_7	-	6	60	nd	1.7± 0.12
9.	T_8	-	8	nd	nd	nd
10.	T_9	2	2	nd	nd	nd
11.	T_{10}	4	4	nd	nd	nd
12.	T_{11}	4	2	nd	nd	nd
13.	T_{12}	2	4	nd	nd	nd
14.	T_{13}	2	6	nd	nd	nd
15.	T_{14}	6	2	nd	nd	nd
16.	T_{15}	6	4	nd	nd	nd

* Average of Five Explants by taking three replications.
nd - Not Determined

Table-15. Effect of Growth Regulators (IBA and BA) and additives on Root and Shoot Induction from Rhizome Explants of Ginseng plant.

Sl. No.	Treatment	Growth Regulator (μM) IBA	BA	Axillary Shoot Induction (%)*	Callus (%)	Shoot Length (c.m.) Mean ± S.D.
1	Control	Nil		20	nd	1.5± 0.23
2.	T_1	2	-	nd	nd	nd
3.	T_2	4	-	nd	nd	nd
4.	T_3	6	-	20	nd	2.2± 0.36
5.	T_4	8	-	60	nd	2.0± 0.17
6.	T_5	10	-	40	nd	1.9± 0.29
7.	T_6	12	-	nd	nd	nd
8.	T_7	14	-	nd	20	nd
9.	T_8	-	4	nd	20	nd
10.	T_9	-	8	20	nd	2.2± 0.05
11.	T_{10}	-	12	40	nd	1.8± 0.3
12.	T_{11}	-	14	nd	nd	nd
13.	T_{12}	-	20	nd	nd	nd
14.	T_{13}	4	4	nd	nd	nd
15.	T_{14}	4	8	nd	nd	nd
16.	T_{15}	8	4	nd	nd	nd

* Average of Five Explants by taking three replications.
nd - Not Determined

Table No. 12 shows that :-

In Thalictrum, IAA treatment is found most effective at concentration of 12μM, which is the optimum value for shoot induction (T_6).

Effect of kinetin no nodal explant of Thallictrum is found not satisfactory as it doesno't affect considerably in axillary shoot induction as well as callus formation (T_8 to T_{12}).

T_{10}(12μM) has little effect on axillary shoot induction.

Most achievement result shows when we used both IAA (an auxin) and kinetin at specific concentration i.e. callus induction observed when both IAA and kinetin is used. Optimum result for callus induction is achieved when both the hormones are taken at equal concentration.

Table No. 13 shows that :-

Treatment of IAA on rhizomatous explants of Ginseng shows no satisfactory result on axillary shoot induction (T_1 to T_7) but it has +ve result on callus induction having optimum concentration at 10μM(T_4,T_5 &T_6).

Kinetin has almost no effect on both axillary shoot induction and callus induction from rhizomatous explant of ginseng (T_8 to T_{12}).

Callus induction was observed when IAA and kinetin both were treated in the medium. Optimum value for callus induction is achieved for these two hormonal combinations.

Table No. 14 shows that :-

IBA growth regulator has little effect on nodal explant of Thallictrum i.e. at 8 μM concentration for axillary shoot induction (T_4).

BA has satisfactory result on axillary shoot induction of nodal explant of Thallictrum and has its optimum value at the concentration of 6μM(T_7).

Combine treatment of IBA and BA has no effect on axillary shoot induction and callus formation of nodal explant of Thallictrum (T_9 to T_{15}).

Table No 15 shows that :-

Treatment of IBA at concentration 6,8 & 10 μM for rhizomatous explant of Ginseng has effect on axillary shoot induction withh its optimum value at 8μM IBA (T_4).

No effect on shoot induction and callus induction by using both IBA and BA for rhizomatous explant of Ginseng plant.

SAILENT ACHIEVEMENTS AND SUMMARY OF PROGRESS

Structural achievements:

A tissue culture laboratory with all infra structures was set up at the Life Sciences Department, Manipur University, for conducting tissue culture related works of the project. Moreover, a green house that is essentially required for functioning of the project was constructed at the complex of DST, Govt. of Manipur at Takyelpat, Imphal as the University authority did not permit erection of the structure at the University campus.

Research achievements:

1. In the present study of tissue culture of Ginseng and Thalictrum plants, we have established a protocol for the induction of shoot callus from the nodal bud of Thalictrum and rhizomatous bud of Ginseng for mass propagation. MS media (Murashige & Skoog 1962) containing 3% sucrose and 0.7% agar supplemented with 2μM IAA induced shoot initiation in Thalictrum plant. Simultaneous treatment of kinetin and IAA treatment (4μM IAA + 8μM Kin.) induced more callus. In Ginseng, two concentrations, 2μM and 4uM IAA enhanced the same axillary shoot induction rate with that of control. Nodal shoot explants of Thalictrum showed higher morphogenic potential than the leaf explants in MS media. In Ginseng, the rhizomatous buds are more efficient than the leaves. When IBA is treated in different concentrations, shoot induction in Ginseng is highest at 8μM IBA. In the concentration of 8μM and 12μM BA, the shoot is also induced in Ginseng (T_7& T_8 of Table –15). Higher concentration of IBA (14μM), and lower concentration of BA (4μM), also induces callus in Ginseng. In Thalictrum, shoot initiation is highest in 6μM BA (T_3 of Table –14). Shoot induction is observed in Thalictrum on 6μM & 8μM IBA also, but no callus formation.

2. Tissue culture for Ginseng had successfully done with zygotic embryos (Choi, Y.E. and Soh, W.Y. 1996) by using MS media. Somatic embryos can be formed directly from culture explant without intervening callus formation (Konar and Nataraja, 1965; Maheswaran and Willium, 1985;Jones and Rost, 1989). But in the present studies, the above observations do not arise because seeds are not available in the natural condition as well as in the pot culture experiments.

3. pH of the medium is usually kept around 5.6 –5.8 which is the specific value for MS media. However, the natural habitat of the plant has soil pH value in alkaline range. This

might also be the reason for much slower growth of Ginseng plant during culture. Moreover, it is very difficult to adjust the media pH in accordance with that of their natural habitat, as higher pH value gives much hardening of the agar that will hinder the tissue growth in the medium.

4. Regarding the distribution of Ginseng plant in their natural habitat, it was found that, there are sharp differences in the mode of distribution of Ginseng plants in the two districts i.e. Senapati and Ukhrul districts. Occurrence is sparse and relative abundance of Ginseng in Datuley hills of Senapati district is much lower than that of Shiroi hills of Ukhrul district. This might be due to more vegetative propagation in the later due to lesser human interference.

5. Occurrence of only one species of Ginseng (*Panax pseudoginseng*) was reported by Deb (1961) in the Shiroi hills, however, this plant is reported for the first time by the project team from Senapati District. Another Ginseng species is also recorded for the first time from Shiroi hills. The species may be a new one as because the morphological and floral characters of this particular species are almost different from that of the *Panax pseudoginseng*. Identification of the plant is still going on with the help of the Botanical Survey of India, Shillong Branch.

Conclusion:

The project was launched in January 1999 with the appointment of project staffs (1 geneticist, 1 Floriculturist and I Laboratory Assistant) with an aim to uplift the economy of the poor tribal people of the state by engaging them in the commercially viable farming of Ginseng and *Thalictrum* through mass propagation from laboratory.

The project was implemented at an arrangement that MASTEC has to look after the administrative works of the project while all technical works of the project to be supported by P.I., Dr. P.Kumar Singh.

All research works related to the project were carried out at the laboratory set up for the purpose at Life Sciences Department, Manipur University, Imphal by the research team under the leadership of Dr.P.Kumar Singh, Assistant Prof. M.U.

During the tenure of the project, as incorporated in the report, the P.I. had claimed that he could achieve Callus of both the plants i.e. Ginseng and *Thalictrum* from the respective explants. However, his claim was not valid, as the investigation team of MASTEC could not witness any formation of Callus of both the plants during the tenure of the research works. Hence, a suitable protocol for propagation of these plants is still not developed.

Tissue culture technique of these is quite a tricky area; further works may give rise to establishment of the desired results.

As the project has a good scope for the future, continuation of the project in the state is sought. Either a change of the present P.I. (Dr.P.Kumar Singh) or initiating fresh project in the same line will certainly help in achieving the goals of the project.

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