PROVENANCE VARIATION ON CERTAIN CHARACTERISTICS AND *IN SITU* GENE CONSERVATION FOREST OF MERKUS PINE (*PINUS MERKUSII* JUNGH. & DE VRIESE)

INTRODUCTION

Merkus pine (*Pinus merkusii* Jungh. & de Vriese) is one of the tropical pine species and only pine species which it has a natural distribution south of the equator. This pine grows extensively in Southeast Asia. The species is one of two native pine species of Thailand where northwestern of country is one of the largest areas of Merkus pine forests.

Normally, Merkus pine is a fast-growing pioneer species and is well adapted to grow on sandy, poor, or dry sites. It is also able to grow on opened and degraded areas. Furthermore, this species is also a multipurpose species. Wood is used for light construction and joinery. More important of the species is the extraction of resin. Resin was earlier of commercial importance in the manufacture of turpentine and a lesser extent, lacquer. In addition, the resin-rich base of the stem can be cut for fire sticks. Nevertheless, the genetic resources found within Thailand could be valuable for future reforestation program of poor and degraded soils and importance to breeding program in the neighboring countries (Indonesia, Laos, and Cambodia).

In 1969, Danish and Thai governments agreed to cooperate in establishment in the Pine Improvement Centre (PIC) at Hot district, Chiang Mai province. The project covered indigenous and exotic pine species of Thailand. Provenance trials of Merkus pine was one of the projects experiment. The provenance trials was established in 1971 at Huey Bong Experimental Area and Gene Conservation Plantations. The objective of the trials was to find the most suitable provenances to be used as potential seed source in the reforestation program in Thailand.

Merkus pine covers approximately 70 % of the total pine forest areas. In contrast, *Pinus kesiya*, which is another native pine species of Thailand, is the most preferred species to grow in plantation and watershed area because of its superior growth performances in the early stage of development. However, in the early development of Merkus pine, seedlings perform a peculiar growth pattern called grass stage. Additionally, the natural stands of Merkus pine are widely exploited by local community, primarily as a source of resin and for fire sticks. For extraction of the resin deep cuts are made through the bark and into the sapwood of mature trees and burn. On the other hand, cutting of fire sticks is still conducted and sold in market as bunch of fire sticks. Both resin tapping and cutting fire stick is considered destructive, as the pine become susceptible to disease or the stem breaks during strong winds. Generally, mature tree of Merkus pine are resistant to fire in dry season, but trees which have been cut for fire sticks and resin collection have had a large portion of the protective bark removed and are prone to damage. Frequent fires also destroy the pine seedlings before they enter the sapling stage. Furthermore, the widespread conversions of forest to farmland have reduced local population of this species and today many stands are fragmented and declined. Those problems effect to reduce of

Merkus pine plus trees, which have good genetic source and is also valuable in economic aspect.

Royal Forest Department (RFD) of Thailand, FAO, and DANIDA Forest Tree Seed Centre (DFSC) realized to those problems in natural stands of Merkus pine in Thailand. In that way, the organizations established *in situ* gene conservation areas of the natural Merkus pine stands in various areas of Northeastern Thailand including *in situ* gene conservation forest at Nong Khu, Sangkha District, Surin Province. From a result of provenance trials in screening phase, it was found that this Merkus pine source in Surin having a good genetic traits and perform the growth better than the other Thai provenances.

In situ gene conservation forest at Nong Khu is one of the important pioneer *in situ* gene conservation works in Thailand, covering an area of 104 ha. The purposes of this establishment were to preserve and conserve good genetic source of Merkus pine under the natural condition, to select good phenotypic trees in the area for selected tree and plus trees selection and for future improvement and breeding programs of Merkus pine in Thailand.

The *in situ* gene conservation forest in Surin is located on the plain, where Merkus pine was a dominant species in the lowland Dry Deciduous Forest and distributed over the area. In the past, the stands were once used for resin tapping and some damaged trees felt down. *In situ* gene conservation forest was partly disturbed by human activities and the number of standing trees decreased and leads to genetic loss.

The objectives of the present study are:

1. To study provenance variability on certain characteristics of Merkus pine in provenance trials

2. To study the stand characteristics and certain morphological characteristics of Merkus pine in *in situ* gene conservation forest

3. To study genetic variability of Merkus pine in *in situ* gene conservation forest

4. To evaluate genetic losses of Merkus pine in *in situ* gene conservation forest

LITERATURE REVIEW

Merkus Pine

Pinus merkusii Jungh. & de Vriese. is in the family Pinaceae, order Coniferae, and subdivision Gymnosperm. The common name of *P. merkusii* is Merkus pine or Tenasserim pine (Mirov, 1967). Thai name of this species is Son Song Bai (Smitinand, 2001). It means two needle (in one fascicle) pine.

Merkus pine was previously proposed as two separated taxa, namely the Insular and the Continental group of provenances. The insular provenances are found in Sumatra island of Indonesia. Otherwise, Continental provenances occur in the Southeast Asia mainland. Evidence of marked variation between these two taxa are seed weight, presence or absence of a grass-stage in the seedlings, nodal habit, needle and cone dimensions, wood density patterns, oleoresin constituents, tree size and bole form, habitat and natural range (Cooling, 1968). The distinct differential characteristic between two taxa can be concluded, as follows;

Insular provenances absence of a grass-stage in the seedlings, which are slender and make rapid height growth with, continues growth throughout the year. Its growth habit is multinodal which has 2-7 whorls. The needles are short, thin shape and light color. Bark may be thin and smooth especially Tapanuli provenance, Atjeh provenance bark usually is thick and fissured. The seed is small. Cone is cylindrical shape and short to medium length, light color. The cone is borne singly, in pairs or in clusters of 3-7 cones.

For continental provenances perform of a grass-stage in the seedlings which posses an exceptional juvenile growth pattern occurrence. Following germination subsequently, the grass stage is an initial period of slow shoot growth with inhibited internodal elongation, and it ends when a period of rapid shoot growth with normal internodal elongation begins (Sirikul, 1990). The slowly developing terminal bud is well protected by long secondary needles and the seedlings also develop a thick secondary cortex in their short, branchless stem (Koskela, 2000). Merkus pine in Thailand consists of two group populations, high-altitude mainland populations and low-altitude mainland populations. So performances of seedling growth in northern Thai populations show a longer lasting grass stage than northeast Thai populations. In northern Thailand, the seedling may stay even up to seven years in the grass stage, but it lasts one to three years in northeastern part of the country. Growth habit of this provenance is uninodal. The needles are medium to long shape, sometime heavy. Bark usually is thick and fissured. The seed is medium to large size. Cone is elongate shape, medium to long size and medium to heavy weight. Usually, it is borne singly, in pairs or sometimes in threes.

1. Natural Distribution of Merkus Pine

Merkus pine is the tropical pine species and a fast growing pioneer species that occurs naturally in the south of the equator and its occurrence in Sumatra is the southernmost occurrence of genus *Pinus*. Thus, the range of Merkus pine is between latitudes 23 $^{o}00^{'}$ N and 2 $^{o}06^{'}$ S and longitude 95 $^{o}30^{'}$ E and 121 $^{o}30^{'}$ E (DFSC, 2000). It has a large altitudinal range from a few feet above sea level to over 1,800 m. above sea level, is found on a diversity of soils over many different parent materials, and under climates (Cooling, 1968). Merkus pine usually grows on acidic soils, typically red or yellow podzols and is competitive on relatively poor soils as alluvial plains, sandstone ridges of granite derivatives. Normally, this pine is not found to grow on site of poor drainage.

The area of natural distribution is in the Southeast Asia mainland (Myanmar, Thailand, Laos P.D.R., Cambodia, and Vietnam) and on the islands of Indonesia (Sumatra) and the Philippines (Luzon and Mindoro). The largest areas of Merkus pine forests are in North – Western Thailand and Eastern Burma and in the Aceh province of Northern Sumatra. The numerous other occurrences are much smaller and widely seperated (Pousujja *et al.*, 1986). The natural distribution of Merkus pine is shown in Figure1.

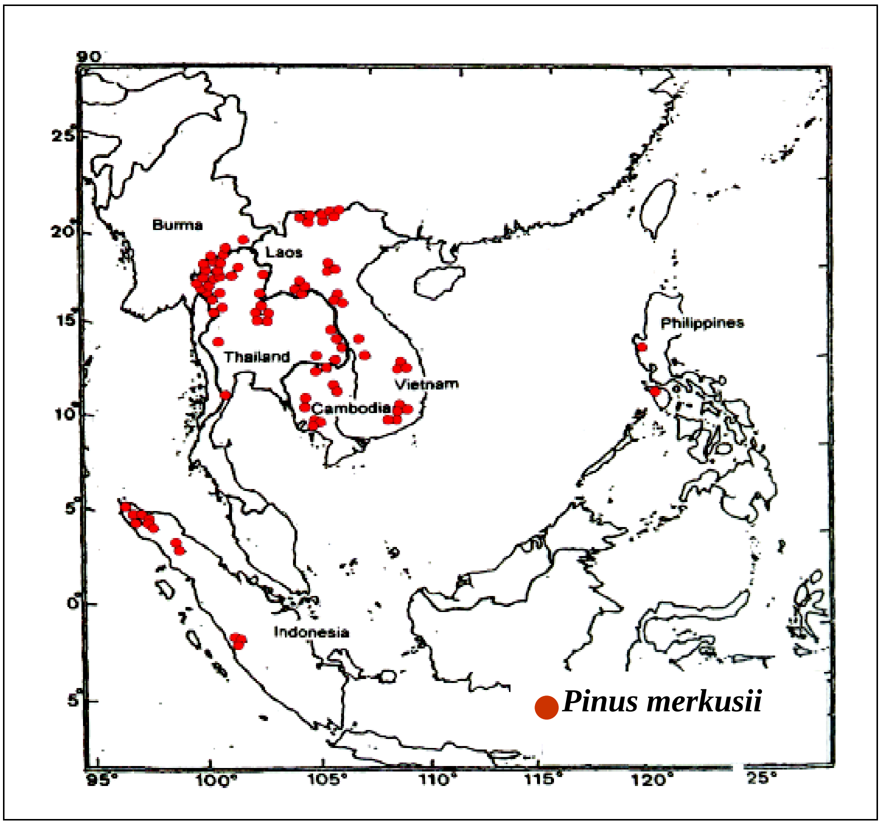


Figure 1 Natural distribution of Merkus pine.

Source: DFSC (2000)

The area of natural distribution on the mainland, the northernmost limit of Merkus pine is located around Lao Kai in northern Vietnam close to the border of Yunnan, and in Muang Ou Nua, a northernmost province of Laos (about 22º - 23º N) (Mirov, 1967; Cooling 1968). It occurs in the southern Shan Hills at elevation of about 150 to 750 m. of Burma, extending south into the adjacent western mountains of northern Thailand. Merkus pine is also found in sporadic patches in northeastern, eastern, and central Thailand. This pine grows in the highlands of Xieng Khouang and Vientiane in central Laos, and Pakse-Paksong in southern Laos. Additionally, in Cambodia, the species grows widely scattered in association with Deciduous Dipterocarpus in the south of the Phanom Dongrak Range at elevations from 100 to 300 m.a.s.l., and farther south in Phanom Khla, Siem Reap, Kampong Thom, Tonle Sap, Pursat, Mondulkiri, and the Kirirom Plateau. In Vietnam, Merkus pine occurs extensively on the plateaus of Kontum, Dalat, Blao, and Lang Hanh at elevations of between 500-1,200 m.a.s.l. (Cooling, 1968).

Among the insular population, the pine is found in two areas in the Philippines, one is on the island of Mindoro and the other is in the Zambales Mountains of western Luzon from 600 m.a.s.l. downwards. On the Mindoro Island, this species occurs in the northwest of the island and in the southwest of the high mountain, from the north to the south of Mount Halcon. In the Zambales Mountains, Merkus pine is found in 3 places which are separated. These 3 places are Candellaria, Masinloc, and Palauig. Surprisingly, the species on Mindoro can grow as low as 60 m above sea level (Mirov, 1967). In addition, in the south, Merkus pine reappears again in the northern part of Sumatra where pines occur in the savanna-like terrain (about 800-2,000 m.a.s.l.) regularly disturbed by occasional forest fire. This pine is found south of the Equator in the Barisan Range of Sumatra at about 1º 41' to 2º 6' S (Santisuk, 1987).

In Thailand, the pine was reported that be scattered or disjunctive distribution consisting of isolated populations intersected by large areas where the species is absent. The natural occurrence of this pine is found thoroughly in the west of northern Thailand, mainly in Lampang, Chiang Mai, Chiang Rai and Mae Hong Son provinces. In the Northern Thailand, Merkus pine grows with *P. kesiya.* The relative distribution of the two pines appears to be controlled mainly by altitude (Cooling, 1968; Armitage and Burley, 1980). The usual pattern of altitudinal distribution is with *P. kesiya* at the higher (often above 1,200 m) and *P. merkusii* at the lower elevation (50-1,000 m) but edaphic conditions may locally cause inversion (Cooling, 1968). In addition, the species occurs sporadically in small groves on the mountain range, lying from Pitsanuloke eastward to Petchaboon and Loei provinces in the northern part of central Thailand. Patches of this pine was also found in the eastern lowland of the country, extending from Surin, Si Sa Ket and Ubol Ratchathani provinces going across the border to the adjacent parts of Cambodia and Laos. It has been shown that Kanchanaburi and Phetchaburi populations are its southern most occurrences in the country (Changtragoon, 1984). The natural distribution of Merkus pine in Thailand is shown in Figure 2.

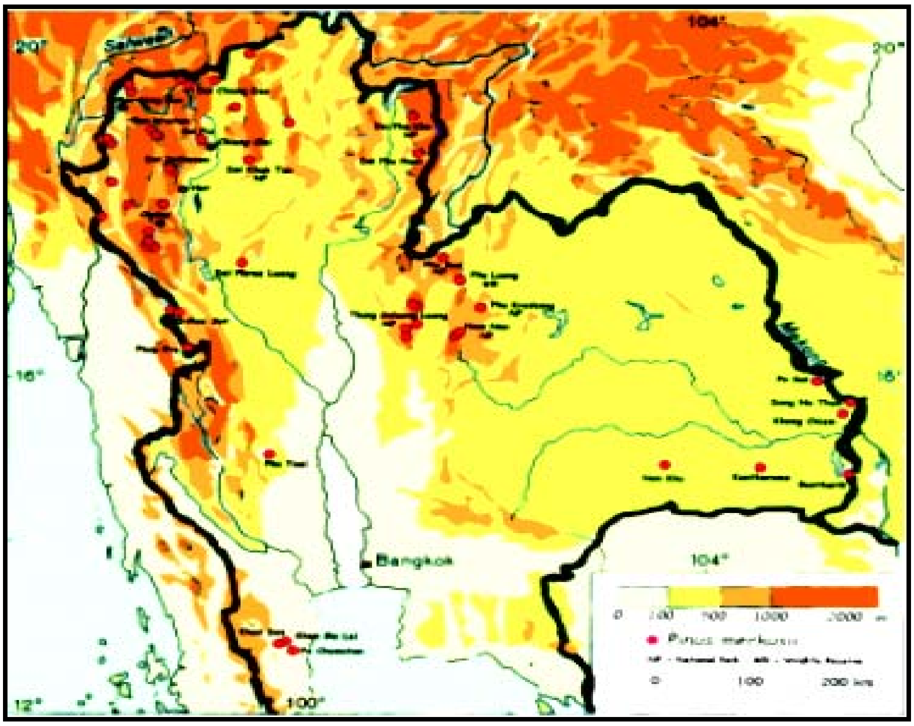


Figure 2 Natural distribution of Merkus pine in Thailand.

Source: DFSC (2000)

Cooling (1968) concluded that Merkus pine in Thailand is found in three main types of forest or habitat, including (1) Mixed *P. merkusii* and Dry Dipterocarp Forest of the higher altitutdes 750-1,000 m in the north and northwest of the country and possibly also of the pine forest near Phetchabun in the north central Thailand. Typically Merkus pine occurs as small groves and scattered individuals in a matrix of deciduous hardwoods, among which *D. obtusifolius* and *D. tuberculatus* often are predominant.   (2) Mixed *P. merkusii* and hardwood forest at low altitudes up to 300 m. The pine grove in the extensive hardwood stands in the Si Sa Ket area. This type is dried deciduous, but it is certainly of much moisture aspect than type (1). Merkus pine is found singly in small groups or stands among a variety of hardwoods, chiefly *Dipterocarps* and including *D. intricatus*, *D. tuberculatus*, *Shorea obtusa*, *Pentacme siamensis* and *D. obtusifolius*. The pines are among the dominants but seldom emerge far beyond the upper hardwood canopy level. (3) Pure or nearly pure *P. merkusii* on sandstone plateau of Phu Kradoeng.

2. Silvicultural Characteristics of Merkus Pine

Generally**,** Merkus pine is an evergreen and a large pine tree with a straight and cylindrical bole. Mature trees normally reach a height of 30 m and a diameter of60-80 cm. Old trees may reach 45 m in height and 140 cm in diameter (Hidayat and Hansen, 2002). When it is a young tree, it has a pyramical crown or conical crown form with well developed apical dominance. Later they attain a flatter and more spreading crown (Werner, 1993).

The bark on the young tree is grey color, later becoming dark color and deeply fissured. Cooling (1968) suggested that in mainland forests, bark is typical of trees grown under relatively open conditions while those with bole continuously shaded by hardwood crowns are often moss and lichen covered and smoother, but the effect on thickness is not clear. Further, it appears that frequent fire may cause foliation of the outer ridged bark of open grown trees resulting in a thinner bark strongly fissured into angular plates, the fissures no longer having a predominantly vertical direction. In Sumatra, Tapanuli provenance bark is thin, mostly smooth, scarely fissured, and light in color. Whereas, Ajeh provenance bark is thick, heavily fissured, and dark color by 10 years of age (De Veer and Govers, 1952).

Merkus pine leaves are spirally arranged at the end of twigs (Phengklai, 1972). This pine has needle like leaves. Two needles are in a fascicle. Needles are slander but rather rigid with persistent basal sheathes. They have dark green color, convex, and finely tooted margin. Usually, the needle size is 16-25 cm in length. In Sumatra provenances, needles are short, thin, light, 16-19 cm long in size and 58-90 mg. per pair. Whereas, in continental provenances, they are medium to long, sometime heavy, 10-25 cm long in size and 103-195 mg. per pair (Cooling, 1968). Some external needle characteristics possible are used for species identification and identifying group of provenances. By using the number of rows of stomataes on adaxial side and the number of stomates per rows per 5 mm, Merkus pine is possible to distinguish Dola Hill and Bulolo provenances from the Thai and the Philippine provenances. Between the Thai provenances and the Philippine, it was suggested to use the number of serrates per side per 5 mm (Janecharat, 1981).

The mainland populations of Merkus pine are clearly uninodal in habit, whereas the Sumatra populations are strongly multinodal (2-7 whorls). On the multinodal trees each branch is itself more ramiform than comparable branches from uninodal trees (Cooling, 1968).

Merkus pine is a monoecious species with unisexual flowers. Male and female organs bear to be male inflorescence and female strobili, respectively. Usually, female strobili are most frequent in the upper third of the crown of the mature tree and usually at the tip of branches. Whereas, male flowers are born in 2-4 cm long inflorescence, mainly in the lower part of the crown. Male and female flowers possibly occur on the same shoot. Flowering occurs in February to March. It is a wind-pollinated species and has been considered principally outcrossing (DFSC, 2000) and selfing will yield empty seed.

Cone may be borne singly, or in twos and threes**.** The cone is cylindrical or elongate-ovoid shape. It is a green color and become orange-brown color when ripening time. The cone size is 5-10 cm long and 2-4 cm wide, up to 10 cm wide after opening (Hidayat and Hansen, 2002). The stalk of cone is about ± 1 cm long. The scales of cones are woody with oblong, smooth and tetragonal in shape. The exposal part of scales is rhomboid and furrowed. The development of cone complete after flowering 12 months in the north-west and after 15 months in the drier north-east of Thailand. Usually, the majority of cone ripens in the period April-June, though all cones in a single cluster may not ripen at same time. Cones are shed soon after releasing the last of the ripe seed, and are not persistent on the tree as is the case with *P. kesiya* (Cooling, 1968). Some silvicultural characteristics of Merkus pine is shown in Figure 3.

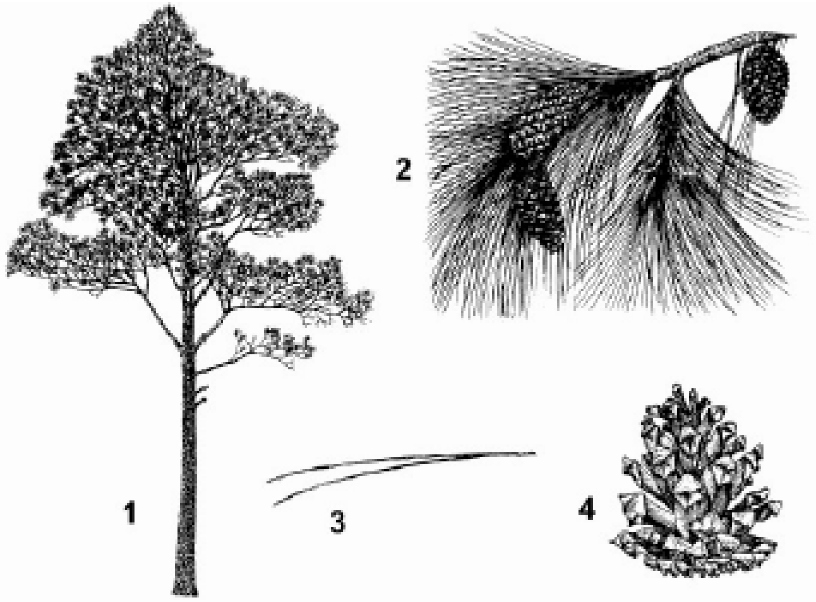


Figure 3 Silvicultural characteristics of Merkus pine.

Source: Hidayat and Hansen (2002)

Seed of Merkus pine has a wing. The wing is attached to seed with "hooks" connected to hygroscopic tissue in the base of the wing. The result is that the seed is firmly held while in a dry condition, favouring wind dispersal, but the wing is quickly released when moist condition suitable for germination are encountered (Hidayat and Hansen, 2002). Each cone scale can support two winged seeds, which are borne at the base of cone scales. The seeds are small size and ovate, slightly flat shape bearing with a thin wing. Hidayat and Hansen (2002) concluded that the wing varies from 22 to 23 mm. in length by 5-8 mm. in width. There are normally 35-40 seeds per cone and 50,000 – 60,000 seeds per kg. DFSC (2000) stated that wind dispersal distance of seeds is probably limited to a maximun of 50 meters. Occasionally strong winds may displace seed further, but dispersal of seeds between separated stands is unlikely. The seeds of Merkus pine are short-lived and a soil seed bank is not built up.

The mature seed of a pine consists essentially of an embryo embedded in the female gametophyte, a fleshy tissue with reserve food. This in turn is enclosed in a seed coat, the outer layer of which, apart from the membranous wing, is hard or stony. While, the embryo is imbedded in the tissue of the female gametophyte commonly called the endosperm. In pines (as in other gymnosperms), double fertilization does not take place as in the angiosperms, and the "endosperm" (female gametophyte) has the haploid number of chromosomes instead of being triploids as in many angiosperms (Mirov, 1967).

Grass stage is a morphological characteristic which posses an exceptional juvenile growth pattern occurrence in several pine species, e.g. *Pinus montezumae* Lamb. and *P.michoacana* Mart. in Mexico highlands (Perry, 1991), *P. palustris* Mill. in southern United States (Brown, 1964), and *P. merkusii* in mainland Southeast Asia (Cooling, 1968) (Figure 4). Following germination subsequently, the grass stage is an initial period of slow shoot growth with inhibited internodal elongation and it ends when a period of rapid shoot growth with normal internodal elongation begins (Sirikul, 1990). The slowly developing terminal bud is well protected by long secondary needles and the seedlings also develop a thick secondary cortex in their short, branchless stem (Koskela, 2000). The formation of the grass stage is under strong genetic control, but environment conditions determine its duration (Brown, 1964).

Figure 4 Grass stage of Merkus pine.

Source: DFSC (2000)

Based on differences in the development of seedling, which is genotypic variation in morphological shoot development pattern among the Southeast Asia populations. Sirikul (1980) separated three groups of provenances:

- High altitude provenances or ecotypes which include Fang, Khun Yuam and Mae Sanaam of Northern Thailand. Seedlings from these provenances were characterized by a pronounced grass stage and poor height growth in the first years followed by rapid increase in height growth at ages 6-7.

- Low altitude provenances or ecotypes with include Si Sa Ket, Ubon Ratchathani and Surin in Northeastern Thailand. Seedlings from low altitude provenances had an intermediate level of the grass stage and moderate height growth in the first years up to ages 1-3.

- Insular provenances, which include Indonesia and the Philippines. The seedlings from insular provenances do not have a grass stage. These provenances developed the best height growth at an early age. At age 10 the insular provenances were surpassed by the northeastern Thai provenances both in height and diameter growth.

From above results, Merkus pine in Thailand consists of two group populations, high-altitude mainland population and low-altitude mainland population. So performances of seedling growth in northern Thai populations show a longer lasting grass stage than northeast Thai populations. In northern Thailand, the seedling may stay even up to seven years in the grass stage, but it lasts one to three years in northeastern part of the country. (Lauer, 1993) suggest that the high-altitude and low-altitude populations may have adapted differently to fire and perhaps also to other environmental stress factors like drought.

3. Morphological Characteristics and Growth of Merkus Pine

Growth, in a restricted sense, is cell division (increase in number) and all cell enlargement (increase in size), which are the irreversible increase over a given period of time. Furthermore, it is the formation of new tissue. Shiozaki (1998) stated that the rate of growth might be expressed as the increase in weight, volume, area or length per unit time. So it may be evaluated by measurements of mass, length or height, surface area, or volume.Growth of tree can be defined according to two principles, biology or mensuration (Tourchob, 1985). Biologically speaking, tree growth refers to the formation of new tissue and increase in size. In mensurational term, tree growth may be defined as a change in tree dimension. These tree dimensions are referred to diameter, height and stem form. While, Mirov (1967) stated that growth is of an intermittent character and different parts of a tree growth at different time.

Generally, the growth pattern of trees at different stages of development differed remarkably with tree age. Kramer and Kozlowski (1960) stated that tree growth patterns in girth or height in general follow the sigmoid curve. A sigmoid growth curve begins with a generally brief period of juvenile acceleration, followed by a period of very rapid growth in the sapling and pole stages, and ending with a relatively long period during which height growth is very low (Baker, 1950).

The growths of tree depend on appreciated factors in order for the trees to grow and to survive. Key factors to control growth of trees are in 2 types. Firstly, there is genetic factor, which can transfer to its offspring. Second, there is environmental factor such as climatic factors, biotic factor, and edaphically factor, etc. Generally, specialty of growth and its shape will be determined by its genetic traits. While floristic composition will be determined by environmental factors. It can be seen that environment is external factor that can be controlled or can be suitably managed for the growth of each tree. So the study on environmental factor will well tell the growth of tree and its response to such factor (Huttametta, 2004). Furthermore, Daniel *et al*. (1979) stated that the growth of a particular plant depends on its capacity to express its genetic potential through physiological functioning within the environment to which it is expressed. While Hocker (1979) also emphasized that other factors affect tree growth including tree age, stand density and site quality.

For tropical rain forest usually grow continuously and may have no real seasonal aspects. Normally, trees under plantation and natural conditions were performed differently in growth patterns. Under the natural condition, tree growth depends much on the environmental factors, i.e. adaptation to climatic factors, competition, and resistance to diseases and insects damaged. On the other hand, under the plantation condition, young planted trees have less competed in tree nutrition, water and light intensity but tree growth is depended much on the spacing used. A trend of growth and development are depended on tree species, provenances, family, and even individuals (Tourchob, 1985).

Merkus pine is as a whole an example of a light demanding pioneer species normally confined to poor well-drained soils (usually podsolic soil). In its natural habitat and under suitable exotic conditions, Merkus pine has generally been found to have fast growth, large dimensions and good form. From the result of study, it was found that Merkus pine was marked morphologically by differences between insular and continental seed source. The continental materials showed a pronounced grass stage and fewer stem malformations. In contrast, the seedlings of the insular provenances are slender and make rapid height growth, which continues throughout most of the year due to absence of grass stage (Cooling, 1968). The Sumatra provenances are capable of very rapid growth indeed according to site quality and yield tables published for the plantations in Java (average annual increment of 14-30 cubic meters per hectare at age 20 according to site). The bulk of evidence suggests slow growth in mainland provenances although they appear to have a period of relatively rapid growth between about the seventh and thirtieth years. The rate of growth is in the order of 1 to 2 m per year in height and 0.7 -1.0 cm in diameter once the grass stage has been passed.

The continental provenance in Thailand is consists of two group populations, high-altitude mainland populations and low-altitude mainland populations. The ones from the eastern lowland (low-altitude) were better growing and survival compared to the provenances from north of Thailand (high-altitude) showing poor growth; this was also due to a lesser degree of grass stage. In Thailand, Merkus pine is often impressive in size and straightness of bole compared to the hardwoods living next to it and in relation to the poor site conditions under which it is often found. It also is the larger dimension and straighter than *P. kesiya*.

Hansen (1999) stated that the maximun height of Merkus pine is about 60 m but the stand height is around 30-35 m. While Cooling (1968) emphasized that mature Merkus pine is usually about 30 m in height and attains diameters in excess of 75 cm. In the Baw Luang area, several trees were 27 to 30 m in height and 65 to 90 cm of diameter. These were predominants or emergents over Dry Dipterocarp Forest. Secondary growth following cultivation was frequent and contained many pines with height up to 20 m and diameter up to 50 cm. For morphological characteristics of the species in the *in situ* gene conservation at Surin province, it was found that average tree height, average length of clear bole, average DBH, average diameter at ground level and average crown diameter were 21.4 m, 14.73 m, 33.64 m, 37.62 cm, and 4.74 m, respectively. Correlation between any pair of characteristics was highly significant (Jenkarnying, 1983). Generally, Merkus pine has average increment rate of stem girth approx. 0.57 inches or 1.45 cm per year (Troup, 1921). However, the younger stems show an appreciably faster diameter increment than the older trees despite widespread fire and a good deal of resin tapping, both of which factors must reduce increment (Cooling, 1968). When the young trees, it has a pyramidal crown form with good development of apical dominance once the grass stage has been passed. It later attains a flatter and more spreading crown. In Thailand, the species is strongly uninodal averaging one whorl of branch per year. Occasional “foxtail” trees are seen in pole stage growth and in such cases several years may pass without the development of a whorl of normal branches though occasional very short malformed branches may be product. Merkus pine in Thailand has thick bark (usually to the order of 6 cm at breast height) on mature trees. Thick bark confers a marked degree of fire resistance on this species. The growth of Merkus pine in Thai plantations was expected that its average increments equal 1 to 2 m per annual in height and 0.7 to 1.0 cm in diameter once the grass stage had been passed.

4. Genetic variability of Merkus Pine

Genetic variability is the state or quality of being variable, or subject to the occurrence of heritable or nonheritable differences in the permanent structures of cells, among individuals of one population, or among population i.e., of having the tendency to vary in form, nature, substance, etc. Rieger *et al.* (1976). To understand genetic diversity within species, one must be able to describe and quantify genetic variation in a population and the pattern of genetic variation among populations. Genetic variation within population is revealed by average number of alleles per locus, average heterozygosity per individual and proportion of polymorphic loci (Hedrick, 1999).

Several factors influence the genetic variation are mutation, recombination, genetic drift, gene flow between populations and natural selection (Hansen, 1999). Rieger *et al*. (1976) stated that genetic variation in a population is controlled by three main factors: the input of new genetic information due to mutation and gene flow; the erosion of this variation by selection and sampling errors; and the protection of the stored variability by cytophysiological devices and ecological factors

Genetic variation of a species can be assessed by different techniques such as study quantitative traits in the field, in provenance trials and from herbarium material, biochemical and gene markers which can be studied in the laboratory for variation in gene level. Furthermore, genetic variation pattern can also be predicted from ecogeographical variation (DFSC, 2000).

In tree improvement, genetic variability is one of the most important aspects. Furthermore, genetic variability in any trees characteristics is considered to be an important research works related to plant biodiversity. Like other living organism, genetic variability of selected tree species varied among and within provenances. Genetic variability of Merkus pine was previously studied in quantitative traits, i.e. the photosynthetic rate of seedling from 3 seed sources by Luukkanen *et al*. (1976). In 1980, Bhumibhamon and Atipanumpai observed the clinal variation on seed weight. Additionally, Bhumibhamon and Janecharat (1981) studied the variation of 12 external needle characters. In addition, variation in nuclear characteristics of Merkus pine from 6 seed sources in Thailand and Indonesia was investigated by Bhumibhamon and Changtragoon (1983). Then in 1984, Changtragoon investigated on provenance variabilities in morphological characteristics, seed, and pollen of 13 year-old Merkus pine from 14 provenances at Huey Bong, Chiang Mai. Additionally, Pothieng (1986) studied variation on certain seed characteristics and seed respiration of Merkus pine from different provenances and different trees of the same provenance, etc.

Furthermore, genetic variation on biochemical characteristics was studied by Changtragoon and Finkeldey (1995) on 11 natural populations of Merkus pine in Thailand. The results revealed that only little genetic diversity at 14 isozyme gene loci. Allelic differentiation among populations is also small, but higher than the differentiation reported from many other conifers. When comparing to the Merkus pine populations in Indonesia, it was noticed that population in Thailand have a lower level of genetic variation (Siregar and Hattemer, 1999). The study also revealed in some populations having high selfing rates which account for the high inbreeding coefficient. The low genetic diversity of Merkus pine in Thailand, which is also found low genetic variation likewise with Vietnam, was explained by bottlenecks and consequently genetic drift in evolutionary history of the population. In addition, ecological factors such as scarcity of foreign pollen available for fertilization of ovules due to low population density, poor synchronization of flowering periods, and over-mature of most stands resulting in limited flower production are mentioned as probable reason for the high breeding. Szmidt *et al*. (1996) studied allozyme variation and showed very low intrapopulational variability but a high level of interpopulational differentiation of Merkus pine in natural populations from Thailand and Vietnam.

Furthermore, Sa-Ardavut *et al.*(1989) study about Merkus pine, the report stated low seed proportion, frequent abortions of female flowers, high proportions of empty seeds, and low seed germination.

Provenance Trial of Merkus Pine

Provenance trials is an experiment in which seeds are collected from number of widely scattered stands and the seedlings are grown under similar conditions. The purpose of the provenance trials establishment is to select the most suitable seed sources that could adapt to the total environment. After provenance trials evaluation, promising provenances could be used as the sources for further tree improvement program including the establishment of seed production area, plus tree selection, *in situ* gene conservation forest, or used as the sources for plantation establishment (Tourchob, 1985).

In Thailand, the study *P. merkusii* from field trial was processed from growth initial of trees. Based on the study of seedling of Merkus pine from different provenance, both Thai provenance and Sumatra provenance, on provenance trial at Huey Bong. Sathaveerapong (1971) reported the provenance variation on germination rate, survival rate, and average height. Seedling height was indicated a clear difference between the non-Sumatra and Sumatra material. Same as the results from field trials of species were studied by Chuntanaparp *et al.* (1974). After ending of third growing season there were significant differences in height in two provenance trials located in Chiang Mai province and Surat province. The provenance of Sumatra origin showed superior height growth due to absence of grass stage. In the case of the Thai provenances, the ones from the eastern lowland were better growing and survival compared to the provenances from north Thailand showing poor growth; this was also due to a lesser degree of grass stage. In 1974, there are no significant differences in survival existed. Significant difference in heights and changes in ranking were observed, the two provenances from northeastern Thailand (#1019 and #1020) were now the tallest, followed by the one of Sumatra origin (#1026) (Hansen, 1999).

HŰbertz and Sirikul (1983) evaluated Merkus pine in Chiang Mai Provenance trial. The trials assessed were survival, height, diameter, stem form and defects, branch characteristics, frequency of insect attacks and flowering. Furthermore, they also found significant differences in stem forms and defects and diameter (#1019 and #1020 still were superior). From study on branch characteristics, it was found that the continental provenances having long annual growth and few whorls with thick branches. In contrast, the insular provenances had many shorter annual growth and thinner branches.

The provenances trial of eight Thai provenances of Merkus pine at Huey Bong Experimental and Gene Conservation Station informed that the Si Sa Ket provenance was the best in height and diameter growth followed by the provenance from Surin. The lowland provenances however, showed a significantly higher wood production than the other provenances (DFSC, 2000). Furthermore, twelve quantitative traits (survival at the age of 1 and 5, diameter, height at the age of 5, 7, and 25, stem form, foxing, branch coarseness, density by pilodyn, and bark thickness) of Merkus pine were used for analysis of eight Thai provenances and established at Huey Bong using multivariate statistics (Hansen, 1999). The results suggested that the eight Thai provenances were divided into four groups. The results reveal important genetic differentiation between provenances of Merkus pine in Thailand, and indicated a pattern related to geographic distance. Two lowland provenances of the northeast differed clearly from the rest. Furthermore, the Khao Maa Lai provenance of the southwest and Khun Yuam of the northwest differed from the main cluster of provenances. For many analyzed traits there seems to be a northwest-southeast clinal variation.

From the study of Changtragoon (1984) in provenance trials plot, the results were found that the survival percentages of 13 year-old *P. merkusii* ranged from 62.96% to 96.30%. The average height, length of clear bole, DBH, diameter at ground level, and crown diameter were 6.07 m, 1.23 m, 10.16 cm, 12.94 cm, and 2.65 m. respectively. Significant variation on all studied characteristics was observed. Phoonsree (2003) studied in provenance trials of 31 year-old and the result showed that #1019 and #1020 had high survival percentage. In contrast, #1022 had lowest survival percentage. Chiang Mai (#1018) was the best on DBH, Height, Biomass, and Volume. Additionally, #1022 also was the poorest on DBH, Height, Biomass, and Volume.

***In Situ* Gene Conservation of Merkus Pine**

In situ gene conservation is a one of two types of gene conservation strategies which have been applied to forest tree species. The *in situ* conservation is defined as maintenance of species within the ecosystem in which it originally evolved for response to natural evolutionary process (Soonhuae *et al*., 1995). The gene conservation forests are natural stands preserved and treated in such a way that further exploitation is prohibited and natural regeneration favored. *In situ* gene conservation forest has advantages over the *ex situ* gene conservation as the gene pool is normally greater. So, special traits tree selection can be made in the Wolfhagen *et al*. (1982).

The reasons of conservation Merkus pine as a result of the pine is native pine species of Thailand. It is sources of timber, as well as supplying pulpwood, resins, turpentine, and various chemicals. The depletion of this pine in Thailand is the cutting and collection of selected part of the very resinous heartwood for fire lighting sticks. Recognizing the threat to this valuable genetic resource, the Royal Forest Department of Thailand, with the assistance of the Danish Government in Pine Improvement program, has taken steps to conserve *in situ* some of the best stands of Merkus pine (RFD, 1989).

In 1979, the continental provenance of Merkus pine was initiated to conserve as *in situ* gene conservation of the best Merkus pine stands which is conserved in the Northeast of Thailand for seed production, selection and possible further breeding. A forest reserve of about 100 ha has been established in Nong Khu, Surin Province. Then gene conservation program of Merkus pine was later expanded to include stands at Khong Chiam, Ubon Ratchathani province in 1981. The proposed area is larger than Nong Ku gene conservation area with 640 ha at Khong Chiam, stand was established to protect Merkus pine and hardwood association. RFD (1989) stated that effort are underway to help the local population understand the importance of these projects to conserve valuable gene pools not only of pines but also of a number of tropical hardwoods and other component of these ecosystems.

The *in situ* gene conservation strategy has many problems. In the beginning, the gene conservation is an attempt to protect the existing endangered stands from extinction, which of course being result of deforestation from the local inhabitants or from outsiders. This is a social problem that challenges to keep the existing stands permanently. People usually invade into the conserved stands for many purposes, in particular to get benefits in term of commercial values from such the stands. In the Northeast with the *P. merkusii in situ* conservation programme, villagers invaded into the conserved area to cut down and process the big lumbers as well as to tap the resin, and collecting other forest products. All these illegal practices are the main problems to keep the conservation stands fruitfully as expected (Kijkar, 1987). The ecosystem of Khong Chiam *in situ* gene conservation forest is threatened and fragmented due to illegal agricultural activities, settlers, clearing, cultivation, forest fires, firewood chopping, illegal logging, and charcoal production. For the stand of Merkus pine in Surin *in situ* gene conservation forest are suffered from destructions. In the study of Jenkarnying (1983) reported that about 30.29 % of the existing pine stand in the forest was debarked while the problems caused by resin tapping, forest fire, branch damaged, leave damaged, and termite attacked were 52.36 %, 46.29 %, 66.03%, 74.33 %, and 2.39 %, respectively.

Therefore, solving the problems can do that the integrated motivation within the conservation stands should somehow to invade and needs good planning, action, close supervision, and cooperation from various related institutes. Cooperation from the powerful head people around the conservation stands proves also very important to protect the stand from being invaded by other people (Kijkar, 1987).

Structural Characteristics of Forest

Structure means the composition of the biological community including species, numbers, biomass, life history and distribution in space of population (Wachrinrat, 2000).

Density is the count of number of individuals of a particular species per unit area (Kershaw and Looney, 1985). While relative density is the density of one species as percent of total plant diversity (Barbour *et al.* 1980).

The frequency of a species is described as the chance of finding a species in a particular trial sample (Goldsmith and Harrison, 1976). It can be measured by noting whether a species is present or not in a series of randomly placed quadrats (Kershaw and Looney, 1985). Frequency relates to the number of times a species occurred in a given number of repeatedly placed small sample plots. Relative frequency is the frequency of one species as a percentage of total plant frequency (Barbour *et al.* 1980).

The degree of dominance of a species is given by its share in the different layers of the vegetation. The relative abundance of the different species may indicate whether each single species has any influence on the system. Addition, the relative dominance is the cover of a particular species as a percentage of total plant cover. Generally, covering of trees canopy is assumed to correlate with trunk cross-section area (Basal area, BA) or with trunk diameter at breast height (DBH) (Wachrinrat, 2000).

Importance value index is defined as the sum of relative dominance, relative density and relative frequency. It can range between 0-300 (Curtis and McIntosh, 1951). This index show ecological success of the abundant species and will provide an indication of the dominant species (Wachrinrat, 2000).

The recognition of more or less continuous layers of vegetation on the basis of height differences is a structural approach to vegetation description and is inherent in most life-form classification (Goldsmith and Harrison, 1976). A structural approach can be described the organization of complex vegetation types. Each layer is described in terms of height and in many instance floristic information. A technique pioneered in the tropics relates a complete visual representation of the stratification of the community in a profile diagram. Profile diagram can also be usefully employed in vegetation of lower stature to illustrate the relationship between topography and the distribution of individuals of a species. Additionally, it also can be used to illustrate details in vertical spacing of species. A method of description of the stratification of forest vegetation which was constructed by Davis and Richards (1933) using to scale, profile diagrams taken from narrow strips. The total height, height to first branch, lower limit of crown and width of crown are used to describe in the method.

Diversity is a measure of variety in ecological communities. The species diversity for a community is a function of the number of different species present, the number of individuals per species, and the total number of individuals of all species in that communities (Southwick, 1976). They are several indices. The first is the Shannon-Wiener index of diversity, H(S) sometimes be called the Shannon-weaver index (Shannoann and Weaver, 1949). This index is used when the relative abundance of the each species is logarithmically related to their significance to the whole system. In case of the relative abundance was assumed to be linearly related to the significance for the system, a useful measure of diversity was presented by Simpson (1949) (Jacobsen, 1983). The Simpson's index of diversity gives very little weight to rare species and is most sensitive to the numbers of abundant species. While Peet (1974) recommended that the reciprocal of the this index as the best index of diversity because of sensitive of Shannon-Wiener index to rare species and this is where sampling error may be most pronounced. Species diversity may be composed of two components. The first one is the number of species in the community, which ecologists often refer to as species richness. Additionally, the second component is species evenness or equitability. Evenness refers to how the species abundance (e.g., the number of individuals, biomass, cover, etc.) is distributed among the species. It is maximum when all species have the same number of individuals and decrease toward zero as the relative abundances of the species diverge away from evenness (Ludwig and Reynolds, 1988). The richness are composed two richness indices as the Margalef (1985) index (R_1) and the Menhinick (1964) index (R_2). Ludwig and Reynolds (1988) recommend using R_1 and R_2 as richness indices only if these assumptions are clearly met. Diversity indices incorporate both species richness and evenness into a single value. In some cases, a given value of a diversity index may result from various combinations of species richness and evenness (Wachrinrat, 2000).

Wolfhagen *et al*. (1982) stated that the *in situ* gene conservation forest is a mixed pine / dipterocarp forest which comprises of about 4,600 trees of *P. merkusii*, with the age ranging from 10 up to 50 years or more. While, Jenkarnying (1983) reported that the forest type of the gene conservation forest at Nong Khu is Dry Dipterocarp Forest with *Pinus merkusii* dominant. In addition, stand structure comprise of three layers. The first layer consisted of pine trees of 20-25 metres height. The second layer consisted of local pine, *Dipterocarpus obtusifolius* Teijsm. ex Miq., *D. tuberculatus* Rpxb., *Irvingia malayana* Oliv. ex A. Benn. , *Mangifera caloneura* Kurz., etc. of 10-20 m. height. The third layer was the ground cover with vegetations like Imperata grass, etc. Poor natural regeneration of *P. merkusii* was observed. Factors affecting to the success in natural regeneration were age of mother trees, forest fire, flooding, etc.

Genetic Markers

Changtragroon and Szmidt (1997) stated that genetic marker screening is based on the survey of genetic diversity as revealed by variation at specific gene loci and provides information about the amount and distribution of genetic diversity within and among populations. Furthermore, analysis of gene marker data permits estimation of outcrossing rates and thus monitoring genetic changes causes by factors affecting reproductive biology of a species. Information gained from genetic marker screening is invaluable for identification of populations which are desirable for conserving and breeding purpose and improving forestry practices which inadvertently alter natural gene pools of domesticated.

The most striking advantage of the use of biochemical and molecular markers (together called genetic markers) in investigations of genetic variation is that it is fast and relatively cheap. The most striking disadvantage of genetic markers is the coarse-grained result which the method gives (Hansen, 1999). They are many molecular markers such as isoenzymes, RFLPs (Restriction Fragment Length Polymorphisms), RAPDs (Randomly Amplified Polymorphic DNA), Microsatellites which have been used to determine genetic diversity, structure, differentiation and mating system of forest tree species. For the limitation of the application of markers for the proposes of study is depended on whether they are dominant or codominant. Isoenzymes are condominant marker thus suitable for analyzing mating system of the forest tree species. Actually in certain specific situations it is possible to study mating system with RAPDs and such analysis can also be done with codominant DNA markers of all sorts. However, DNA markers such as RFLPs, microsatellites and RAPD are widely used to determine genetic diversity of the forest populations because they are usually more polymorphic and detect more loci than isozymes (Changtragroon and Szmidt, 1997).

Microsatellites are short tandem repeats of nucleotides, from 1-6 base pair (bp) long (Goldstein and Pollack, 1997). It is now the commonest term used to describe tandem repeats of short sequence motifs. Microsatellites have been found in every organism investigated so far. They may be highly polymorphic, especially if long and uninterrupted, and they are therefore useful genetic marker (Goldstein and ShlÖtterer, 1999). The high rate of mutation at these microsatellite loci leads to extensive allelic variation and high level of heterozygosity. They are codominant DNA markers, so it can distinguish heterozygous individuals from homozygotes. Additionally, they are characterized by high levels of polymorphism.

Microsatellite markers or simple sequence repeat (SSR) markers in Merkus pine were studied and developed to investigate genetic diversity and mating system in the established SSO in Indonesia. The results showed that of the 10 microsatellite loci isolated, five were codominant and polymorphic, two were monomorphic, two were multiband and one locus was not amplified. The number of alleles observed for each locus ranged from three to six. The value of H_E ranged from 0.389 to 0.728. Three loci were deviated significantly from HWE, due to an excess of homozygous. Null alleles may be expected to be present at these loci (Nurtjahjaningsih *et al.* 2005).

Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is now one of the most widely used techniques in genetic and molecular biology. It is a powerful and widely used technique that has greatly advanced our ability to analyze genes (Winter *et al.*, 2002). PCR is a chain reaction because the number of new DNA strands is double in each cycle, and the new and the old strands serve as templates in the next cycle (William and Michael, 2003). The methodology was first devised by Kary Mullis in 1984, but has become widely and extensively used in virtually every molecular genetics laboratory. The essence of the technique is the amplification of a selected area of the DNA sequence of interest (Hancook, 1999). PCR generates many copies of a specific DNA sequence through a series of reactions in a test tube (*in vitro*), and amplifies small DNA sequence in a population of other DNA molecules (William and Michael, 2003).

Each PCR contains four important components (Winter *et al*., 2002):

- Template DNA containing the target DNA sequence to be amplified. The template DNA is usually a complex mixture of many different sequences, as is found in genomic DNA, but any DNA molecule that contains the target sequence can be used.

- Oligonucleotide primers, each PCR requires a pair of the primers. These are short single-stranded DNA molecules (typically 20 bases) obtained by chemical synthesis. Their sequences bind by complementary base-pairing to opposite strands of the template DNA at either end of the sequence to be amplified.

- DNA polymerase, this enzyme copies the target sequence and is thermostable. *Taq* DNA polymerase is the most common using for PCR. The enzyme is from *Thermus aquaticus*, a bacterium presents in hot springs. The enzyme binds to single-stranded DNA and synthesis a new strand complementary to the original strand. It require a short region of double-stranded DNA to get started. This is provided by the oligonucleotide primers in PCR. In this way the primers direct the DNA polymerase to copy only the target DNA sequence.

- Deoxynucleotide triphosphates (dNTPs), these molecules correspond to the four bases present in DNA (adenine, guanine, thymine and cytosine) ad are substrates for the DNA polymerase. Each PCR requires four dNTPs (dATP, dGTP, dTTP, dCTP) which are used by the DNA polymerase as building blocks to synthesize new DNA.

The PCR reaction involves three basic steps (Figure 5) which take place at different temperatures and together result in the synthesis of target DNA (William *et al*., 2003; Winter *et al*., 2002).

Step 1 (Denaturation), the reaction is heated at 90-95^{o}C with usually about 5 minutes. At this temperature the double helix is destabilized and the DNA molecules separate into single strands capable of being copied by the DNA polymerase. This DNA does not have to be purified and can come from any number of sources, including genomic DNA, forensic sample.

Step 2 (Primer annealing), the reaction is cooled to a temperature that allows binding of the primers to the single-stranded DNA without permitting the double helix to reform between the template strands. The temperate used varies (typically 40-60 ^{o}C) and is determined by the sequence and the number of bases in the primers (usually about 15-30 nucleotide long).

Step 3 (Extension), this stage is carried out at the temperature at which the DNA polymerase is most active (for *Taq* is 72^{o}C). The DNA polymerase directs by the position of the primers and copies the intervening target sequence using the single-stranded DNA as a template. This is the *Taq* polymerase to extend the primers by adding nucleotides in the $5' \rightarrow 3'$ direction.

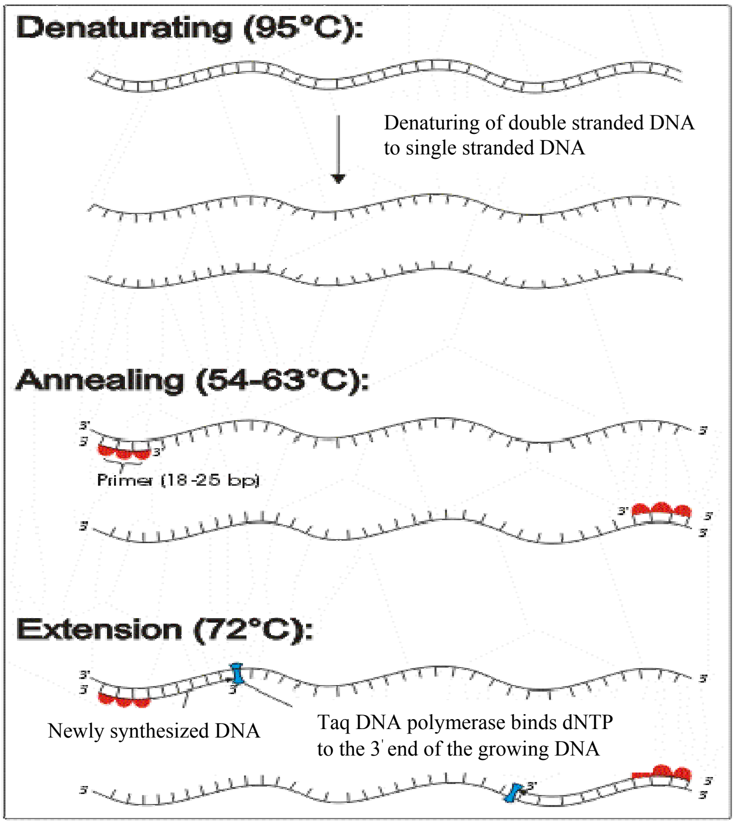


Figure 5 Three basic steps of PCR reaction.

Source: Forthum (2006)

A total of 20-40 PCR cycles are carried out depending on the abundance of the target sequence in the template DNA. Sequences up to several thousand base pairs can be amplified. To deal with the large number of separate incubations needed, the PCR is carried out using a microprocessor controlled heating block known as a thermal cycler (Winter *et al*., 2002).

Although PCR is a valuable technique, it does have limitations. Some information about the nucleotide sequence of the target DNA must be known, and even minor contamination of sample with DNA from other sources can cause difficulties. So PCR reactions must always be run with carefully designed and appropriate controls (William and Michael, 2003). A warning is that the more cycles and rounds of PCR that are carried out, the greater the number of errors that will be introduced. The polymerase can mistakes and it this happens and then further rounds of PCR are carried out, the errors are also amplified (Hancook, 1999).

Genetic Losses

In evolution process, natural selection, genetic drift, mutation, hybridization will have some effects on genetic losses. So genetic losses are in fact losses of genetic resource that effect directly to losses of genetic variation including losses of individual genes, and the loss of particular combinations of genes. It causes changes in allele frequency of population. The primary factors contributing to loss of genetic variation are directly or indirectly related to human impacts. Since the human population is growing rapidly the impacts of these factors are continually increasing. Human pressure and need for more land for cultivation, shifting cultivation by conversion of forests to farmland. As a result in habitat and forest genetic resources are lost. Furthermore, human induce exotic species to plant in the forest and thus the native species are invaded by exotic species. Additionally, they over-exploit and cause pollution. Habitat loss, induced species, over-exploitation, and pollution effect reduce in overall numbers and separation into small, isolated population. Normally, small and fragmented isolated population or threatened population often displays much low levels of genetic variation as a result of inbreeding and genetic drift (chance).

Frankham *et al.* (2002) stated that small populations become inbred at a faster rate than do large population. Inbreeding is a mating system which, in contrast to out or crossbreeding, involves the breeding together of individuals more closely related than mates chosen at random from a population. Its degree in any particular population or mating is a function of the relationship between the mating partners (inbreeding coefficient). The closet form of inbreeding is self-fertilization; other forms are sib matings and half-sib matings. All forms of this process result in an increase of homozygosity, i.e. lead to genetic fixation. Inbreeding in normally outbreeding populations generally leads to a loss of reproductive fitness referred to as inbreeding depression. In plants, morphological and physiological mechanism favoring inbreeding is common, e.g., failure of flowers to open or opening of flowers only after the pollen has been shed (Rieger *et al.*, 1976).

When small population reproduces, the subsequent generation is derived from a sample of parental gametes. Each offspring receives one allele, selected at random from each parent. Just by chance, some alleles, especially rare ones, may not be passed on to the offspring and may be lost. The frequencies of alleles that are transmitted to the following generation are likely to differ from those in the parents. Over multiple generate allele frequency charge, or drift, from one gene. So the next, a process genetic drift results in random fluctuations in allele frequency, diversification among population, fixation and loss of genetic variation (Frankham *et al.*, 2002). In summarization, genetic drift is any change, either directed or undirected in gene frequency in a population. Frankham *et al.* (2002) stated that genetic variation is lost by genetic drift in small population and by directly selection.

Furthermore, environment changes are also the destruction agent of genetic variation. The environmental changes include disease, pests, parasite competitors and predators, population, etc.

Genetic variation is required for populations to adapt to environmental change. When genetic losses occur, the species is lost of potential for future adaptive evolution for a long term. While genetic losses effect reduction of reproductive fitness in short term.

METERIALS AND METHODS

Site Selection

The present study was conducted in two locations (Figure 6), including Merkus pine provenance trials at Huey Bong Experimental Area and Gene Conservation Plantations, Hot District, Chiang Mai and the *in situ* gene conservation Merkus pine forest, Sangkha District, Surin Province. To study provenance variation on survival percentages and certain morphological characteristics of Merkus pine, data were collected from Huey Bong Provenance Trials. In determining the stand characteristics and certain morphological characteristics of Merkus pine in the *in situ* gene conservation forest, data were conducted from Nong Khu *in situ* gene conservation Merkus pine forest. Furthermore, this forest was studied on genetic variation of Merkus pine using microsatellite markers. Additionally, the evaluation of genetic losses of the species in the forest was also done.

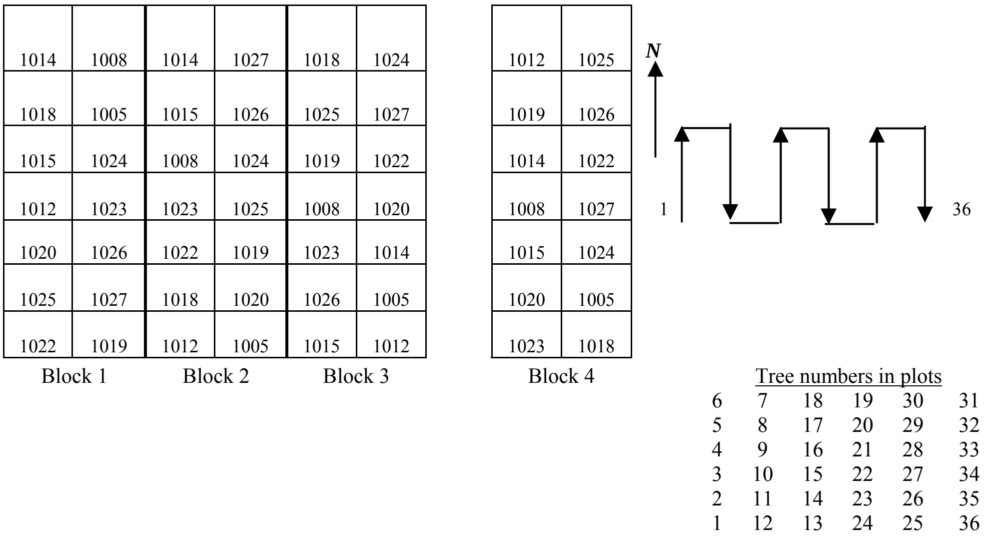
Figure 6 Locations of Merkus pine Provenance trials at Hot District, Chiang Mai Province and *In situ* Gene Conservation Merkus pine Forest at Sangkha District, Surin Province.

1. Merkus Pine Provenance Trials

The provenance trials is located at the Huey Bong Experimental Area and Gene Conservation Plantations, Hot District, Chiang Mai Province in the northern Thailand, next to the highway linking between Chiang Mai and Mae Sariang. The exact position of the station is 18 ° 10 N, 98 ° 25 E and the elevation above sea level is 790 m. Mean annual rainfall is 1,191 mm and the mean annual temperature is 23 C°.

The native stand was Dry Dipterocarp Forest where pine is commonly seen growing in association with other native hardwoods, including *Shorea obtusa*, *Dipterocarpus tuberculatus*, *Dipterocarpus obtusifolius*, *Phyllanthus emblica*, etc. The undergrowth in the area are *Imperata cylindrical*, *Cycas siamensis*, ground orchids, and others. The great soil group is the Red Yellow Podzolic with the soil depth between 150-200 cm. The top soil is sandy loam with the pH between 5.8- 6.0. The top soil contains of organic matters 0.93-1.76 %; phosphorus 1.3-3.0 ppm; potassium 3.7-4.5 ppm; calcium 1.06-1.08 me/100g of soil; magnesium 1.11 me/100g of soil, and cation exchange capacity 2.25-2.28 me/100g of soil (Tourchob, 1985).

The provenance trials was established with six month olds seedlings at 3x3 m^2 spacing. The trial was laid out as a Ramdomized Complete Block Design (RCBD) with 6 x 6 trees per plot. There is no isolation between plots or blocks but a single row of Merkus pine was planted round the outer border of the whole experiment. A plan for the trial is shown in Figure 7.



Block 1		Block 2		Block 3		Block 4	
1014	1008	1014	1027	1018	1024	1012	1025
1018	1005	1015	1026	1025	1027	1019	1026
1015	1024	1008	1024	1019	1022	1014	1022
1012	1023	1023	1025	1008	1020	1008	1027
1020	1026	1022	1019	1023	1014	1015	1024
1025	1027	1018	1020	1026	1005	1020	1005
1022	1019	1012	1005	1015	1012	1023	1018

Tree numbers in plots

6	7	18	19	30	31
5	8	17	20	29	32
4	9	16	21	28	33
3	10	15	22	27	34
2	11	14	23	26	35
1	12	13	24	25	36

Figure 7 Experimental design of Merkus pine provenance trials at Huey Bong Experimental Area and Gene Conservation Plantations, Hot Distinct, Chiang Mai.

Source: Hansen (1999)

An experimental plots of provenance trial consist of fourteen provenances. Eight of these are from the natural forests of Thailand, four originated from the Philippine, one was from Papua New Guinea (Sumatra origin) and one was from Zambia (Vietnam origin). Details of seed sources are seen in Table 1. The last two provenances in the Table 1, 1026 and 1027, are coming from outside the natural distribution area of the species.

Table 1 Natural seed sources of Merkus pine in provenance trials at the Huey Bong Experimental Area and Gene Conservation Plantations, Hot District, Chiang Mai Province

Provenances No.	Seed sources	Lat.	Long.	Elevation (m)
1005	Tha Yang, Phetchaburi, S., Thailand	12°45′ N	99°15′ E	30
1008	Phu Kradung, Loei, NE., Thailand	16°51′ N	101°47′E	1,300
1012	Khun Yuam, Mae Hong Son, NW., Thailand	18°50′ N	97°56′ E	600
1014	Mae Tha, Lamphun N., Thailand	18°21′ N	99°20′ E	800
1015	Fang, Chiang Mai N, Thailand	19°52′ N	99°15′ E	5-600
1018	Hot, Chiang Mai NW., Thailand	18°04′ N	98°10′E	1,100
1019	Sangkha, Surin NE., Thailand	14°43′ N	103°50′E	180
1020	Huey Ta, Si Sa Ket NE., Thailand	14°50′ N	104°32′E	150
1022	Santa Cruz, Zambales Philippines	15°45′ N	120°02′E	13-1,600
1023	Santa Cruze, Zambales Philippine	15°47′ N	120°01′E	7-900
1024	Panas, Mindoro Occ. Philippine	13°03′ N	120°51′E	12-1,600
1025	Kipkipan, Mindoro Occ. Philippine	13°04′ N	120°50′E	7-900
1026	Bulolo, Papua-New Guinea ex Sum.	7° S	146°E	18-2,100
1027	Dola Hill, Zambia	12° S	12°E	700

Source: Changtragoon (1984)

2. ***In Situ* Gene Conservation Merkus Pine Forest**

The *in situ* gene conservation forest is located in Tab Tan Sub-district, Sangkha District, Surin Province. The exact position of the forest is 14°43′ N and 103° 50′ E beside highway linking between Surin and Sangkha (35-38 kilometer). The forest covers approximately 3.52 ha (2,200 rai). This forest is located in national reserved forest boundary, north of area is located in Huey Kam Pod reserved forest and south of area is located in Left Bank Huey Sam Ran reserved forest. The north of forest is located near Ban Rom Yen and forest planting plot of Surin logging company, Ltd. The south of area is located near Ban Kog Yang and Ban Krahom. The eastern forest is closely Ban Nong Ku and Ban Ta Pun. The west of forest is located next with Ban Lert Aroon (Figure 8).

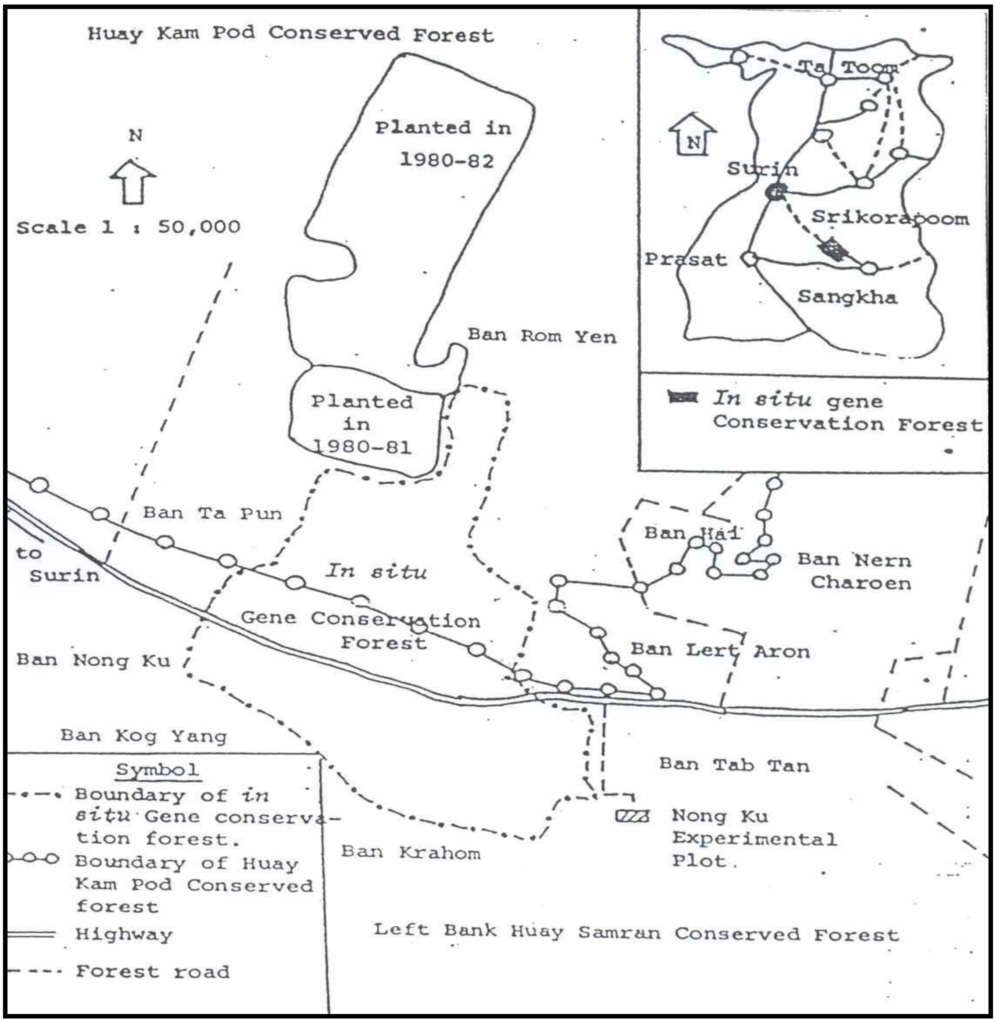


Figure 8 Location of *in situ* Gene Conservation Forest of Merkus pine at Sangkha District, Surin Province.

Source: Jenkarnying (1983)

Previously, Nong Khu *in situ* Gene Conservation Merkus pine Forest has been used to be tree planting experimental site in 1969. After the provenance trials of Merkus pine at Huey Bong, Chiang Mai Province was evaluated. Royal Forest Department (RFD) of Thailand and DANISH/FAO FOREST TREE SEED CENTER of Denmark collaborated to establish the *in situ* gene conservation forest of Merkus pine at Nong Khu, Surin Province in August 1980. Tab Tan Gene Conservation Station has managed this forest. In December 1980, this station was integrated with Nong Khu Station and formed Nong Khu Tree Plantation Experimental Station. The station handled planting for tree species experiment and managed the Merkus pine *in situ* gene conservation forest.

Methods

1. Data Collection in Merkus Pine Provenance Trials

In provenance trials at Chiang Mai province, all trees of 33 year-old Merkus pine in blocks 1, 2, and 3 were measured. Total height and length of clear bole were measured using Haga altimeters. For DBH and D_0 measurement were done using diameter tapes. In addition, crown diameter was measured using diameter tape at two positions at 90 degrees. Additionally, survival percentages of each provenance were also recorded.

2. Data Collection in the *In Situ* Gene Conservation Forest

For the *in situ* gene conservation forest at Surin province was surveyed for check the number and position of Merkus pine in the area. Moreover, trees (Merkus pine) were measured in many morphological characteristics. Total height was measured using Haga altimeter. DBH and D_0 were measured using diameter tape. In addition, crown diameter was also measured using diameter tape at two positions at 90 degrees. Furthermore, all damages were also recorded.

The four sample plots with a size of 40 x 40 m^2 were established in the *in situ* gene conservation forest (Figure 9). The sample plots were studied by applying Relevé methode of Mueller-Dombois and Ellenburg (1974) which are categorized as following:

1. Each sample plot was divided into 16 quadrates (10 x 10 m^2) to study all trees. In each quadrate, every tree having a diameter at breast height (DBH, 1.30 m above ground level) greater than 4.5 cm was measured and recorded its local name. Total height (H) of trees was measured by using the Haga altimeter. The DBH of trees were measured with the diameter tape.

2. Plots sized 4 x 4 m^2 were established on the four corners of each sample plot. Plants having a height higher than 1.30 m but a diameter at breast height smaller than 4.5 cm were recorded local name and total height of saplings was also measured.

3. Plots sized 1 x 1 m^2 were established on the four corners of each sample plot. Plants having a height less than 1.30 m were recorded local name and total height of seedlings.

4. Plots sized 10 x 40 m^2 were established in each of sample plot to study the profile diagram and crown projection diagram of plant community. The crown cover, crown shape and tree position were sketched.

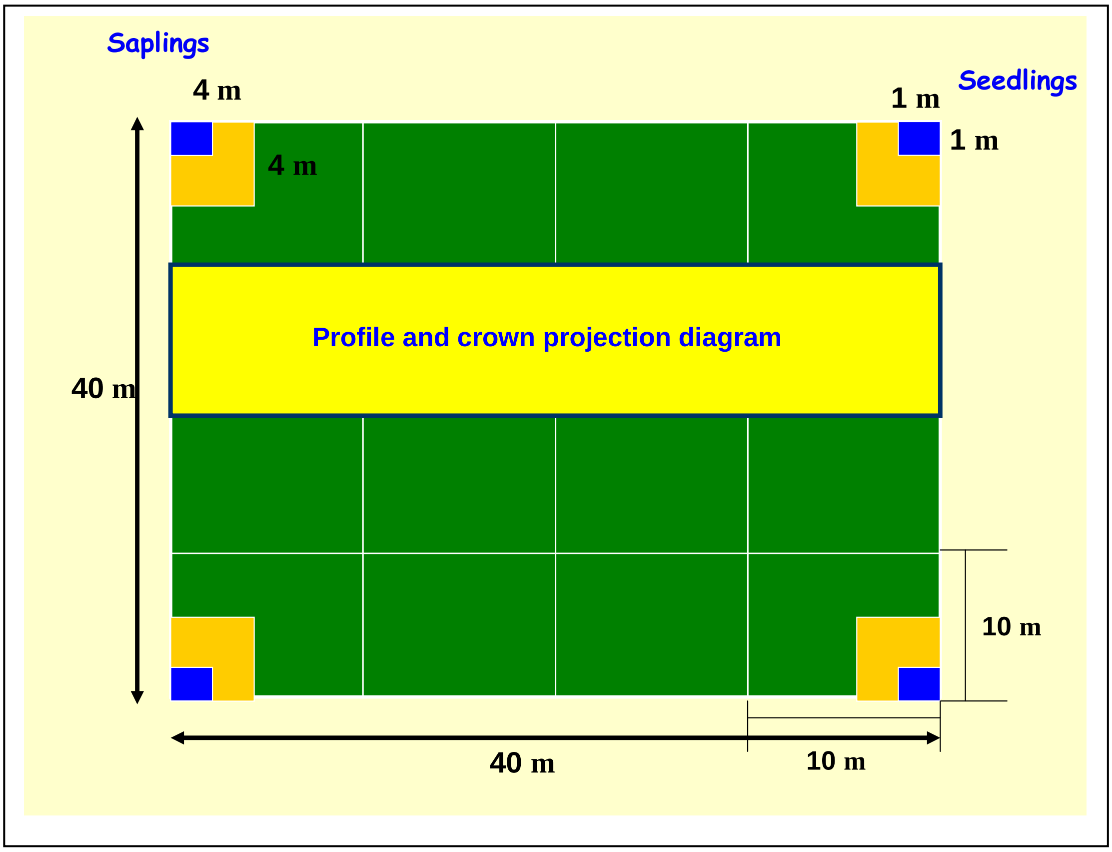


Figure 9 The sample plot with a size of 40 x 40 m^2 was set out in the *in situ* gene conservation Merkus pine forest for stand structure of the forest.

In addition, the concepts of villagers surrounding the *in situ* gene conservation Merkus pine forest area were studied using the designed questionnaires. It was open-end questionnaire. The contents of questionnaires were comprised of five sections. There were general information, socio-economic characteristics, the concepts of community on forest resource, the concepts of community on forest resource conservation, and the concepts of community on the *in situ* gene conservation Merkus pine forest at Tab Tan Sub-district, Sang Kha District, Surin Province. The respondents reside in Ban Nong Khu, Ban Kog Kra Don, Ban Ta Pong, Ban Kog Yang, Ban Krahom and Ban Lert Aron. All villages are in Tab Tan Sub-district, Sang Kha District, Surin Province.The samples to be used for the study were the household heads or the representatives of the sample household.

The simple random sampling was used and the suitable sample size was counted by Taro Yamane method (Yamane, 1973) as follow:

$$n = \frac{N}{1 + Ne^2}$$

Where, n = Sample size
N = Total population size
e = sampling error

A total population size of six villages was 680 households. The households were consisted of 120 households of Ban Nong Khu, 160 households of Ban Kog Kra Don, 91 households of Ban Ta Pong, 40 households of Ban Kog Yang, 100 households of Ban Krahom and 187 households of Ban Lert Aron. The sampling error was decided as 0.05. So the sample size which was counted using Yamane method was 255 interviewees. The questionnaires were used for collecting data by interviewing 280. In addition, the sample size of each village was also counted. The results of counting were 50 interviewees for Ban Nong Khu, 58 interviewees for Ban Kog Kra Don, 38 interviewees for Ban Ta Pong, 26 interviewees for Ban Kog Yang, 51 interviewees for Ban Krahom and 57 interviewees for Ban Lert Aron

Furthermore, the needles of Merkus pine in the forest were randomly collected through the area for using in genetic variation study. The needled samples were composed 32 individual mature trees.

3. Genetic Variation Study Using Microsatellite Markers

The Genomic DNA of each samples was extracted from their young needle tissue of Merkus pine using a modified cetyltrimethyl ammonium bromide (CTAB) method (Changtragoon *et al*., 1996) as followed:

3.1 Rinse 2-5 g frozen young needles with distilled water to remove foreign material.

3.2 Grind the tissue to a very fine powder with mortor and pestle under liquid nitrogen. Transfer frozen powdered tissue into a 50 ml capped centrifuge tube, add 700 µl extraction buffer (2x CTAB buffer: 2% CTAB, 1% PVP, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8.0, 0.2% 2-mercaptoethanol).

3.3 Incubate the samples in water bath for 1 hr at 60^{o}C.

3.4 Add 700 µl of 24:1 Chloroform: isoamyl alcohol, shake well about 10 min.

3.5 Centrifuge the tubes at 120,000 g for 10 min at 4^{o}C to resolve phases.

3.6 Transfer the aqueous phase to a fresh tube, add 700 µl of 24:1 Chloroform: isoamyl alcohol, shake well about 5 min.

3.7 Centrifuge the tubes at 120,000 g for 10 min at 4^{o}C

3.8 Transfer the aqueous phase to a fresh tube, add 700 µl of 24:1 Chloroform: isoamyl alcohol, shake well about 5 min.

3.9 Centrifuge the tubes at 120,000 g for 10 min at 4^{o}C

3.10 Transfer the aqueous phase to a fresh tube, add 700 µl of 24:1 Chloroform: isoamyl alcohol, shake well about 5 min.

3.11 Centrifuge the tubes at 120,000 g for 10 min at 4°C

3.12 Add 1x of volume of cold absolute isopropanol to precipitate, mix, and place at -20 °C for 20 min.

3.13 Centrifuge the tubes at 120,000 g for 10 min at 4°C.

3.14 Pour off liquid being careful, add 600 µl of cold 70% Ethanol, shake.

3.15 Centrifuge the tubes at 120,000 g for 10 min at 4°C.

3.16 Pour off liquid being careful not to lose pellet (barely visible), add 600 µl of cold 70% Ethanol, shake.

3.17 Centrifuge the tubes at 120,000 g for 5 min at 4°C.

3.18 Pour off liquid being careful not to lose pellet (barely visible).

3.19 Dry pellet with air drying method for 20-30 min.

3.20 Resuspend samples with 50-100 µl of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0)

3.21 Keep DNA at temperature of -20°C.

Check quality and quantified Genomic DNA by 0.8 % of agarose gel electrophoresis (1.2 g of agarose and 150 ml of TAE buffer; 0.04 M Tris-acetate, 0.001 M EDTA) with fluorescence of ethidium bromide which cover DNA method. DNA should be free from any contaminants, e.g. residual polysaccharides, proteins, RNA, remnants of phenol, chloroform, etc. 5 ng DNA were used for SSR method.

For the amplification of DNA samples, total of seven microsatellite marker (Table 2) were used by PCR technique. They were referred from Nurtjahjaningsih *et al.* (2005). The PCR amplification was carried out using a PCR thermal cycler (MJ Research, PTC-2000 Teltier Thermal Cycler). Amplified fragments were detected on non denaturing polyacraylamide gel (Gel-scan 2000) in 0.6X TBE buffer mix with Ethidium bromide for 45 min at 1,000 volt of gel current. 6% polyacraylamide is composed of 30% Acraylamide (19 g of Acraylamide and 1 g of Bisacraylamide), 5X TBE, Deonized water, 10% APS and TEMED. The combination of PCR reaction volume and thermocycler profile was as following:

PCR-reaction mixture:
2.5 µl Template DNA (5-10 ng)
1.0 µl 10xPCR buffer II
0.2 $MgCl_2$ (25 mM $MgCl_2$)
1.0 dNTP (0.2 mM dNTP)
0.5 F Primer (0.5 µM)
0.5 R Primer (0.5 µM)
0.1 µl Yea Taq (0.25 U of Yea *Taq*.)
4.2 µl H_2O
10 µl final volume

Thermocycler profile:

95°C	5 min.	
94°C	45 sec.	x 30 cycles
Primer annealing temperature (52 °C, 56 °C, 59 °C)	45 sec.	
72°C	1 min.	
72°C	12 min.	
8°C	forever	

Table 2 Characteristics of Merkus pine SSR markers, locus, product size, annealing temperature, repeat motif and primer sequences

Locus	Product Size (bp)	T_a (C^o)	Repeat motif	Primer sequence 5′→3′	GeneBank Accession no.
pm01	111-117	56	$(TG)_{12}$	F:AGAGAAGGCACGATTTTGTC R:TCCCACTAATCACTTTGAAAG	AB201535
pm02	92	56	$(TG)_{10}$	F:CTCTAAGTAGGACAAGGCCT R:TCCCACTAATCACTTTGAAAG	AB201536
pm03	112-118	52	$(TG)_9$	F:GAGTCTAATTGCAAACCCCA R:TGGAGATCTACCACTTTTTC	AB201537
pm04	284-309	52	$(AC)_8(AT)_4$	F:GAATCTAAGCATATGAAATGAG R:CTTGTTAATGCTACTAGTTATG	AB201538
pm05	132	59	$(AT)_2(GT)_{11}$	F:GCTTCAATCTATTGACCCCAT R:TAAAGGGGCAGCTGCTACAACCAATGG	AB201539
pm06	81-99	52	$(AT)_2(GT)_{11}(AT)_2$	F:CCTTCTCATTTCGATATGCAC R:ATTAAAGGTTATATGGGGCT	AB201540
pm07	181-193	59	$(GT)_5CT(GT)_5(AT)_5$	F:GAACAATCATTGCGGGTCCCG R:TATGCTGCGTTTATATGTATAAGTGTC	AB201541

Remark T_a : annealing temperature, GeneBank Accession no. is found at available source: NCBI’s website

Source: Nurtjahjaningsih *et al*. (2005)

Data Analysis

1. Provenance Variation of Merkus Pine in Provenance Trials

All morphological characteristics within provenance and between provenances were analyzed using an Analysis of Variance and Duncan's New Multiple Range Test by statistic program (SPSS program).

2. Merkus Pine in the *In Situ* Gene Conservation Forest

The morphological characteristics of Merkus pine in the forest such as total height, DBH, etc., were analyzed. Furthermore, the morphological characteristics of Merkus pine were classified and studied about number distribution in each class. The distribution was also compared with Jenkarnying's study. The relation between the results in year of 1983 and 2005 were tested with Wilcoxon-signed rank test (SPSS program). On the other hand, the damages of Merkus pine were also investigated.

3. Structural Characteristics of the Forest

The studies were analyzed using the quantitative ecological methods as follow:

3.1 Importance Value Index (IVI) the high IVI value indicates being dominance and important species. The I.V.I (Cottam, 1949) of plot was determined as:

$$\text{I.V.I} = \text{R.D. (Relative Density)} + \text{R.F. (Relative Frequency)} + \text{R.}D_0 \text{ (Relative Dominance)}$$

Where,

$$\text{Relative Density} = \frac{\text{Density of species } i\textit{th}}{\text{Total plant densities}} \times 100$$

$$\text{Density} = \frac{\text{Number of all individuals of species } i\textit{th}}{\text{Total size of quadrate used}}$$

$$\text{Relative Frequency} = \frac{\text{Frequency of species } i\textit{th}}{\text{Total frequency of all species}} \times 100$$

$$\text{Frequency} = \frac{\text{Number of quadrates that species } i\textit{th} \text{ occurred}}{\text{Number of all quadrates}}$$

$$\text{Relative dominance} = \frac{\text{Total basal area of species } i\textit{th}}{\text{Total basal area of all species}} \times 100$$

Relative density was determined from all standing trees with DBH larger than 4.5 cm in all the 40 x 40 m^2 plots. Relative frequency was determined for sixteen 10 x 10 m^2 subplots set by regularly subdividing the 40 x 40 m^2 plot. Relative dominance was obtained from the basal area at breast height, computed as $\pi D^2/4$, of each tree in the whole plot.

3.2 Species Diversity diversity indices were investigated by the Fisher's Index of species diversity, (α) (Fisher *et al.*, 1943; Shinozaki, 1983), Shannon-Wiener Index of species diversity (H) (Shannon and Weaver, 1949) and Simpson's Index (D) (Simpson, 1949). Diversity Index (N_0, N1, N2) (Hill, 1973; Ludwig and Reynolds, 1988) was also analyzed. Furthermore, the investigation was carried out the richness of the forest by the richness index analysis, in forms of Richness $Index_1$ (R_1) of Margalef's Index (Margalef, 1958) and Richness $Index_2$ (R_2) or Menhinick's Index (Menhinick, 1964). Then the evenness of the site was studied by the evenness index analysis, in forms of Evenness Index (E) (Pielou, 1969).

Fisher's Index of Diversity, (α) $[S = \alpha \ln(1 + \frac{n}{\alpha})]$

Where, S = number of species in a sample area
n = number of individuals in the same area
α = Fisher's Index of Diversity

Shanon-Wiener Index of Diversity, H $[H = -\sum_{i=1}^{N}(pi \log_2 pi)]$

Where, p_i = proportion of the number of species ith to the total number of individuals of all species
N = total number of species in the sample area

Simpson's Index, D $[D = 1 - \frac{\sum_{i=1}^{N} n_i(n_i - 1)}{N(N - 1)}]$

Where, n_i = total number of each species
N = total number of individuals of all species

Richness $Index_1$, R_1 $[R_1 = \frac{S - 1}{\ln(n)}]$

Richness $Index_2$,R_2 $[R_2 = \frac{S}{\sqrt{n}}]$

Where, S = total number of all species
N = total number of individuals of all species

Diversity Index

N_0 = S
N_1 = $e^{H'}$
N_2 = $\frac{1}{\lambda}$

Where, S = total number of all species in sample plot (N_0)
N_1 = number if abundance species in the same area
N_2 = number of very abundance species in the same area, which normally $N_2 < N_1 < N_0$
H' = Shannon's Index

$$H' = -\sum_{i=1}^{S}\left[\left(\frac{n_i}{n_i}\right)\ln\left(\frac{n_i}{n_i}\right)\right]$$

Where, n_i = The number of individuals belonging to the i*th* of S species in the sample
n = The total number of individuals in the sample
λ = Simpson's Index (adjusted by Hill, 1973)

$$\lambda = \frac{\sum_{i=1}^{N} n_i(n_i - 1)}{N(N-1)}$$

Where, n_i = Total number of each species
N = Total number of individuals of all species

Evenness Index

$$E_1 = \frac{H'}{\ln(S)} = \frac{\ln(N_1)}{\ln(N_0)}$$

$$E_2 = \frac{e^{H'}}{S} = \frac{N_1}{N_0}$$

$$E_3 = \frac{e^{H'} - 1}{S - 1} = \frac{N_1 - 1}{N_0 - 1}$$

$$E_4 = \frac{1/\lambda}{e^{H'}} = \frac{N_2}{N_1}$$

$$E_5 = \frac{(1/\lambda)-1}{e^{H'}-1} = \frac{N_2-1}{N_1-1}$$

Where, S = Total number of all species in sample plot (N_0)
N_1 = Number of abundance species in the same area
N_2 = Number of very abundance species in the same area, which normally $N_2 < N_1 < N_0$
H' = Shannon’s Index
λ = Simpson’s Index

4. Genetic Variation

The data was provided for analysis. The banding patterns of DNA sample were translated to be genotype data. A band is classified into homozygote displaying at the locus. While sample performs two bands to be classified into heterozygote at the locus (Srijumroen, 2004). Example of data translation from DNA bands to be genotype data are shown in Table 3.

Table 3 Example of data translation from bands of DNA to be genotype data

Bands of microsatellite markers			Genotype data			
Sample 1	Sample 2	Sample 3	Allele	Sample 1	Sample 2	Sample 3
	———	———	1	22	11	13
———			2			
		———	3			

Genotype at all microsatellite loci were recorded according to each individual and used for calculating population genetic parameters. The genetic variation within population was determined using the common genetic variation parameters calculating. The parameters were used for analysis be composed Allele Frequencies, Mean number of Alleles per Locus, Observed Heterozygosity (H_0), Expected Heterozygosity (H_e) and Population Inbreeding Coefficient or Fixation index (Fstatatistics). All analyses were conducted with TFPGA version 1.3 (Tool For Population Geneticc Analyses) and GenAlEx version 6 (Genetic Analysis in Excel). TFPGA is a windows program for the analysis of allozyme and molecular population genetic data. This program calculates descriptive statistics, genetic distances, and F-statistics. It also performs tests for Hardy-Weinberg equilibrium, exact tests for genetic differentiation, Mantel Test, and UPGMA Cluster Analysis. Addition features include the ability to analyze hierachical data sets as well as data from either codominant markers such as allozymes or dominant markers such as AFLPs or RAPDs (Miller, 1997). GenAlEx is a user-friendly cross-platform package for population genetic analysis that runs withing Microsoft Excel. The program enables population genetic data analysis of codominant, haploid and binary genetic data providing analysis tools applicable to plants, animals and microorganisms. GenAlEX was originally developed as a tool to assist us in our teaching of population genetic analysis, and is now widely used by university teachers and researchers around the world (Peakall and Somose, 2006).

5. **The Concepts of Community on the Forest Area**

A total of 37 variables were analyzed using SPSS computer program. The statistic analysis techniques were employed for data analysis including percentage, maximum, minimum, mean to determine socio-economic and the concepts of community surrounding the *in situ* gene conservation Merkus pine forest area on forest resource, forest resource utilization, forest resource conservation and the *in situ* gene conservation Merkus pine forest. The results of data will provide the basic information of proper management direction about people’s participation in conservation this forest area in maintaining the existing gene pool of Merkus pine.

RESULTS AND DISCUSSION

The study on provenance variation on survival percentages and some morphological characteristics of Merkus pine, Merkus pine characteristics in the *in situ* gene conservation, Structural characteristics of the forest, the concepts of village surrounding the forest and genetic variation of Merkus pine in the *in situ* gene conservation Merkus pine forest were showed and discussed, as follows:-

Provenance Variation on Survival Percentages and Certain Morphological Characteristics

1. Survival Percentages

Under natural conditions, the survival percentages are decreased accordingly with the age. It was found that the provenance with highest survival percentages of Merkus pine was northeastern Thailand origin (provenance Nos. 1020, and 1019), and followed by trees of Mae Hong Son (provenance No. 1012), Sumatra origin and other Northern Thailand origins, respectively. The Philippine origin had however the lowest survival percentages. In the present study, the survival percentages ranged from 10.19 % to 85.25 %, as illustrated in Figure 10.

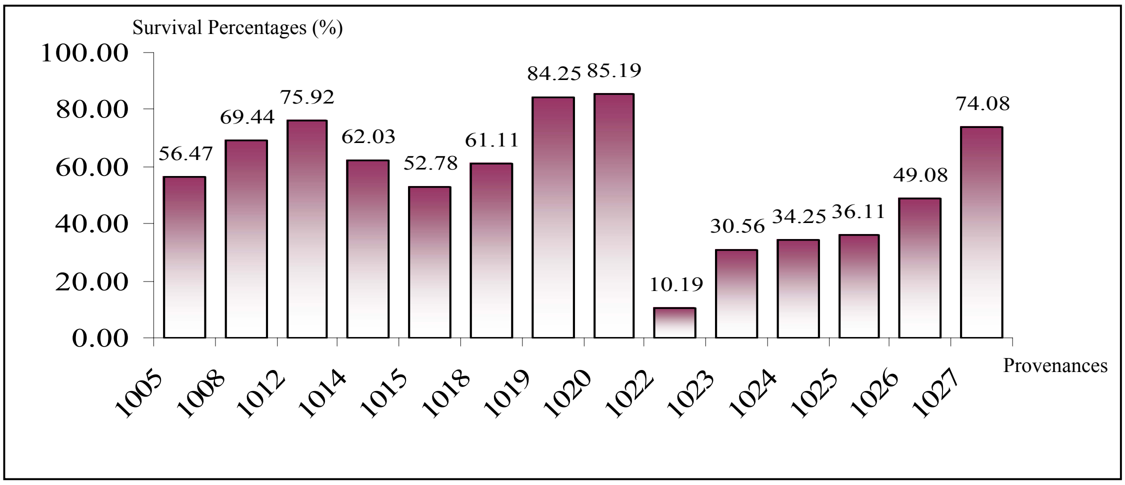


Figure 10 Survival percentages of 33 year-old Merkus pine at Huey Bong Provenance Trials in Chiang Mai.

Furthermore, Table 4 shows Analysis of Variance on survival percentages. The results indicated the highly significant difference at 99% level in survival percentages among provenances. Variation in survival percentages performance within the provenance or between blocks was found the significant difference at 95% level.

Table 4 Analysis of Variance on survival percentages of 33 year-old Merkus pine at Huey Bong Provenance Trials in Chiang Mai.

SOV	df	SS	MS	F
Between provenances	13.00	2445.62	188.13	14.84**
Within provenances	2.00	96.33	48.17	3.8*
Error	26.00	329.67	12.68	

** Highly significant difference at 99% level

* Significant difference at 95% level

The result of survival percentage of 33 year-old Merkus pine showed that the ranking was still remained the same as previously reported by Changtragoon (1984) and Phoonsree (2003). Changtragoon (1984) stated that the highest survival percentages was northeastern origin (provenances No. 1019, No. 1020, and No. 1008), and followed by trees of Sumatra origin and northern Thailand origin. In addition, Phoonsree (2003) presented that the highest survival percentages were Surin provenace (1019), and followed by Sri Sa Ket (1020) and the lowest survival percentages was No. 1022 from the Philippines. But the range of survival percentages in this study was lower than 13 year-old trees, there was in the range from 62.96 % to 96.30 % (Changtragoon, 1984). Additionally, this result was found that was near with the results of Phoonsree (2003), which is showed in the range of 16.67 % to 85.42 %. The percentages on survival was decreased in later years may be dued to the increasing of age and the improper silvicultural treatments.

2. **Total Height**

Average height of Merkus pine in provenance trials were measured during 1971-1984. It indicated that the average height of all provenances in early period was increased gradually development. In the early period of tree growth, Sumatra origin showed the best performances for 8 years and was substituted by provenances No. 1019 and No. 1020 which were originated from NE Thailand (Changtragoon, 1984). Then in 2004 (17.50 m), the height growth differed slighthy with in 2002 (17.25 m) (Phoonsree, 2003). This means that the height growth increased only.

In the part of performance in height growth of 33 year-old trees was found that the best provenances were Surin (No. 1019), and followed by Sri Sa Ket (No. 1020) in the NE Thailand. The provenances from No. 1008, No. 1015, and No. 1024 had the moderate height growth. In contrast with the 13 year-old trees, they were poor height growth (Changtragoon, 1984). And No. 1022 showed the lowest height growth which like as 31 year-old trees (Phoonsree, 2003). The average height growth of 33 year-old of Merkus pine is shown in Table 5.

Table 5 Average height of 33 year-old Merkus pine at Huey Bong Provenance Trials in Chiang Mai.

Provenance No.	Average height (m.)
1019	20.14 ± 1.24 d
1020	18.95 ± 0.67 de
1024	18.30 ± 0.72 cde
1027	18.30 ± 0.60 cde
1005	18.25 ± 0.91 cde
1008	18.03 ± 1.62 bcde
1015	17.47 ± 1.03 bcd
1014	17.47 ± 0.84 bcd
1026	17.31 ± 0.04 bcd
1025	17.29 ± 1.23 bcd
1018	17.10 ± 0.79 bcd
1023	16.11 ± 2.21 abc
1012	15.74 ± 0.86 ab
1022	14.53 ± 2.36 a
Average	17.50 ± 1.70

Remark: Values are expressed as means ± S.D. Mean followed by the same letter (s) in a column do not differ significantly at 0.05 level by Duncan's New Multiple Range Test.

From Analysis of Variance of height growth (Table 6), it was found that variation among provenances was highly significant differences at 99% level. On the other hand, variation in height growth performances within the provenance or between trees was found to be non-significant difference.

Table 6 Analysis of Variance of height growth in 33 year-old Merkus pine at Huey Bong Provenance Trial in Chiang Mai.

SOV	df	SS	MS	F
Between provenances	13.00	75.79	5.83	3.92^{**}
Within provenances	2.00	4.17	2.08	1.40^{ns}
Error	26.00	38.64	1.49	

** Highly significant difference at 99% level

^{ns} Non-significant

3. Length of Clear Bole

The length and stem straightness are under genetic control (Changtragoon, 1984). Under natural condition, the length of clear bole depends on the ability of natural pruning. Moreover, the spacing of planting trees is also control the length clear bole of trees. Narrow spacing would stimulate height growth and narrow crown development.

For the present study, trees were planted with a spacing of 3x 3 m^2. They were not treated with artificial pruning and thinning. The result on length of clear bole of 33 year-old varied from 8.34 to 11.21 m. When comparing between 13 year-old trees (0.61-2.01 m) (Changtragoon, 1984), it was found that development of Merkus pine in this trials has increasing. The longest clear bole was found in Provenance No. 1026. As well as No. 1020 and No. 1019 were the longest clear bole group. The provenance No. 1015 had the shortest length of clear bole (Table 7). This result confirmed well with 13 year-old trees by which provenance No. 1020 showed the longest clear bole while provenance No. 1015 showed the shortest one (Changtragoon, 1984).

Table 7 Average length of clear bole of 33 year-old Merkus pine at Huey Bong Provenance Trials in Chiang Mai.

Provenance No.	Average length of clear bole (m.)
1026	11.21 ± 0.79 d
1020	11.11 ± 1.05 d
1019	11.06 ± 0.57 d
1005	10.57 ± 0.90 cd
1024	10.31 ± 0.52 bcd
1027	9.84 ± 1.01 abcd
1025	9.76 ± 1.09 abcd
1023	9.43 ± 1.65 abc
1008	8.87 ± 0.09 ac
1022	8.79 ± 0.70 ab
1012	8.62 ± 0.24 a
1014	8.41 ± 1.05 a
1018	8.65 ± 1.09 a
1015	8.34 ± 0.38 a
Average	9.64 ± 1.27

Remark: Values are expressed as means ± S.D. Mean followed by the same letter (s) in a column do not differ significantly at 0.05 level by Duncan's New Multiple Range Test.

The variation of length of clear bole among provenances was found to be highly significant difference at 99 % level while variation within provenance or between trees showed non-significant (Table 8).

Table 8 Analysis of Variance on length of clear bole in 33 year-old Merkus pine at Huey Bong Provenance Trials in Chiang Mai.

SOV	df	SS	MS	F
Between provenances	13.00	43.77	3.37	4.84^{**}
Within provenances	2.00	3.94	1.97	2.83^{ns}
Error	26.00	18.09	0.70	

**Highly significant difference at 99% level, ^{ns} Non-significant

4. Diameter at Ground Level

Provenance No. 1019 has the largest diameter diameter at ground level (27.91 cm). In contrast, the smallest diameter at ground level was found in provenance No. 1022 (18.87 cm). The average of diameter size was 24.64 cm (Table 9). The result was also confirmed well with 13 year-old trees by which the provenance which highest diameter at ground level was No. 1019 (15.7433 cm) and the smallest diameter was observed in provenance No. 1022 (8.43 cm) (Changtragoon, 1984).

Table 9 Average diameter at ground level of 33 year-old Merkus pine at Huey Bong Provenance Trials in Chiang Mai.

Provenance No.	Average diameter at ground level (cm.)
1019	27.91 ± 3.21 d
1015	27.74 ± 2.05 d
1005	26.59 ± 2.70 cd
1014	25.97 ± 1.67 cd
1020	25.91 ± 0.34 cd
1018	25.74 ± 0.90 cd
1008	25.65 ± 3.60 cd
1027	25.49 ± 1.80 cd
1012	24.58 ± 1.94 cd
1024	23.44 ± 0.85 bc
1026	23.30 ± 0.94 bc
1025	23.21 ± 1.84 bc
1023	20.46 ± 3.61 ab
1022	18.87 ± 0.27 a
Average	24.64 ± 3.07

Remark: Values are expressed as means ± S.D. Mean followed by the same letter (s) in a column do not differ significantly at 0.05 level by Duncan's New Multiple Range Test.

Base on the Analysis of variation, it was found that provenance variation in diameter at ground level of 33 year-old Merkus pine was highly significant while differences between trees was non-significant (Table 10). The analysis of variation confirmed well with the previous study (Changtragoon, 1984). This means that the trend of development of Do remained the same at the later part of tree development.

Table 10 Analysis of Variance of diameter at ground level in 33 year-old Merkus pine at Huey Bong Provenance Trials in Chiang Mai.

SOV	df	SS	MS	F
Between provenances	13.00	259.61	19.97	4.26^{**}
Within provenances	2.00	5.18	2.59	0.55^{ns}
Error	26.00	121.87	4.69	

**Highly significant difference at 99% level, ^{ns} Non-significant

5. Diameter at Breast Height

Average DBH of all provenances recorded in the years 1978, 1981, and 1983 were 5.03, 7.50 and 10.16 cm, respectively (Changtragoon, 1984). DBH growth rate was consistent. In 2003, DBH was 20.83 cm (Phoonsree, 2003) and was 22 cm in 2004.

Based on the measurement in 2004 (Table 11), Surin provenance (No. 1019) had the biggest stem size (25.22 cm) which is similar with 13 year-old trees (Changtragoon, 1984), and followed by trees of Fang provenance (Chiang Mai, No. 1015) while Sumatra origin (No. 1023) showed the smallest stem size.

Table 11 Average diameter at breast height of 33 year-old Merkus pine at Huey Bong Provenance Trials in Chiang Mai.

Provenance No	Average DBH (cm.)
1019	25.22 ± 2.24 d
1015	25.20 ± 2.23 d
1005	23.58 ± 2.05 cd
1014	23.55 ± 1.10 cd
1027	23.49 ± 1.53 cd
1008	23.35 ± 3.46 cd
1018	23.29 ± 0.86 cd
1020	21.99 ± 2.00 cd
1012	21.67 ± 1.80 cd
1026	21.36 ± 0.89 bc
1024	20.59 ± 1.06 bc
1025	20.23 ± 1.63 bc
1023	17.93 ± 2.90 ab
1022	16.48 ± 0.34 a
Average	22.00 ± 2.94

Remark: Values are expressed as means ± S.D. Mean followed by the same letter (s) in a column do not differ significantly at 0.05 level by Duncan's New Multiple Range Test.

Results showed that DBH of Merkus pine among 14 provenances has the highly significant difference at 99 % level while within provenance or between trees showed non-significant (Table 12). The trend of differences was also the same as recorded when trees were13 year-old trees (Changtragoon, 1984).

Table 12 Analysis of Variance of diameter at breast height in 33 year-old Merkus pine at Huey Bong Provenance Trials in Chiang Mai.

SOV	df	SS	MS	F
Between provenances	13.00	251.74	19.37	5.16^{**}
Within provenances	2.00	3.97	1.99	0.53^{ns}
Error	26.00	97.63	3.76	

**Highly significant difference at 99% level, ^{ns} Non-significant

6. Crown Diameter

Stiell (1966) stated that development of crown form is subjected on the genetic background, but spacing and age may influence to crown diameter. The width of crown among 14 provenances had differed remarkably. Based on analysis of variation it was found to be non-significant among and within provenance (Table 13). The result of 33 year-old Merkus pine differs with 13 year-old, which showed the highly significant variation in crown diameter.

For young Merkus pine, it shows a pyramidal crown form with will developed stem. The spacing of 3 x 3 m^2 may be enough for crown extension but when the trees getting older, it will become a flatter and more spreading crown. The spacing used was narrow spacing for crown development and crown development will be better if genetic thinning is used.

Table 13 Analysis of Variance of crown diameter in 33 year-old Merkus pine at Huey Bong Provenance Trials in Chiang Mai.

SOV	df	SS	MS	F
Between provenances	13.00	22.70	1.75	1.17^{ns}
Within provenances	2.00	0.25	0.12	0.08^{ns}
Error	26.00	38.84	1.49	

^{ns} Non-significant.

Table 14 showed that provenance from Phu Kra Deung, No. 1008 has the widest crown diameter while provenance of Santa C., Philippine (No. 1022) showed the narrowest crown diameter. From the results of 33 year-old differed with 13 year-old (the widest crown diameter was No. 1020 and the narrowest crown diameter was No. 1022).

Table 14 Average crown diameter of 33 year-old Merkus pine at Huey Bong Provenance Trials in Chiang Mai.

Provenance No.	Average crown diameter (m.)
1008	5.28 ± 1.56
1025	5.18 ± 2.39
1015	5.16 ± 0.77
1024	4.92 ± 0.66
1018	4.39 ± 1.07
1005	4.37 ± 0.45
1019	4.14 ± 0.88
1027	4.07 ± 0.47
1012	4.02 ± 0.89
1014	3.71 ± 0.47
1023	3.48 ± 1.92
1026	3.48 ± 0.76
1020	3.24 ± 0.55
1022	2.89 ± 1.58
Average	4.17 ± 1.23

The present study showed that the northeastern provenances (Nos. 1019 and 1020) especially Surin provenance showed the best both survival percentages and morphological characteristics. The results were the similar with the previous studies done by Changtragoon (1984) and Phoonsree (2003), etc. It was reported that Merkus pine from Surin source performs a good genetic traits and overall better than the other Thai provenances. This study strongly confirmed the importance of *in situ* gene conservation forest establishment in Surin province.

Structural Characteristics of the *In Situ* Gene Conservation Forest

The plant data in the forest were collected from sample plots I, II, III and IV which are located in the *in situ* gene conservation forest of Merkus pine at Sang Kha Distinct, Surin Province. The quantitative characteristics of trees with DBH larger than 4.5 cm and higher than 1.3 m, sapling with DBH smaller than 4.5 cm and higher than 1.3 m, and seedlings with DBH smaller than 4.5 cm and lower than 1.3 m are shown in Tables 14, 15 and 16, respectively.

1. Plant Composition

From the study, it was found that a total number of tree species was 56 species in 6,400 m^2 of the study area. There were 44, 21, 32 and 31 tree species in plots I, II, III and IV, respectively (Table 15). The area of each plot was 1600 m ^{2}. The common species were 14 tree species in all plots. For Merkus pine, it was found to occupy in all plots.

When comparison with other pine forests, the forest had number of tree species more than pine forest in Phu Kradung National Park, Loei province (2 tree species in 6400 m^2 as *Pinus merkusii* and *Quercus helferiana*) (Pothitan, 1999). As the result of site and environmental condition which differ together. The studied forest was lowland pine forest mixed with hardwood species. Normally the species richness is more than highland nearly pure pine forest. While the number of tree species in the forest also was more than Hill Forest in Phu Kradung National Park (40 species in 6400 m^2) (Pothitan, 1999). Otherwise, the forest had the number of trees nearly with *Pinus kesiya* plantation at Phrao Watershed Management Unit, Chiang Mai province (40-72 species of trees in 12,000 m^2) (Yongsawai, 2002).

The present study showed a total number of saplings to be comprised with 39 species in the 256 m^2 of the study area. There were 19, 14, 9 and 15 sapling species in plots I, II, III and IV, respectively (Table 16). The area of each plot was 64 m^2. From the study, it was found that Merkus pine sapling did not occur in any sample plots. The result was the same as the previous study of Jenkarnying (1983) namely the Merkus pine sapling was absent in the sample plots. This meant that it was difficult for natural pine seedlings to grow up to be the sapling stage.

For seedlings study, there were 16 seedling species in the area of 16 m^2. In plots I, II, III and IV, the seedlings were comprised of 6, 5, 4 and 4 species, respectively. The area of each plot was 4 m^2. For Merkus pine, it was found that only one plot has only one Merkus pine seedling in total number of 35 seedlings. This meant that the natural pine seedling was relatively small as it may be caused by poor seed crop or poor seedling development under the natural condition. The results are shown in following Table 17.

2. Density of Plants

The density of trees in the forest was found to be 1,756.25 trees. ha^{-1}. While the density of tree in plots I, II, III and IV were 1,387.50, 1,606.25, 2,537.50 and 1,493.75 trees. ha^{-1}, respectively. Those results were shown in Table 15. Tree density of Merkus pine were 87.50, 62.50, 56.25, 31.25 trees. ha^{-1} in plots I, II, III and IV, respectively. The average tree density of Merkus pine in the forest was the 59.38 trees. ha^{-1}.

When comparison with the investigation was carried out by Jenkarnying (1983), the density of trees in the present study showed more number than those that the previous studied (183.75 trees. ha^{-1}). For the tree density of Merkus pine in the present study, it was found to be lower than those recorded in the year of 1983 (137.5 trees. ha^{-1}). So the result of comparison was stated that the density of Merkus pine is reduced due to number of Merkus pine trees was decreased. Decreasing in the number of Merkus pine trees maybe affected with the density of other tree species to increase.

For the study on density of saplings in each plot, there were 5,468.75, 2,656.25, 3,593.75 and 8,437.5 trees. ha^{-1} in plots I, II, III and IV, respectively. The sapling density of the forest was 5,039.06 trees. ha^{-1} (Table 16).

The occurrence of seedlings per area in the forest was 21,875 trees. ha^{-1} with 25,000, 17,500, 22,500 and 22,500 trees. ha^{-1} in plots I, II, III and IV, respectively, as shown in Table 17. While density of Merkus pine seedling was 2,500 trees. ha^{-1} in plot II and was 625 trees. ha^{-1} in the forest. The present study showed more density of Merkus pine seedlings than those recorded in the year 1983 (112.5 trees. ha^{-1}, Jenkarnying, 1983).

Based on the present study, it can be concluded that natural regeneration of Merkus pine in the *in situ* gene conservation forest relatively poor as caused by poor seed crops, small number of natural seedling and sapling. Results in maybe caused by forest fire and flooding which occur in the forest several times in due course. Furthermore, mother trees of remained Merkus pine were relatively old. However, comparison with 1983 (Jenkarnying, 1983), the natural regeneration at present is somehow better than those recorded in 1983 as caused by better forest fire and flood control.

3. Total Basal Area and Percentages of Basal Area

Total basal area (BA) value of tree species in the forest was 20.219 m^2 per ha with 10.128, 3.521, 3.907 and 2.662 m^2 per ha in Plots I, II, III and IV, respectively (Table 15). The total basal area of trees in the forest was lower than value recorded in 1983 (36.207 m^2 per ha) (Jenkarnying, 1983). For total basal area of Merkus pine in the forest, it was found to be 5.792 m^2 per ha. The result was somehow less than BA recorded in 1983 which was 11.45 m^2 per ha (Jenkarnying, 1983). As the result, trees in the forest had been reduced in large number as affected by several factors as previously stated.

The percentage of basal area per ha of natural pine trees with DBH larger than 4.5 cm and higher than 1.3 m was 0.316 percentages. In addition, the percentages of trees of this particular size in plots I, II, III and IV were 0.633, 0.220, 0.244 and 0.166 percentages, respectively (Table 15). All results related to density of plants.

Table 15 Quantitative characteristics of trees in the *in situ* gene conservation forest, Surin Province

Quantitative characteristics	Plot I	Plot II	Plot III	Plot IV	Average
Number of species (per sample plot area*)	44	21	32	31	56**
Number of trees (per sample plot area*)	222	257	406	239	1124**
Density of trees (per ha)	1387.50	1606.25	2537.50	1493.75	1756.25
Average DBH (cm)	10.54	10.27	8.85	9.45	9.60
Basal area per ha	10.128	3.521	3.907	2.662	20.219**
Percentage of Basal area per ha	0.633	0.220	0.244	0.166	0.316
Average Height (m)	8.53	7.90	6.82	7.01	7.45

Remarks: * Area of sample plot was 1,600 m^2
** The combined data of all plots

Table 16 Quantitative characteristics of saplings in the *in situ* gene conservation forest, Surin province

Quantitative characteristics	Plot I	Plot II	Plot III	Plot IV	Average
Number of species (per sample plot area*)	19	14	9	15	39**
Number of sapling (per sample plot area*)	35	17	23	54	129**
Density of sapling (per ha)	5468.75	2656.25	3593.75	8437.5	5039.06
Average Height (m)	4.39	2.12	3.15	2.66	3.15

Remarks: * Area of sample plot was 64 m^2
** The combined data of all plots

Table 17 Quantitative characteristics of seedlings in the *in situ* gene conservation forest, Surin province

Quantitative characteristics	Plot I	Plot II	Plot III	Plot IV	Average
Number of species (per sample plot area*)	6	5	4	4	16**
Number of sapling (per sample plot area*)	10	7	9	9	35**
Density of sapling (per ha)	25000	17500	22500	22500	21875

Remarks: * Area of sample plot was 4 m^2

** The combined data of all plots

4. DBH Class Distribution

Most pine trees in the *in situ* gene conservation forest showed that the most DBH of trees are between 4.6- 9.6 cm. More number of trees was reduced in higher DBH size or more stem counts in the smaller size classes. In summation, the DBH diameter class of trees in the forest was demonstrated in the inversed J-shape or L-shape distribution pattern. Actually, this pattern is shown as balance maintenance. These indicated that the forest is a stationary stage or regenerating well. The result differed with pine forest in Phu Kradung National Park, Loei province. DBH class distribution of Phu Kradung pine forest did not display as the inversed J-shape or L-shape distribution patterns (Pothitan, 1999). The diameter distribution pattern of each plot is illustrated in Figure 11. Furthermore, the result was found that size class distribution of Merkus pine in each plot was found between 29.60 and 59.6 cm which is medium size class of DBH trees in the forest.

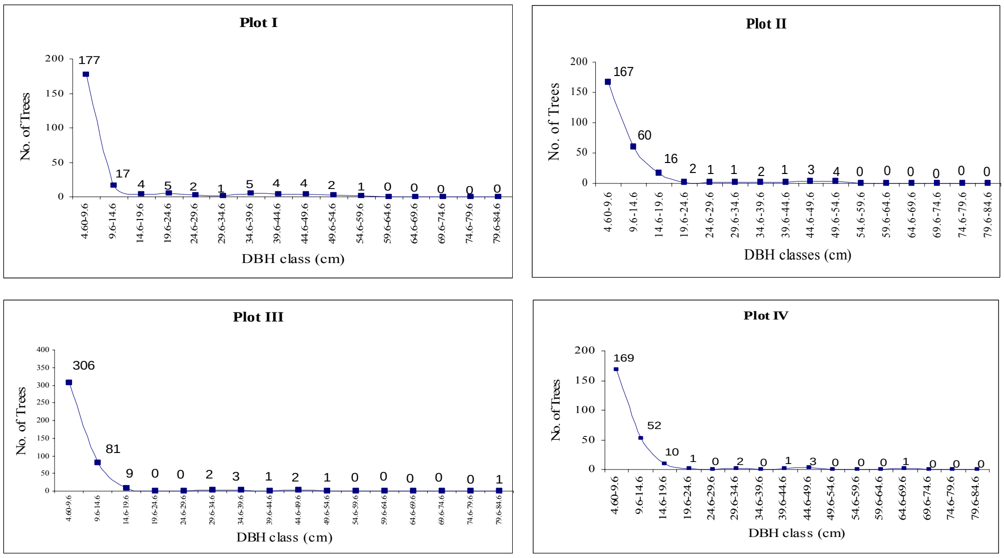


Figure 11 DBH distribution of trees with DBH larger than 4.5 cm (class interval 5 cm) in the *in situ* gene conservation forest.

5. Importance Value Index

The Important Value Index (IVI) was used to determine dominance and important trees in plant community. The index is outcome from summation of relative density, relative frequency and relative dominance in case of tree species. The result of relative density and relative frequency summation is used to consider the sapling and seedling.

Result showed that the trees species that have the highest relative density in plot I was *Parinari anamense* and plots II, III and IV was *Dipterocarpus obtusifolius.* Additionally, the tree species that showed the highest relative frequency in plot I was *Parinari anamense* while plots II, III was *Dipterocarpus obtusifolius* and plot IV was *Calophyllum polyanthum*. For the highest relative dominance of tree species in plot I was *Parinari anamense* when plots II and III was *Pinus merkusii* and plot IV was *Dipterocarpus obtusifolius*. Base on the sum of relative density, relative frequency and relative dominance, it was found that the highest Importance Value Index (IVI) in plot I was *Parinari anamense* and plots II, III and IV was *Dipterocarpus obtusifolius*. The results of all plots were showed in Tables 18-21, respectively.

Table 18 Relative density (RD), Relative frequency (RF), Relative dominance (RDo) and Importance Value Index (IVI) of trees of the top ten highest IVI in Plot I in the *in situ* gene conservation forest

Scientific names	RD	RF	Rdo	IVI
Paranari anamense Hance	9.009	7.042	25.186	41.237
Pinus merkusii Jungh. & de Vriese	6.306	7.042	21.730	35.079
Carallia brachiata (Lour.) Merr.	7.207	3.521	22.169	32.898
Anisoptera costata Korth.	5.856	5.634	3.985	15.475
Aporosa villosa (Wall. ex Lindl.) Baill.	2.252	3.521	9.374	15.147
Dalbergia cochinchinensis Pierre	4.505	4.225	4.472	13.201
Xylopia vielana Pierre	5.856	4.930	0.508	11.294
Microcos tomentosa Sm.	5.856	3.521	0.358	9.735
Shorea roxburghii Gc. Don	3.604	4.930	1.082	9.615
Peltophorum dasyrachis (Miq.) Kurz	4.054	4.930	0.485	9.468

Table 19 Relative density (RD), Relative frequency (RF), Relative dominance (RDo) and Importance Value Index (IVI) of trees of the top ten highest IVI in Plot II in the *in situ* gene conservation forest

Scientific names	RD	RF	Rdo	IVI
Dipterocarpus obtusifolius Teijsm. ex. Miq.	42.802	17.391	27.739	87.932
Pinus merkusii Jungh. & de Vriese	3.891	6.522	48.647	59.060
Shorea roxburghii Gc. Don	13.619	14.130	6.180	33.929
Aporosa villosa (Wall. ex Lindl.) Baill.	12.062	11.957	3.255	27.273
Calophyllum polyanthum Wall. ex. Choisy	4.280	9.783	0.968	15.031
Sindora siamensis Teijsm. & Miq.	5.058	4.348	2.178	11.585
Vatica harmandiana Pierre	3.502	6.522	0.716	10.739
Buchanania lanzan Spreng.	1.946	5.435	0.446	7.826
Dipterocarpus intricatus Dyer	1.556	4.348	0.682	6.586
Paranari anamense ance	2.335	3.261	0.835	6.430

Table 20 Relative density (RD), Relative frequency (RF), Relative dominance (RDo) and Importance Value Index (IVI) of trees of the top ten highest IVI in Plot III in the *in situ* gene conservation forest

Scientific names	RD	RF	Rdo	IVI
Dipterocarpus obtusifolius Teijsm. ex. Miq.	31.527	11.029	24.775	67.331
Pinus merkusii Jungh. & de Vriese	2.217	5.147	28.575	35.939
Paranari anamense Hance	12.315	9.559	8.715	30.589
Shorea roxburghii Gc. Don	12.069	10.294	5.359	27.722
Dipterocarpus intricatus Dyer	2.463	5.882	15.546	23.891
Anisoptera costata Korth.	3.695	6.618	2.094	12.406
Calophyllum polyanthum Wall. ex. Choisy	5.172	5.147	1.628	11.947
Tristaniopsis burmanica (Griff.) Peter Gc. Wilson & J.T. Waterh. var. burmanica	3.448	4.412	1.313	9.173
Vatica harmandiana Pierre	2.709	4.412	0.732	7.853
Carallia brachiata (Lour.) Merr.	2.956	3.676	1.149	7.781

Table 21 Relative density (RD), Relative frequency (RF), Relative dominance (RDo) and Importance Value Index (IVI) of trees of the top ten highest IVI in Plot IV in the *in situ* gene conservation forest

Scientific names	RD	RF	Rdo	IVI
Dipterocarpus obtusifolius Teijsm. ex Miq.	45.607	12.500	32.584	90.690
Calophyllum polyanthum Wall. ex. Choisy	18.828	13.542	6.716	39.086
Pinus merkusii Jungh. & de Vriese	2.092	4.167	28.607	34.865
Paranari anamense Hance	5.858	11.458	6.960	24.276
Anisoptera costata Korth.	0.418	1.042	12.926	14.386
Aporosa villosa (Wall. ex Lindl.) Baill.	2.510	6.250	1.003	9.763
Shorea roxburghii Gc. Don	2.510	5.208	0.665	8.384
Dalbergia cochinchinensis Pierre	1.255	3.125	3.429	7.809
Buchanania lanzan Spreng.	1.674	4.167	0.342	6.182
Tristaniopsis burmanica (Griff.) Peter Gc. Wilson & J.T. Waterh. var. burmanica	2.092	3.125	0.666	5.883

For the tree species that have the highest relative density, relative frequency in the forest was *Dipterocarpus obtusifolius*. Otherwise, the tree species that perform the highest relative dominance in the forest was *Pinus merkusii* as Merkus pine had large diameter while the number of *Dipterocarpus obtusifolius* was found to be more than other species. The summation of relative density, relative frequency and relative dominance was performed that the tree species that show the highest IVI in the forest was *Dipterocarpus obtusifolius*, and followed by *Pinus merkusii*, *Parinari anamense*, *Shorea roxburghii*, *Carallia brachiata*, respectively as shown in Table 22.

Table 22 Relative density (RD), Relative frequency (RF), elative dominance (RDo) and Importance Value Index (IVI) of trees of the top ten highest IVI in the *in situ* gene conservation forest

Scientific names	RD	RF	RDo	IVI
Dipterocarpus obtusifolius Teijsm. ex Miq.	30.872	9.227	13.909	54.008
Pinus merkusii Jungh. & de Vriese	3.381	5.794	28.646	37.821
Parinari anamense Hance	8.007	7.940	15.362	31.309
Shorea roxburghii Gc.Don	8.719	8.369	2.741	19.829
Carallia brachiata (Lour.) Merr.	2.491	2.146	11.327	15.964
Calophyllum polyanthum Wall.ex Choisy	7.028	6.652	1.417	15.098
Aporosa villosa (Wall.ex Lindl.) Baill.	3.915	5.150	5.451	14.516
Anisoptera costata Korth.	2.580	3.863	4.103	10.545
Dipterocarpus intricatus Dyer	1.779	3.433	3.281	8.493
Vatica harmandiana Pierre	2.224	3.433	0.338	5.996

Jenkarnying (1983) concluded that *Pinus merkusii* shows the highest IVI in the forest (133.480), and next with *Dipterocarpus tuberculatus* (44.397), *D. obtusifolius* (36.944), *Irvingia malayana* and *Shorea floribunda*, respectively. This indicated that there was more number of Merkus pine occurred and distributed all over the forest area. At the present, the tree species was decreased in number and grown as small groups. Furthermore, *D. obtusifolius* and other species had opportunity to regenerate and replace Merkus pine.

The sum of relative density and relative frequency to IVI performance indicated that the sapling species with the highest Importance Value Index in plot I was *Miliusa mollis* and *Melodorum fruticosum* while plots II, III and IV were *Madhuca kerrii, Cratoxylum cochinchinense* and *Gomphia serrata*, respectively. So the dominance and important sapling species in the *in situ* gene conservation forest were *Cratoxylum cochinchinense*, and followed by *Miliusa mollis*, *Anisoptera costata*, *Madhuca kerrii* and *Melodorum fruticosum*, respectively (Table 23). *C. cochinchinense* is the pioneer species. This is indication that the forest area was opened wide, so the pioneer species invaded the area.

Table 23 Relative density (RD), Relative frequency (RF) and Importance Value Index (IVI) of saplings of the top ten highest IVI in the *in situ* gene conservation forest

Scientific names	RD	RF	IVI
Cratoxylum cochinchinense (Lour.) Blume	12.500	6.061	18.561
Miliusa mollis Pierre var. mollis	9.375	4.545	13.920
Anisoptera costata Korth.	7.031	4.545	11.577
Madhuca Kerrii H.R. Fletch	5.469	6.061	11.529
Melodorum fruticosum Leur.	7.813	3.030	10.843
Gomphia serrata (Gaertn.) Kanis	5.469	4.545	10.014
Dipterocarpus obtusifolius Teijsm. ex Miq.	3.125	6.061	9.186
Vatica harmandiana Pierre	7.031	1.515	8.546
Memecylon pauciflorum Blume	3.906	4.545	8.452
Calophyllum polyanthum Wall. ex Choisy	4.688	3.030	7.718

The present study showed that the seedling species with the highest Importance Values Index in Plots I and IV were *Miliusa mollis*, Plot II was *Garcinia cowa*, Plot III was *Memecylon pauciflorum*. In summary, it could be concluded that the seedling species with highest IVI of the forest was *Miliusa mollis*, and followed by *Memecylon pauciflorum*, *Garcinia cowa*, respectively and other results are shown in Table 24. The IVI values of Merkus pine seedling was 7.205 which was the least value of IVI. It is possible to conclude that Merkus pine seedling in the forest had low IVI due to low number of seedling as may be caused by poor seed crops and poor natural seedlings in the studied site. To follow the concept of *in situ* gene conservation forest, poor natural regeneration need better management practices and more effort to improve the better natural regeneration.

Table 24 Relative density (RD), Relative frequency (RF) and Importance Value Index (IVI) of seedlings in the *in situ* gene conservation forest

Scientific name	RD	RF	IVI
Miliusa mollis Pierre var. mollis	25.714	13.043	38.758
Memecylon pauciflorum Blume	22.857	8.696	31.553
Garcinia cowa Roxb. ex DC.	5.714	8.696	14.410
Calophyllum polyanthum Wall. ex Choisy	5.714	8.696	14.410
Atalantia monophylla (DC.) Correa	5.714	8.696	14.410
Syzygium cumini (L.) Skells	5.714	8.696	14.410
Cinnamomum sp.	2.857	4.348	7.205
Gomphia serrata (Gaertn.) Kanis	2.857	4.348	7.205
Cratoxylum cochinchinense (Lour.) Blume	2.857	4.348	7.205
Pterocarpus macrocarpus Kurz	2.857	4.348	7.205
Sindora siamensis Teijsm. & Miq.	2.857	4.348	7.205
Parinari anamense Hance	2.857	4.348	7.205
Melodorum fruticosum Lour.	2.857	4.348	7.205
Pinus merkusii Jungh. & de Vriese	2.857	4.348	7.205
Vatica harmandiana Pierre	2.857	4.348	7.205
Aporosa villosa (Wall. ex Lindl.) Baill.	2.857	4.348	7.205

6. Vertical Stratification

Four sample areas of 10x40 m^2 were randomly sampled. The profile and crown projection diagram of trees with DBH larger than 4.5 cm was studied (Figure 12-15).

From the investigation, it was found that Plot I was comprised with 25 tree species and can be classed into three canopy layers (H<10m, 10-20m, >20m). Additionally, the top canopy (>20 m) was *Pinus merkusii* which had distinct height performance from other tree species, followed by *Carallia brachiata* and *Syzygium cumini* while crown cover percentages of this profile was 64.22 %.

Plot II was occupied with 13 tree species and can be occupied into two stratified layers (H<10m, 10-20m). This plot showed that the emergent tree species were *Shorea roxburhii* and *Dipterocarpus obtusifolius*. The height of tree in this plot had continuous more than the Plot I. In addition, the crown area covered approximately 33.04 %. The cover was less than Plot I because amount of trees were small and the size of trees had only small and medium size.

Plot III had 15 tree species. Based on the height of trees, it could be classified into three layers. The top and second layers were *P. merkusii*. The pine performed distinctively in height when compared with other tree species. The percentage of crown cover on the area was 80.75 %.

In Plot IV, it was found that the tree species is composed of 16 species and could be classified into two stratified layers. The results showed that *P. merkusii* performs as emergent species and the cover of crown was 69.18%.

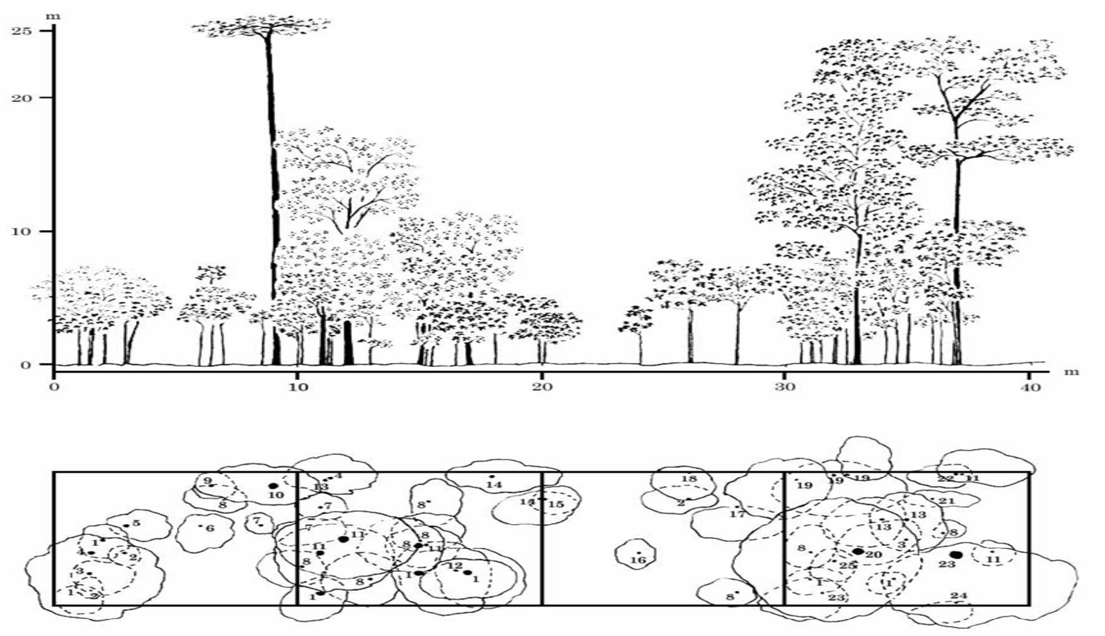


Figure 12 Profile diagram and crown projection diagram of the Plot I in the Merkus pine *in situ* gene conservation forest, Surin Province.

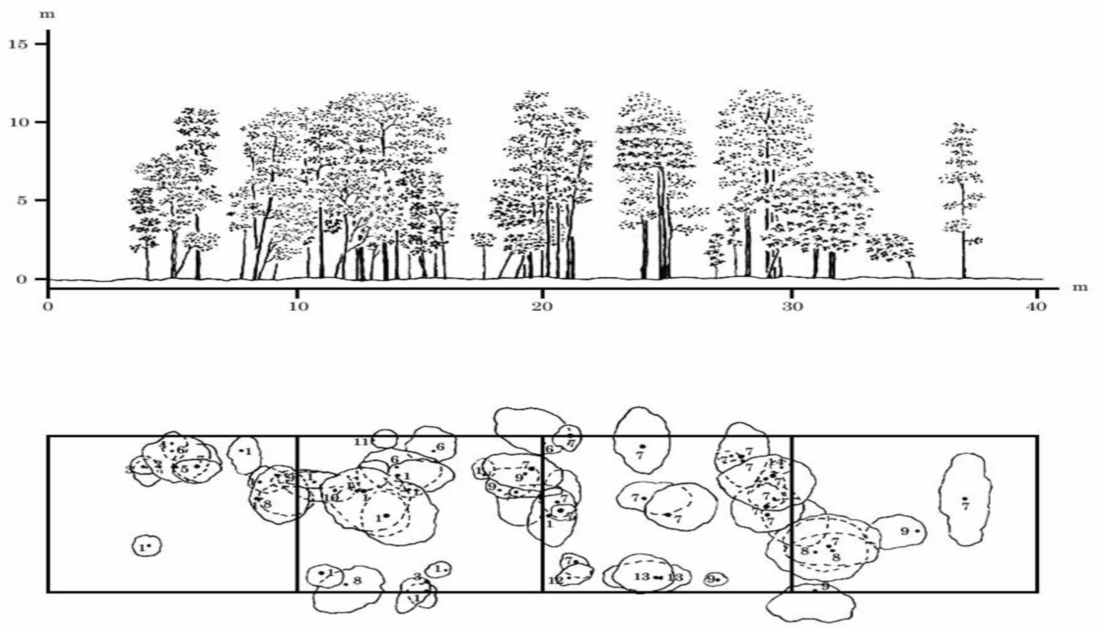


Figure 13 Profile diagram and crown projection diagram of the Plot II in the Merkus pine *in situ* gene conservation forest, Surin Province.

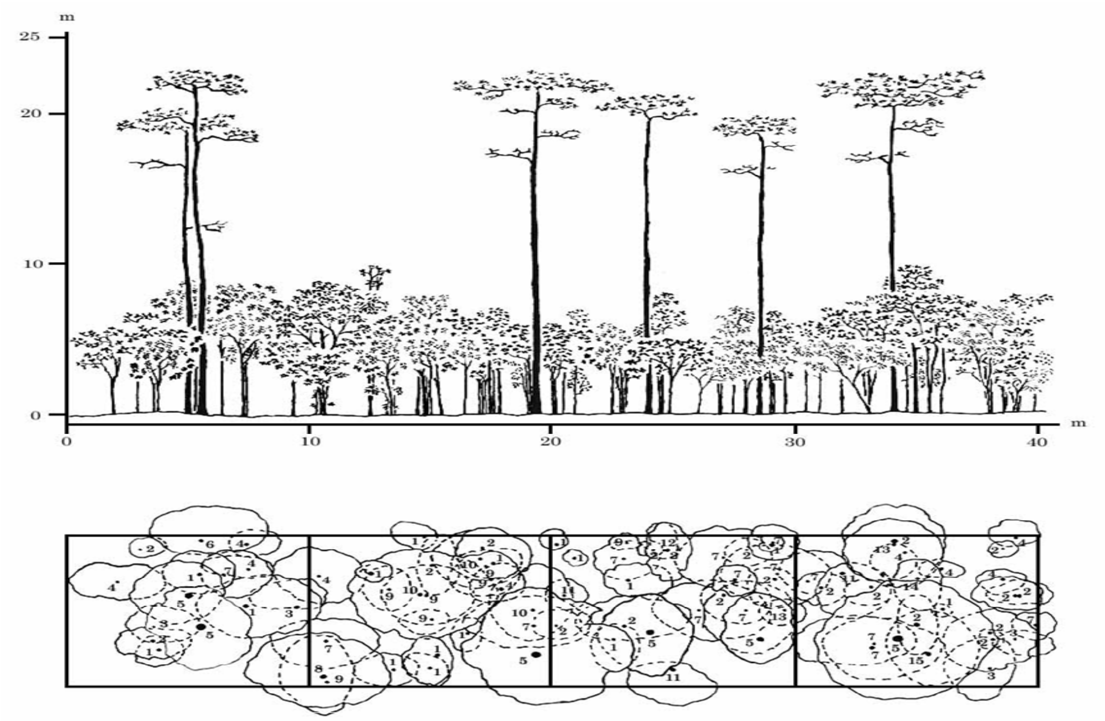


Figure 14 Profile diagram and crown projection diagram of the Plot III in the Merkus pine *in situ* gene conservation forest, Surin Province.

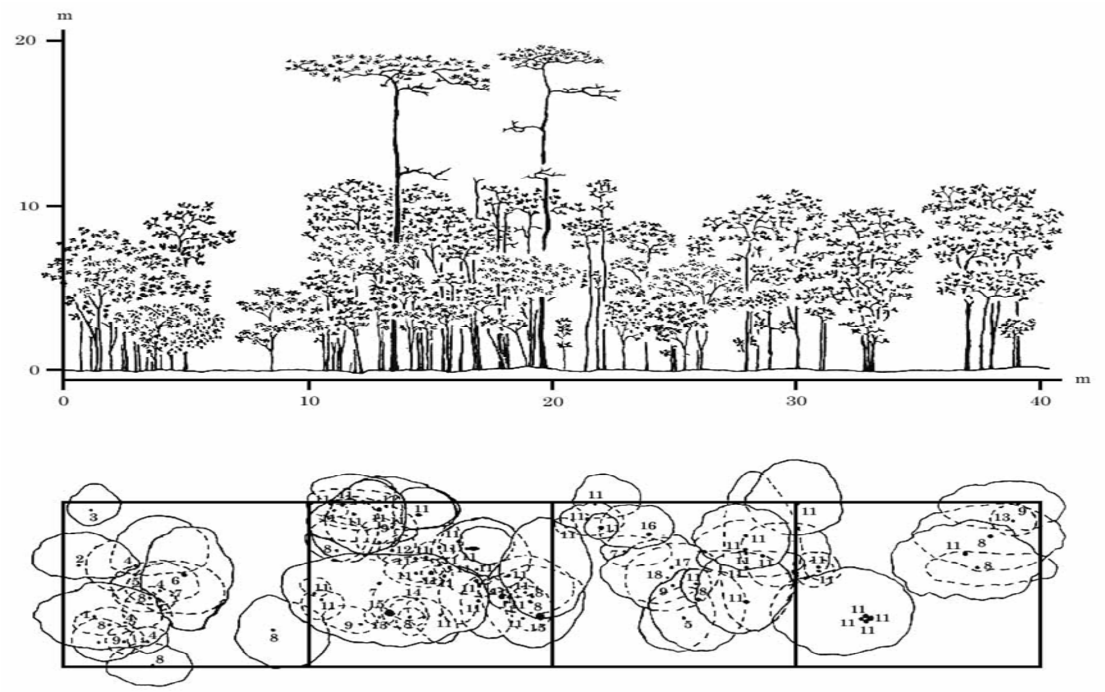


Figure 15 Profile diagram and crown projection diagram of the Plot IV in the Merkus pine *in situ* gene conservation forest, Surin Province.

In conclusion, the vertical structure of forest was consisted of three layers in the area of *P. merkusii* occurrence. Additionally, the vertical structure was classed into two layers in the area of other tree species occurrence but Merkus pine was absent. The emergent one layer of the forest (20-30m) was *P. merkusii*. The height of the pine species was between 22-26 m and some trees might was more than 30 m. Furthermore, this layer also consisted of broad leaved species such as *Carallia brachiata* and *Syzygium cumini*. The second layer was 10-20 m of height. The layer was comprised with *P. merkusii*, *Shorea roxburhii* and *Dipterocarpus obtusifolius*. The height of lower layer was less than 10 m. The layer was composed with many tree species such as *Canarium subulatum*, *D. obtusifolius*, *Parinari anamense* and *Calophyllum polyanthum*. From the survey over all the forest area, it was found that the ground cover with grass species. For the average percentages of crown closer was approximately 30-80 %.

Jenkarnying (1983) reported that stand structure comprised of three layers. The first layer consisted of Merkus pine of 20-25 metres height. The second layer consisted of Merkus pine, *Dipterocarpus obtusifolius*, *D. tuberculatus, Irvingia malayana, Mangifera caloneura* of 10-20 m. height. The third layer was the ground cover with vegetations like *Imperata* grass, etc. From the study in sample plots, it was found that crown closure was approximately 60-70 %.

When comparing to Jenkarnying's study, it was able to conclude that the vertical structure of the forest remains original characteristic performance namely the forest comprised of three layers but number of trees of each species changed in each layer. The present study confirmed that Merkus pine was still emergent species of the forest. More crown cover was observed in the present study. The crown cover ranged from 30-80 % which is near with the past study (60-70%) (Jankarnying, 1983).

7. Species Diversity in the Forest

Diversity indices were investigated by Fisher's Index (α), Shannon's Index (H) and Simpson's Index (D). It revealed that Fisher's Index (α), Shannon's Index (H) and Simpson's Index (D) of trees in the *in situ* gene conservation forest were 12.394, 2.896 and 0.878, respectively. When, Hill's diversity number of trees, N_1 and N_2 were 18.091 and 8.218, respectively. The diversity indices are shown in Table 25.

Actually, the Fisher's Index is a constant proportion of number of species to number of individuals. If it is high, it means that there is high diversity. When the *in situ* gene conservation forest had the numbers of species near with Dipterocarp stand (56 and 53 species, respectively). Therefore, the *in situ* gene conservation forest had also Fisher's Index value close with Dipterocarp stand, Prachaup Khiri Khan (Fisher's Index as 12.606) (Wachrinrat *et al.*, 1999). Otherwise, the forest had higher Fisher's Index than Hill forest and Pine forest at Phu Kradung National Park, Loei province especially the Pine forest (Fisher's Index as 8.661 and 0.434, respectively) (Pothitan, 1999). The present study had higher numbers of species than two forests (44 and 2 tree species, respectively). However, this forest had lower index value when comparing to study at the Dry Evergreen Forest at Namprom Basin (Fisher's index as

19.680, Visaratana, 1987). From result of this index, it indicated that the forest has medium species diversity.

As regards, Shanon's Index, the forest had the index close to the value studied in DDF, Sakaerat, *S. obtuse* type (Shanon's Index as 2.490±0.368, Sahunalu and Dhanmanonda, 1995). In addition, the forest had less Shanon's Index than Moist Evergreen Forest, Khao Chong, Trang (6.027±0.197) (Kiratiprayoon, 1986). The Shanon's Index revealed the average degree of "uncertainly" in predicting to what species an individual is chosen at random from a collection of S species and N individuals will belong. This average uncertainly increases as the species become even. If the index shows high value, it means that there is high diversity. From the result, it concluded that the forest had low diversity when Shanon's Index has taken into consideration with previously studied.

The result of Simpson's Index comparing with other forests, it was found that the forest had quite high diversity. The forest had Simpson's Index near with Dipterocarp stands, Prachaup Khiri Khan (0.869) (Wachrinrat *et al.*., 1999). Furthermore, the forest had this index value rather near with Ban Thung Soong Community Forest, Krabi province (0.926). Sawatdee (2002) stated that the community forest performs high diversity. The present study had higher index than Phu Kradung Pine forest with the value of 0.228 (Pothitan, 1999).

Richness Indices were calculated by the Margalef (1958) Index (R_1) and Menhinick (1964) Index (R_2), and it was found that the R_1 and R_2 of trees in the forest were 7.830 and 1.670, respectively. Additionally, the evenness indices which were classified into five values as E_1, E_2, E_3, E_4 and E_5 were 0.719, 0.323, 0.311, 0.454 and 0.422, respectively. The richness and evenness indices were also illustrated in Table 25. Both indices displayed moderated values, so it affected perform the moderate diversity index. Summary, the forest performed medium species diversity.

Table 25 Species diversity, Richness and Evenness Index of trees with DBH > 4.5 cm in four plots and the *in situ* gene conservation forest

Index	Plot I	Plot II	Plot III	Plot IV	Forest
Fisher's Index (a)	16.459	5.410	8.486	9.496	12.394
Shanon-Wiener's Index (H)	4.907	2.972	3.723	3.077	4.178
Simpson's Index (D)	0.962	0.777	0.862	0.752	0.878
Shanon's Index ($H_{(s)}$)	3.402	2.060	2.580	2.133	2.896
N_0	44	21	32	31	56
N_1	30.000	7.846	13.199	8.436	18.091
N_2	25.986	4.491	7.225	4.029	8.218
Richness index					
R_1	7.959	3.604	5.161	5.478	7.830
R2	2.953	1.310	1.588	2.005	1.670
Evenness index					
E_1	0.899	0.677	0.744	0.621	0.719
E_2	0.682	0.374	0.412	0.272	0.323
E_3	0.674	0.342	0.394	0.248	0.311
E_4	0.866	0.572	0.547	0.478	0.454
E_5	0.862	0.510	0.510	0.407	0.422

Merkus Pine in the *In Situ* Gene Conservation Forest

The importance of Nong Khu *in situ* gene conservation forest of Merkus pine was recognized by FAO. This was considered as the pilot study and should be applied to other species and other locations. The study on provenance trials of Merkus pine in the present study has also showed the promising provenance at Nong Khu, Surin Province. It is also importance to illustrate the changes in the *in situ* gene conservation forest about 20 years ago after establishment.

1. Amount and Number of Remaining Merkus Pine in the Forest

From the intensive survey, it was found that Merkus pine in the forest was remained as remnants which are scattered. In each group consisted of 3-5 Merkus pine trees unlike the previous study in 1983. The pine trees distributed all over the forest area (Jenkarnying, 1983). The existing pine stands were debarked and uprooted for resin tapping and fire-stick. Furthermore, mature pine trees were easily felled down by wind because of those actions. Additionally, forest fire burning incidentally occurred in the forest. It damaged to mature pine and especially seedling. Moreover, forest fire was one cause of poor natural regeneration of Merkus pine in the forest. Same as flooding was also one of poor natural regeneration causes. Above factors were the main causes to reduce Merkus pine in the forest.

Position and distribution of all Merkus pine trees in the forest were surveyed and marked intensively (Figure 21). The study showed that total number of Merkus pine in the area was 960 trees. When comparing with the recorded found in the year 1983, it was 4,601 trees (Jenkarnying, 1983). The number of Merkus pine trees was reduced about 3,641 trees or approx. 4 times (79.13 % of 4601 trees). In addition, the percentage of damage from debarking and resin tapping in 2005 was 26.04 % and fire burning was 0.73 %. As previously recorded, 4,601 trees were debarked, resin tapping and forest fire about 30.29 %, 52.36% and 46.29 %, respectively (Jenkarnying, 1983).

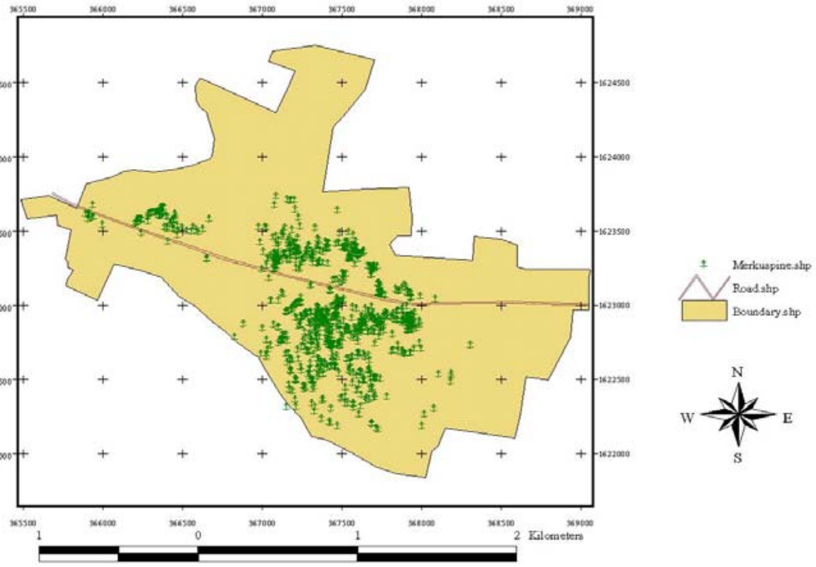


Figure 16 Position and distribution of all Merkus pine trees in the *in situ* gene conservation Merkus pine forest, Surin province.

2. **Morphological Characteristics of Merkus Pine**

Morphological characteristics of trees (Merkus pine) such as total height (H), Diameter at breast height level (DBH), Diameter at ground level (D_0) and crown diameter (CD) in the *in situ* gene conservation forest were analyzed. The results were shown, as follow:-

2.1 Total Height (H) the height of all Merkus pine (960 trees) was divided into 8 classes. The class interval was 5 m. The results showed that the majority was found in the height class between 20-25 m (46.67%), and followed by 15-20 m (29.06%) and 25-30 m (17.40%), respectively. The highest tree was 38.5 m which is remained at 35-40 m height class (0.31%). The average height of trees was 21.92±4.06 m. The results are shown in Table 26. The average height was however slightly higher than was reported in 1983 (21.47±4.29 m.). In addition, average height of Merkus pine in this forest was less than normal height of Thai Merkus pine with height 30 m (Jenkarnying, 1983).

Table 26 Distribution of Merkus pine trees in each height classes in 1983 and 2005 of the *in situ* gene conservation forest, Surin province

	Number of existing trees in various years			
	1983		2005	
Height class (m)	No. of trees	Percentages (%)	No. of trees	Percentages (%)
0-5^{-}	0	0	0	0
5-10^{-}	20	0.43	4	0.42
10-15^{-}	293	6.37	40	4.17
15-20^{-}	1,191	25.88	279	29.06
20-25^{-}	2,270	49.47	448	46.67
25-30^{-}	754	16.39	167	17.40
30-35^{-}	63	1.37	19	1.98
35-40^{-}	4	0.09	3	0.31
Total	4,601	100	960	100

The pattern of height distribution of tree population in 2005 performed similarly with tree population in 1983 (Jenkarnying, 1983). It is shown in Figure 22A. For analysis with Wilcoxon-signed Rank Test between height of 1983 and 2005, the result was found the significant difference at 95% level.

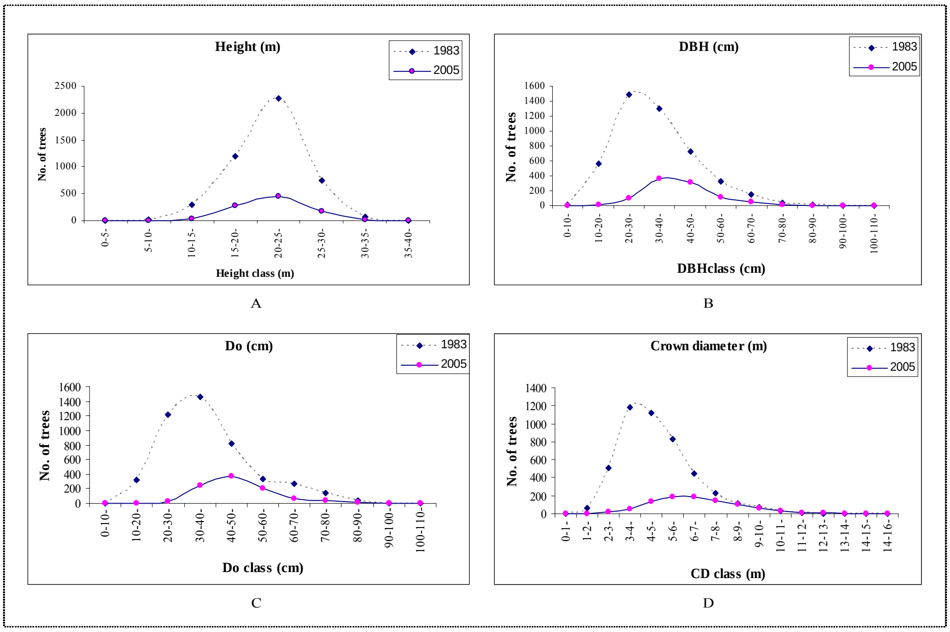


Figure 17 Number distribution of Merkus pine in each morphological characteristics which is comparison between year of 1983 and 2005.

2.2 Diameter at Breast Height Level (DBH) from Table 27, all Merkus pine (960 trees) was consisted of 11 classes. Each class interval was 10 cm. The study showed that most trees was located at the DBH between 30-40 cm (38.02%), and followed by 40-50 cm (31.98%) and 50-60 cm (11.98%), respectively. The biggest DBH of tree was 89.55 cm. The average DBH was 41.55 ± 10.81 cm which bigger than year of 1983 (33.64±13.19 m) as reported by Jenkarnying (1983). Comparison between 2 studied years, it was found that pattern of number distribution in each DBH classes of existing trees are changed namely the existing trees (2005) distributed in bigger size class than 1983 (Jenkarnying, 1983) as shown in Figure 22B. For analysis with Wilcoxon-signed Rank Test between DBH of 1983 and 2005, the result was found to be significant difference at 95% level.

Table 27 Distribution of Merkus pine trees in each DBH classes in 1983 and 2005 of the *in situ* gene conservation forest, Surin province

DBH class	Number of existing trees in various years			
	1983		2005	
(cm)	No. of trees	Percentages (%)	No. of trees	Percentages (%)
0-10^{-}	7	0.15	1	0.10
10-20^{-}	566	12.30	11	1.15
20-30^{-}	1,487	32.32	97	10.10
30-40^{-}	1,305	28.36	365	38.02
40-50^{-}	719	15.63	307	31.98
50-60^{-}	319	6.93	115	11.98
60-70^{-}	145	3.15	47	4.90
70-80^{-}	42	0.91	14	1.46
80-90^{-}	10	0.22	3	0.31
90-100^{-}	0	0	0	0
100-110^{-}	1	0.02	0	0
Total	4,601	100.00	960	100.00

2.3 Diameter at Ground Level (Do) Do data of all pine trees (960 trees) was classed into 11 classes and each class interval was 10 cm. The result showed that the most Do of trees was between 40-50 cm (38.33%), and followed by 30-40 cm (24.79%) and 50-60 cm (20.73%), respectively. The biggest Do of tree was 98.92 cm. The average Do of tree population was 46.88±11.67 cm which bigger than average Do in 1983 (37.62±14.78 cm) (Jenkarnying, 1983). The results are shown in Table 28. The comparison between 1983 (Jenkarnying, 1983) and 2005 showed that the pattern of distribution in Do classes of existing trees (year 2005) are changed from 1983's pattern (Figure 4C). Analysis with Wilcoxon-signed Rank Test between Do of 1983 and 2005, the result showed the significant difference at 95% level.

Table 28 Distribution of Merkus pine trees in each Do classes in 1983 and 2005 of the *in situ* gene conservation forest, Surin province

Do Class (cm)	Number of existing trees in various years			
	1983		2005	
	No. of trees	Percentages (%)	No. of trees	Percentages (%)
0-10^{-}	2	0.04	1	0.10
10-20^{-}	314	6.82	6	0.63
20-30^{-}	1,217	26.45	32	3.33
30-40^{-}	1,465	31.84	238	24.79
40-50^{-}	824	17.91	368	38.33
50-60^{-}	332	7.21	199	20.73
60-70^{-}	270	5.87	67	6.98
70-80^{-}	136	2.95	38	3.96
80-90^{-}	34	0.74	8	0.83
90-100^{-}	6	0.13	3	0.31
100-110^{-}	1	0.02	0	0
Total	4,601	100.00	960	100.00

2.4 Crown Diameter (CD) the CD of Merkus pine was classed into 16 classes. The class interval was 1 m. The result of the study was displayed that the most CD was between 6-7 m (19.48%), and followed with 5-6 m (19.06%) and 7-8 m (15.21%), respectively. The widest of crown was 15.25 m which is in 15-16 m class (0.10%) (Table 29). The average CD was 6.68±2.15 m which wider CD recorded in the year 1983 (Figure 22D). Jenkarnying (1983) concluded that the average CD was 4.74±1.73 m. While the result of analysis of Wilcoxon-signed Rank Test between DBH of 1983 and 2005, the result was indicated that the significant difference at 95% level.

Table 29 Distribution of Merkus pine trees in each crown diameter classes in 2005 of the *in situ* gene conservation forest, Surin province

CD Class (m)	Number of existing trees in various years			
	1983		2005	
	No. of trees	Percentages (%)	No. of trees	Percentages (%)
0-1^{-}	0	0	1	0.10
1-2^{-}	65	1.41	2	0.21
2-3^{-}	504	10.95	19	1.98
3-4^{-}	1,182	25.69	51	5.31
4-5^{-}	1,122	24.39	135	14.06
5-6^{-}	826	17.95	183	19.06
6-7^{-}	450	9.78	187	19.48
7-8^{-}	225	4.89	146	15.21
8-9^{-}	111	2.41	106	11.04
9-10^{-}	69	1.5	60	6.25
10-11^{-}	26	0.56	34	3.54
11-12^{-}	13	0.28	15	1.56
12-13^{-}	5	0.11	13	1.35
13-14^{-}	3	0.06	5	0.52
14-15^{-}	0	0	2	0.21
14-16^{-}	0	0	1	0.10
Total	4,601	100.00	960	100.00

From above-cited table, it can be concluded that Merkus pine trees in the forest had development in crown diameter. Because of Merkus pine in 2005 had the crown diameter more than 14 m while the pine in 1983 (Jenkarnying, 1983) had the crown diameter less than 14 m. The differences may caused by the changing in the density of Merkus pine. In the past, Merkus pine covered densely the forest area. When, some pine trees were lost with several factors. This is cause of low density of Merkus pine in the forest. So it is chance to spreading crown of the remainder pine trees. In addition, when it is a young tree, it has a pyramical crown or conical crown form with well developed apical dominance. Later they attain a flatter and more spreading crown.

Conclusion, the report was shown that only pattern of height distribution of tree population in 2005 performed similarly with tree population in 1983. Otherwise, the patterns of number distribution in each DBH, Do and CD classes of existing trees (year 2005) are changed from 1983's pattern. The average of morphological characteristics (H, DBH, Do and CD) in 2005 was higher than in 1983.

3. Genetic Variation of Merkus Pine

Genomic DNA was extracted from individual young needle tissue of Merkus pine using a modified cetyltrimethyl ammonium bromide (CTAB) method. It was found that quantity and quality of DNA was sufficient.

DNA was amplified using seven microsatellite markers following as suitable condition by PCR technique. Then DNA bands were separated and detected. The results of amplification were found that three primer-pairs (pm01, pm03, pm07) can be amplified and be codominant and polymorphic while two microsatellite markers (pm04, pm06) were multiband. Otherwise, the results on study of Nurtjahjaningsih *et al.* (2005) was found that those primer-pairs showed to be codominant and polymorphic and banding pattern performed 5 and 6 alleles, respectively. In addition, two primer-pairs (pm02, pm05) were not amplified.

The investigation was shown that the highest number of alleles per locus were 5 alleles. This is occurred on locus pm01 (Figures 18). The lowest numbers of alleles per locus were 2 alleles. This occurrence was on pm07 (Figure 19). Locus pm03 was shown as 4 alleles. The figure of banding pattern using primer pm03 was shown in Figures 20.

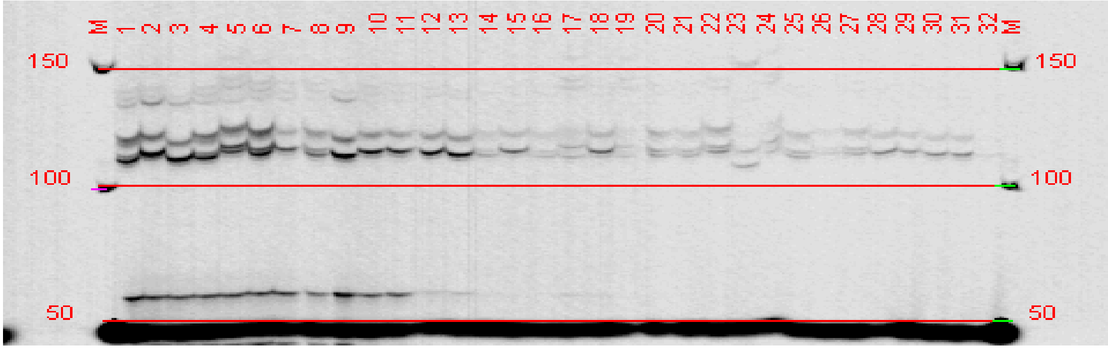


Figure 18 Banding patterns of DNA amplification by microsatellite markers on 32 individuals of Merkus pine in the *in situ* gene conservation forest using primer pm01.

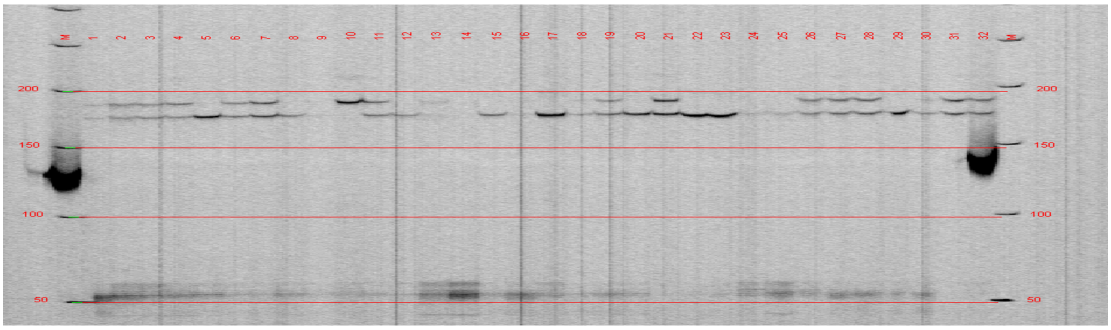


Figure 19 Banding patterns of DNA amplification by microsatellite markers on 32 individuals of Merkus pine in the *in situ* gene conservation forest using primer pm07.

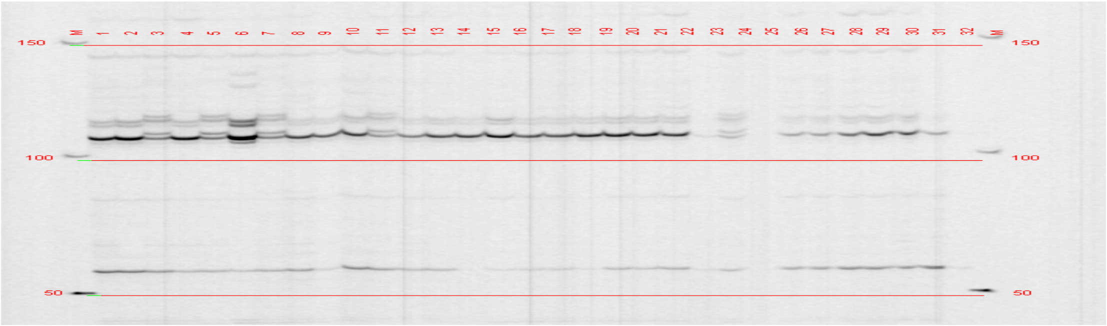


Figure 20 Banding patterns of DNA amplification by microsatellite markers on 32 individuals of Merkus pine in the *in situ* gene conservation forest using primer pm03.

The genetic variation parameters within population were analyzed by TFPGA version 1.3 and GenAlEX 6. The results of analysis on allele frequencies in each locus are shown as Table 30. The results of average allele per locus were found that locus pm01 and pm07 were equivalent (0.200). Locus pm03 had the highest of average allele frequencies (0.500).

Table 30 Analysis on allele frequencies.

Allele	Locus		
	Pm01	Pm03	Pm07
1	0.016	0.672	0.016
2	0.328	0.328	0.375
3	0.172		0.188
4	0.266		0.250
5	0.219		0.172
Average	0.200	0.500	0.200

The values of number of alleles per locus, Observed heterozygosity (H_0), Expected heterozygosity (H_e) and Fstatistics were calculated. The number of alleles observed for each locus ranged from two to five. While the research of Nurtjahjaningsih *et al.* (2005), it was shown that the number of alleles observed for each locus ranged from three to six on five microsatellite markers (pm01, pm02, pm03, pm04, pm05, pm06, pm07). The average number of alleles per locus in the present study was 3.67. The value of Ho of each locus ranged from 0.441 to 0.732. The value of Ho on locus pm01 was highest (0.732), and followed by locus pm 03 (0.650), locus pm 07 (0.441), respectively. Those values were similar with the study of Nurtjahjaningsih *et al.* (2005). There were ranged from 0.389 to 0.728. The average of Ho in this study was 0.608. This value is quite high. In addition, the value of He of each locus ranged from 0.586 to 0.969. The average of He in this study was 0.829. So, the Fstatistic was 0.265. The results of genetic variation parameters of Merkus pine in Surin population are shown as Table 31.

Table 31 The number of alleles and heterozygosities and Fstatistics were calculated for 32 individuals from the *in situ* gene conservation Merkus pine forest, Surin Province.

Locus	$^*N_A$	N_A	Ho	He	Fstatistics
pm01	3	5	0.732	0.969	0.245
pm03	4	4	0.650	0.931	0.302
pm07	3	2	0.441	0.586	0.247
Average		3.67	0.608	0.829	0.265

Remark: $^*N_A$-Number of alleles which were studied by Nurtjahjaningsih *et al.* (2005)
N_A -Number of alleles
Ho -The observed heterozygosity
He -The expected heterozygosity

Nevertheless, the species had been threatening with many factors, which can reduce its genetic variation. Furthermore, the pine had also been facing with scarcity of pollen available for fertilization of ovules during the flowering time due to low population density. In addition, most Merkus pine trees are over-mature and produce less flower and seeds. Those occurrences are however affects to high empty seed bearing. A consequence is genetic loss occurrence on the pine in this forest. It is also risk to lost good genetic resource of the species. Chance of genetic variation reduction of Merkus pine is able to occur in the forest. This is risk to inbreeding depression. Therefore, the intensive management should be conducted for genetic variation maintaining or adding the genetic variation level.

From the study on isozyme marker of Changtragoon and Finkeldey (1995), it was found that mean number of alleles per locus of Nong Khu Merkus pine population in Thailand was 1.6 and expected heterozygosity (He) was 0.051. Furthermore, average number of alleles per locus of Thailand was 1.6 and expected heterozygosity was 0.037. The results revealed only little genetic diversity at 14 isozyme gene loci (average He = 0.058). Allelic differentiation among populations is also small (0.034), but higher than the differentiation reported from many other conifer.

Genetic structure and variation of Merkus pine from two natural populations in Sumatra and one artificial population in Java was evaluated using isoenzyme as genetic marker. 8 isoenzyme loci were used to determined population. Analysis was concluded that a higher level of genetic variation of Merkus pine in Indonesia and was noticed when comparing to the populations of Thailand (Siregar *et al.*, 1999).

The Concepts of community on the Forest Area

The investigation on the concepts of community to the *in situ* gene conservation Merkus pine forest was focused on 6 villages surrounding the forest area. The community is composed of Ban Nong Khu, Ban Kog Kra Don, Ban Ta Pong, Ban Kog Yang, Ban Krahom and Ban Lert Aroon. The villages are located at Tab Tan sub-district, Sang Kha District, Surin Province. A total of samples were 280 respondents which are consisted of 50 interviewers of Ban Nong Khu, 58 interviewers of Ban Kog Kha Don, 38 interviewers of Ban Ta Pong, 26 interviewers of Ban Kog Yang, 51 interviewers of Ban Krahom and 57 interviewers of Ban Lert Aroon. The samples to be used for the study were the household heads or the representatives of the sample household. The results of the study are presented into 5 sections, as followed;

1. Section 1: General Information

The most of respondents were female (51.4%). Their ranges of age were 12-89 years and their average age was 45 years. The education level of the most interviewees was at Phatom 4 (Primarily School) (48.9%), and followed by Phatom 6 (Primarily School) (22.5%). The most of respondents were local people (50.4%). The range of migration year was between 2500 and 2547 while the most respondents migrated in the area in 2500 (9.3%). The range of resettlement duration was therefore 1 to 48 years. Detailed results of section 1 are shown in Table 32.

2. Section 2: Socio-Economic Characteristics

The most of main occupation of respondents was agriculture land use (60.4%), and followed by general employee (21.1%), several occupations (11.4%), respectively. In addition, their supplementary occupation of the most was general employee (63.2%) and followed by small shop (12.1%). Most respondents faced several problems, including (financial, land uses, debt, etc.), and followed by single problem of financial, land use and debt problem, respectively. The results of socio-economic study are shown in Table 33.

3. Section 3: The Concepts of Community on Forest Resource

Concepts of the most of respondent to the natural forest benefits were that forest give multipurpose use (94.3%) especially being forest minor product source, major income and minor income, timber harvesting and biodiversity. The most of interviewee thought that they got benefits from forest in several aspects (74.3%) especially minor forest products harvesting and being a recreation area. Thai forest resource is decreasing rapidly. This is worried by the most of respondent (85.7%) while remainder samples were not concerned with the problem (2.1%). Most interviewee thought that the main factor of the rapid decrease of Thai forest resource consist of several factors (41.8%) especially people influences and natural factors. Everybody has participated in destruction on forest where is located near the community. Most of them thought that they will help to solve the forest existing problems by means of activities related with forest resource conservation (71.1%).

Table 32 General Information of respondents

Characteristics	Number of respondents	Percentages (%)
Gender		
Male	134	47.9
Female	144	51.4
Age (12-89 yrs.)		
12-20	11	4
21-30	24	8.6
31-40	65	23.1
41-50	60	21.3
51-60	48	17.1
61-70	25	9.1
71-80	1	0.4
81-90	4	1.5
Education		
Little formal education	33	11.8
Phatom 4 (Primarily School)	137	48.9
Phatom 6 (Primarily School)	63	22.5
Matayom 1-3 (Secondary School)	22	7.9
Matayom 4-6 (Secondary School) or Por wor chor (Diploma)	18	6.4
Por wor sor (Upper Diploma)	0	0
Bachelor degree	2	0.7
Higher than Bachelor degree	0	0
Origin		
Local	141	50.4
Migration from other Tam bon in Sang Kha District	7	2.5
Migration from other Sub-district in Surin Province	90	32.1
Migration from other province	20	7.1
Year to migration		
(2500-2547)	102	36.4
The most was 2500	26	9.3
The frame of migration (year)		
(1-48 years)	100	35.7
The most duration was 48 yrs.	22	7.9

Table 33 Socio-economic of respondents

Characteristics	Number of respondents	Percentages
Main occupation		
Agricultural land use	169	60.4
Small shop	8	2.9
General employee	59	21.1
Government officer	2	0.7
Private business	1	0.4
Student	6	2.1
Several occupations	32	11.4
Supplementary occupation		
Agricultural land use	17	6.1
Small shop	34	12.1
General employee	177	63.2
Government officer	0	0
Private business	1	0.4
Student	6	2.1
Several occupations	27	9.6
Main problem of the family		
Financial problem	43	15.4
Land use problem	37	13.2
Debt problem	23	8.2
Pilferage problem	0	0
Member in the family separate for occupation	11	3.9
Several problems	155	55.4

4. Section 4: The Concepts of Community on Forest Resource Conservation

From interviewing with yes or no question, it was shown that villagers concluded that responsibility to forest is not only forest officer's function, but people should participate (86.4%). Forest sustainable use would lead to the forest conservation (78.6%). Most respondents did not agree with the statement that deforestation is not affecting to human and other organism (62.1%). They also considered that encroaching the forest by conversion forest area to farmland is illegal though it is the human basic need (75%). Most interviewee approved of the statement that forest conservation is also include soil and water protection (89.6%). Most of them (75.7%) did not consider that the forest conservation without utilization is only the forest preservation. In addition, most interviewees (84.6%) did not approve with statement that forest resource belong public so people are useable as their wish. To their opinion, forest conservation is also genetic resource conservation (88.6%). The species in the conservation area should not be used freely (72.9%). Most respondents (72.9%) approved that villager's participation is able to contribute the succession of forest conservation. Those results are shown in Table 34.

5. Section 5: The Concepts of Community on the *In Situ* Gene Conservation Merkus Pine Forest, Tab Tan Sub-district, Sang Kha District, Surin Province

Most villagers knew that Merkus pine is multipurpose species (58.9%), and then the species can be used for fuel (18.2%) while only 12.9% did not know about its utilization potentials. However, most of them did not know that Merkus pine in the *in situ* gene conservation forest is the best genetic resource (47.1%). To those who know, most of them got information from forest officers (24.3%) as well as from other information sources (media, relatives and other villagers, etc). Most of interviewee did not know that Merkus pine in Thailand is threatening to be endangered species (41.8%). While some part of knower was gathered information from forest officer (17.1%) and media (12.5%), respectively. Most respondent (87.8%) thought that the species should be conserved because of several reasons (87.8%). However, they did not know that the forest area is important (44.6%). To those who know, they got information from media.

Table 34 The concepts of community on forest resource

Concepts of community on forest resource conservation	Number of respondents	Percentages (%)
Forest overseeing is only the function of forest officer. People can not participate		
Yes	12	4.3
No	242	86.4
Proper forest utilization is the forest conservation		
Yes	220	78.6
No	36	12.9
Deforestation is not affecting to human and other organism		
Yes	76	27.1
No	174	62.1
Forest invading for conservation from the forestland to farmland is not illegal. Because it is necessary for life		
Yes	36	12.9
No	210	75
Forest conservation will help soil and water protection		
Yes	251	89.6
No	6	2.1
Forest conservation is forest keeping without use		
Yes	36	12.9
No	212	75.7
Forest resource is public treasure. So you can use forest resource		
Yes	12	4.3
No	237	84.6
Forest conservation is genetic resource conservation		
Yes	248	88.6
No	5	1.8
Species conservation area can be get benefit by free		
Yes	42	15
No	204	72.9
People's participation contribute the succession of forest conservation		
Yes	253	90.4
No	5	1.8

Most interviewed villager mentioned that they have been utilized the forest area especially harvesting timber and gathering minor forest products. Most villager's opinion showed that the *in situ* gene conservation Merkus pine forest was suffering with several problems especially trees cutting, resin tapping and flooding during the rainy season.

From interviewing the respondents, it was found that most of them considered the forest management and conservation at medium level (37.9%), and followed by good level (37.5%), worst level (11.8%), and very good level (6.1%), respectively. Most respondent thought that everybody should participates in managing and preserving this forest area especially the forest officer and people should cooperate in forest protection. Most respondent agreed that they had participated on forest conservation (81.1%) while some of them did not participate (10.4%). Interviewees showed their interest to conservation this forest (61.8%).

In the present study, not only forest officer to forest protection and conservation are everybody's concern. The majority of interviewee thought that deforestation is affecting to human and other organisms. Additionally, they thought that forest conservation should be sustainable uses and the forest can protect soil, water, and protecting genetic resource. This means that people participation would contribute to the success of forest conservation.

As for Merkus pine, most of respondents stated that the species is multipurpose species. But they did not inform that this provenance has the best genetic performance. As well as Merkus pine is endangered species. The villagers thought that the species should be conserved as it provides many benefit to them.

In practices, villagers nearby have harvesting wood and gathering minor forest products. Forest management and conservation was conducted in a moderate level. However, most interviewee concluded that everybody could participate in conserving the forest area. Integrated cooperation between forest officers and people will lead to better forest conservation.

CONCLUSION

1. The study on provenance variation on survival percentages and certain morphological characteristics of Merkus pine provenance trials showed that the northeastern provenances (Nos. 1019 and 1020) especially Surin provenance showed the best both in fitness and some morphological characteristics (total height, DBH, Do) while length of clear bole and crown diameter of this provenance had moderate performances. Analysis of variance between 14 provenances showed highly significant difference at 99% level except crown diameter. The results were similar when comparing with the previous studies that Merkus pine from Surin source performs a good genetic traits and better than the other provenances. This superior stand was later on supported by FAO to conserve the genetic materials in this forest. This study strongly confirmed the importance of *in situ* gene conservation forest in Surin province. It is also importance to illustrate the changes in the *in situ* gene conservation forest after 20 years of establishment.

2. The study was indicated that the forest is a stationary stage or regenerating well. In addition, the highest IVI of trees was *Dipterocarpus obtusifolius*. For Merkus pine, which had been being the most dominance and importance species, became below importance species at present. In contrast, the vertical structure of the forest remains original characteristic performance namely the forest comprised of the three layers but changing of species maybe occurred in each layer.

3. From the intensive survey, it was found that Merkus pine in the *in situ* gene conservation forest was remained as remnants which are scattered. Each group consisted of 3-5 Merkus pine trees. The study showed that total number of Merkus pine in the area was 960 trees. After the establishment of *in situ* gene conservation forest, the number of existing Merkus pine was changed from 4,601 trees in 1983 to 960 trees in 2003 as caused by illegal cutting, over resin tapping, and fire burning. Comparison with previous years, it was found that pattern of number distribution in each class on morphological characteristic of existing trees were changed and significant difference when comparing with previous years. Based on the present study, it can be concluded that Merkus pine in the *in situ* gene conservation forest had poor natural regeneration as a result of forest fire, flooding and old age mother trees. The result of basal area of Merkus pine at present was less than those recorded in 1983 owing of amount reducing of Merkus pine.

4. Genetic variation of Merkus pine population in the *in situ* gene conservation forest at Surin provenance using seven microsatellite markers to detect by PCR technique. Only three microsatellite markers (pm01, pm03 and pm07) can be amplified. The investigation was shown that the highest number of alleles per locus were 5 alleles. Additionally, the lowest numbers of alleles per locus were 2 alleles. The results of average allele per locus were found that the number of alleles observed for each locus ranged from two to five. In addition, the average number of alleles per locus was 3.67. The value of Ho of each locus ranged from 0.441 to 0.732. The average of Ho in this study was 0.608. This value is quite high. In addition, the value

of He of each locus ranged from 0.586 to 0.969. The average of He in this study was 0.829. So, the Fstatistic was 0.265.

5. The majority of respondents though that forest resource, the *in situ* gene conservation Merkus pine forest area and Merkus pine give many advantages to them. They realized on damages to the *in situ* gene conservation forest and the Merkus pine in this area. In addition, the most of them would like to participate on forest conservation. Surprisingly, public relations about forest management and conservation were insufficiency. Therefore, people participation activities, dissemination about forest management and more conservation works are required.

6. The Merkus pine was disturbed and remained only 960 trees in the present. This existing Merkus pine (960 trees) performed small amount of trees. Furthermore, they were still damaged with debarking, uprooting, fire burning, flood affecting, and other factors. The most factors were made by human. Furthermore, Merkus pine had relatively poor natural regeneration. From the result of analysis on genetic variation, it was found that its Ho was quite high. Nevertheless, the species had been threatening with many factors, which can reduce its genetic variation. Furthermore, the pine had also been facing with insufficient pollen available for fertilization of ovules during the flowering time due to low population density. In addition, most Merkus pine trees are over-mature and produce less flower and seeds. Those occurrences are however affects to high empty seed bearing. A consequence is genetic loss occurrence on the pine in this forest. It is also risk to lost good genetic resource of the species. Chance of genetic variation reduction of Merkus pine is able to occur in the forest. This is risk to inbreeding depression which is a one of factors of species extinction.

7. Regarding the Merkus pine trees in the *in situ* gene conservation, the species was under critical status. Otherwise, the stand characteristics of the forest were changed and had moderate species diversity level. This means that the forest was remained normal situation but proper management practices is needed for the remaining Merkus pine.

RECOMMENDATION FOR FUTURE WORK

1. As villager from 6 villagers nearby had strong impacts on the existing of the remaining forest. People participation activities required carrying capacity workshops and the need of youth program to consider the importance of pine trees.

2. Merkus pine in the *in situ* gene conservation forest had relatively poor natural regeneration maybe due to forest fire, flood and old aged mature trees. Long term flowering and seed crop production studies are needed. Intensive management practices would help in maintaining the existing forests.

3. Restoration of Merkus pine is needed and it should be better to divide the area into zones. Seedlings of each zone should be planted in that particular site.

4. Further study on genetic marker should be added more amount of sample size and number of primer-pairs.

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Appendix

QUESTIONNAIRES

STUDY ON
CONCEPT OF COMMUNITY ON THE *IN SITU* GENE CONSERVATION
MERKUS PINE FOREST, SANG KHA DISTRICT, SURIN PROVINCE

As a Part of the Master Degree Thesis under the Title
PROVENANCE VARIATION ON CERTAIN CHARACTERISTICS AND
IN SITU GENE CONSERVATION FOREST OF MERKUS PINE
(*PINUS MERKUSII* JUNGH. & DE VRIESE.)

Conducted by

Miss Kanjana Popromsree
Graduate Student
Department of Silviculture, Graduate School, Kasetsart University

Name of the Interviewee…………………………………………………………..
Address…………………………………………………………………………….
Name of village……………………………………………………………………
Date………………………………………………………………………………..

Write the description on the blanks and mark respective column with ticks (✓).

<u>Section 1</u>: General Information.

1. Gender

(…) Male (…) Female

2. Age…………………………………Years

3. Education Background

(…)	Little formal education	(…)	Por wor sor (Upper Diploma)
(…)	Phatom 4 (Primary School)	(…)	Bachelor degree
(…)	Phatom 6 (Primary School)	(…)	Higher than Bachelor degree
(…)	Matayom 1-3 (Secondary School)		
(…)	Matayom 4-6 (Secondary School) or Por wor chor (Diploma)		

4. Origin

(…) Local
(…) Migrant
  (…) From other Tambon in Sang Kha District
  (…) From other District in Surin Province
  (…) From other Province

Years of migration…………………Time frame of migration…………………...years

Section 2: Socio-economic Characteristics

1. Main occupation
   - (…) Farming
   - (…) small shop owner
   - (…) General employee
   - (…) Several occupations
   - (…) Government officer
   - (…) Other private business
   - (…) Student

2. Supplementary occupation
   - (…) Farming
   - (…) Small shop
   - (…) General employee
   - (…) Several occupations
   - (…) Government officer
   - (…) Other private business
   - (…) Student

3. Main problems in the Family
   - (…) Financial problem
   - (…) Land use problem
   - (…) Debt problem
   - (…) Steal problem
   - (…) Members in the family separate for occupation
   - (…) Several problems

Section 3: Understandability about the forest resource

1. How much you know what is the advantage of natural forest?
   (can answer more than one choice)

   - (…) Forest provide timber for uses, such as construction and furniture, etc.
   - (…) Forest is a source of minor forest product such as fuel, mushroom, vegetable, medicinal plants, etc.
   - (…) Source of major income and minor income
   - (…) Genetic and Biodiversity sources
   - (…) Youth and people can study about forest
   - (…) Forest contribute to higher rainfall and fresh air
   - (…) Forest can be a shelter to prevent natural disaster
   - (…) Forest can prevent soil erosion

(…) Forest can be relieved flood disaster
(…) Forest contribute to water flow consistent by throughout the year
(…) Provide multipurpose uses

2. Have you ever been utilize the natural forest?

(…) No, I don't
(…) Yes, I do.

If you use, what do you utilize from it?
(can answer more than one choice)

(…) Minor forest product gathering such as fuel, mushroom, vegetable, and medicinal plants, etc.
(…) Livestock feeding area
(…) Timber harvesting for construction, furniture, etc.
(…) Recreation area
(…) Knowledge source
(…) Multipurpose uses

3. How do you feel about the rapid decrease of Thai forest resource?

(…) No response to the depletion
(…) Be worry with the losses

4. Do you think that what is the main factor of the rapid decrease of Thai forest resources?

(…) Natural factors such as diseases, insects, storms, forest fires, etc.
(…) People
(…) Government officer
(…) Private timber companies
(…) Several factors

5. Do you think that who is the destroyer the forest near your home ?

(…) Forest officer
(…) Agriculture officer
(…) Administration officer
(…) Policeman
(…) Head of Sub-district
(…) Head of village
(…) Tambon Administration Organization Council Member (Orbor Tor)
(…) Sawmill owner
(…) Timber shop
(…) Abbot
(…) People
(…) Everybody

6. How do you can help to solve the forest decreasing problem?

(…) Preserve the remaining forests
(…) Use the forest production in conjunction with forest conservation
(…) Reforestation and to make ness for people

Section 4: Understandability about the forest resource conservation

Answer the following sentences with yes or no. If you think that it is correct sentences, please mark on the blanks with tick (✓) and If you think that it is wrong sentences, please mark on the blanks with tick (X).

…..1. Forest planning is done by forest authorities without people participation.
…..2. Proper forest utilization is the forest conservation
…..3. Deforestation may not affect human.
…..4. Changing forest to farmland is not illegal as it is important to life.
…..5. Forest will protect soil and water.
......6. Forest conservation is maintaining forest without uses.
…..7. Forest resource is a common property. So you can make use as you pleased.
…..8. Forest conservation helps conservation of forest genetic resources.
…..9. Species conservation area can be get benefit by free.
…..10. People participation can contribute to the success of forest conservation.

Section 5: Understandability about the *in situ* gene conservation Merkus pine forest at Sang Kha district, Surin province

1. Do you know about the benefit of Mekus pine?

(…) No, I don't
(…) Yes, I do

If you know, how the species benefit you ?
(can answer more than one choice)

(…) Timber for construction and furniture
(…) Terpene
(…) Fuelwood
(…) Pulp and newsprint
(…) Multiplepurpose uses

2. Do you know that Merkus pine in this *in situ* gene conservation Merkus pine forest is the best genetic source in Thailand ?

(…) No, I don't
(…) Yes, I do

If you know, where are the information sources?

(…) Forest officer
(…) Government officer
(…) Mass media such as radio, TV and newspaper, etc.
(…) Relatives and other villagers
(…) Other sources

3. Do you know that Thai Merkus pine have been threatening with decreasing problem to be endangered species?

(…) No, I don’t
(…) Yes, I do

If you know, where are the information sources?

(…) Forest officer
(…) Government officer
(…) Mass media such as radio, TV and newspaper, etc.
(…) Relatives and other villagers
(…) Other sources

4. According to the decrease of Merkus pine trees, do you think that it is necessary to conserve this species or not?

(…)No, I don’t
(…) Yes, I do.

Give mote details (can answer more than one choice)

(…) To exist this species for further multi-purpose uses
(…) To conserve this species for our next generation
(…) To be a source of gene pool for future propagations
(…) To conserve this species in maintaining the biodiversity
(…) Because human realize the importance to maintain the other organisms on the earth
(…) This species has right to exist and reproduction on the earth
(…) Several reasons

5. Do you know the important of *in situ* Merkus Pine gene convervation forest?

(…) No, I don’t
(…) Yes, I do

If you know, where are the information sources?

(…) Forest officer
(…) Government officer
(…) Mass media such as radio, TV and newspaper, etc.
(…) Relatives and other villagers
(…) Other sources

6. Have you ever been taking benefit from this forest?

(…) No
(…) Yes,

What kind of benefits? (Can answer more than one choice)

(…) Woods and timbers
(…) Resin and terpene tapping
(…) Farming in this forest
(…) Raising Livestock in this forest
(…) Collect minor forest product
(…) Multipurpose uses

7. Do you think that what are the main problems in this forest?

(…) Forest encroachment for settlements
(…) Forest encroachment for new farm areas
(…) Forest encroachment for extending farm areas
(…) Illegal logging and resin tapping
(…) Floods occur during rainy season
(…) Forest fire caused by human
(…) Trees are damaged from livestock
(…) Several problems

8. Do you think that which problems are facing the remaining of Merkus pine?

(…) Illegal logging
(…) Fire sticks
(…) Poor method of resin tapping
(…) Forest fires damage
(…) Flooding
(…) Wind and storm damages
(…) Insects and diseases damages
(…) Livestock damages
(…) Several problems

9. What is the present status of forest management?

(…) Worst	(…) Medium
(…) Good	(…) Excellent

10. Do you think that who are involving in managing this forest?
(can answer more than one choice)

(…) Forest officers
(…) Head of sub-district
(…) Head of village
(…) Tambon Administration Organization Council Member (Orbor Tor)
(…) People
(…) Everybody

11. Do you participate in forest managing?

(…) No
(…) Yes

12. Would you like to participate in conserving this forest?

(…) No
(…) Yes

Further suggestion

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"Thank you very much for your valuable information"

Interviewer…………………………………………………………………………………
Position……………………………………………………………………………………
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