มอร์โฟเมตรีของใบ ความแปรผันทางพันธุกรรม และวงศ์วานทางวิวัฒนาการของกวาวเครือขาว *Pueraria mirifica* ในประเทศไทย

นายตฤณ สุวรรณวิจิตร

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LEAF MORPHOMETRY, GENETIC VARIATION, AND PHYLOGENY OF WHITE KWAO KRUA *Pueraria mirifica* IN THAILAND

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Thesis title	LEAF MORPHOMETRY, GENETIC VARIATION, AND PHYLOGENY OF WHITE KWAO KRUA *Pueraria mirifica* IN THAILAND
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เก็บตัวอย่างของกวาวเครือขาว โดยเก็บใบ ฝัก และดอก จากแหล่งต่างๆ ในประเทศไทยมาใช้ในการวิเคราะห์ลักษณะทางมอร์โฟเมตรีและทางพันธุกรรมเพื่อหาความสัมพันธ์ว่าสอดคล้องกับข้อมูลของสารเคมีในหัวกวาวเครือขาวหรือไม่ ผลของการวิเคราะห์ทางมอร์โฟเมตรี พบว่าสามารถแบ่งกลุ่มกวาวเครือขาวได้ โดยมอร์โฟเมตรีของใบสามารถแยกสายพันธุ์ออกเป็น 5 กลุ่ม ส่วนมอร์โฟเมตรีของฝักแสดงให้เห็นว่าสามารถแบ่งได้เป็น 2 กลุ่ม และมอร์โฟเมตรีของดอกสามารถแยกสายพันธุ์ได้เป็น 3 กลุ่ม ทั้งนี้สามารถสรุปได้ว่าผลของมอร์โฟเมตรีสามารถบ่งบอกความแตกต่างแต่ละสายพันธุ์ได้ค่อนข้างต่ำ หลังจากนั้นนำข้อมูลมาวิเคราะห์สหสัมพันธ์ต่อค่าละติจูด และลองจิจูด เพื่อดูถึงความสัมพันธ์ และแนวโน้มการเปลี่ยนแปลงทางมอร์โฟเมตรีของพืชชนิดนี้ในประเทศไทย พบว่าทั้งมอร์โฟเมตรีของใบ ฝัก และดอก ล้วนมีความสัมพันธ์ และแนวโน้มต่อค่าละติจูด และลองจิจูดที่แตกต่างกัน

ในส่วนการวิเคราะห์ทางพันธุกรรม พิจารณาผลจากลำดับเบสและอาร์เอพีดี ในส่วนของลำดับเบส ทำการเพิ่มชิ้นส่วนและหาลำดับเบสบนบริเวณ ITS, *trn*L และ *trn*L-F พบว่าลำดับเบสทั้งหมดที่ได้จากทุกสายพันธุ์แสดงความแปรผันทางพันธุกรรมที่ต่ำ อย่างไรก็ตามค่าไดเวอร์เจนท์ของลำดับเบสของ ITS มีค่าอยู่ระหว่าง 0-25.2% ซึ่งสูงกว่าค่าไดเวอร์เจนท์ของอีก 2 บริเวณ ส่วนผลของอาร์เอพีดีนั้นพบว่า เกิดแบนด์ที่แตกต่างกันทั้งหมด 93 ชิ้น มีค่าเฉลี่ยของระยะห่างทางพันธุกรรมอยู่ในช่วง 0-0.4381 จากการวิเคราะห์วงศ์วานทางวิวัฒนาการโดยเอ็นเจ 4 วงศ์วาน (3 วงศ์วานโดยลำดับเบสและ 1 วงศ์วานโดยอาร์เอพีดี) พบความแตกต่างของแต่ละวงศ์วาน ถึงแม้ว่าจะพบดีเอ็นเอโพลีมอร์ฟิซึมที่ต่ำ แต่วงศ์วานของอาร์เอพีดีสามารถนำมาใช้ในการจัดกลุ่มสายพันธุ์กวาวเครือขาวได้สอดคล้องตามจังหวัดและภูมิภาคของประเทศไทยได้

ส่วนการวิเคราะห์ทางองค์ประกอบสารเคมีพวกไอโซฟลาโวนอยด์ สามารถแบ่งกลุ่มสายพันธุ์กวาวเครือขาวออกเป็น 2 กลุ่ม พบว่าความแปรผันทางองค์ประกอบของสารเคมีของแต่ละสายพันธุ์มีค่าต่ำ และยังพบอีกว่าความแปรผันดังกล่าวไม่มีความสัมพันธ์ และแนวโน้มใดๆ ต่อค่าละติจูด และลองจิจูด กล่าวโดยสรุปได้ว่าผลจากการวิเคราะห์ทั้ง 3 รูปแบบแสดงให้เห็นถึงความแปรผันทางสายพันธุ์ของกวาวเครือขาวในประเทศไทยที่ต่ำ

สาขาวิชา....เทคโนโลยีชีวภาพ..... ลายมือชื่อนิสิต.....ตฤณ สุวรรณวิจิตร.....

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TRIN SUWANVIJITR: LEAF MORPHOMETRY, GENETIC VARIATION, AND PHYLOGENY OF WHITE KWAO KRUA *Pueraria mirifica* IN THAILAND. THESIS ADVISOR: ASST. PROF. CHANPEN CHANCHAO, Ph.D., CO-ADVISOR: ASSOC. PROF. WICHAI CHERDSHEWASART, Ph.D., 156 pp.

Pueraria mirifica (leaf, pod, and flower) were collected from many localities (cultivars) throughout Thailand for morphometric and genetic analyses in order to evaluate the evolutionary relationship to the chemical (isoflavonoid) analysis. In morphometric analysis, the results showed that all cultivars were moderately classified. The leaf morphometry could separate the cultivars into 5 groups. The pod morphometry indicated that the cultivars belong into 2 groups. In addition, the flower morphometry could classify the cultivars into 3 groups. It could summarize that *P. mirifica* cultivars have low variation in morphometric approach. Due to correlation analysis, patterns of characterization of *P. mirifica* in Thailand were determined. The leaf, pod, and flower morphometry of *P. mirifica* presented that the morphological traits are correlated to angular distances on latitude and longitude.

In genetic analysis, direct sequencing and RAPD were used. About direct sequencing, PCR products of ITS, *trn*L, and *trn*L-F could be amplified and sequenced. All obtained sequences indicated low level of genetic variation among the cultivars in Thailand. However, the sequence divergence of ITS (0-25.2%) was higher than that of other 2 regions. Due to RAPD, total of 93 polymorphic bands were generated. The average of genetic distance was varied from 0 to 0.4381. According to 4 NJ phylogenies (3 phylogenies by direct sequencing and 1 phylogeny by RAPD), the obtained topologies were different. Although the genetic polymorphism among cultivars was low, the RAPD tree could moderately illustrate the cultivar classification depending on provinces and regions of Thailand.

In chemical (isoflavonoid) content analysis, it showed 2 classified groups. Remarkably, variation of chemical contents among cultivars was also low. Furthermore, there was no significant correlation in isoflavonoid contents against latitude and longitude. In summary, all 3 analyses indicated low variation among cultivars of *P. mirifica* in Thailand.

Field of study…Biotechnology….. Student's signature…Trin Suwanvijitr……

Academic year…2006…… Advisor's signature…Chanpen Chanchao……

Co-advisor's signature…Wichai……

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ABBREVIATIONS

A, G, C and T	4 types of nitrogenous bases; Adenine, Guanine, Cytosine and Thymine, respectively that containing in dNTP
bp	base pair
˚C	Degree Celsius
cm	Centimetre
CpDNA	Chloroplast DNA
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylene diamine tetra-acetic acid
g	Gram
hr	Hour
ITS	Internal transcribed spacer
kb	kilobase
M	Molar
mg	milligram
min	minute
ml	milliliter
mM	Millimolar
ng	nanogram
NJ	Neighbour Joining
nm	Nanometre
nrDNA	nuclear ribosomal DNA
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
rpm	revolution per minute
RT	room temperature
sec	second
Tris	Tris (hydroxyl methyl) aminomethane
trnL-F	transfer RNA-Leucine and Phenylalanine intergenic spacer of chloroplast DNA

UV	Ultraviolet
V	Volt
v/v	volume by volume
w/v	weight by volume
µg	microgram
µl	microlitre
µM	microMolar

CHAPTER I
INTRODUCTION

Pueraria mirifica Airy Shaw & Suvatabandhu (with a Thai name of "White Kwao Krua") which is classified in Family Fabaceae and subtribe Glycininae (the same subtribe as soy bean *Glycine max* L.) is an indigenous Thai climbing herbal leguminous plants. Its underground tuberous roots are widely used for folk medical therapy such as rejuvenating qualities (Kerr, 1932), estrogen supplements or replacement (Suntara, 1931), anti-tumor activity (Panriansaen, 2005), and cancer prevention (Murkies *et al*., 1998). This plant species is widely distributed in Thailand and Myanmar especially in the deciduous and mountainous forests in the northern and the western regions of Thailand. It is particularly abundant in Chiangmai (in the north) and Kanchanaburi (in the west) provinces (Panriansaen, 2000). Its tuberous roots are always harvested by native people for a traditional drug preparation. Recently, *P. mirifica* became popular among Asian people, especially Japanese, Chinese and Thai. for consuming. In addition, chemicals in tuberous roots have been commercially applied for dietary supplement and cosmetics in order to promote physical appearance in breast enlargement, induction of menstruation in women, as well as improvement of the complexion or skin refreshment.

Nowadays, the status of *P. mirifica* is moderately threatened by rapid and vast deforestation and human invasion that causes decreasing in the population and intra-specific variation. Thus, it should be conserved, cultivated and propagated urgently.

It has been reported that its tuberous roots contain many interesting chemicals identified as phytoestrogens including miroestrol derivatives (Chansakaow *et al*., 2000[a]). In 1949, Sukhavachana found that phytoestrogens are mostly similar to female sex hormone or estrogen in quality and structure. Later, isoflavonoids (daidzin, daidzein, genistin, genistein, puerarin, mirificin, kwakhurin, and kwakhurin hydrate) were identified (Pisetpakasit, 1976; Ingham *et al*., 1986; 1988; 1989; Tham *et al*., 1998; and Chansakaow *et al*., 2000[b]). These chemicals extracted from a tuberous root have a bioactivity on cultured cells. For example, crude extract at high dosage (1,000 mg/ml) can inhibit the growth of both types of cancer cells, an estrogen receptor positive (ER+) human mammary adenocarcinoma or MCF-7 (Cherdshewasart *et al*.,

2004[a]) and cervical cancer cells or HeLa cells (Cherdshewasart *et al.*, 2004[b]). A number of researches are conducted in human (in menopausal women as a clinical trial) and also in experimental animals such as mice (Jones and Pope, 1960; Malaivijitnond *et al*., 2003[a]), rats (Benson *et al.*, 1961; Malaivijitnond *et al*., 2003[b] and 2004), monkeys (Trisomboon *et al*., 2004; 2006[a]; and 2006[b]). *P. mirifica* root extract contains high estrogenic activity so it can affect physical appearance, mating behavior, and mental properties in animals and human. Phytoestrogens particularly affect reproductive organs and anatomical traits of female animals and human such as enlargement of uterus, vagina and breast, prolongation of menstrual cycle, and increase of Follicle stimulating hormone (FSH) and Leutinizing hormone (LH) in blood. Moreover, powder of crude extract from *P. mirifica* can be used as estrogen replacement for menopausal women (Cain, 1960; Muangman and Cherdshewasart, 2001).

Indeed, there are 2 interesting species in the genus *Pueraria*. The first species is *P. lobata* (kudzu) which is native to China, Korea and Japan but is introduced as a notorious climbing weed in the US. Other species is *P. mirifica* which are beneficial in estrogenic-liked properties and in other pharmaceutical applications. Chemical and genetic variation has been more studied in genus *Pueraria* than in *P. lobata*. Although a lot of reports were conducted on *P. mirifica* which is native to Thailand, they are mostly focused on pharmaceutical analysis and bioassay. Few works were based on biological variation.

Recent studies found that *P. mirifica* collected from different locations in Thailand showed different chemical patterns by Thin Layer Chromatography (TLC) (Panriansaen, 2000). In addition, by High Performance Liquid Chromatography (HPLC), it presented that *P. mirifica* collected from 28 provinces could exhibit a distinguish variation of isoflavone contents (Subtang, 2002). Furthermore, by MCF-7 proliferation assay, *P. mirifica* collected from 28 provinces exhibited variation in proliferative effect on MCF-7 cells (Cherdshewasart *et al.*, 2004[a]). Chemical constituents and morphological characteristics of *P. mirifica* populations from various regions in Thailand are different (Panriansaen, 2000 and Subtang, 2002). This may be related to physical and biological environments such as soil components, pH, humidity, temperature, sunlight, and weeds, etc. Also, it is possible that it may be related to genetics.

As mentioned above, crude extracts of tuberous roots collected from various locations perform different bioactivities so it is our interest to find out whether it is related to morphometric and genetic variation or not. Mature leaf was used for morphometric analysis. Principal Component Analysis (PCA) method of Factor analysis and Between-groups linkage method of Cluster analysis in SPSS program for statistical computation were performed. Also, partial sequences of non-coding regions in the nuclear genome (nuclear ribosomal ITS) and in chloroplast genome (*trn*L and *trn*L-F) will be analysed. Both analyses will be used to determine the intra-specific relationship among collected cultivars. This work should be benefit in defining the relationship between chemical components, morphometry and genetics of *P. mirifica* from different locations. Finally, phylogenetic trees by NJ method in PAUP program were constructed.

CHAPTER II
LITERATURE REVIEWS

Pueraria mirifica Airy Shaw et. Suvatabandhu (synonym: *Pueraria candollei* Wall. ex Benth var. *mirifica* Airy Shaw et. Suvatabandhu) is a tropical herbal plant (Lakshnakara, 1952) which is classified in family Fabaceae or leguminous plants like soy bean and peas (Suvatti, 1978). People in various parts of Thailand know *P. mirifica* in different local names such as Thong Kwao, Thong Krua, Hua Kwao, Chan Krua, and White Kwao Krua, most known name. 'Kwao Krua' is commonly used for plants in many different genera and species especially for white Kwao Krua (*P. mirifica*), red Kwao Krua (*Butea superba*), and black Kwao Krua (*Mucuna colletii*). *P. mirifica* was identified in the genus *Pueraria* and named *Pueraria mirifica* Airy Shaw & Suvatabandhu in February 1947. There are a few plant species that look like *P. mirifica*, e.g. *P. lobata* or Kudzu which is mostly distributed in China, Korea and Japan. Plant morphology and outstanding organs of *P. mirifica* are illustrated in Figures 2.1-2.2.

Taxonomy of *Pueraria mirifica* is identified and classified by Suvatti in 1978.

Kingdom Plantae
Subkingdom Tracheobionta
Superdivision Spermatophyta
Division Magnoliophyta
Class Magnoliopsida
Order Fabales
Family Fabaceae
Subfamily Faboideae
Tribe Phaseoleae
Subtribe Glycininae
Genus *Pueraria*
Species *P. mirifica*

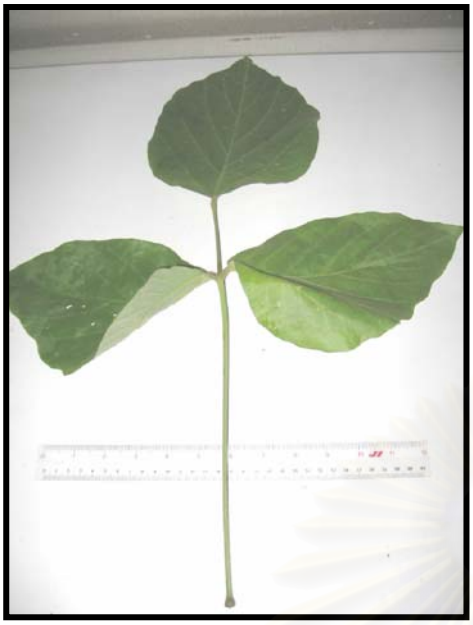
(A)

(B)

Figure 2.1. *P. mirifica* plants in the field.

(A) Root and stem of a young plant in Nakorn Ratchasima province

(B) The growing plants in Ratchaburi province



(A)

(B)

(C)

(D)

Figure 2.2. Parts of *P. mirifica* plant.

(A) Compound leaf of Chiangrai cultivar

(B) Inflorescences or flower bunches of Kanchanaburi cultivar

(C) Young pods of Kanchanaburi cultivar

(D) Tubers of Doi Tao and Chiangdao cultivars (from Chiangmai province)

2.1 Botanical characteristic, ethnobotany, and application of *P. mirifica*

P. mirifica is a perennial leguminous herb. It is a climbing plant that wraps around large trees or spreads on the ground. It has pinnate tri-foliate compound leaf. Roots are long tuberous or rather globular. The size varies on cultivars or environmental factors. Flowers compose of five petals that look like bluish-purple butterfly and bloom during February to April. Each flower composes of one standard, two keels, and two wings similar to leguminous flower. Inflorescences or flower bunches, about 15-40 cm long, are racemose type. Pods are typically short or small, slender and covered with brown hairs. They contain 1-5 dark brown or black seeds when completely mature. In 1986, Smitasiri *et al*. reported that *P. mirifica* is closely related to Kudzu or *P. lobata* (Willd.) that is the native species in southern Japan and southeastern China. The different aspects between them are leaf laminar and petiole. Kudzu's leaf and its petiole are typically slightly smaller and more hairy than *P. mirifica*.

The underground tuberous root extract of *P. mirifica* have been long recorded as domestic consumption to promote youth in both male and female consumers (Suntara, 1931). In the past, Thai menopausal women in some regions take it for traditional remedies which include the tuber powder of *P. mirifica* to relieve vasomotor symptoms (hot flashes and night sweats).

Recently, *P. mirifica* root powder is extracted and admixed in industrial cosmetics such as a firming breast lotion or cream, eye gel, and skin moisturizer. The benefits of skin application are mostly used for anti-wrinkle and breast firming. Additionally, this plant powder was manufactured as a food supplement for anti-aging (Dweck, 2002).

2.2 Chemical constituents and bioassay of root extract from *P. mirifica*

The root or tuber extract of *P. mirifica* contains a large number of chemical constituents mostly classified as phytoestrogens that its structure and effect is similar to female sex hormone, estrogen. Miroestrol was first isolated and analyzed to be the most active compound. Its effects are most similar to estrogen. It can affect estrogen-receptor cells in mammalian. Normally, it is found in little amount of roughly 1.5 mg/ 100 g dry weight of tuberous root powder (Bound and Pope, 1960). Other chemical constituents are coumestans, isoflavones, chromenes, steroids, sugar alcohols, minerals, sugars, lipids, and others (Nilandihi, 1957). Coumestans, isoflavones, and chromenes can induce estrogenic activity. Recently, deoxymiroestrol was isolated and could provide higher estrogenic potency (about 10 folds) than miroestrol (Chansakaow *et al*., 2000[a]). Isoflavones have 5 remarkable members of puerarin, daidzin, genistin, daidzein and genistein in the extracted powder of *P. mirifica* tuber (Cherdshewasart *et al.*, 2006). Five compounds of a tuber from various locations of Thailand were distinguished by HPLC fingerprint analysis. It revealed that there was the great chemovariation of isoflavones among a tuber from different locations (Subtang, 2002). Briefly, the important chemical constituents are summarized and listed in Table 2.1. Moreover, figure 2.3 presents the chemical structures of chemical constituents in *P. mirifica* tuber extract and figure 2.4 shows the comparison of the structures of isoflavonoid nucleus and estrogen.

Most researchers are interested in the effect of tuber extracts to animal model and cell culture. For instance, in female rats, crude extract affects reproductive system distinctly. It can enlarge uterine and vagina and it also can increase LH and FSH level in blood (Malaivijitnond *et al*., 2004). In female monkeys, the maximized dose (1,000 mg of *P. mirifica* extract) can affect a menstrual cycle length. Follicular phase and menstrual cycles are longer than normal condition (Trisomboon *et al*., 2004). Furthermore, there are many researches on the effect of crude extract to cultured cells. For example, high dose of the tuber extract (100 and 1,000 mg/ml) can inhibit the growth of MCF-7 cell or an estrogen receptor positive (ER +) human mammary adenocarcinoma (Cherdshewasart *et al*., 2004[a]).

Table 2.1. The important chemical constituents in the root extract of *P. mirifica.*

Category	**Chemical constituents**	**References**
Chromenes	Miroestrol Deoxymiroestrol Isomiroestrol	Ingham *et al*., 1986
Coumestans	Coumestrol Mirificoumestan Mirificoumestan glycol Mirificoumestan hydrate	Ingham *et al*., 1986; 1988
Isoflavones	Daidzin Daidzein Genistin Genistein Kwakhurin Kwakhurin hydrate Mirificin Puerarin Puerarin 6'- monoacetate	Ingham *et al*., 1986; 1989
Steroids	β-sitosterol Stigmatosterol	Hayodom, 1971

Isoflavone and Isoflavone glycosides

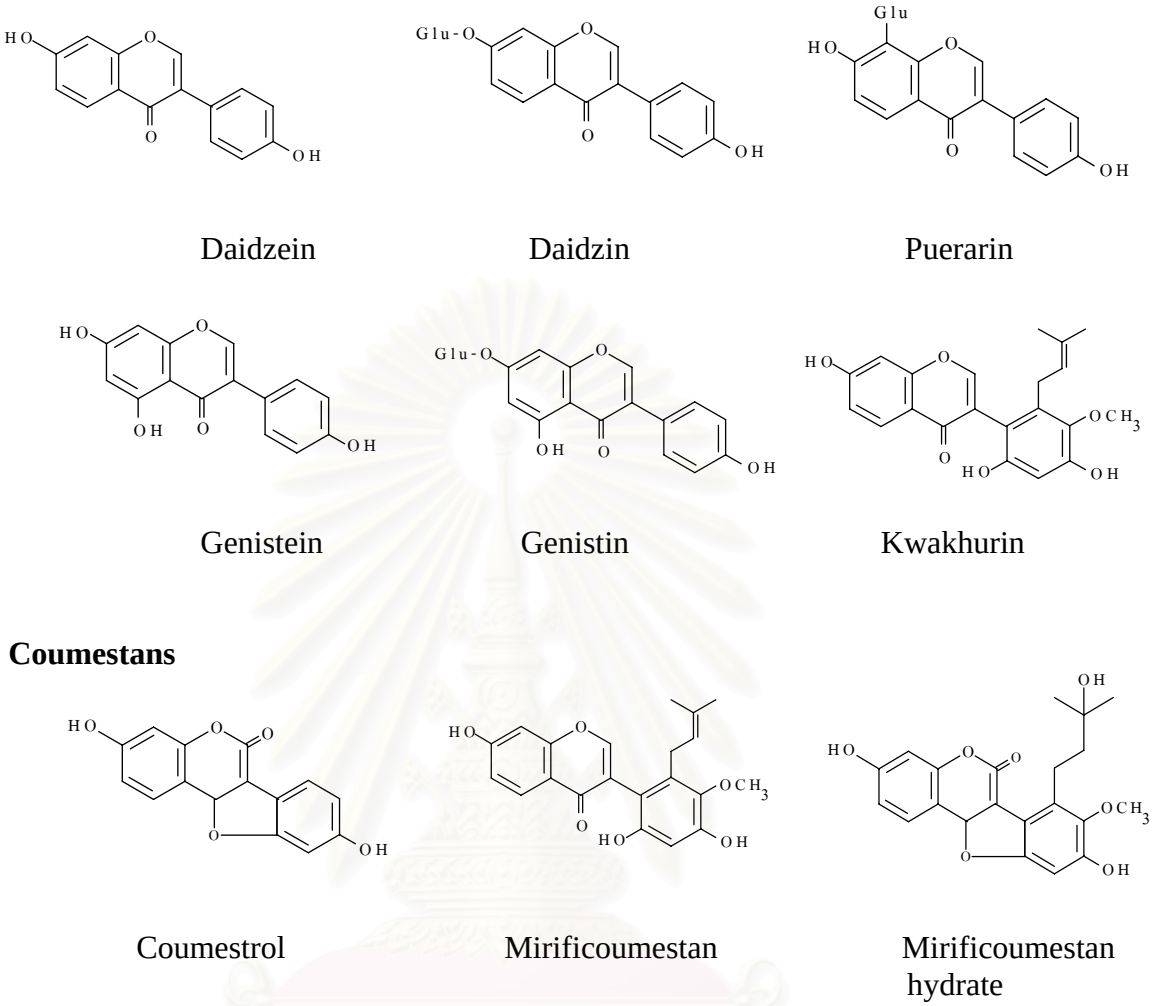
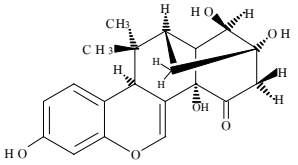
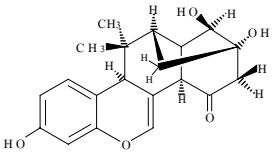


Figure 2.3. The structures of chemical constituents in *P. mirifica* root extract. (Ingham *et al*., 1986)

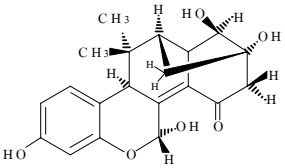
Chromenes



Miroestrol

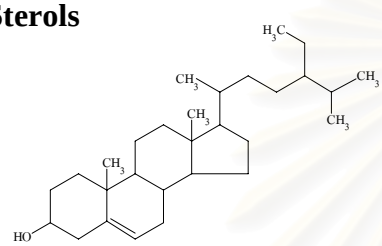


Deoxymiroestrol

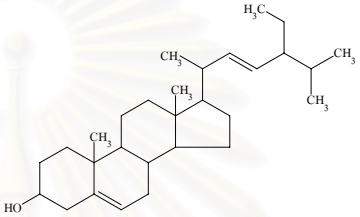


Isomiroestrol

Sterols

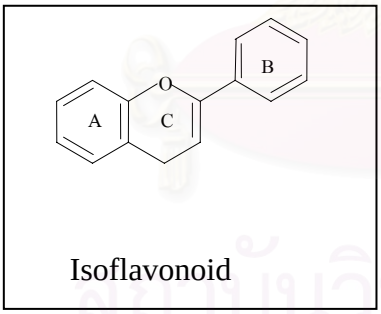


β-sitosterol

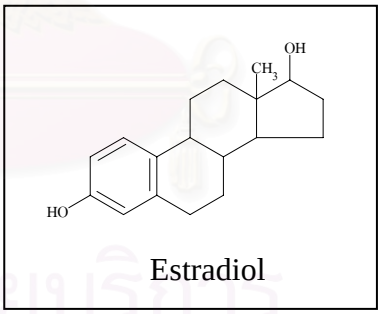


Stigmasterol

Figure 2.3. The structures of chemical constituents in *P. mirifica* root extract. (continued)



Isoflavonoid



Estradiol

Figure 2.4. The comparison of the structures of isoflavonoid nucleus and estrogen.

2.3 Distribution and propagation of *P. mirifica*

P. mirifica is an herbal plant that has long been used in traditional medicine in Southeast Asia, especially in Thailand and Myanmar. Panriansaen (2000) presented that this plant could grow on the mountainous forest and sandy soil at roughly 80-800 meters above the sea level. Moreover, *P. mirifica* herb typically shares the same habitats to *Butea superba* (red Kwao Krua), teak, and bamboo but it was not distributed in high-dense and evergreen forests. The vine of *P. mirifica* elongates for climbing over trees or spreads on the ground in an open area. In Thailand, Panriansaen (2000) also reported that *P. mirifica* is an endemic species that mostly grew in the deciduous or dry forest areas. It is widely distributed in the North, the Northeast, the Central, and the West, especially in Kanchanaburi and Chiangmai provinces. After it has long been surveyed and collected, *P. mirifica* could be mainly found in 3 areas that are 1) farmland, field, or orchard, 2) natural areas such as the mountainous forest, and 3) national park. Morphological variation between provinces or locations is also found. The distinct difference between *P. mirifica* from 2 provinces (Kanchanaburi and Chiangmai) was flower color. Flowers of Chiangmai cultivar was darker blue than flowers from Kanchanaburi cultivar. After that, Subtaeng (2002) presented that *P. mirifica* was widely distributed in at least 28 provinces in Thailand (Figure 2.5). Also, there was the isoflavonoid chemovariation in collected tubers and there was high chemical variety by HPLC analysis.

Sexual reproduction takes place by seed and asexual reproduction is by mean of underground rhizomes and tissue culture.

No.	Province
1	Chiang Rai
2	Chiang Mai
3	Mae Hong Son
4	Phayao
5	Nan
6	Lampang
7	Phrae
8	Lamphun
9	Uttaradit
10	Sukhothai
11	Tak
12	Phitsanulok
13	Phetchabun
14	Kamphaeng Phet
15	Nakhon Sawan
16	Uthai Thani
17	Sakon Nakhon
18	Nong Bua Lam Phu
19	Chaiyaphum
20	Nakhon Ratchasima
21	Saraburi
22	Lopburi
23	Kanchanaburi
24	Phrachinburi
25	Ratchaburi
26	Phetchaburi
27	Prachuap Khiri Khan
28	Chumphon

Figure 2.5. Locations that Subtaeng (2002) surveyed and collected *P. mirifica* tubers.

2.4 Morphometric study

Morphometry is the measurement of morphological characters or structures and shapes of organisms. Later, it will be analyzed by statistical computation. Morphometry has been used in order to study taxonomic diversity or variation of many organisms such as honeybees, gastropods, fish, etc.

2.4.1. Morphometry in plants

In plants, morphological characters such as leaf, seed, fruit, flower, etc. have been commonly used. For example, Creed (1997) reported the morphological variation in sea grass *Halodule wrightii* in Rio de Janeiro, Brazil. He found that leaf width, leaf length, sheath length, rhizome diameter, and root density showed higher variation between populations than within the populations.

Perez (2003) presented morphological variation or polymorphism of style (a stalk of female reproductive organ) and perianth (the outer and sterile whorls of a flower) in 7 species of *Narcissus* which were collected from Spain, Portugal, and Morocco. There was a significant relation between perianth and style although there was widely range in flower morphology.

Swamy *et al.* (2004) studied on seed morphometry by using scanning electron microscope (SEM) of 10 epiphytic orchid species from the Western Ghats of Karnataka in Southern India. Characters of seeds such as seed surface, size, shape, visibility of embryo, testa (seed coat) cells and structure, curvature, and ridges are varied. Moreover, seed colors were ranged from pale yellow to yellow, brown, and white.

Agustin (2006) studied morphometry of organs and fruit parameters of cherimoya (*Annona cherimola* Mill.) in Mexico. The multivariate analyses were performed by Principal Component Analysis method (PCA) of Factor analysis and then statistically clustered by the unweighted pair-group method arithmetic average (UPGMA) of Cluster Analysis. Finally, cherimoya could be separated into 4 groups based on Cluster analytical dendrogram.

2.4.2. Morphometry of *Pueraria*

In genus *Pueraria*, the study on morphometry is very rare. In *P. mirifica*, Panriansaen (2000) used some seed characters and shapes of tuberous roots to study

morphometry in populations from Chiangmai and Kanchanaburi only. Both areas were selected because of their abundance. Morphological differences in flower color, seed size, and tuber shape were found.

2.5 Genetic variation in plants

Methods in molecular biology have been used to study genetic diversity. It mostly aims on genetic materials especially DNA that could be found in all living organisms' cells. There are 3 types of DNA, 1) nuclear DNA (nrDNA), 2) chloroplast DNA (cpDNA) in all plants and 3) mitochondrial DNA (mtDNA) in all Eukaryotes. Genetic analysis of polymorphism in DNA level is considered to be a direct method to investigate inter- and intra-specific genetic variations of living organisms. Nowadays, a lot of molecular approaches have been used for the genetic diversity purpose such as Random Amplified Polymorphic DNA (RAPD), direct DNA sequencing, etc. Also, both types of molecular techniques were used to detect and investigate the variation of *P. mirifica* in this thesis.

2.5.1. RAPD in studying genetic variation in plants

Because of its advantages such as low cost, easiness and rapidity, RAPD technique has been recently and widely used in genetic variation and phylogenetic studies. In 1995, Mienie *et al.* used RAPD to identify and investigate 37 cultivars of South African soybean (*Glycine max* L.) in order to improve commercial seed production and crop certification. Total of 120 random primers and 60 combination primers were used in the reactions. Fourteen primers that could indicate repeatable polymorphisms were selected to identify individual cultivars. Then, amplified fragments were scored as present or absent (1 or 0). Frequency of detected polymorphism was low but cultivar investigation could be distinguished with a combination of the band patterns amplified by selected primers.

Thompson *et al.* (1998) conducted the study of genetic diversity of soybean (*Glycine max*) in North America. Accession numbers of the amplified sequences were obtained. Eighteen cultivars were determined to be ancestors while 17 cultivars were identified to be new cultivars. All of them were maintained in the USDA Soybean Germplasm Collection. The genetic relationship among 35 genotypes was calculated from 281 RAPD markers by using simple matching coefficient (SMC) and by expressing as Euclidean distances. The genetic distance among all genotypes was 0.56 and finally cluster analysis was identified as distinct groups from ancestors.

Bautista *et al.* (2001) used RAPD, RFLP, and SSLP to analyze phylogenetic relationships between cultivated and wild rice in Asia. The result presented that wild cultivars and cultivated rice are apparently varied from each other.

Baranek *et al.* (2002) evaluated the genetic diversity in 19 *Glycine max* in the Czech National Collection of Soybean Genotypes. RAPD technique was used and only 22 of 40 random primers showed the polymorphism that was acceptable for an effective characterization of these accessions. One hundred and twenty two reproducible RAPD fragments were generated and 55 bands of them were polymorphic (46%). The result could be useful for the cultivar selection and plant breeding.

Lakshmi *et al.* (2002) performed molecular phylogeny and genetic polymorphism in 9 species of mangroves (Rhizophoraceae) collected from the Indian sub-continent. In the past, the taxonomic relationships of the mangroves were usually investigated by morphological analysis. Sometimes, it was unclear and ambiguous because of some complicated and overlapped characteristics. Later, it was analyzed by using 3 techniques: RAPD, restriction fragment length polymorphism (RFLP), and RFLP of polymerase chain reaction (PCR-RFLP) products of chloroplast genes. Some primers were represented as markers. Finally, phylogenetic trees were constructed and analyzed. The result indicated that the sampled mangroves were totally classified into 3 clusters.

Xu and Gai (2003) carried out the genetic diversity of soybeans by RAPD method and could clarify the genetic difference of wild and cultivated soybeans growing in China. There were 21 wild soybeans and 27 cultivated soybeans. It showed that wild soybeans had higher genetic variation than cultivated soybeans. It indicated that genetic variation had been reduced by domestication of wild varieties. Based on genetic similarity coefficient, all of the accessions were classified into 2 major clusters: wild and cultivated varieties. In addition, the results indicated that geographical differentiation played an important role in genetic polymorphism of both wild and cultivated plants.

Wu *et al.* (2004) investigated the existing population and local distribution of *Oryza granulata* in Yunnan Province of Southwestern China in order to evaluate and conserve this endangered wild rice species. The genetic diversity among population and within population was determined by using RAPD and inter-simple sequence repeat (ISSR) as molecular markers. In studying among populations, the percentage of

polymorphic bands was 59% for RAPDs and 64% for ISSRs. Moreover, in studying within populations, the percentage generated by 2 techniques were 26% in the first population while the percentage were 21% (RAPD) and 22% (ISSRs), respectively in the second population. It also reported that genetic variation among populations of rice is much higher than genetic variation within populations.

Radmann *et al.* (2006) characterized and analyzed genetic variation of 10 main cultivars of strawberry in Brazil by 26 RAPD markers. Considering 19 selective primers, 14 primers could present polymorphism. From total of 116 bands, 84 bands were polymorphic. The strawberry cultivars were classified into 2 main groups: industry cultivars and fresh fruit market cultivars. Genetic similarity in the group of fresh market cultivars (44-74%) was less than the similarity in the other group.

2.5.2. DNA sequencing of chloroplast DNA transfer RNA-Leucine and phenylalanine region (cpDNA *trn*L-F) and nuclear ribosomal DNA internal transcribed spacer region (nrDNA ITS) in plants

Nowadays, a great number of intergenic spacer between chloroplast DNA transfer RNA-leucine and phenylalanine region (*trn*L-F) from various organisms are recorded in GeneBank database and other databases. This region can be practically used and can be probably combined to nuclear ribosomal DNA internal transcribed spacer region (ITS) in order to reveal phylogeny and genetic variation. Both regions are non-coding and conserved. Many researches on phylogeny have been reported by using both regions. For example, Alvarez and Wendel (2003) performed phylogenetic analysis by using ribosomal ITS sequences of 18S–5.8S–26S nuclear ribosomal cistron and constructed plant phylogenetic trees in order to interprete the genetic relationship. In addition, Taberlet *et al.* (1991) designed primers from the cpDNA, especially in *trn*L-F region in order to study phylogeny. The maps of ITS, *trn*L and *trn*L-F regions are displayed in the figure 2.6 and 2.7.

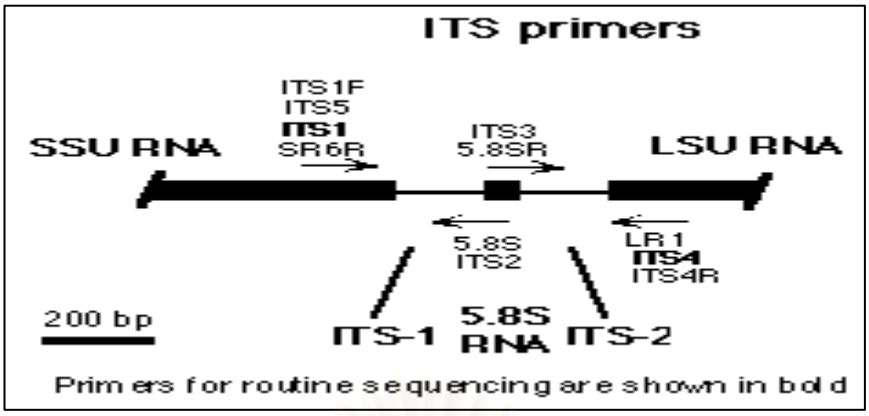


Figure 2.6. Map of nuclear ribosomal DNA internal transcribed spacer (ITS) region in eukaryotes (White *et al*., 1990).

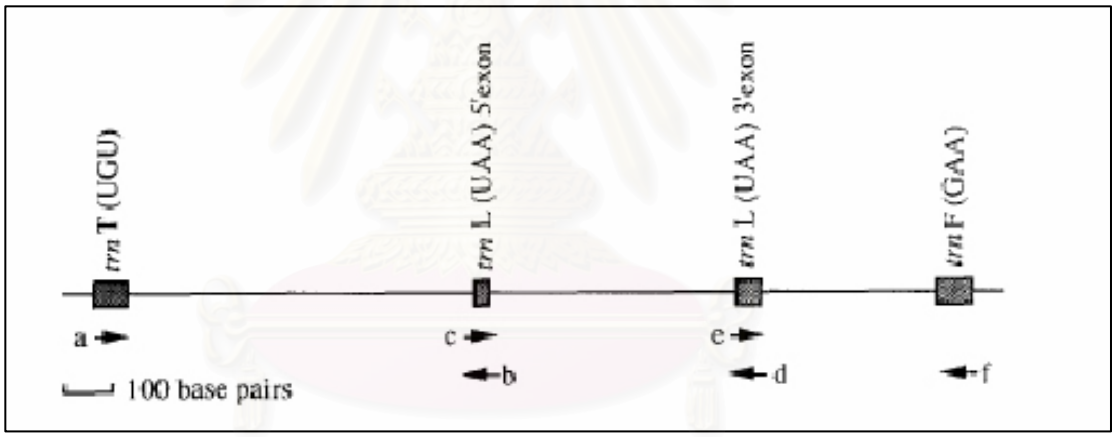


Figure 2.7. Map of chloroplast DNA *trn*L-F region in plants. Universal primers (a-f) were designed in phylogenetic analysis (Taberlet *et al.*, 1991).

In early 1990s, molecular phylogenetic researches have been mostly relied on cpDNA *rbc*L sequence but nowadays they have been relied on nrDNA internal transcribed spacer (ITS), cpDNA *trn*L-F, and mtDNA instead. ITS regions of nrDNA are always used in studying different taxonomic levels, particular at intraspecific level because of relatively rapid evolutionary rates. The cpDNA (e.g. *trn*L-F) in plants are usually conserved in terms of genome sizes, structures, gene contents, and linear

orders. Chloroplast genome evolves at a slower rate than nuclear genome but some regions change more rapidly than the average. Briefly, both regions mentioned above are evolutionarily varied. For instance, Taberlet *et al.* (1991) designed 6 primers to amplify 3 non-coding regions of chloroplast DNA: *trn*L-F and *rbc*L. In order to develop universal primers, those designed primers were tried on amplifying DNA from various plant species. The primers worked well on tested DNA of many organisms including algae, bryophytes, pteridophytes, gymnosperms, and angiosperms. It implied that primers were universal for organisms belonging in wide taxa.

Zimmer *et al.* (2002) conducted phylogenetic analyses of nuclear ITS, and chloroplast DNA *trnL* intron, *trnL-trnF* intergenic spacer region, and *trnE-trnT* intergenic spacer region in Gesnerioideae (Gesneriaceae). Live samples were grown and collected at the Smithsonian's National Museum of Natural History Botany Research Greenhouses, Maryland, USA. By analyzing nrDNA and cpDNA sequences, monophylly of clades of plants in this Family were revealed. The data was strongly supported by statistic calculation.

Schuettpelz and Hoot (2004) investigated phylogeny and biogeography of plants in genus *Caltha* (Ranunculaceae) which consists of 10 species of perennial herbs. The plants are widely distributed throughout the moist temperate and cold regions of the northern and southern hemispheres. The cpDNA and nrDNA sequences were used to indicate the monophyly (grouping in one branch) of genus *Psychrophila* and *Caltha*, to trace the evolutionary history of diplophylly (grouping in 2 branches), and to explore biogeographical hypotheses of the genus. The analysis of these data resulted in a well supported phylogeny.

Yulita *et al.* (2005) performed the molecular phylogeny of plants in genus *Hopea* and *Shorea* (Dipterocarpaceae) by determining the chloroplast DNA *trn*L–F and nuclear ITS regions. Plant materials from the Australian National Herbarium and Harvard University Herbaria were used. The inferred phylogenetic relationships between 2 genera could indicate that *Shorea* is paraphyletic while *Hopea* is potentially monophyletic.

Ellison *et al.* (2006) analysed phylogeny of 255 species of leguminous plants in genus *Trifolium* based on nuclear ITS and chloroplast *trnL* intron sequences. Representatives were from 11 genera of vicioid clade (tribes of Cicereae, Trifolieae,

and Vicieae) and *Lotus* as an outgroup. Disharmony between the nrDNA and cpDNA sequences suggested that there was hybrid speciation.

2.5.3. Genetic variation of plants in genus *Pueraria*

Researches on genetic variation of plants in genus *Pueraria* are quite rare. Pappert *et al.* (2000) reported genetic variation of *P. lobata* (Kudzu) of 20 populations in southeastern U.S. by using 14 allozyme loci analysis. The polymorphism of loci is 92.9% and overall genetic variation is 0.29. The average proportions of polymorphic loci and genetic variation within populations were 55.7% and 0.213, respectively.

Heider *et al.* (2004) carried out researches on *P. montana* in the topic of genetic diversity of a neglected crop in North Vietnam. The objective of this study was to develop an appropriate molecular marker to analyze the genetic variation of 5 accessions of *P. montana* that were collected in Bac Kan province, North Vietnam in order to know a basic understanding of genetic differentiation patterns in this target location. By using RAPD marker, this species illustrated a high polymorphic level of variation with 54.3%.

Sun *et al.* (2005) presented the genetic variation of *P. lobata* (Kudzu) that have been a noxious weed in the U.S. and plants in 4 closely related taxa (*P. edulis, P. montana, P. phaseoloides, and P. thomsoni*) collected from China and the U.S. The data was revealed by Inter-Simple Sequence Repeat Analysis (ISSR) method. The genetic diversity in both native (China) and invasive areas (the U.S.) could apply to the effective biological control standard. ISSR results showed a clear separation of these 5 species. High genetic differentiation was found in *P. lobata, P. montana*, and *P. thomsoni* collected from China. High genetic diversity and low population differentiation was found in *P. lobata* from the U.S. The obtained data supported a hypothesis of multiple introductions from Japan or China into the U.S. It brought to the subsequent gene exchange and recombination theories.

Until present, there is still no research on genetic variation of *P. mirifica.* Most researches have been focused on bioassay and pharmaceutical tests.

2.5.4. Relevance of plant morphometry and molecular analysis

There are some reports that apply morphometry together with molecular analysis to support each other in order to determine the plant variation. For example,

Hoey *et al.* (1996) performed the phylogenetic analysis of 17 wild and cultivated pea of genus *Pisum* in Germany based on 16 morphological characters, allozyme and, RAPD markers. The results showed that cladograms could confirm the close relationships among wild species. Morphological characters, allozyme, and RAPD markers were all together used to organize the pea taxa groupings.

Bailey *et al*. (2002) carried out the systematic and relationship of Halimolobine plant (Family Brassicaceae). Thirty three species from various locations were analyzed by using 3 loci (*trnL-F* region, nrDNA ITS, and *pistillata* intron) and morphological traits (17 morphological characters). The consensus tree contained 5 well-supported halimolobine subclades. Although there is variation in morphological character for classification of this group, the majority of these characters provide some grouping information within the halimolobine clade.

Chowdhury *et al.* (2002) identified 48 exotic germplasm lines of Thai soybean. All genotypes were classified by 37 morphological markers which could fully generate discrimination of the cultivars. The similarity index using Dice coefficient between cultivars was varied from 0.00 to 0.92 (an average of 0.45). The UPGMA cluster analysis showed 2 groups, first group of 32 cultivars and second of 16 cultivars. DNA of 48 cultivars was amplified by RAPD in order to identify cultivars and determine level of genetic similarity. From all of 80 random primers, 37 primers could reproduce polymorphic RAPD patterns. It indicated that high level of genetic similarities existed in these exotic cultivars. Then, the data were computed by using UPGMA method. Each genotype was clearly identified and separated from others. RAPD based dendrogram revealed that 48 cultivars could be classified into 4 groups.

Szczepaniak *et al.* (2002) combined 35 morphological characters and AFLP method to analyze intraspecific variation of 4 populations of *Elymus repens* (L.) or gould (Family Poaceae) collected from different habitats in Poland. Four pairs of selective primers were used to detect 279 AFLP bands. Also, 104 bands presented polymorphic patterns between populations (37.28%). Cluster analysis based on AFLP fingerprint data showed the individual arrangement in population. The analyses of variance (ANOVA and AMOVA) indicated significantly morphological and genetic differentiation among populations. This study showed that common analysis of genetic diversity and morphology are powerful tools for low-level taxonomic research.

Perez *et al.* (2003) used flower characters to reveal morphological variation of plants in genus *Narcissus*. The features were style and perianth (petals or outer whorled parts of flower) of *Narcissus* 7 species collected from Spain, Portugal, and Morocco. Significant relation between perianth morphology and style polymorphism of the studied species exhibited a wide-ranged morphological features. Based on molecular analysis and phylogeny of chloroplast *trn*L-F sequence, it could be concluded that 2 heterostylous species (2 or 3 different morphological types of flowers) had an independent origin. Also, it supported the convergence hypothesis of heterostyly in *Narcissus* spp. Furthermore, the second phylogeny of chloroplast *trn*T-L sequence could help to elucidate some evolutionary transitions in heterostylous species. Style dimorphism appeared in 2 types, the distyly (2 style forms) and style monomorphism.

Compton *et al*. (2004) conducted 9 different classifications of small plants in genus *Cyclamen*. Samples were collected from various sites worldwide. These classifications were generated by morphometric and cladistic analyses which were based on morphology, cytology, and DNA sequence of nrDNA ITS and cpDNA *trn*L intron. Only nrDNA sequence data revealed good resolution. When these 3 data sources were combined together, they provided the stronger resolution and the support for constructing 3 major clades.

Stuessy *et al.* (2006) presented the phylogenetic implications of plants in subfamily Barnadesioideae (Asteraceae) in Argentina. Generic and specific levels were based on morphological features of corollas (the overall structure of petals of a flower including shape and vascularization) and DNA sequences of ITS region and *trnL* intron. Molecular phylogeny is more compatible to evolutionary inferences than the morphological phylogeny. Highly significant correlation was obtained.

CHAPTER III
MATERIALS AND METHODS

3.1 Materials

3.1.1 Morphometric study equipments

- Vernia caliper
- Ruler

3.1.2 Genetic study materials

3.1.2.1 Equipments

- Autoclave: model Conbraco (Conbraco Ind. Inc., USA)
- Automatic micropipette: P10, P20, P200, and P1,000 (Gilson, France)
- Centrifuge/vortex: model Centrifuge FVL-2400 (BIOSAN, Latvia)
- Electronic UV transilluminator (Ultra lum Inc., USA)
- Electrophoresis chamber set: model Mupid (Advance Co. Ltd., Japan)
- Freezer -20°C
- Incubator (Memmert, Germany)
- Magnetic stirrer: model PC-320 (Corning, USA)
- Maxima ultra pure water: model Maxima UF (ELGA, England)
- Microcentrifuge: model Centrifuge pico (SORVALL®, Germany)
- Microcentrifuge tubes (0.5 and 1.5 ml)
- Microwave oven: model Sharp carousel R7456 (Sharp, Thailand)

- PCR machine: model GeneAmp® PCR system 2400 (Applied Biosystem, Singapore)

- PCR machine: model GeneAmp® PCR system 9700 (Applied Biosystem, Singapore)

- pH meter: model Cybersean 500 (Eutech cybernatics, Singapore)

- Pipette tips (10, 200, and 1,000 µl)

- Polaroid camera: model Direct screen instant camera DS 34 H-34 (Peca products, UK)

- Power supply: EC 5 70-90 LVD CE (E-C Apparatus corporation, USA)

- Thin-wall microcentrifuge tube (0.2 ml)

- Vortex: model MS I Minishaker (IKA-Works, Inc., USA)

- Vortex mixer: model KMC-1300V (Vision scientific Co, Ltd., Korea)

- Whatman laboratory sealing film (Whatman international Ltd., England)

3.1.2.2 Chemicals

- Absolute Ethanol, M.W. = 46.07 (Merck, Germany)

- Agarose gel (Research organics, USA)

- Boric acid (Research organics, USA)

- DNA *Hin*dIII marker, catalog# SM0101 (Fermentas Life Science, Germany)

- GeneRuler™ 100 bp DNA Ladder, catalog# SM1143 (Fermentas Life Science, Germany)

- DNeasy® plant mini kit, catalog# 69104 (QIAGEN UmbH, Germany)

- Ethedium bromide

- Ethanol 95%, M.W. = 46 (Thailand)

- Ethylene diamine tetra-acetic acid (EDTA), M. W. = 292.2 (Serve feinbiochemica GmbH & Co., USA)

- Nucleospin® DNA plant kit, catalog# 740570.50 (Machery-Nagel, Finland)

- PCR Master mix (*i*-Taq) solution, catalog# 25028 (*iNtRON* BIOTECHNOLOGY, Korea)

- Primers or oligonucleotides (Bioservice unit, Thailand.)

- QIAquick® PCR purification kit, catalog# 28104 (QIAGEN GmbH, Germany)

- Tris-(Hydroxymrtyl)-aminomethane, M.W. = 121.14 (Pharmacia Biotech, USA)

3.1.3 Plant materials and sampling collection

Pueraria mirifica samples were collected from 5 parts of Thailand (the North, the Northeast, the Center, the West, and the South) during May to October in 2006.

For morphometric analysis, mature leaves, old brown pods, and blooming flowers were collected. Total of 39 cultivars were sampled within 27 provinces of Thailand (Figure 3.1). Fifty leaves of each cultivar (about 1 meter far from shoot) was collected for measurement with 9 parameters. Ten pods per each site of total 14 cultivars within 11 provinces (Figure 3.2) were taken for measurement with 3 parameters. Furthermore, 10 flowers per each location of total 11 cultivars within 8 provinces (Figure 3.3) were used for measurement with 7 parameters. Moreover, *P. lobata* (Kudzu) from Japan was also used as an outgroup in order that interspecific differentiation of these sampled could be analysed. Leaves and pods of *P. lobata* were obtained to fit the above purpose. Unlike leaves and pods, *P. lobata* flowers could not be collected because it was not the blooming season at the time of harvesting. Numeric data was recorded and used for further statistical computerization.

For genetic analysis, young leaves were collected. Three young leaves were collected from each cultivar. Leaves were stored at -20^{o}C.

Table 3.1. List of sampling collection of *P. mirifica* in Thailand and *P. lobata*. The √ symbol represents a collected and analyzed sample.

No.	Cultivar	Leaf	Pod	Flower
1	CM1	√	√	
2	CM2	√		
3	CM3	√	√	√
4	CM4	√	√	√
5	CR	√		
6	LPang	√	√	
7	MHS	√		
8	LPoon	√		
9	Nan	√		
10	PY	√	√	
11	P1	√		
12	P2	√		
13	P3	√	√	
14	UTRD	√		
15	KPP	√	√	
16	LBR	√	√	
17	NKSW	√		
18	PBoon	√		
19	PSNL	√		
20	SR1	√	√	
21	SR2	√		√
22	SKHT1	√		
23	SKHT2	√		
24	UTTN	√		
25	KC1	√	√	√
26	KC2	√	√	√
27	KC3	√		
28	PCHBR	√	√	√
29	PJKRK	√	√	√
30	RB1	√		√
31	RB2	√		√
32	RB3	√		
33	RB4	√		
34	Tak	√	√	
35	CHYP	√		√
36	NKRSM	√		
37	SKNK	√		
38	CHPn	√		√
39	SRTN	√		
40	*P. Lobata*	√	√	
Total		40	15	11

No.	Province	Code name
1-4	Chiang Mai	CM1-4
5	Chiang Rai	CR
6	Lampang	LPang
7	Mae Hong Son	MHS
8	Lamphun	LPoon
9	Nan	Nan
10	Phayao	PY
11-13	Phrae	P1-3
14	Uttaradit	UTRD
15	Kamphaeng Phet	KPP
16	Lopburi	LBR
17	Nakhon Sawan	NKSW
18	Phetchabun	PBoon
19	Phitsanulok	PSNL
20-21	Saraburi	SR1-2
22-23	Sukhothai	SKHT1-2
24	Uthai Thani	UTTN
25-27	Kanchanaburi	KC1-3
28	Phetchaburi	PCHBR
29	Prachuap Khiri Khan	PJKRK
30-33	Ratchaburi	RB1-4
34	Tak	Tak
35	Chaiyaphum	CHYP
36	Nakhon Ratchasima	NKRSM
37	Sakon Nakhon	SKNK
38	Chumphon	CHPn
39	Surat Thani	SRTN

Figure 3.1. Map for leaf collection of 39 cultivars of *P. mirifica* among 27 provinces in Thailand.

No.	Province	Code name
1-3	Chiang Mai	CM1, 3, 4
4	Lampang	LPang
5	Phayao	PY
6	Phrae	P3
7	Kamphaeng Phet	KPP
8	Lopburi	LBR
9	Saraburi	SR1
10-11	Kanchanaburi	KC1-2
12	Phetchaburi	PCHBR
13	Prachuap Khiri Khan	PJKRK
14	Tak	Tak

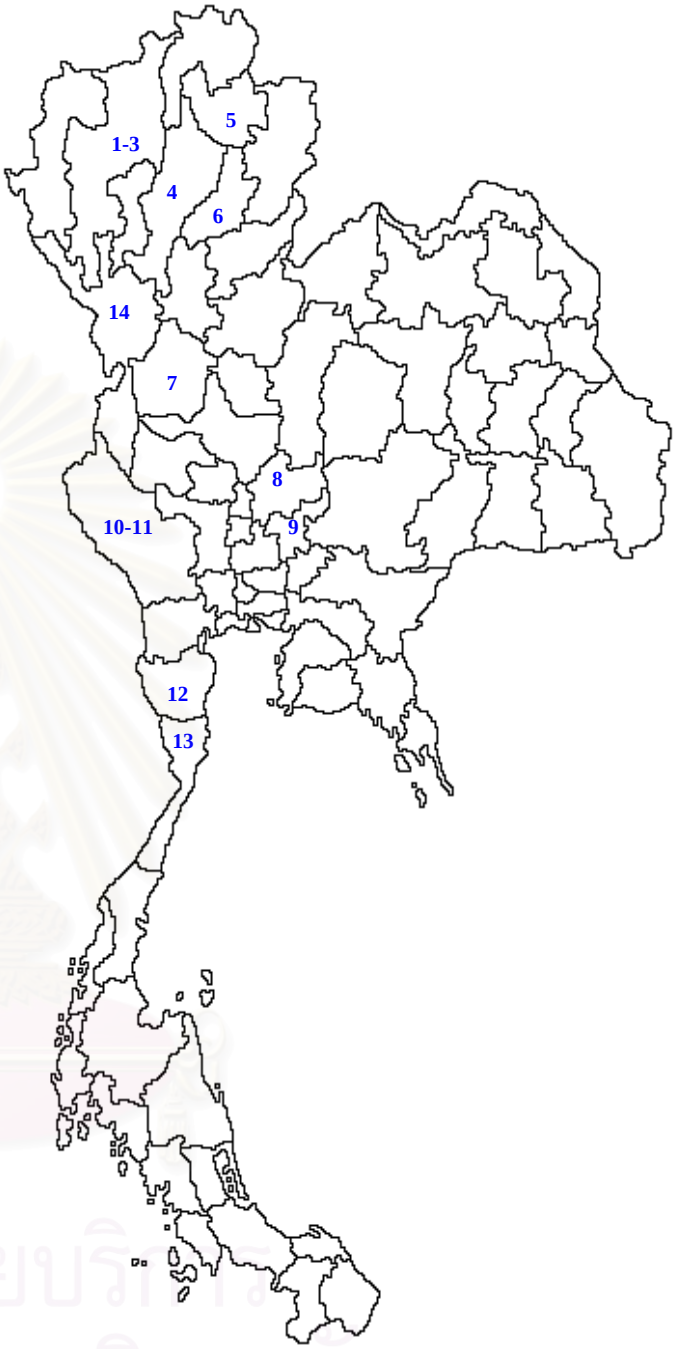


Figure 3.2. Map for pod collection of 14 cultivars of *P. mirifica* among 11 provinces in Thailand.

No.	Province	Code name
1-2	Chiang Mai	CM3-4
3	Saraburi	SR2
4-5	Kanchanaburi	KC1-2
6	Phetchaburi	PCHBR
7	Prachuap Khiri Khan	PJKRK
8-9	Ratchaburi	RB1-2
10	Chaiyaphum	CHYP
11	Chumphon	CHPn

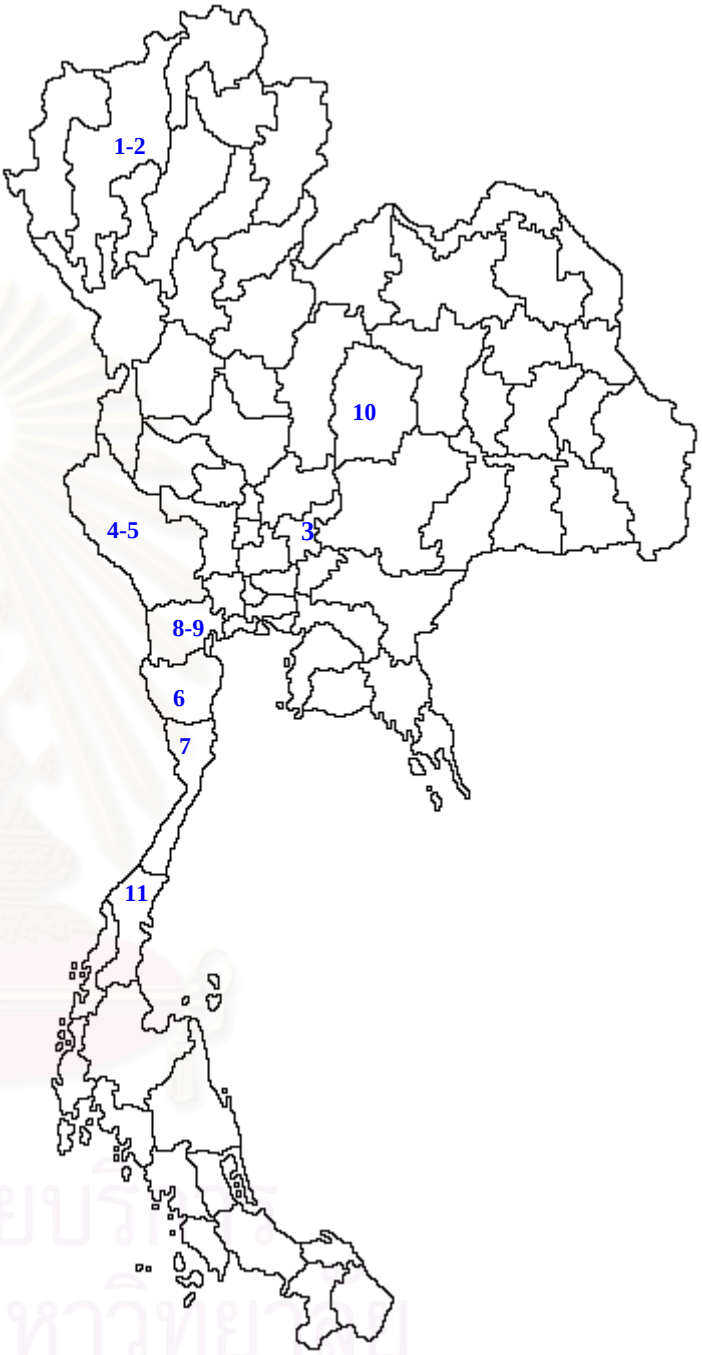


Figure 3.3. Map for flower collection of 11 cultivars of *P. mirifica* among 8 provinces in Thailand.

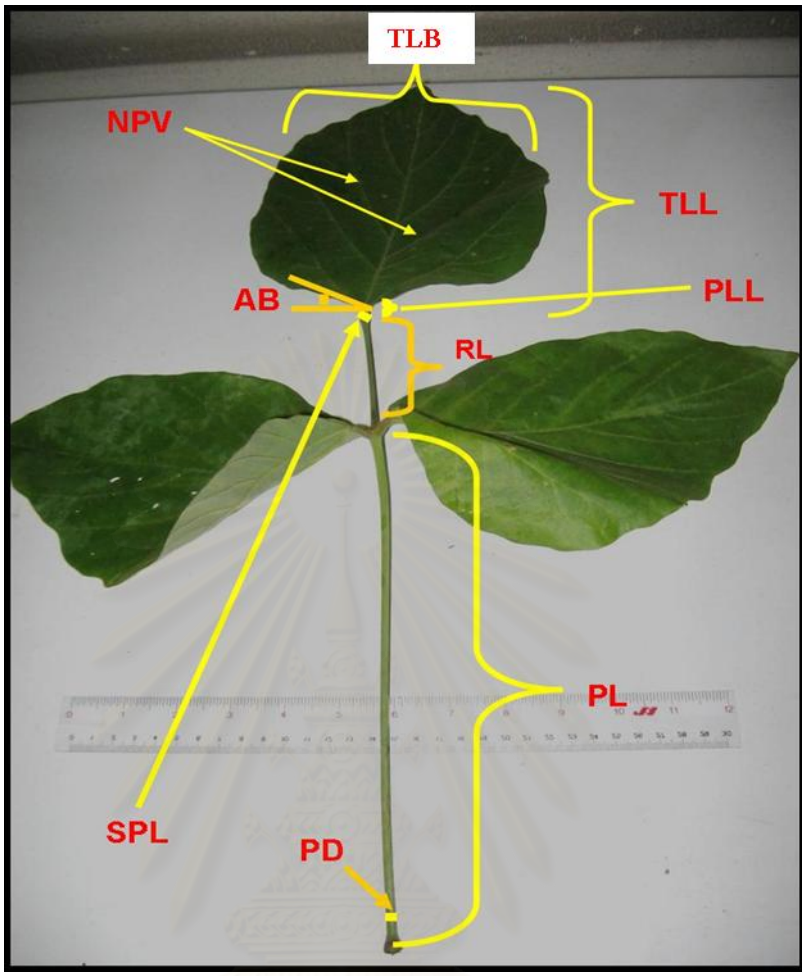


Figure 3.4. Nine morphometric parameters for measurement of collected *P. mirifica* leaf. Abbreviations used above are PL (petiole length), PD (petiole diameter), RL (rachis length), PLL (petiolet length), TLL (terminal leaflet length), TLB (terminal leaflet breadth), SPL (stipule length), (AˆB)^{o} - angle of first leaf border, and NPV (number of pairs of primary veins).

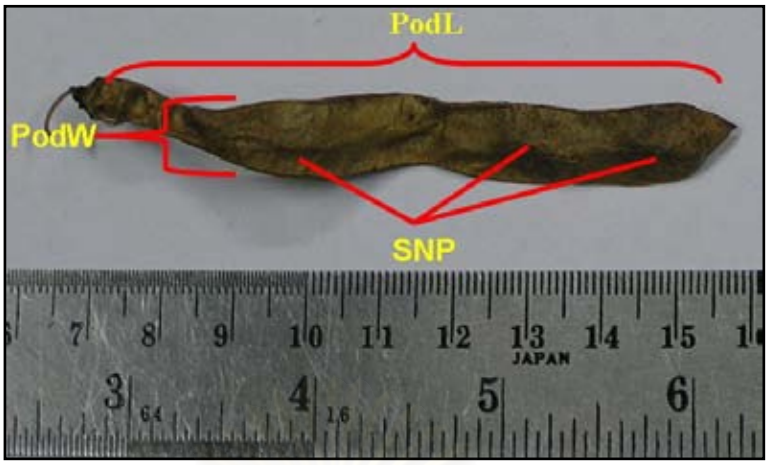
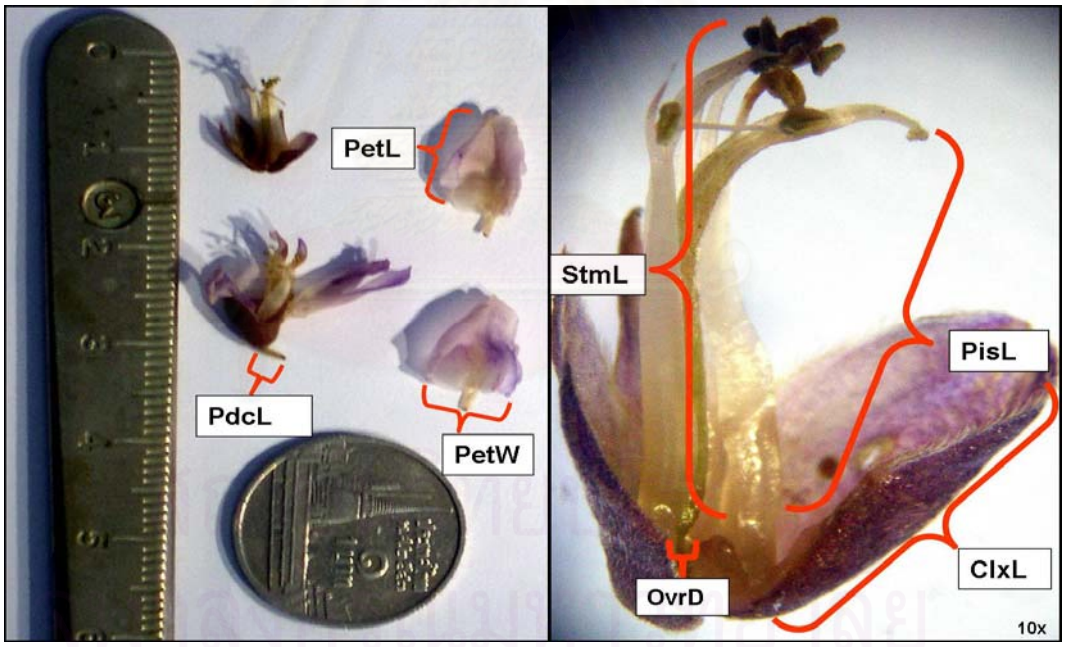


Figure 3.5. Three morphometric parameters for measurement of collected *P. mirifica* pod. Abbreviations used above are PodL (pod length), PodW (pod width), SNP (seed numbers per pod).



(A) (B)

Figure 3.6. Seven morphometric parameters for measurement of collected *P. mirifica* flower (A and B). Abbreviations above are PdcL (pedicel length), PetW (petal width), PetL (petal length), StmL (stamen length), PisL (pistil length), OvrD (ovary diameter), and ClxL (calyx length). Figure 3.6 (B) was from a stereomicroscope with the magnification of 10x when compared to Figure 3.6 (A).

3.2 Methods

3.2.1 Morphometric measurement and data analysis

Mature leaves were measured by 9 parameters and pods were measured by 3 parameters. In addition, flowers were measured by 7 parameters. Numerical data was recorded and applied to further statistically calculate and analyze.

Statistical analyses for investigating via Principal Component Analysis (PCA) method of Factor analysis (SPSS program for Windows) on the data using all parameters or characters were used. Parameters with higher factor loading scores from various factors would be used in order to provide reduction in the number of parameters. The selected parameters would be used for further cluster analysis in SPSS. After that, between-groups linkage method of the Cluster analysis would be used to calculate the relationship between groups and classify clusters.

3.2.2 Molecular and phylogenetic analyses

3.2.2.1 DNA extraction

Young leaf samples were kept in -20 ºC freezer for long preservation. DNA extraction was firstly done by using 2 optional Kits.

3.2.2.1.1 DNeasy plant mini kit (Qiagen, catalog# 69104)

DNA extraction was performed as followed. Young leaves were ground into powder with liquid nitrogen, and 400 µl of buffer AP1 and 4 µl of 100 mg/ml RNase A solution were added. Mixture was vortexed, incubated at 65°C for 10 min, and occasionally mixed by inverting. Then, 130 µl of buffer AP2 was added to this lysate, mixed, and incubated for 5 min on ice. Later, it was centrifuged at 20,000x g (14,000 rpm) for 5 min. The lysate was applied to a QIAshredder spin-column and centrifuged at 20,000x g for 2 min. The flow-through fraction was transferred to a new tube and mixed with 1.5x volume of buffer AP3. The mixture of 650 µl was applied to the DNeasy mini spin-column, and then was centrifuged at 6,000x g for 1 min. The flow-through was discarded. The remaining sample was added to the spin column and repeatedly centrifuged at 6,000x for 1 min. The column was placed in a new tube and 500 µl of buffer AW was added, and then was centrifuged for another 1 min. Another 500 µl of AW buffer was added again, and then was centrifuged at 20,000x g for

2 min. The spin column was transferred to a new tube and 100 µl of buffer AE was added onto the DNAeasy membrane of the column. The column was incubated at RT for 5 min and then centrifuged at 6,000x g for 1 min in order to elute DNA. Finally, DNA was stored at -20°C.

3.2.2.1.2 Nucleospin® DNA plant kit (Machinery-Nagel, catalog# 740570.50)

Briefly, dry weight of young leaves (100 mg) were ground with liquid nitrogen by a pestle and motar. After that it was transferred into a new microcentrifuge tube. The 400 µl of buffer C0 was added to the homogenized powder in order to break cells. RNase A solution (10 µl) was added and then incubated at 60^{o}C for 30 min. The lysate was applied to a Nucleospin® Filter column and then the centrifugation of the mixture was done at 11,000x g for 5 min. After that, the clear flow through was collected. Clear lysate (300 µl) was transferred to a new microcentrifuge tube. Buffer C4 (300 µl) and absolute ethanol (200 µl) were added (C4 buffer and absolute ethanol must be premixed before used). The sample was loaded into a provided Nucleospin® Plant column and centrifuged at 11,000x g for 1 min. The flow-through was discarded. Furthermore, a silica membrane in the column must be washed. It was firstly washed by 400 µl of buffer CW and centrifuged at 11,000x g for 1 min. Then, about 700 µl of buffer C5 were used to wash a silica membrane for the second time. For the third wash, 200 µl of buffer C5 was applied to the column and then centrifuged at the same speed for 2 min in order to remove buffer and make the silica membrane dry completely. Finally, highly pure DNA was eluted from the membrane by adding 50 µl heated buffer CE, 2x. Finally, the eluted DNA was kept at -20^{o}C until use.

3.2.2.2 Agarose gel electrophoresis

Extracted DNA was checked by electrophoresis of 0.8-1% (w/v) agarose gel in 1x TBE buffer (0.05 M Tris, 0.05 M Boric acid, and 0.65 M EDTA) as an electric running buffer. An electrophoresis was usually operated at 100 V for 40 min. Lamda *Hin*d III marker was used as a standard DNA marker. Loading sample composed of 5 µl of the extracted DNA and 1 µl of loading dye (6x loading dye buffer: 0.25% bromophenol blue, 40% (v/v) glycerol, and diluted in lx TBE running buffer). After that, the gel was stained by 10 µg/ml Ethedium bromide (EtBr) solution for 5-10 min

and destained in d-H_2O for about 20 min. DNA would be visible and photographed under UV light of a UV transilluminator.

3.2.2.3 PCR amplification

Double-stranded DNA of internal transcribed spacer in nuclear ribosomal DNA (ITS region) and 2 regions of the non-coding regions of transfer RNA-Leucine-Phenylalanine in the chloroplast DNA (*trn*L-F) were amplified by using specific primers shown in the Table 3.2. Each PCR reaction mixture contained 10 µl of 2x *iTaq* Mastermix (comprising all the reagents except the template DNA and primers), 2 µl of both 10 µM forward primer and reverse primer, 3 µl of 100 ng genomic DNA template, and 3 µl d-H_2O to reach total volume of 20 µl.

PCR amplification was carried out in GeneAmp PCR 2400 or 9700 System thermal cycler machine (Applied Biosystems). The PCR program was as follows: 94°C for 2 min 30 sec and 40-45 cycles of 94°C for 1 min, 55-60°C for 1 min, and 72°C for 3 min. Then, the final extension at 72°C for 10 min was performed. PCR products were detected by 1% agarose gel submerged in 1x TBE buffer at 100 V for 40 min. Finally, the gel was visualized on UV transilluminator after being soaked in EtBr for 5 min and destained in d-H_2O for about 20 min.

Table 3.2. Lists of 5 primers used for PCR amplification and direct sequencing.

Primer name	Direction	Sequence (5´ to 3´)	Reference
ITS_1	forward	TCCGTAGGTGAACCTGCGG	White *et al.*, 1990
ITS_4	reverse	TCCTCCGCTTATTGATATGC	White *et al.*, 1990
*trn*L(UAA)5'exon primer_c	forward	CGAAATCGGTAGACGCTACG	Taberlet *et al.*, 1991
*trn*L(UAA)3'exon primer_d	reverse	GGGGATAGAGGGACTTGAAC	Taberlet *et al.*, 1991
*trn*F(GAA) primer_f	reverse	ATTTGAACTGGTGACACGAG	Taberlet *et al.*, 1991

3.2.2.4 Purification of the PCR products

Before direct sequencing, PCR products were purified by using QIAquick PCR purification kit (Qiagen, catalog# 28104).

Briefly, 5x volume of buffer PB (Binding buffer) were added to PCR product and mixed. After that, the mixture was applied to a QIAquick spin column which was placed in a 2 ml collection tube. It was centrifuged at 13,000 rpm for 1 min. Later, the flow-through was discarded. Buffer PE (Wash buffer) of 0.75 ml was added into the column. It was centrifuged at 13,000 rpm for 1 min. The flow-through was discarded again and the column was additionally centrifuged at 13,000 rpm for 1 min. The column was transferred to a new 1.5 ml microcentrifuge tube and 30 μl of buffer EB (Elution buffer) was added onto the center of the membrane in the column. Then, the tube was incubated at RT for 1 min before being centrifuged at 13,000 rpm for 1 min again. The elution was saved and stored at -20^{o}C.

3.2.2.5 Direct DNA sequencing

Purified PCR product was sent to Bioservice Unit (BSU), National Science and Technology Development Agency (NSTDA), Thailand for DNA sequencing.

3.2.2.6 Random Amplified Polymorphic DNA technique (RAPD)

Five RAPD primers below were selected and presented in Table 3.3 (Mienie *et al.*, 1995).

Table 3.3. Lists of 5 arbitrary primers for RAPD.

Primer	Sequence (5' to 3')	Reference
OPA-07	GAAACGGGTG	Mienie *et al.* (1995)
OPA-12	TCGGCGATAG	Mienie *et al.* (1995)
OPD-02	GGACCCAACC	Mienie *et al.* (1995)
OPD-16	AGGGCGTAAG	Mienie *et al.* (1995)
OPE-01	CCCAAGGTCC	Mienie *et al.* (1995)

One PCR reaction mixture contained 5 μl of 2x *iTaq* Mastermix (comprising all the reagents except the genomic DNA and primers), 2 μl of 10 μM RAPD primer, 2 μl of 20 ng genomic DNA, and added with 1 μl d-H_2O to reach total volume of 10 μl.

PCR amplification of RAPD was also conducted in GeneAmp PCR 2400 or 9700 System thermal cycler machine (Applied Biosystems). A PCR reaction was performed as follows: 94°C for 2 min 30 sec and 45 cycles of 94°C for 1 min, 36°C for 1.5 min, and 72°C for 3 min. At last, the final extension was performed at 72°C for 10 min. PCR products were detected by 2% agarose gel submerged in 1x TBE buffer at 80 V and 1 h 20 min for electrophoresis procedure. Finally, a gel was visualized on UV transilluminator after being soaked in EtBr solution for 5 min and being destained in d-H_2O for about 20 min.

3.2.2.7 Phylogenetic analysis

All obtained sequences were aligned by multiple sequence alignment programs i.e. Clustal X or Clustal W (http://www.ebi.ac.uk/clustalw). The data matrix of DNA sequences were analyzed in Phylogenetic Analysis Using Parsimony (PAUP) program version 4.0b10. Phylogenetic analyses are performed by using neighbor-joining (NJ) method based on Kimura 2 parameter (K2P) genetic distance. Then, Bootstrap analysis with 1000 replicates was conducted in PAUP in order to evaluate supporting for nodes.

For RAPD analysis, record of 1 (presence of band) and 0 (absence of band) of each amplification of each primer was prepared. The data matrix was analyzed by Nei-Li genetic distance in PAUP. NJ phylogenetic tree building and Bootstrap support were estimated based on Nei-Li distance for RAPD fragments.

CHAPTER IV
RESULTS

4.1 Morphometric variation

4.1.1 Factor analysis

In order to reduce the morphometric characters or parameters, the data of all parameters were calculated and analyzed by the Factor analysis in SPSS program. Then, the selected data would be used for Cluster analysis. The Principal Component Analysis (PCA) method of Factor analysis was performed by using the raw data of each morphometric parameter of each sampling cultivar. Then, Factor loading scores could be obtained from the output of this statistic calculation. Only Eigen values that its value is higher than 1.0 would be applied for further analysis (Between-groups linkage method of Cluster analysis).

4.1.1.1 Factor analysis of leaf morphometry

The Factor analysis was conducted and then displayed the output. There were 9 leaf morphometric parameters. There are listed as below.

1. Petiole length – PL
2. Petiole diameter – PD
3. Rachis length – RL
4. Petiolet length – PLL
5. Terminal leaflet length – TLL
6. Terminal leaflet breadth – TLB
7. Number of pairs of primary veins – NPV
8. Stipule length - SPL
9. Angle of first leaf border - (AˆB)^{o}

After the Factor analysis using the data of the 9 morphometric parameters was performed, it could be divided into 2 new factors or groups. Each group contained parameters with Eigen values higher than 1.0. The first factor was accounted for 42.4

% of total variance and was mainly associated with 6 parameters (PD, TLB, PL, RL, TLL, PLL and AˆB). The second factor composed of NPV and SPL. This factor was accounted for 14.6 % of total variance.

Figure 4.1 shows a scatter plot of 2 new factor scores generated by Principal Component Analysis (PCA). The Principal Components were obtained from the raw data of 9 morphometric parameters. All parameters were measured from each leaf. *P. mirifica* in Thailand were coded by regions. Considering figure 4.1, it presents a plot of factor 1 (X-axis) versus factor 2 (Y-axis). A graph shows no grouping structure within *P. mirifica* in Thailand.

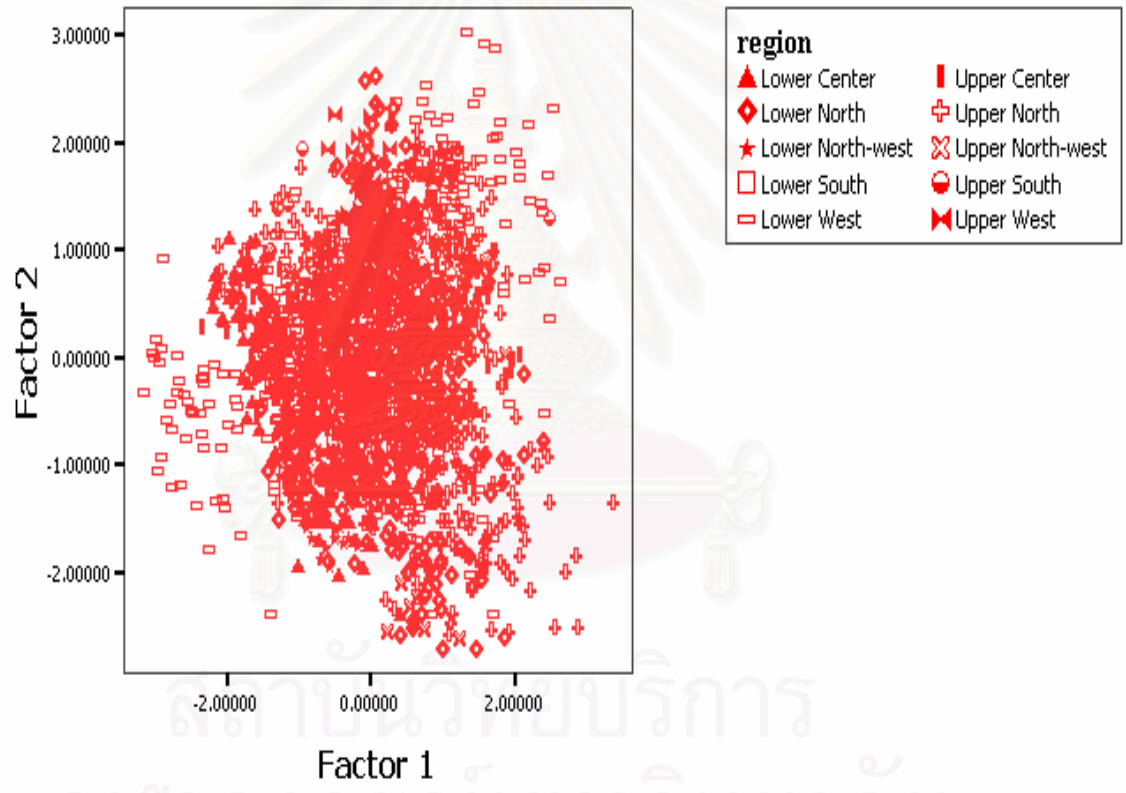


Figure 4.1. Position of each region in Thailand. Factor axes of 1 and 2 were derived from Factor analysis of leaf morphometric analysis: ordinate; factor 1 and abscissa; factor 2.

4.1.1.2 Factor analysis of pod morphometry

Factor analysis was not conducted for pod morphometry because only 3 pod parameters were obtained as listed below. Three-dimensional scatter plot of the raw data was constructed instead.

1. Pod length – PodL
2. Pod width – PodW
3. Seed numbers per pod - SNP

Figure 4.2 shows the scatter plot of the raw data of 3 parameters. All parameters were measured from each *P. mirifica* pod. Fourteen cultivars of *P. mirifica* in Thailand were coded by abbreviated names. Figure 4.2 presents the plot of PodL (X-axis), PodW (Y-axis), and SNP (Z-axis), respectively. A graph also shows no clustering structure within *P. mirifica* in Thailand. Fourteen cultivars of *P. mirifica* were unclearly separated from each other.

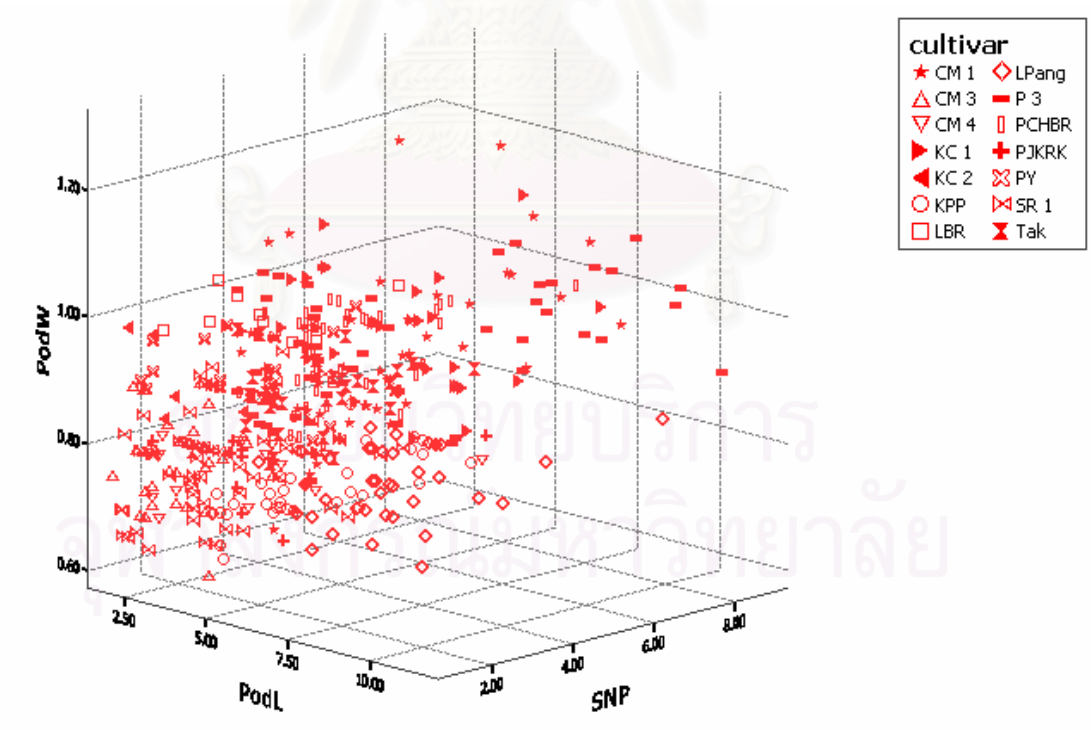


Figure 4.2. Position of each *P. mirifica* cultivar in Thailand for pod morphometric analysis. The 3-dimensional scatter plot was constructed on the axes of X-axis (PodL), Y-axis (PodW), and Z-axis (SNP).

4.1.1.3 Factor analysis of flower morphometry

Factor analysis was performed and provided the statistic output. There were 7 flower morphometric parameters as listed below.

1. Pedicel length – PdcL
2. Petal width – PetW
3. Petal length – PetL
4. Stamen length – StmL
5. Pistil length – PisL
6. Ovary diameter – OvrD
7. Calyx length – ClxL

After Factor analysis using the means of 7 morphometric parameters was performed, it could also be divided into 2 groups. Each group contained parameters with Eigen values higher than 1.0. The first new group or factor was accounted for 49.9 % of total variance and was mostly associated to 6 parameters (PetL, StmL, PisL, ClxL, PetW, and OvrD). The second new factor composed of only PdcL. This factor was accounted for 15.8 % out of total variance.

Figure 4.3 shows a scatter plot of 2 factor scores generated by Principal Component Analysis (PCA). The Principal Components were obtained from the raw data of all 7 parameters. All parameters were measured from each flower. The 11 cultivars of *P. mirifica* in Thailand were coded by abbreviated names. Figure 4.3 illustrated a plot of factor 1 (X-axis) versus factor 2 (Y-axis). The graph showed no grouping of *P. mirifica* in Thailand. Notably, KC1 cultivar from Thongphaphum district in Kanchanaburi province was clearly separated from the rest.

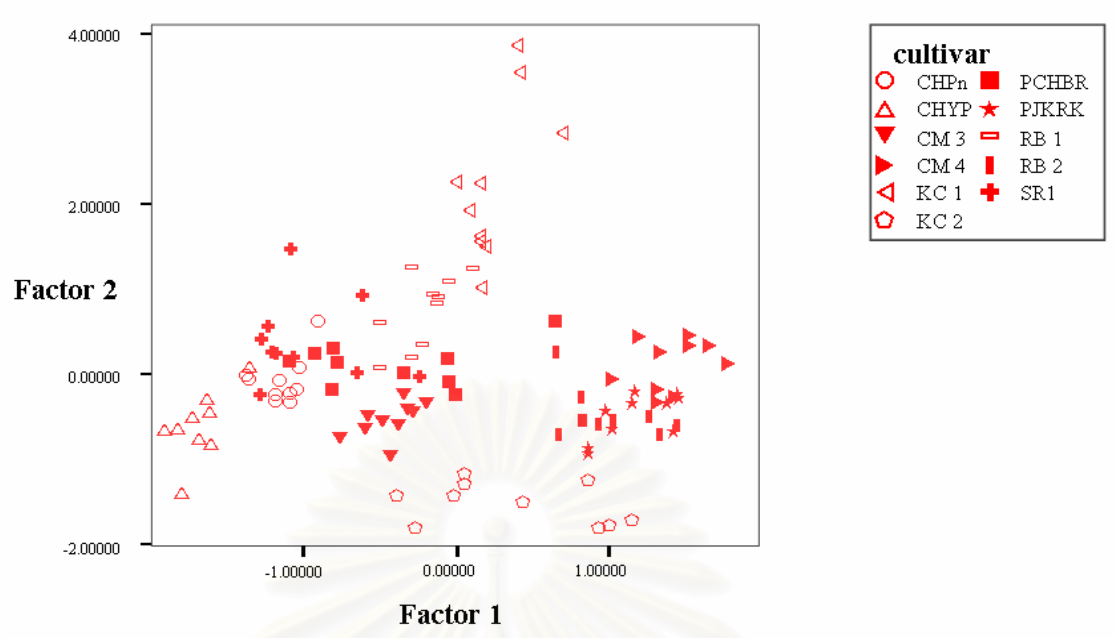


Figure 4.3. Position of each *P. mirifica* cultivar in Thailand for flower morphometric analysis. Factor axes of 1 and 2 were derived from Factor analysis: ordinate; factor 1 and abscissa; factor 2.

4.1.2 Cluster analysis

Figure 4.4-4.6 represent 3 dendrograms or trees which were constructed by using the Between-groups linkage method of Cluster analysis on Squared Euclidian distances. The factor scores of mean values from Factor analysis were used for leaf and flower morphometries while the standardized mean data were used for pod morphometry. The data were classified by collected locations. The figures present the dendrograms grouped by all *P. mirifica* cultivars from different locations in Thailand. All dendrograms revealed that all collected *P. mirifica* could be clustered differently.

4.1.2.1 Cluster analysis of leaf morphometry

Considering a dendrogram of figure 4.4, it indicated that 39 cultivars of *P. mirifica* were classified into 5 groups or main branches. The 1st upper group composed of 24 cultivars. The 2nd group composed of 5 cultivars (Chiang Mai 1, Ratchaburi 4, Chiang Mai 4, Sakon Nakhon, and Lampang cultivars). The 3rd group composed of 4 cultivars (Chiang Rai, Phrae 3, Phayao, and Chiang Mai 3 cultivars). Moreover, the 4th group also composed of 5 cultivars (Phrae 1, Nakhon Sawan, Lopburi, Ratchaburi 1, and 2 cultivars). Lastly, only Uthai Thani cultivar was distinctly classified into the lowest branch or in the 5th group.

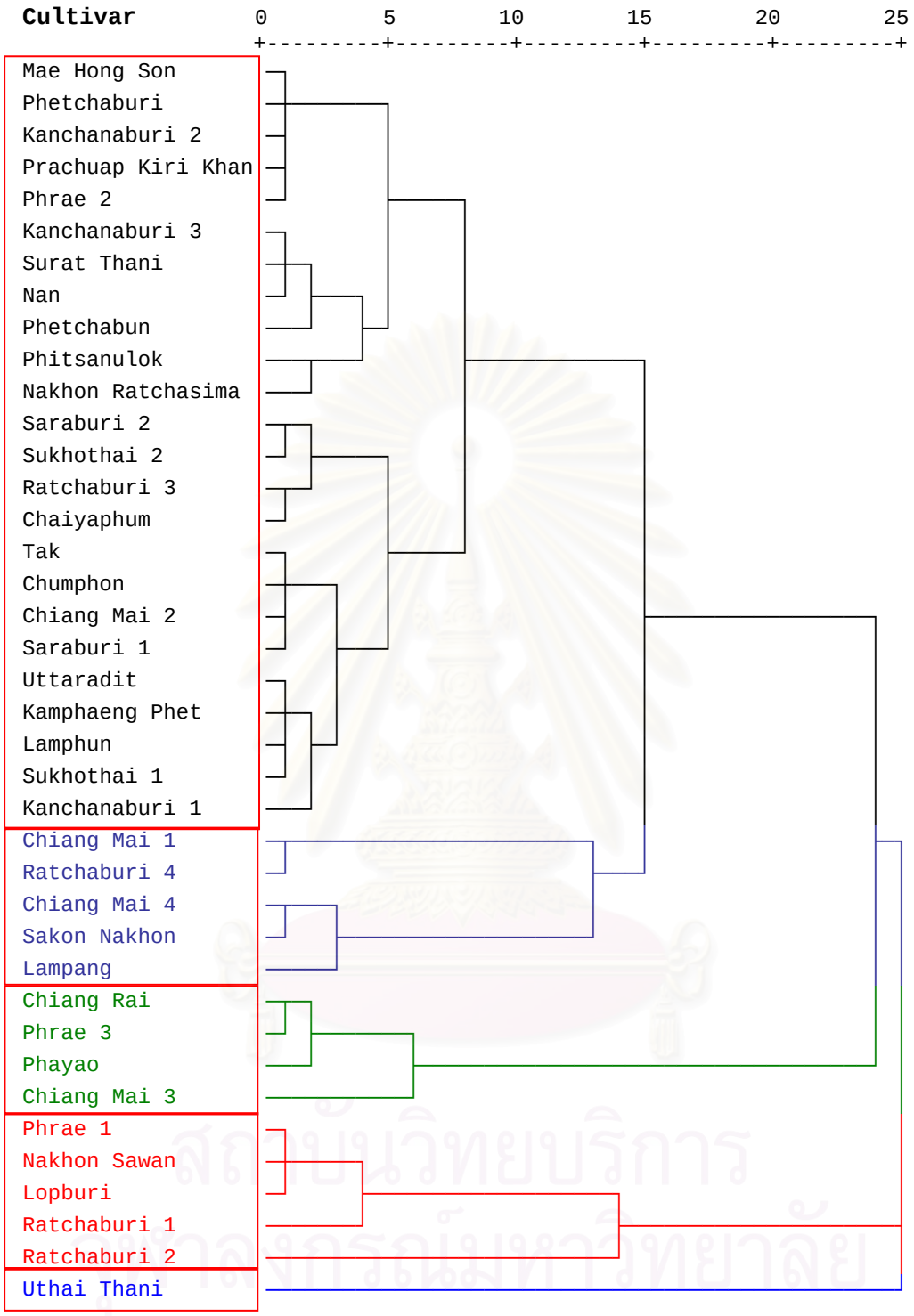


Figure 4.4. Leaf morphometric dendrogram created by Between-groups linkage method of Cluster analysis. *P. mirifica* is classified by collected location names.

4.1.2.2 Cluster analysis of pod morphometry

From a dendrogram of figure 4.5, it indicated that all 14 cultivars of *P. mirifica* were classified into 2 main branches or groups. The 1st upper group composed of 8 cultivars whereas the 2nd lower group composed of other 6 cultivars.

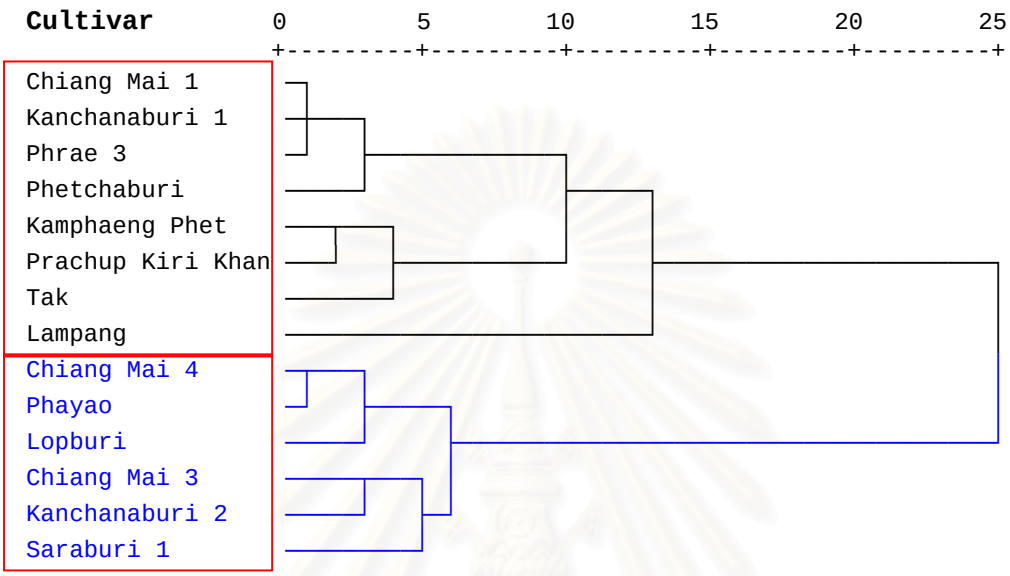


Figure 4.5. Pod morphometric dendrogram created by Between-groups linkage method of Cluster analysis. *P. mirifica* is classified by collected location names.

4.1.2.3 Cluster analysis of flower morphometry

Due to a dendrogram of figure 4.6, it indicated that 11 cultivars of *P. mirifica* were classified into 3 groups. The 1st upper group composed of 4 cultivars (Prachuap Kiri Khan, Ratchaburi 2, Chiang Mai 4, and Kanchanaburi 2 cultivars). The 2nd middle group or cluster composed of 6 cultivars. Furthermore, only Kanchanaburi 1 cultivar was clearly separated into the last lower group.

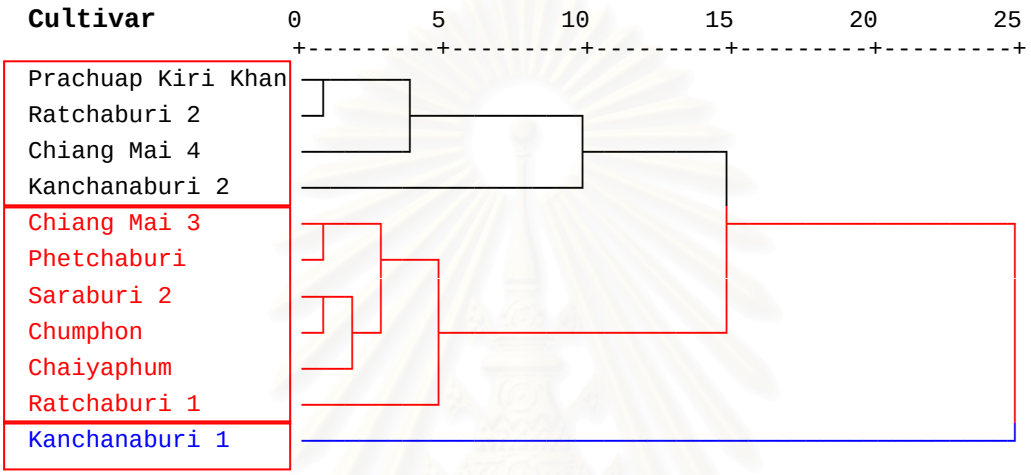


Figure 4.6. Flower morphometric dendrogram created by Between-groups linkage method of Cluster analysis. *P. mirifica* is classified by collected location names.

4.1.3 Characterization of *P. mirifica* in Thailand

Clinal pattern of characterization of *P. mirifica* cultivars in Thailand could be revealed. By considering morphometric data, factor scores of leaf together with flower morphometries and the standardized data of pod morphometry were plotted against latitude and longitude. The characterization of cultivars from the South to the North and the West to the East are indicated in the scatter-plotted graphs (Figure 4.7-4.20). The results of correlation analyses of the factor scores against latitude and longitude were evaluated.

4.1.3.1 Leaf morphometry

Due to figure 4.7-4.10, there was statistically significant correlation between factors 1 and latitude & longitude as well as between factor 2 and latitude & longitude ($P \leq 0.05$). Results of correlation analysis in Table 4.1 revealed that both factor 1 (PD, TLB, PL, RL, TLL, PLL, and AˆB parameters) and factor 2 (NPV and SPL parameters) significantly correlated to latitude and longitude.

It could infer that *P. mirifica* leaves (PD, TLB, PL, RL, TLL, PLL, and AˆB parameters) trend to increase in size from the South to the North of Thailand (R = 0.229). On the other hand, the trend of *P. mirifica* leaves (NPV and SPL parameters) decreass in size from the South to the North (R = - 0.097). Moreover, the trend of all 9 parameters decrease in size from the West to the East of Thailand (R = - 0.175 and - 0.169, respectively).

Table 4.1. Correlation analysis of geographic trends in leaf morphometric factors of *P. mirifica* in Thailand. It was derived from Principal Component Analysis (Factor analysis).

Independent variable or predictor	Dependent variable	R value	P significance
Latitude	Factor 1	0.229 **	0.000
	Factor 2	- 0.097 **	0.000
Longitude	Factor 1	- 0.175 **	0.000
	Factor 2	- 0.169 **	0.000

** Correlation is significant at the 0.01 level (2-tailed).

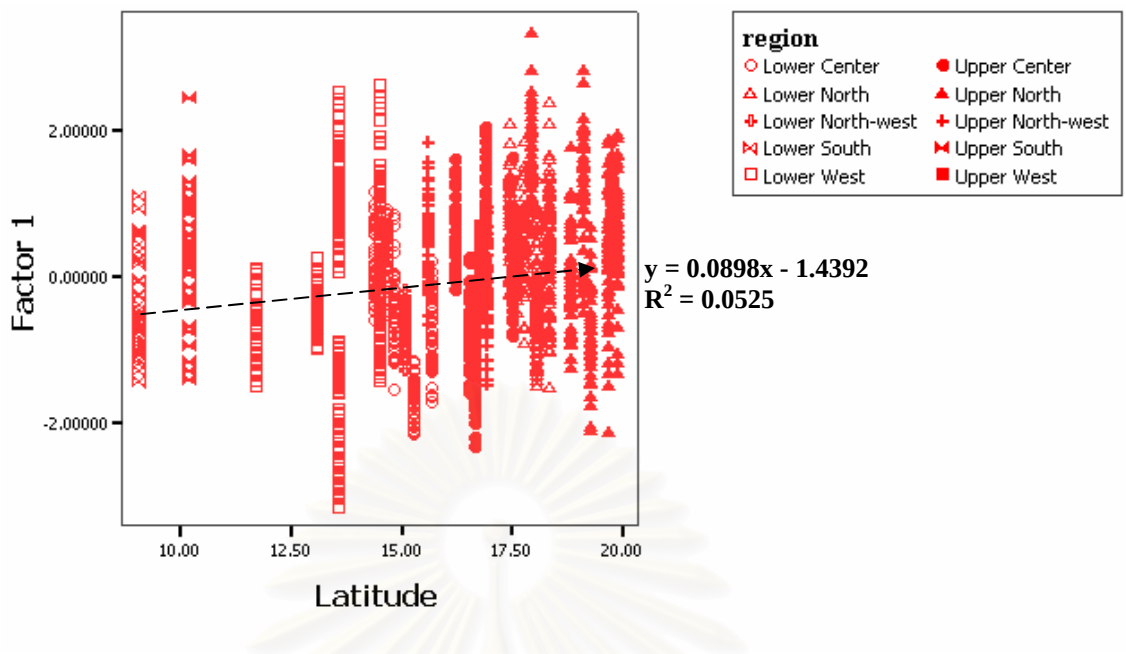


Figure 4.7. Geographic trends in leaf morphometry of *P. mirifica* in Thailand: abscissa; latitude; the factor score 1 derived from PCA. Value labels refer to regions.

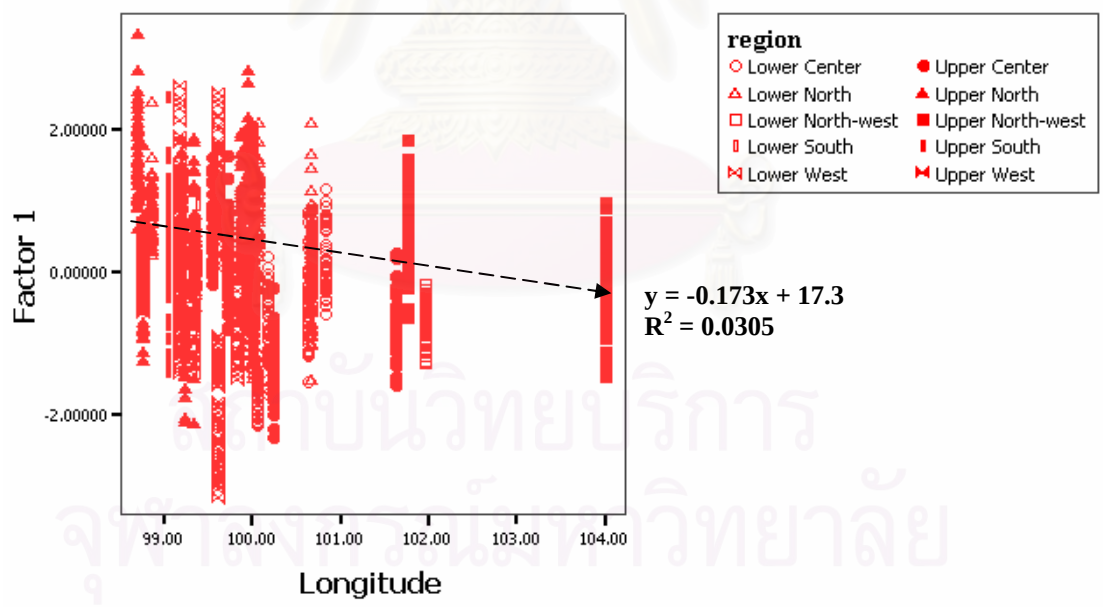


Figure 4.8. Geographic trends in leaf morphometry of *P. mirifica* in Thailand: abscissa; longitude; the factor score 1 derived from PCA. Value labels refer to regions.

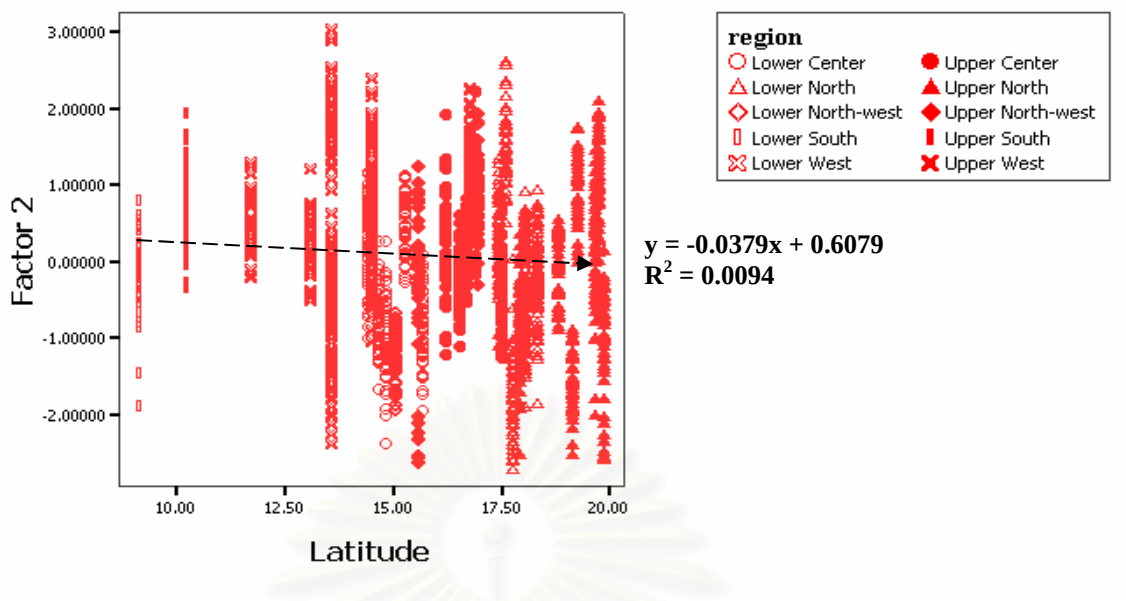


Figure 4.9. Geographic trends in leaf morphometry of *P. mirifica* in Thailand: abscissa; latitude; the factor score 2 derived from PCA. Value labels refer to regions.

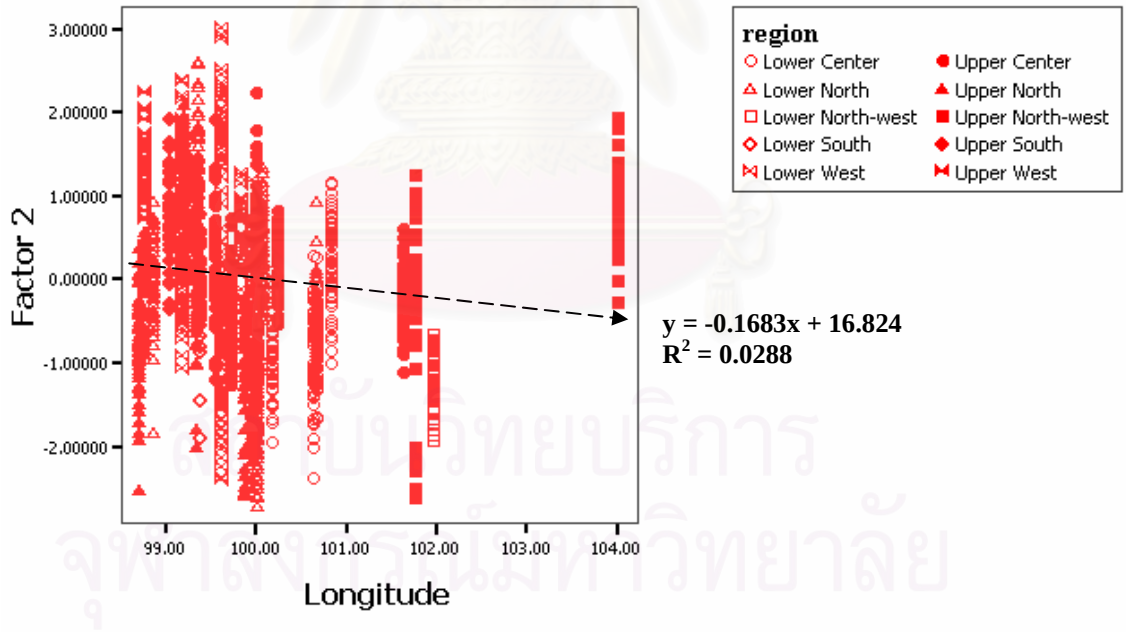


Figure 4.10. Geographic trends in leaf morphometry of *P. mirifica* in Thailand: abscissa; longitude; the factor score 2 derived from PCA. Value labels refer to regions.

4.1.3.2 Pod morphometry

Six scatter plots of the standardized data (Zscore) of PodL, PodW, and SNP parameters against latitude and longitude were illustrated (Figure 4.11-4.16). There was statistically significant correlation in the Z score of PodL ($P \leq 0.05$). Unlike the Z score of PodL, there was no significant correlation in both latitude and longitude ($P \geq 0.05$) for the Z score of PodW and SNP. Correlation analysis in Table 4.2 showed that the Z score of PodL significantly correlated to latitude and longitude (R = 0.209 and - 0.096, respectively).

It showed that *P. mirifica* pods (PodL) trend to increase in length from the South to the North but decrease in length from the West to the East of Thailand.

Table 4.2. Correlation analysis of geographic trends in pod morphometric factors of *P. mirifica* in Thailand derived from standardized data (Zscore).

Independent variable or predictor	Dependent variable	R value	P significance
Latitude	Zscore of PodL	0.209 **	0.000
	Zscore of PodW	0.083	0.087
	Zscore of SNP	- 0.006	0.899
Longitude	Zscore of PodL	- 0.096 *	0.046
	Zscore of PodW	- 0.002	0.973
	Zscore of SNP	0.012	0.812

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

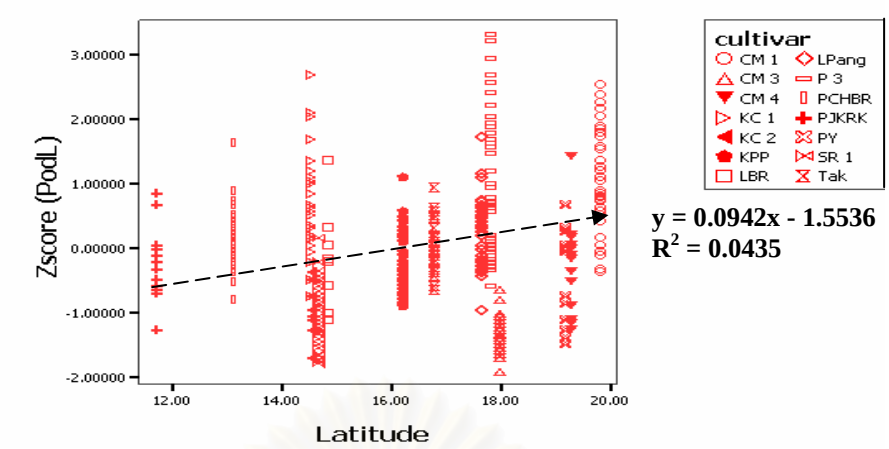


Figure 4.11. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; latitude; the Zscore of PodL. Value labels refer to cultivars.

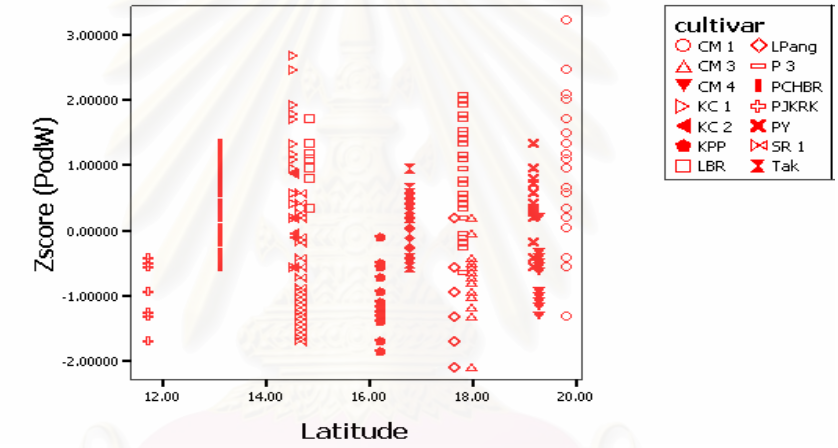


Figure 4.12. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; latitude; the Zscore of PodW. Value labels refer to cultivars.

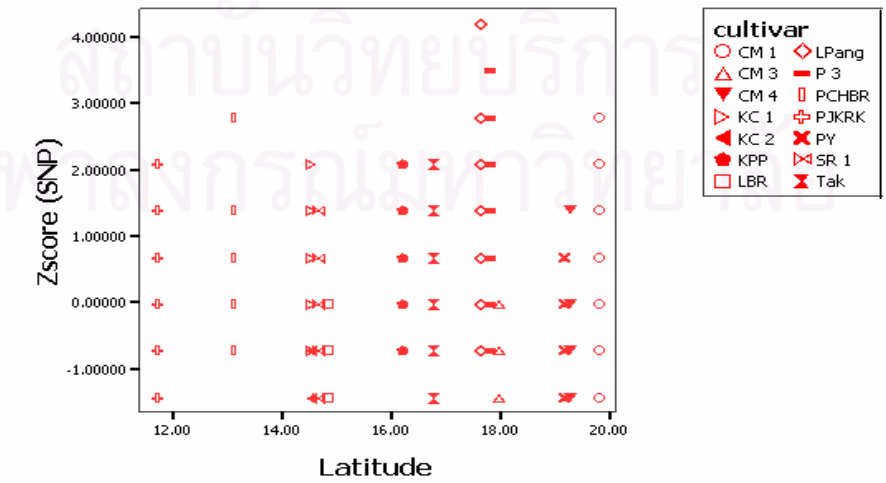


Figure 4.13. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; latitude; the Zscore of SNP. Value labels refer to cultivars.

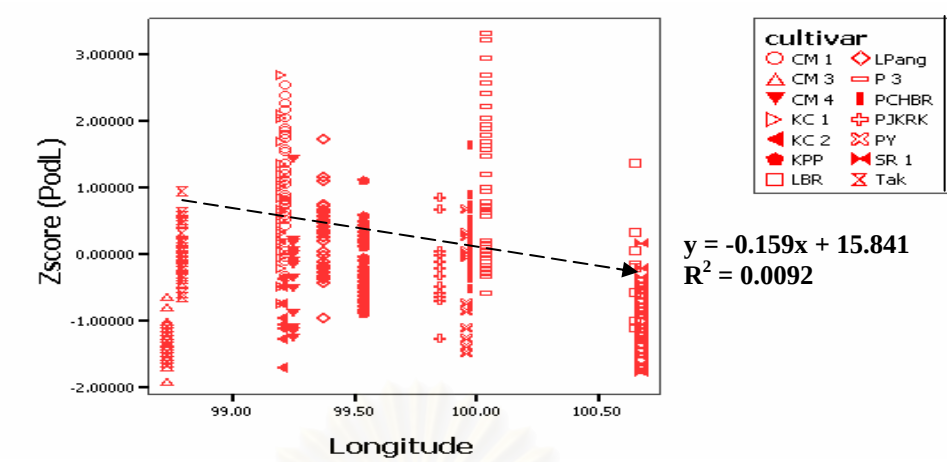


Figure 4.14. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; longitude; the Zscore of PodL. Value labels refer to cultivars.

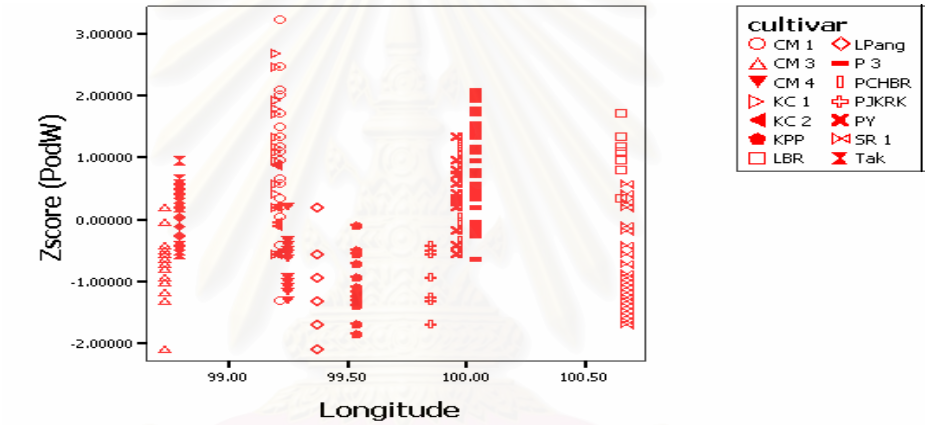


Figure 4.15. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; longitude; the Zscore of PodW. Value labels refer to cultivars.

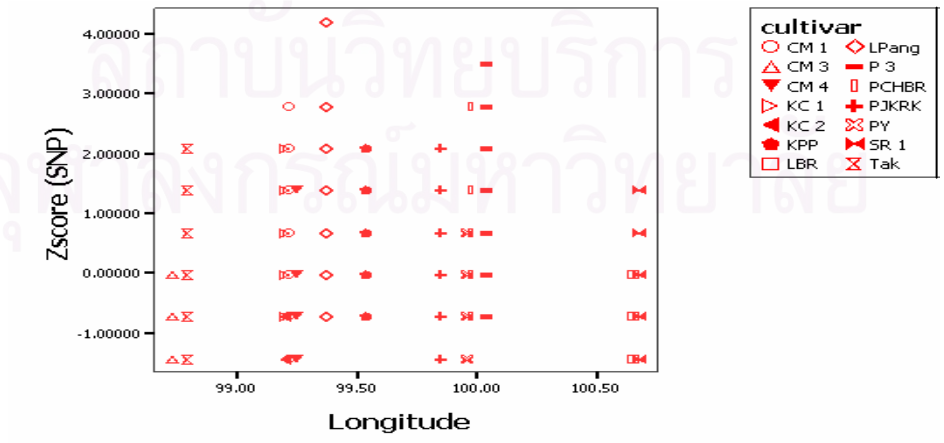


Figure 4.16. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; longitude; the Zscore of SNP. Value labels refer to cultivars.

4.1.3.3 Flower morphometry

There was no statistically significant correlation between factor 1 (PetL, StmL, PisL, ClxL, PetW, and OvrD parameters) and factor 2 (PdcL) on latitude ($P \geq 0.05$). Moreover, factor 2 shows no significant correlation on longitude. Correlation analysis in Table 4.3 presents that only factor 1 was significantly correlated to longitude ($R = -0.468$). Figure 4.17-4.20 present the scatter plots of factor scores (1 and 2) against latitude and longitude.

It indicates that the size of factor 1 (PetL, StmL, PisL, ClxL, PetW, and OvrD) of the flower trends to decrease from the West to the East of Thailand.

Table 4.3. Correlation analysis of geographic trends in flower morphometric factors of *P. mirifica* in Thailand derived from Principal Component Analysis (PCA) method.

Predictor or independent variable	Dependent variable	R value	P significance
Latitude	Factor 1	0.171	0.074
	Factor 2	- 0.027	0.779
Longitude	Factor 1	- 0.468 **	0.000
	Factor 2	- 0.081	0.400

** Correlation is significant at the 0.01 level (2-tailed).

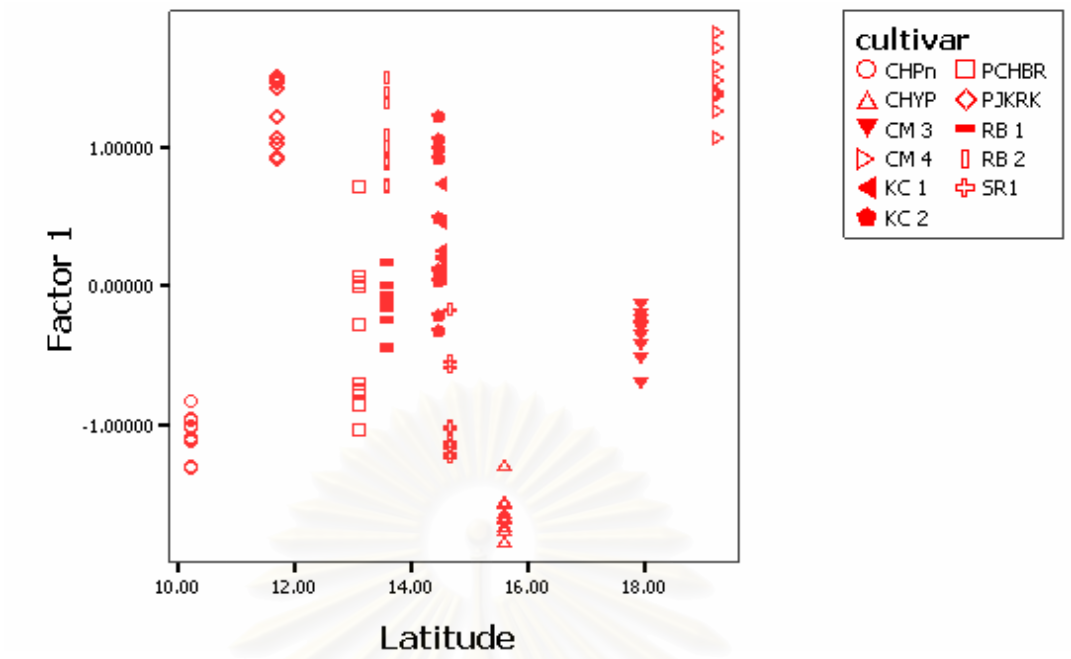


Figure 4.17. Geographic trends in flower morphometry of *P. mirifica* in Thailand: abscissa; latitude; the factor score 1 derived from PCA. Value labels refer to cultivars.

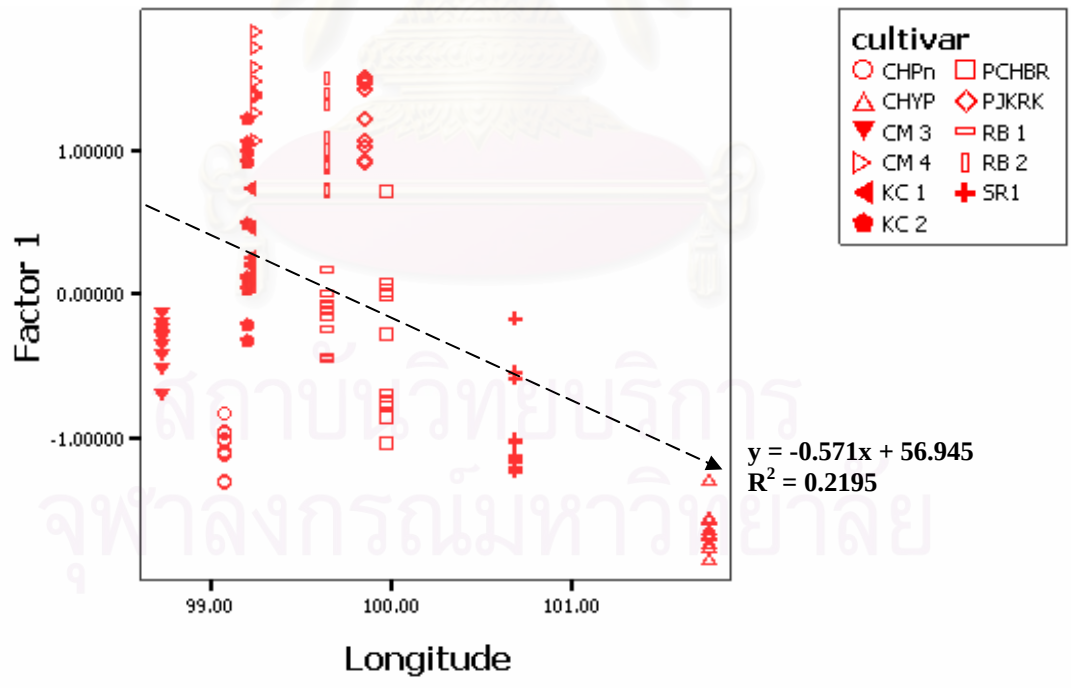


Figure 4.18. Geographic trends in flower morphometry of *P. mirifica* in Thailand: abscissa; longitude; the factor score 1 derived from PCA. Value labels refer to cultivars.

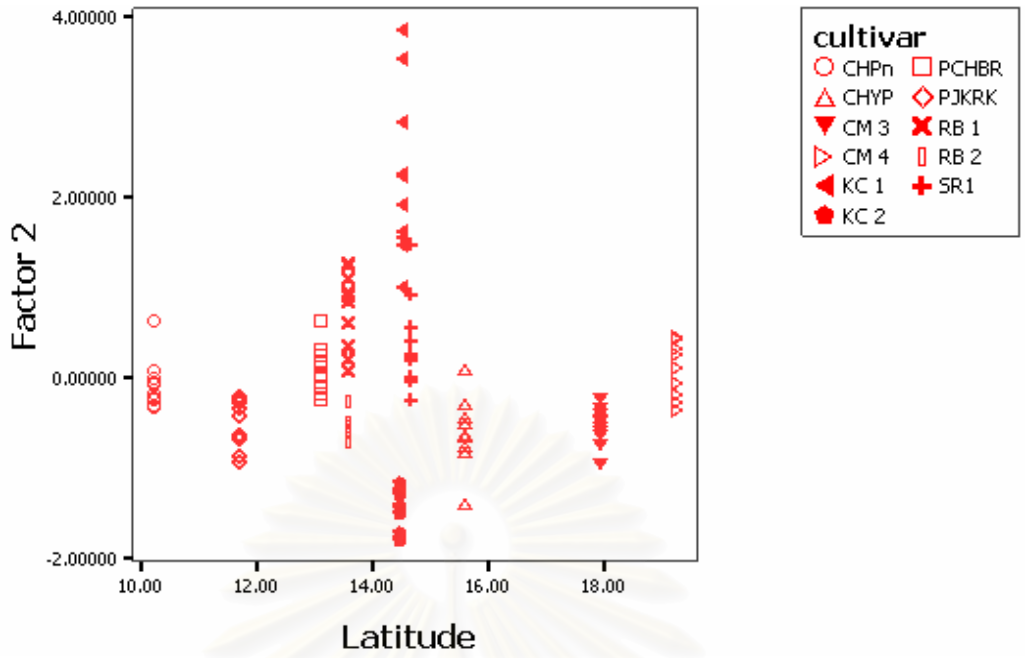


Figure 4.19. Geographic trends in flower morphometry of *P. mirifica* in Thailand: abscissa; latitude; the factor score 2 derived from PCA. Value labels refer to cultivars.

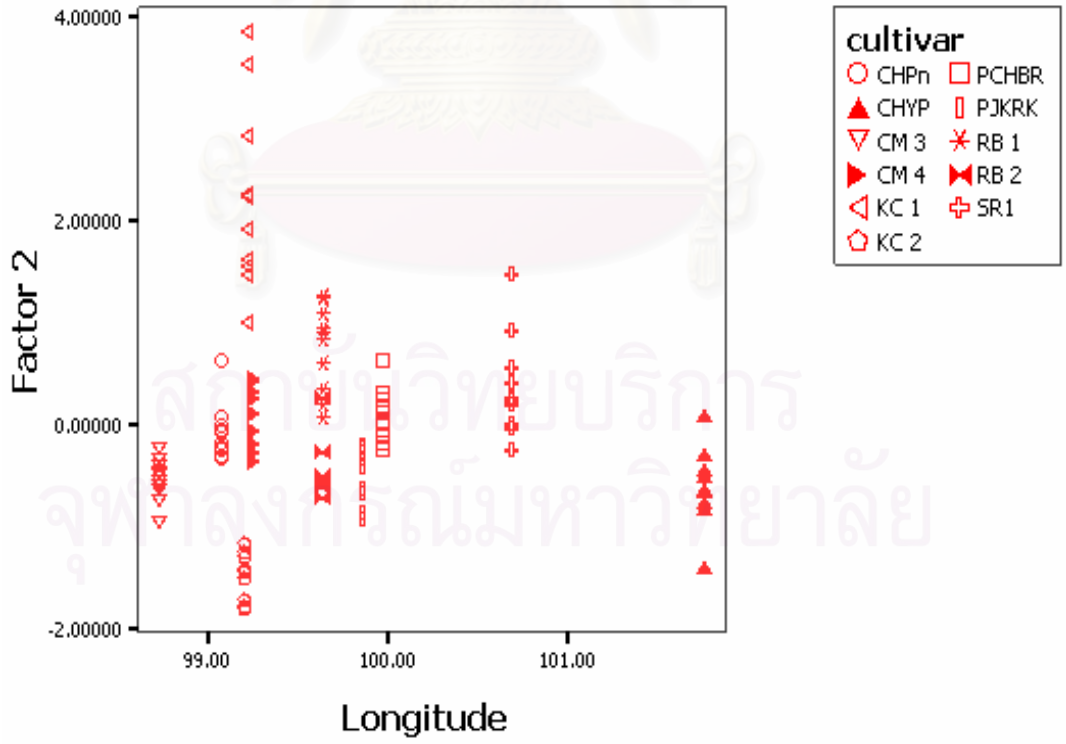


Figure 4.20. Geographic trends in flower morphometry of *P. mirifica* in Thailand: abscissa; longitude; the factor score 2 derived from PCA. Value labels refer to cultivars.

4.2 Genetic variation

4.2.1 DNA extraction

Genomic DNA of *P. mirifica* fresh young leaf (100 mg) was isolated by DNeasy® Plant Mini kit (QIAGEN, catalog # 69104) or NucleoSpin® Plant kit (MACHEREY-NAGEL, catalog # 740 570.50). Genomic DNA at high molecular weight (roughly 23 kb in length) was observed on 0.8% agarose gel (Figure 4.21).

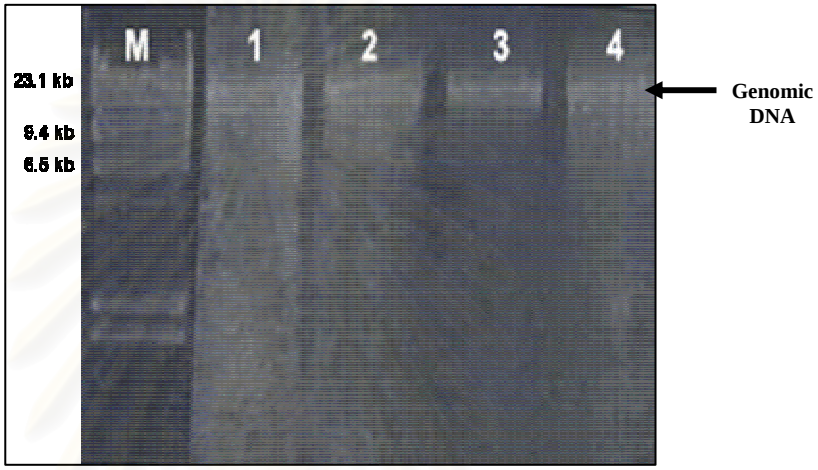


Figure 4.21. Genomic DNA on 0.8% agarose gel. Lane M represents Lamda *Hin*d III as DNA marker. Lanes 1-4 contain genomic DNA of *P. mirifica* leaf samples from CM1-4 cultivars (from Chiang Mai province) in Thailand.

4.2.2 PCR amplification

PCR technique is an approach for DNA amplification on specific target sequence by synthesized primers that can polymerize or extend complementary strands of DNA. After 1.0% agarose gel electrophoresis and EtBr staining, PCR products will be observed under UV light on UV transilluminator. A product size was estimated and compared to DNA standard as marker. In this research, PCR products were amplified under optimum condition by nrDNA ITS, cpDNA *trn*L, and *trn*L-F primers. Single sharp bands of expected PCR products were visible on agarose gel (Figure 4.22-4.24). Moreover, in Table 4.4, it summarizes results of PCR amplification of ITS, *trn*L, and *trn*L-F regions of all *P. mirifica* samples in Thailand and *P. lobata* as outgroup.

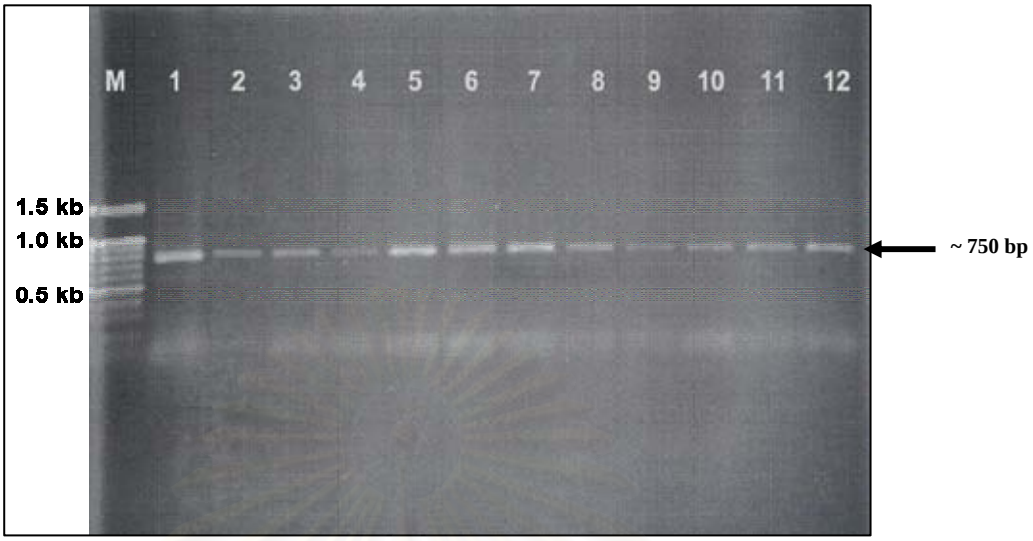


Figure 4.22. PCR products of ITS on 1.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-12 contain PCR products of ITS at about 750 bp in length from *P. mirifica* leaf samples from different locations in Thailand. DNA was amplified by ITS_1 and ITS_4 primers.

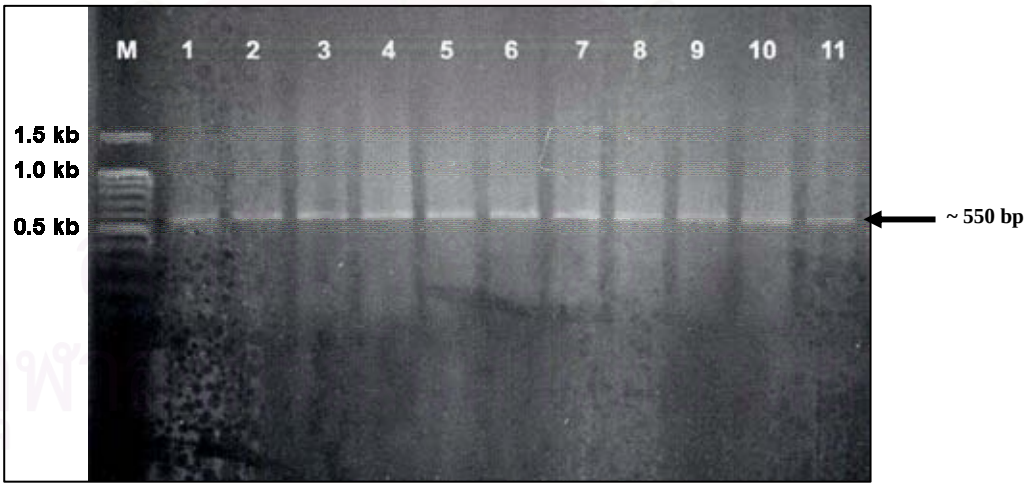


Figure 4.23. PCR products of *trn*L on 1.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-11 contain PCR products of *trn*L at about 550 bp in length from *P. mirifica* leaf samples from different locations in Thailand. DNA was amplified by c and d primers.

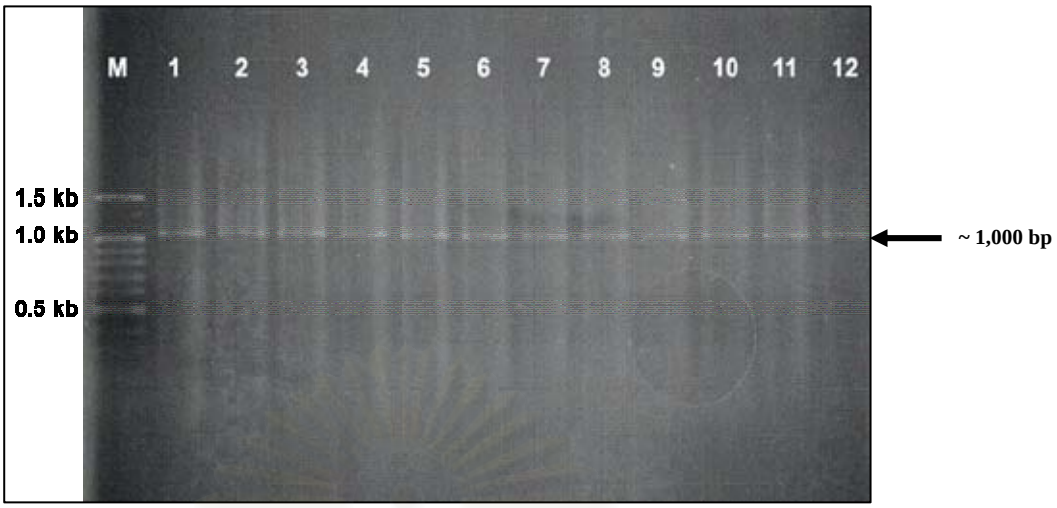


Figure 4.24. PCR products of *trn*L-F on 1.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-12 contain PCR products of *trn*L-F at about 1,000 bp in length from *P. mirifica* leaf samples from different locations in Thailand. DNA was amplified by c and f primers.

Table 4.4. Summary of PCR amplification of ITS (ITS_1 and ITS_4 primers), *trn*L (c and d primers) and *trn*L-F (c and f primers) regions of all *P. mirifica* samples in Thailand and *P. lobata* from Japan as outgroup. The √ symbol indicates to a sequence-analyzed sample that was amplified and detected by agarose gel electrophoresis.

Cultivar	**ITS**	***trn*L**	***trn*L-F**
CM1	√	√	√
CM2		√	
CM3	√	√	√
CM4	√	√	√
CR		√	√
LPang	√	√	√
MHS	√	√	
LPoon	√	√	√
Nan	√	√	
PY	√	√	
P1	√	√	√
P2	√	√	√
P3	√	√	√
UTRD		√	
KPP	√	√	√
LBR	√	√	√
NKSW		√	
PBoon	√	√	√
PSNL	√	√	
SR1	√	√	√
SR2	√	√	√
SKHT1	√	√	√
SKHT2	√	√	√
UTTN	√	√	√
KC1	√	√	√
KC2	√	√	√
KC3	√	√	√
PCHBR	√	√	√
PJKRK	√	√	√
RB1	√	√	√
RB2	√	√	√
RB3	√	√	√
RB4		√	
Tak	√	√	√
CHYP	√	√	√
NKRSM	√	√	√
SKNK		√	√
CHPn	√	√	√
SRTN	√	√	√
P. lobata	√	√	√
Total	**34**	**40**	**32**

4.2.3 Sequence analysis

All PCR products of nrDNA ITS, cpDNA *trn*L and *trn*L-F of *P. mirifica* from 39 collected sites in Thailand and *P. lobata* from Japan as an outgroup were purified and directly sequenced. Sequences of designed regions were completely trimmed and the consensus was kept. ITS region at 687 bp in length, *trn*L region at 397 bp in length, and *trn*L-F region at 731 bp in length were shown in Figure 4.25-4.27. Percentages of nitrogenous base composition (A, C, G, and T) of 3 regions were displayed in Table 4.5-4.7. The A+T contents of these 3 regions were approximately 41.5, 67.4, and 71.1, respectively. On the other hand, *trn*L and *trn*L-F regions contained high amount of A and T (67.4 and 71.1) but 41.5 in ITS region instead. There were a lot of base transitions and transversions occured. The pairwise similarity percentages of these sequences are more than 70% (Table 4.8-4.10). The pairwise and multiple sequences alignment comparisons can demonstrate nucleotide variation in the form of single base pair substitution. The percentages of sequence divergences of these regions are variable (0-25.2%) as presented in Table 4.11-4.13. Considering the similarity percentages and sequence divergences, ITS sequences of each cultivar of *P. mirifica* that provided lower similarity percentages (72-100 %) and higher sequence divergence (0-25.2%) are more variable than the other 2 chloroplast regions. The cpDNA at *trn*L and *trn*L-F regions are less variable with higher similarity percentages (92-100% and 93-100%). And less sequence divergence (0-7% and 0-4.7%) was obtained.

```
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        10         20         30         40         50         60
KC2             GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
CHPn            GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
CM3             GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
PCHBR           GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
KC1             GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
MHS             GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
SR1             GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
CM1             GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
CM4             GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
PJKRK           GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
Tak             GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
RB3             GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
SKHTH1          GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
KC3             GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
CHYP            GGTCGGGCCG GGCTTCTTCA GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
SRTN            GGTCGGGCCG GGCTTCTTCA GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
LBR             GGG-GGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
Nan             GGGCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
P1              G-TCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
UTTN            G-TCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
SKHTH2          GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
PSNL            GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTCT GCCTGTTGCG TTGGGGCGGG
PBoon           GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
PY              GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
LPang           GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
NKRSM           GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
SR2             G-TCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
Lobata          G---GGGCCG GGCTTCTTCC GTC----CTC CCCCTGCTCT GCCTGTTGCG TTGGGTCGGG
RB1             GGTCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
KPP             GGTCGGGCCG GGCTTCTTCA GTCCGGCCTC CCCTTGCTTT GCCTGTTGCT TTGGGGCGGG
P2              G-TTGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
RB2             G-TCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
LPoon           G-TCGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
P3              GG-CGGGCCG GGCTTCTTCC GTCCGTCCTC CCCTTGCTTT GCCTGTTGCG TTGGGGCGGG
Clustal Co      *   ****** *********  ***    *** *** **** * *********  ***** ****
```

Figure 4.25. A 687 bp character matrix of 33 cultivars of *P. mirifica* and 1 cultivar of *P. lobata* based on nrDNA ITS sequences. Asterisk symbols (*) show that all samples provide nitrogenous base (A, C, G, and T) consensus or identity.

```
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       70         80         90        100        110        120
KC2             GGGGTGG--- -----ACACA AAGCAAGCAT CGACT-TGCC TGGCCTCCTC TCCTCCTTCC
CHPn            GGGGTGG--- -----ACACA AACCAAGCAT CGACT-TGCC TGGCCGCCTC TCCTCCTTCC
CM3             GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
PCHBR           GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
KC1             GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
MHS             GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
SR1             GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
CM1             GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
CM4             GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
PJKRK           GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
Tak             GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
RB3             GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
SKHTH1          GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
KC3             GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
CHYP            GGGGTGG--- -----ACACG AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
SRTN            GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
LBR             GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
Nan             GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
P1              GGGGTGG--- -----ACACA AAGCAAGCAT CGTC-CTGCC TGGCCTCCTC TCCTCCTTCC
UTTN            GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
SKHTH2          GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
PSNL            GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
PBoon           GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
PY              GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
LPang           GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
NKRSM           GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
SR2             GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
Lobata          GGGGAGGGAG GGGGGACACA AAGCAATGTT GTTCTGCGCC CGACCTCTCC TCCTCCTTCC
RB1             GGGGTGG--- -----ACACA AAACAAGCAT CGTCT-TGGC TGGCCTCCTC TCCTCCTTCC
KPP             GGGGTGG--- -----ACACG AAACAAGCAT CGGC-CTGCC TGGGCTCGTC TCCTCCTTCC
P2              GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGGC TGGGCTCCTC TCCTCCTTTC
RB2             GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGGC TGGCCTCCTC TCCTCCTTCC
LPoon           GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
P3              GGGGTGG--- -----ACACA AAGCAAGCAT CGTCT-TGCC TGGCCTCCTC TCCTCCTTCC
Clustal Co      **** **         ****  ** ***   *    *   * *  *  * *  * ******** *

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       130        140        150        160        170        180
KC2             C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
CHPn            C-TCCCAACA CAACCCCCGG CCCTTCCTGC GCGAAGGAAC TCCATTCTGT TAGGT---GC
CM3             C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
PCHBR           C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
KC1             C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
MHS             C-AACAAACA CAACCCCCGA CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
SR1             C-AACAAACA CAACCCCCGA CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
CM1             C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
CM4             C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
PJKRK           C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
Tak             C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
RB3             C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
SKHTH1          C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
KC3             C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
CHYP            C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
SRTN            C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
LBR             C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
Nan             C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
P1              C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT CAAGT---GC
UTTN            C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
SKHTH2          C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
PSNL            C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
PBoon           C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
PY              C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
LPang           C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
NKRSM           C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
SR2             C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
Lobata          CCAACAAACA CAAACCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT CAAGTTGTGC
RB1             C-AACAAACA CAACCCCCGG CGCTTCTTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
KPP             C-AACTAACA CAACCCCCGG CGCTTCGGGC GCCAAGGAAC TCCAATCTGT CAAGG---GC
P2              C-AACAAAAA CAACCCCCGG GGCTTCCTGG GCCAAGGAAC TCCAATCTGG TAAGG---GG
RB2             C-AACAAAAA CAACCCCCGG GGCTTCGTGG GCCAAGGAAC TCCAATCTGT TAAGT---GC
LPoon           C-AACAAACA CAACCCCCGG GGCTTCGGGG GCCAAGGAAC TCCAATCTGT TAAGG---GG
P3              C-AACAAACA CAACCCCCGG CGCTTCGTGC GCCAAGGAAC TCCAATCTGT TAAGT---GC
Clustal Co      *   * ** * *** *****    ****  *  ** ******* **** ****   * *    *
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Figure 4.25. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       190        200        210        220        230        240
KC2             GACTCCCAAC GGCCCGGACA CAGAGCCCCG CGGGCGTTGT CACGACCCAC ATTATT-ATA
CHPn            GACTCCCTCC GGCCCGGACA CAGAGCCCCA CCCGCGTTGT CTCGACCCAC ATTATT-TTA
CM3             GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
PCHBR           GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
KC1             GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
MHS             GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
SR1             GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
CM1             GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
CM4             GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
PJKRK           AACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
Tak             AACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
RB3             AACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
SKHTH1          GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
KC3             GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
CHYP            GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
SRTN            GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
LBR             GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
Nan             GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
P1              GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
UTTN            GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
SKHTH2          GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
PSNL            GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
PBoon           GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
PY              GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
LPang           GACTCCCAAC GGCCCGGAAA CGGTGCCCCG CGGGCGTTGT CACGACCCAC ATTATT-ATA
NKRSM           GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
SR2             GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTTGT CACGACGCAC ATTATT-ATA
Lobata          GACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGCGTCGT CACGACACAC ACGATTTATA
RB1             GACTCCCAAC GGCCCGGAGA CGGTGCCCCC CGGGCGTTGT CACGACACAC ATTATT-ATA
KPP             GACTCCCAAC GGCCCGGAGA CGGAGACCCG CGCGCGTTGT CACTACCCAC ATTATT-ATA
P2              GACTCCCAAC GGGCCGGAAA CCGGGGCCCG CGGGCGTTGT CACCACCCAC ATTATT-ATA
RB2             GACTCCCAAC GGCCCGGAAA CGGGGCCCCC CGGGCGTTGT CACGACCCAC ATTATT-ATC
LPoon           GACTCCCAAC GGCCCGGAAA CGGGGCCCCG GGGGGGTTGT CCCGACCCAC ATTATT-TTA
P3              AACTCCCAAC GGCCCGGAGA CGGTGCCCCG CGGGGGTTGT CCCCACCAAC ATTATT-TTT
Clustal Co       ******  * ** ***** * * * * ***     * ** ** * * **  ** *  ***  *

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       250        260        270        280        290        300
KC2             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTACCGAAAT
CHPn            CATAATGACT CTCGCCCACG GATATCTCTG CTCTTGCATG GATGAATAAC GAACCGAACT
CM3             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
PCHBR           CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
KC1             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
MHS             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
SR1             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
CM1             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
CM4             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
PJKRK           CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
Tak             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTA-CGAAAT
RB3             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
SKHTH1          CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
KC3             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
CHYP            CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
SRTN            CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
LBR             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
Nan             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
P1              CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
UTTN            CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
SKHTH2          CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
PSNL            CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
PBoon           CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
PY              CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
LPang           CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAACGAAAT
NKRSM           CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
SR2             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
Lobata          CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAGAAC GTAGCGAAAT
RB1             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGCATC GATGAAAAAC GTATCGAAAT
KPP             CAAAATGACT CTCGGTCACG GATATCTCGG TTCTTGCATC GGTGAAGAAC ATAACGAAAT
P2              CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGGATC CATGAAAAAC GTAACGAAAT
RB2             CAAAATGACT CTCGGCAACG GATATCTCGG CTCTTGGATC GATGAAGAAC GTAACGAAAT
LPoon           CAAAATGACT CTCGGCAACG GAAATCTCGG TTCTTGCATC AATAAAAAAC GTACCGAAAT
P3              CAAAATGACT CTCGGCAACG GAAATTTCGG CTCTTGGATC CATGAAAAAC GGAACGAAAT
Clustal Co      ** ******* ****   *** ** ** ** *  ***** **    * ** ***   * **** *
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Figure 4.25. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       310        320        330        340        350        360
KC2             GAGATACTTG GTGTGAATTG AATAATCCCG TGAACCATCC AGTCTTTGAA CGCAAGTTGC
CHPn            GAGATACTTG CTGGGAATTG ATTAATCACG TGCACCATCC AGTCTTTGAT TGCAAGTTGC
CM3             GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
PCHBR           GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
KC1             GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
MHS             GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
SR1             GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
CM1             GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
CM4             GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
PJKRK           GCGATACTTG GTGTGAATTG CAAAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
Tak             GCGATACTTG GTGTGAATTG CAAA-TCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
RB3             GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
SKHTH1          GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
KC3             GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
CHYP            GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
SRTN            GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
LBR             GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
Nan             GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
P1              GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
UTTN            GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
SKHTH2          GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
PSNL            GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
PBoon           GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
PY              GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCC AGTCTTTGAA CGCAAGTTGC
LPang           GCGATACTTG GTGTGAATTG CAAAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
NKRSM           GCGATACTTG GTGTGAATTG CAAAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
SR2             GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
Lobata          GCGATACTTG GTGTGAATTG CAGAATCCCG TGAACCATCG AGTCTTTGAA CGCAAGTTGC
RB1             GCGATACTTG GGGTGAATTG CAGAATCCCG TGAACCATCT AGTCTTTGAA CGCAAGTTGC
KPP             GCGATACTTG GTGTGGATTG CAAAATCCCA TGAACCATCA AGTCTTTGAA CGCAAGTTGC
P2              GGGATACTTG GTGGGAATTG GAAAATCCCG TGAACCATCC AGTCTTTGAA CGCAAGTTGC
RB2             GCGATACTTG GTGTGAATTG CAAAATCCCG TGAACCATCC AGTCTTTGAA CGCAAGTTGC
LPoon           GGGATACTTG GGGGGAATTG CAAAATCCCG GGAACCATCA AGTCTTTGAA CGCAAGTTGG
P3              GGGAAACTTG GGGGGAATTG GAAAATCCCG GGAACCATCC AGTCTTTGAA CCCAAGTTGG
Clustal Co      * ** *****   * * ****    * ** *   * ******  *********    *******

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       370        380        390        400        410        420
KC2             GCCCGAACCC ATT-AAGTGG AGGGAACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
CHPn            GTCCGACCCC ATT-AAGTGG AGGGAACGCC TGCCTGGCTG GCAGACATCG TTACCCCCAC
CM3             GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
PCHBR           GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
KC1             GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
MHS             GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
SR1             GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
CM1             GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
CM4             GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
PJKRK           GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
Tak             GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
RB3             GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
SKHTH1          GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
KC3             GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
CHYP            GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
SRTN            GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
LBR             GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
Nan             GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
P1              GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
UTTN            GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
SKHTH2          GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
PSNL            GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
PBoon           GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
PY              GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG GCACACATCG TTACCCCCAC
LPang           GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
NKRSM           GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
SR2             GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
Lobata          GCCCGAAGCC ATTTAGGCAG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCAAC
RB1             GCCCCAAACC ATT-ATGTGG AGGGGACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
KPP             GCCCGAACCC ATT-ACGTGG AGGGGAAGCC TGCCTGGGTG TGACACATCC TTACCCCCAC
P2              GCCCGAAGCC ATT-AAGTGG AGGGCACGCC TGGCTGGGTG GCACACATCG TTACCCCCAC
RB2             GCCCGAAGCC ATT-AGGTGG AGGGCACGCC TGCCTGGGTG TCACACATCG TTACCCCCAC
LPoon           GCCCGAAGCC ATT-AGGGGG AGGGAACGCC TGCCTGGGGG TCACAAATCG TTACCCCCAC
P3              GCCCCAAACC ATT-AAGGGG AAGGGACCCC TGGCTGGGTG GCACACATCG TTACCCCCCC
Clustal Co      * ** *  ** *** * *  * * ** *  ** ** ****  *   * * ***  *******  *
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Figure 4.25. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        430        440        450        460        470        480
KC2             GCAAACAAAT GT---CTCAC ACAACAGA-- ---CGTTCTG CGTAGTAAGG TGCACGCTGA
CHPn            CCAAACAAAT GT---CTGAC ACGACAGA-- ---CGTTCTG TGTAGTAAGG TGGATGCTGA
CM3             GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
PCHBR           GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
KC1             GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
MHS             GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
SR1             GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
CM1             GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
CM4             GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
PJKRK           GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
Tak             GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
RB3             GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
SKHTH1          GCAAACAAAT GT---CTCAC ACGACAAA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
KC3             GCAAACAAAT GT---CTCAC ACGACAAA-- ---CGATCTG CGTAGTAGGG TGCACGCTGA
CHYP            GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
SRTN            GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
LBR             GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
Nan             GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
P1              GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
UTTN            GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
SKHTH2          GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
PSNL            GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
PBoon           GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
PY              CCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGGACGCTGA
LPang           GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
NKRSM           GCAAACAAAT GT---CTCGC ATCACAGA-- ---CGTTCTG CGTAGTATGG TGCACGCTAA
SR2             GCAAACAAAT GT---CTCAC ACGACAGA-- ---CGTTCTG CGTAGTAGGG TGCACGCTGA
Lobata          GCAAACAGAC GT---CCCAC ACGACGGC-- ---CGTT--G CGTGGTAGGG TGCACGCTGA
RB1             GCAAACAAAT GT---CTCAC ACGACAAA-- ---CATTCTG CGTAGTAAGG TGCACGCTGA
KPP             GCAAACAAAT GT---CTCAG ACGACAAA-- ---CATTCAG CGTATGAGGG TGCACGCTGA
P2              CCAAACAAAT GG---CTCAC ACCACAAAA- ----GTTCTG CGTAATAAGG TGGACGCTGA
RB2             GCAAACAAAT GTTTTCTCAC ACAAAAGA-- ---TCTTCTG AGTAGGATGG AGGACGATCA
LPoon           CCAAACAAAG GT---CTCAA ACAAAAAA-- ---CGTTCTG CGAAAAAGGG GGCACGTTAA
P3              CCAAAAAAAT GG---CTCAC ACGAAAAAAG TTCTGTTCTG TTAGGGAGGG GGCAAACTTA
Clustal Co       **** * *  *    *     *  *              *  *        * **  * *   * *

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        490        500        510        520        530        540
KC2             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCAAG TTCGTGGCCG AGTGAGCCGT
CHPn            CCTACCTCCA GCACCGTCTC GCGGTTGGTT GAAAAAAAAG TTCGTGCCCG AGTGAGCCGT
CM3             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTACGCCGT
PCHBR           CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTACGCCGT
KC1             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTACGCCGT
MHS             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTACGCCGT
SR1             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTACGCCGT
CM1             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTACGCCGT
CM4             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTACGCCGT
PJKRK           CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTACGCCGT
Tak             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCAAG TTCGTGGCCG AGTACGCCGT
RB3             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTACGCCGT
SKHTH1          CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTACGCCGT
KC3             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAAACAAC TTCGTGGCCG ACTACGCCGT
CHYP            CCTCCCGCGA GCACCGTCTC GTGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTGCGCCGT
SRTN            CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTGCGCCGT
LBR             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTGCGCCGT
Nan             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTGCGCCGT
P1              CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTGCGCCGT
UTTN            CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTGCGCCGT
SKHTH2          CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTGCGCCGT
PSNL            CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTGCGCCGT
PBoon           CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTGCGCCGT
PY              CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAAACAAG TTCGTGGCCG AGTGCGCCGT
LPang           CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTGCGCCGT
NKRSM           CCTCACGCGA GCACCGTCTC GTGGTTGGTT GAAAATCGAA TTCGTGGCCG AGTGCACCGT
SR2             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCGTGGCCG AGTGCGCCGT
Lobata          CCTCCCGCGA GCGGCGTCTC GCGGTTGGTT GAAAATCGAG TTCGCGGCCG AGCACGCCGT
RB1             CCTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCGAG TTCATGGCCG AGAACGCCGT
KPP             CCTCCCGCGA GCACCGGCTC GCGGTTGGGT GAAAATCAAA ATCGAGGCCG AGGGCGACGG
P2              ACTCCCGCGA GCACCGTCTC GCGGTTGGTT GAAAATCCAG TTCCTGGCCC AATGCCCCCT
RB2             CCCCCCGGGA GCGCCTCCTC GCGGGTGGAA AAAAAAATAC TTCGCGGACC AAAGCGCCCT
LPoon           CCTCCCGGGA GAACCGTCTC GGGGTTGGTT AAAAATCAAT TTCGGGGCCA AATGCCCCGG
P3              CCTCCAGCAA GGACCGGCGT TGGGTTGAAA AAAAATTTCG TGCCCGATCG AGCGCGCCTA
Clustal Co       *       * *   *  *     ** **     ****        *  *  *  *      *
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Figure 4.25. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       550        560        570        580        590        600
KC2             GATAAAATGG TGGATGAACA ACGCTCGATA CCAATCAAGC GCCGCGACTC GGTCGGCTCC
CHPn            GATAAAATGG TGGATGAACA ACGCTCGATA CCAATCACGC GCCGCTACTC GGTCGGCTCC
CM3             GG-AAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
PCHBR           GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
KC1             GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
MHS             GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
SR1             GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
CM1             GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
CM4             GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
PJKRK           GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
Tak             GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
RB3             GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
SKHTH1          GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
KC3             GATAAAATGA TGGATGAGCA ACGCTCGAGA CCAATCACGC GGCGCGACTC GTTCGGCTCC
CHYP            GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
SRTN            GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
LBR             GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
Nan             GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGTTCC
P1              GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
UTTN            GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
SKHTH2          GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
PSNL            GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
PBoon           GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
PY              GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
LPang           GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
NKRSM           GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
SR2             GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
Lobata          GATAAAATGG TGGATGAGCA ACGCTCGAGA CCAATCACGC GCTGCGACTC GGTCCGCGAA
RB1             GATAAAATGG TGGATGAGAA ACGCTCGAGA CCAATCACGC GCCGCGACTC GGTCGGCTCC
KPP             GATAAAATGG AGGAGGAGCA ACACTCAAGA CCAATCACGC GCCGCGACTC GGACGGGTCC
P2              GATAAAATGG GGGAAGAACA ACGCTCGAAA ACAATCACGC GCCGCTACTC GGTCGGGTCC
RB2             GAAAAAATGG GGGAAGAACA ACCCTCAAGA AACATCACGC GCCACGACTC CGGCTGCTCA
LPoon           GAAAAAATGG GGGATAAGCA ACGTTCGAAA CCAATCACGG GCCGGGACTC GGTCGGTTCC
P3              AATGGAGGGA TGAACAAGCA ACGGTCCAAA TCAATCACCG GCCCTGGCTC GGTTCGGTCC
Clustal Co            *  *   * *  *  * **  ** * *    ****    *      ***      *

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       610        620        630        640        650        660
KC2             GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC ACTCTCAACG
CHPn            GGACTCATCC C-------TC ACCCTATATT GACTCCTCTT C-CGTGAGAC ACTCTCTACC
CM3             GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC GCTCTCAACG
PCHBR           GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC GCTCTCAACG
KC1             GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC GCTCTCAACG
MHS             GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC GCTCTCAACG
SR1             GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC GCTCTCAACG
CM1             GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC GCTCTCAACG
CM4             GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC GCTCTCAACG
PJKRK           GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC GCTCTCAACG
Tak             GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC GCTCTCAACG
RB3             GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC GCTCTCAACG
SKHTH1          GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC GCTCTCAACG
KC3             GGACTCCTCC A-------CG ACCCTATAAT GGCTCTTCTT C-GGGGAGAA GATCTCAACG
CHYP            GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC GCTCTCAACG
SRTN            GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC GCTCTCAACG
LBR             GGACTCCTCC A-------CG ACCCTATAGT GCCTCCTCTT C-GGGGAGAC GCTCTCAACG
Nan             GGACTCCTCC A-------CG ACCCTATAGT GCCTCCTCTT C-GGGGAGAC CCTCTCAACG
P1              GGACTCCTCC A-------CG ACCCTATAGT GCCTCCTCTT C-GGGGAGAC GCTCTCAACG
UTTN            GGACTCCTCC A-------CG ACCCTATAGT GCCTCCTCTT C-GGGGAGAC GCTCTCAACG
SKHTH2          GGACTCCTCC A-------CG ACCCTATAGT GCCTCCTCTT C-GGGGAGAC GCTCTCAACG
PSNL            GGACTCCTCC A-------CG ACCCTATAGT GCCTCCTCTT C-GGGGAGAC GCTCTCAACG
PBoon           GGACTCCTCC A-------CG ACCCTATAGT GCCTCCTCTT C-GGGGAGAC GCTCTCAACG
PY              GGACTCCTCC A-------CG ACCCTATAGT GCCTCCTCTT C-GGGGAGAC GCTCTCAACG
LPang           GGACTCCTCC A-------CG ACCCTATAGT GCCTCCTCTT CCGGGGAGAC GCTCTCAACG
NKRSM           GGACTCCTCC A-------CG ACCCTATAGT GCCTCGTCTT C-GGGGAGAC GATCTCAACG
SR2             GGACTCCTCC A-------CG ACCCTATATA GCCTCTCCTT C-TGGGAGAC ACTCTCTACA
Lobata          GGACTCCCTG ATTGATGACG ACCCTACAGT GCG-CCTCCT CTCCGGAGAC GCTCTCTACG
RB1             GGACTCCTCC A-------CG ACCCTATAGT GCCTCTTCTT C-GGGGAGAC ACTCTCAACG
KPP             GGATTCCTCC A-------CC ACCCTAAAGT GACTCCTCTT C-CGGGAGAC GCTCACACTC
P2              GGAATCCTCC A-------CG AACCTATAGT GGCTCCTCTT C-CGGGAAAA GCTCTCAACG
RB2             CGACTCCCCC A-------CC AAACTATAGC GCCTCTTCTT G-GGGGAGAT CCTCTCAAAA
LPoon           GGATTCCTCC A-------CA ACCCTATAGG GCCCCCCCTT C-GGGGGAAA GTCTCCAACA
P3              TCATCCCTAC C-------CT AACCT----G GCCTGTCTTT CGGGGGAGGG ATTCTCTCTA
Clustal Co        *  *               *  **      *        *      *         *
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Figure 4.25. (continued)

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                ....|....| ....|....| ....|..
                      670        680
KC2             AGACCTCAGG -TCAGGCGGG GCTACCC
CHPn            AGACCTCACG -TCAGGCGGG GCTACCC
CM3             AGACCTCAGG -TCAGGCGGG GCTACCC
PCHBR           AGACCTCAGG -TCAGGCGGG GCTACCC
KC1             AGACCTCAGG -TCAGGCGGG GCTACCC
MHS             AGACCTCAGG -TCAGGCGGG GCTACCC
SR1             AGACCTCAGG -TCAGGCGGG GCTACCC
CM1             AGACCTCAGG -TCAGGCGGG GCTACCC
CM4             AGACCTCAGG -TCAGGCGGG GCTACCC
PJKRK           AGACCTCAGG -TCAGGCGGG GCTACCC
Tak             AGACCTCAGG -TCAGGCGGG GCTACCC
RB3             AGACCTCAGG -TCAGGCGGG GCTACCC
SKHTH1          AGACCTCAGG -TCAGGCGGG GCTACCC
KC3             AGACCTCATG -TCAGGCGGG GCTACCC
CHYP            AGACCTCAGG -TCAGGCGGG GCTACCC
SRTN            AGACCTCAGG -TCAGGCGGG GCTACCC
LBR             AGACCTCAGG -TCAGGCGGG GCTACCC
Nan             AGACCTCAGG GTCAGGGGGG GCTACCC
P1              AGACCTCAGG -TCAGGCGGG GCTACCC
UTTN            AGACCTCAGG -TCAGGCGGG GCTACCC
SKHTH2          AGACCTCAGG -TCAGGCGGG GCTACCC
PSNL            AGACCTCAGG -TCAGGCGGG GCTACCC
PBoon           AGACCTCAGG -TCAGGCGGG GCTACCC
PY              AGACCTCAGG -TCAGGCGGG GCTACCC
LPang           AGACCTCAGG TTCAGGGGGG GCTACCC
NKRSM           AGACCTCAGG -TCAGGCGGG GCTACCC
SR2             AGACCTCTCG -TGAGGCGGG GGTACCC
Lobata          AGACCTCAGG -TCAGGCGGG GCTACCC
RB1             AGACCTCAGG -TCAGGCGGG GCTACCC
KPP             AGACCTCAAG -TCAAGGGGG GGTACCC
P2              AAACCTCCAG -GCAGGGGGG GCTACCC
RB2             AGACCTCTCG -GGAGGGGGG GCCCCCC
LPoon           AAACCTCCGG -TCGGGCGGG GTTACCC
P3              AAACCT-AAG -TCAGGGGGG GGCGCCC
Clustal Co      * ****   *      * *** *   ***
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Figure 4.25. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         10         20         30         40         50         60
CM3             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
PY              TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
PSNL            TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTATGAAAAG
RB2             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
RB3             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
P1              TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
SKHT2           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
SRTN            TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
SR1             TCGGGGGAAG CTGTTCTAAC GAACGGGGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
UTTN            TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
SKNK            TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
CR              TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
Tak             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
KC1             TCAATGGAAG CTGTTCTAAC AGACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
CM4             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
LBR             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
PCHBR           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
KC2             TCTGGGGTCC CCAAATAAAA AAACGGGGTG GACGATTTTT TCTTTTTGCA TTAAGAAAAT
SR2             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
Lobata          TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
P3              TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
P2              TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
SKHT1           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
CM1             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
NKRSM           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
CHYP            TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
RB1             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
PBoon           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
PJKRK           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
KPP             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
LPang           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
KC3             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
LPoon           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
CHPn            TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
UTRD            TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
CM2             TCTCTGGTCC CTGTTTTCCC AAAGGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
RB4             TCTATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
MHS             TCTCTGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
Nan             TCTCTGGAAG CTGTTCTAAC AAAGGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAGAG
NKSW            TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
Clustal Co      **   **    *           * ** **  **********  ********* *** *** *
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Figure 4.26. A 397 bp character matrix of 39 cultivars of *P. mirifica* and 1 cultivar of *P. lobata* based on *trn*L sequences. Asterisk symbols (*) show that all samples provide nitrogenous base (A, C, G, and T) consensus or identity.

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        70         80         90        100        110        120
CM3             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
PY              AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
PSNL            AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
RB2             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
RB3             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
P1              AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
SKHT2           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
SRTN            AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
SR1             AATCCTTCCA TCAAAATTCG GGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
UTTN            AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
SKNK            AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
CR              AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
Tak             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
KC1             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
CM4             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
LBR             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
PCHBR           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
KC2             AATACTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
SR2             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
Lobata          AATCCGTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
P3              AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGACATACT
P2              AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
SKHT1           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
CM1             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
NKRSM           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
CHYP            AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
RB1             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
PBoon           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
PJKRK           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
KPP             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
LPang           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
KC3             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
LPoon           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
CHPn            AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
UTRD            AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
CM2             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
RB4             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
MHS             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
Nan             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
NKSW            AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
Clustal Co      *** * **** *********   ********* ********** ********** **** *****
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Figure 4.26. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       130        140        150        160        170        180
CM3             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
PY              ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
PSNL            ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
RB2             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
RB3             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
P1              ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
SKHT2           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
SRTN            ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
SR1             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
UTTN            ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
SKNK            ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
CR              ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
Tak             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
KC1             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
CM4             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
LBR             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
PCHBR           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
KC2             ATTTCAATTG ATTAATGAAG ATCC-TTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
SR2             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
Lobata          ATTTCAATTG ATTAATGAAG ATCCATTTGT GATAAAAATA TTCACAAATG AAAGATGTGA
P3              ATTTCAATTG ATTAATGAGG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
P2              ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
SKHT1           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
CM1             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
NKRSM           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
CHYP            ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
RB1             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
PBoon           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
PJKRK           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
KPP             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
LPang           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
KC3             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
LPoon           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
CHPn            ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
UTRD            ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
CM2             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
RB4             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
MHS             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
Nan             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
NKSW            ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
Clustal Co      ********** ******** * **** ***** *** ****** ********** **********
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Figure 4.26. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      190        200        210        220        230        240
CM3             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
PY              ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
PSNL            ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
RB2             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
RB3             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
P1              ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
SKHT2           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
SRTN            ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
SR1             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
UTTN            ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
SKNK            ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
CR              ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
Tak             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
KC1             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
CM4             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
LBR             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
PCHBR           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
KC2             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
SR2             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
Lobata          ATCAA-----  TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
P3              ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
P2              ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
SKHT1           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
CM1             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
NKRSM           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
CHYP            ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
RB1             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
PBoon           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
PJKRK           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
KPP             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
LPang           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
KC3             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
LPoon           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
CHPn            ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
UTRD            ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
CM2             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
RB4             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
MHS             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
Nan             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
NKSW            ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
Clustal Co      *****      ********** ********** ********** ********** **********
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Figure 4.26. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       250        260        270        280        290        300
CM3             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
PY              ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
PSNL            ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
RB2             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
RB3             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
P1              ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
SKHT2           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
SRTN            ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
SR1             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
UTTN            ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
SKNK            ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
CR              ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
Tak             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
KC1             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
CM4             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
LBR             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
PCHBR           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
KC2             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
SR2             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
Lobata          ATCATAATCT GATAGATCCC TTGAAGAACT GATTAATCAG ACGAGAATAA AGATAGAGTC
P3              ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
P2              ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
SKHT1           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
CM1             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
NKRSM           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
CHYP            ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
RB1             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
PBoon           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
PJKRK           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
KPP             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
LPang           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
KC3             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
LPoon           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
CHPn            ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
UTRD            ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
CM2             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
RB4             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
MHS             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
Nan             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
NKSW            ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
Clustal Co      ********** ********** *******     ******** ********** **********
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Figure 4.26. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       310        320        330        340        350        360
CM3             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
PY              CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
PSNL            CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
RB2             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
RB3             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
P1              CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
SKHT2           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
SRTN            CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
SR1             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
UTTN            CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
SKNK            CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
CR              CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
Tak             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
KC1             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
CM4             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
LBR             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
PCHBR           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
KC2             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
SR2             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
Lobata          CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
P3              CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
P2              CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
SKHT1           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
CM1             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
NKRSM           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
CHYP            CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
RB1             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
PBoon           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
PJKRK           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
KPP             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
LPang           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
KC3             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
LPoon           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
CHPn            CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
UTRD            CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
CM2             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
RB4             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
MHS             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
Nan             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
NKSW            CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
Clustal Co      ********** ********** ********** ********** ********** **********
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Figure 4.26. (continued)

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                ....|....| ....|....| ....|....| ....|....|
                       370        380        390        400
CM3             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
PY              TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
PSNL            TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
RB2             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
RB3             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
P1              TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
SKHT2           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
SRTN            TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
SR1             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
UTTN            TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
SKNK            TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
CR              TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
Tak             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
KC1             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
CM4             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
LBR             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
PCHBR           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
KC2             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
SR2             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
Lobata          TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
P3              TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
P2              TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
SKHT1           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
CM1             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
NKRSM           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
CHYP            TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
RB1             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
PBoon           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
PJKRK           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
KPP             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
LPang           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
KC3             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
LPoon           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
CHPn            TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
UTRD            TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
CM2             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
RB4             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
MHS             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
Nan             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
NKSW            TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCA
Clustal Co      ********** ********** ********** *******
```

Figure 4.26. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        10         20         30         40         50         60
P1              TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
SKHT2           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
SRTN            TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
RB2             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
RB3             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
CM1             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
NKRSM           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
CHYP            TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
RB1             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
CM3             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
CHPn            TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
KC3             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
LPoon           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
KPP             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
LPang           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
PBoon           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
PJKRK           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
P2              TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
SKHT1           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
PCHBR           TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
KC2             TCTGGGGTCC CCAAATAAAA AAACGGGGTG GAGCTTTTTT TCTTTTTGGA TTAAGAAAAT
SR2             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
Lobata          TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
P3              TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
LBR             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
SR1             TCGGGGGAAG CTGTTCTAAC GAGCGGGGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
UTTN            TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
SKNK            TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
CR              TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
Tak             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
KC1             TCAATGGAAG CTGTTCTAAC AGACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
CM4             TCAATGGAAG CTGTTCTAAC AAACGGAGTT GACGATTTTT CCTTTTTGCA TTAGGAAAAG
Clustal Co      **   **    *      **     *** **  **   *****  ********* *** *****
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Figure 4.27. A 731 bp character matrix of 31 cultivars of *P. mirifica* and 1 cultivar of *P. lobata* based on *trn*L-F sequences. Asterisk symbols (*) show that all samples provide nitrogenous base (A, C, G, and T) consensus or identity.

```
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        70         80         90        100        110        120
P1              AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
SKHT2           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
SRTN            AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
RB2             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
RB3             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
CM1             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
NKRSM           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
CHYP            AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
RB1             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
CM3             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
CHPn            AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
KC3             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
LPoon           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
KPP             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
LPang           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
PBoon           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
PJKRK           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
P2              AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
SKHT1           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
PCHBR           AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
KC2             AATACTTCGA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
SR2             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
Lobata          AATCCGTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
P3              AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGAGAAA CATATATATA CTGACATACT
LBR             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
SR1             AATCCTTCCA TCAAAATTCG GGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
UTTN            AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
SKNK            AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
CR              AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
Tak             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
KC1             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
CM4             AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
Clustal Co      *** * ** * *********   ********* ****** *** ********** **** *****

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       130        140        150        160        170        180
P1              ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
SKHT2           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
SRTN            ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
RB2             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
RB3             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
CM1             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
NKRSM           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
CHYP            ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
RB1             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
CM3             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
CHPn            ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
KC3             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
LPoon           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
KPP             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
LPang           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
PBoon           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
PJKRK           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
P2              ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
SKHT1           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
PCHBR           ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
KC2             ATTTCAATTG ATTAATGAAG ATCC-TTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
SR2             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
Lobata          ATTTCAATTG ATTAATGAAG ATCCATTTGT GATAAAAATA TTCACAAATG AAAGATGTGA
P3              ATTTCAATTG ATTAATGAGG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
LBR             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
SR1             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
UTTN            ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
SKNK            ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
CR              ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
Tak             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
KC1             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
CM4             ATTTCAATTG ATTAATGAAG ATCCATTTGT GATCAAAATA TTCACAAATG AAAGATGTGA
Clustal Co      ********** ******** * **** ***** *** ****** ********** **********
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Figure 4.27. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      190        200        210        220        230        240
P1              ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
SKHT2           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
SRTN            ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
RB2             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
RB3             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
CM1             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
NKRSM           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
CHYP            ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
RB1             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
CM3             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
CHPn            ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
KC3             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
LPoon           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
KPP             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
LPang           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
PBoon           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
PJKRK           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
P2              ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
SKHT1           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
PCHBR           ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
KC2             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
SR2             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
Lobata          ATCAA----- TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
P3              ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
LBR             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
SR1             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
UTTN            ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
SKNK            ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
CR              ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
Tak             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
KC1             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
CM4             ATCAAATCAA TTCCAAGTTG AAGAAAAGAT GGAATATTCA TTGATCAAAT TATTCACTCC
Clustal Co      *****      ********** ********** ********** ********** **********

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      250        260        270        280        290        300
P1              ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
SKHT2           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
SRTN            ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
RB2             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
RB3             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
CM1             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
NKRSM           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
CHYP            ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
RB1             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
CM3             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
CHPn            ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
KC3             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
LPoon           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
KPP             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
LPang           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
PBoon           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
PJKRK           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
P2              ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
SKHT1           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
PCHBR           ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
KC2             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
SR2             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
Lobata          ATCATAATCT GATAGATCCC TTGAAGAACT GATTAATCAG ACGAGAATAA AGATAGAGTC
P3              ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCCG ACGAGAATAA AGGTAGAGTC
LBR             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
SR1             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
UTTN            ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
SKNK            ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
CR              ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
Tak             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
KC1             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
CM4             ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
Clustal Co      ********** ********** *******     ****** * ********** ** *******
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Figure 4.27. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      310        320        330        340        350        360
P1              CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
SKHT2           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
SRTN            CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
RB2             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
RB3             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
CM1             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
NKRSM           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
CHYP            CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
RB1             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
CM3             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
CHPn            CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
KC3             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
LPoon           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
KPP             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
LPang           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
PBoon           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
PJKRK           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
P2              CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
SKHT1           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
PCHBR           CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
KC2             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
SR2             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
Lobata          CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
P3              CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
LBR             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
SR1             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
UTTN            CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
SKNK            CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
CR              CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
Tak             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
KC1             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
CM4             CTATTCTACA TGTCAATACC GACAACAATG AAATTTATAG TAAGAGGAAA ATCCGTCGAC
Clustal Co      ********** ********** ********** ********** ********** **********

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      370        380        390        400        410        420
P1              TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
SKHT2           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTCTTTA ACTTTCTAAT
SRTN            TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
RB2             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
RB3             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
CM1             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
NKRSM           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
CHYP            TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
RB1             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
CM3             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
CHPn            TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
KC3             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
LPoon           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
KPP             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
LPang           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
PBoon           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
PJKRK           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
P2              TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
SKHT1           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
PCHBR           TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
KC2             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
SR2             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
Lobata          TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GACCTGTTTA ACTTTCTAAT
P3              TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
LBR             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
SR1             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
UTTN            TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
SKNK            TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
CR              TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
Tak             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
KC1             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
CM4             TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
Clustal Co      ********** ********** ********** ********** * *** **** **********
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Figure 4.27. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       430        440        450        460        470        480
P1              TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
SKHT2           TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
SRTN            TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
RB2             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
RB3             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
CM1             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
NKRSM           TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
CHYP            TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
RB1             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
CM3             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
CHPn            TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
KC3             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
LPoon           TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
KPP             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTAT--
LPang           TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTAT--
PBoon           TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
PJKRK           TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
P2              TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
SKHT1           TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
PCHBR           TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
KC2             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
SR2             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
Lobata          TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
P3              TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTAGG TGTTTTATTC AGCTTTATAT
LBR             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
SR1             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
UTTN            TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
SKNK            TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
CR              TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
Tak             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
KC1             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
CM4             TTTTTCCCAT ATCCTCTCTA TCTTTAAGTC GTTATTTATG TGTTTTATTC AG-TTTATAT
Clustal Co      ********** ********** ********** ******** * ********** ** *****


                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       490        500        510        520        530        540
P1              TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
SKHT2           TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
SRTN            TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
RB2             TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
RB3             TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
CM1             TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
NKRSM           TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
CHYP            TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
RB1             TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
CM3             TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
CHPn            TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
KC3             TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
LPoon           TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
KPP             ---------T CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
LPang           ---------T CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
PBoon           TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
PJKRK           TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
P2              TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
SKHT1           TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
PCHBR           TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
KC2             TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
SR2             TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
Lobata          TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
P3              TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCGAA CGTTTCTTAT
LBR             TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
SR1             TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
UTTN            TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
SKNK            TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
CR              TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
Tak             TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
KC1             TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
CM4             TCAGTTTATT CTTTCACAAC TAAATTGGAA TTTGTCTTTT TATTTTCAAA AATTTCTTAT
Clustal Co            * ********** ********** ********** ******* **   ********
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Figure 4.27. (continued)

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       550        560        570        580        590        600
P1              CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
SKHT2           CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTTTAA
SRTN            CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
RB2             CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
RB3             CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
CM1             CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
NKRSM           CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA GACAGAGAAT TTTTTTT-AA
CHYP            CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
RB1             CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
CM3             CATAATTACC AGTCACAGGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-A-
CHPn            CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAC CACAGAGAAA TTTTTTTTAA
KC3             CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
LPoon           CATAATTACC AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
KPP             CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
LPang           CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
PBoon           CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
PJKRK           CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
P2              CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
SKHT1           CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
PCHBR           CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
KC2             CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
SR2             CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
Lobata          CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA GACAGAGAAT TTTTTTTTAA
P3              CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CCCAGAGAAT TTTTTTT-AA
LBR             CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
SR1             CATATTTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AT
UTTN            CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
SKNK            CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
CR              CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CCCAGAGAAT TTTTTTT-AA
Tak             CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
KC1             CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
CM4             CATAATTACA AGTCACAAGT TTTGAAATAT ATATGTGAAA CACAGAGAAT TTTTTTT-AA
Clustal Co      ********* ******* ** ********** **********  *******  ******* *

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                       610        620        630        640        650        660
P1              TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
SKHT2           TGATAAACGT ACAA-TGAAT ATCTTATTTT TGAGC-AGGG AATTCTCATA TGCGTGATTA
SRTN            TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
RB2             TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
RB3             TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
CM1             TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGC-AGGG AATTCTCATA TGCGTGATTA
NKRSM           TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGC-AGG- AATTCTCATA TGCGTGATTA
CHYP            TGATAA-CGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
RB1             TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
CM3             TGATAA-CGT ACAA-TGAAT ATCTT-TTTT TGAGC-AGG- AATTCTCATA TGCGTGATTA
CHPn            TGATAA-CGT ACAA-TGAAT ATCTTA-TTT TGAGC-AGG- AATTCTCCTA TGCGTGATTA
KC3             TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGC-AGG- AATTCTCATA TGCGTGATTA
LPoon           TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
KPP             TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
LPang           TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
PBoon           TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
PJKRK           TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
P2              TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
SKHT1           TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
PCHBR           TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
KC2             TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
SR2             TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
Lobata          TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
P3              TGATAAACGT ACAAATGAAT ATCTTATTTT TGACGCAGG- AATTCTCATA TGCGTGATTA
LBR             TGATAA-CGT ACAA-TGAAT ATCTTATTTT TGAGC-AGG- AATTCTCATA TGCGTGATTA
SR1             TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
UTTN            TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGGG AATTCTCATA TGCGTGATTA
SKNK            TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
CR              TGATAACCGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
Tak             TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
KC1             TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
CM4             TGATAAACGT ACAAATGAAT ATCTTATTTT TGAGCAAGG- AATTCTCATA TGCGTGATTA
Clustal Co      ****** *** **** ***** *****  *** ***   ***  ********** **********
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Figure 4.27. (continued)

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                     670        680        690        700        710        720
P1              -CCAAACA-T AC--AAATA- TTACTACTAC TGAAACTAAC TTACAA-C-T TTTATTTTTC
SKHT2           -CAAAACAAT AC--AAATA- TTTCTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
SRTN            ACAAAACATT AC-AAAATAA TTACTACTAC TGAAACTAAC TTCCAA-C-T TTTATTTTCC
RB2             ACAAAACAAT AC--AAATA- TTACTACTAC TGAA-CTAAC TTACAA-CTT TTTATTTTTC
RB3             ACAAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
CM1             -CAAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
NKRSM           ACAAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
CHYP            ACAAAACCAT ACAAAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
RB1             -CAAACCAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
CM3             -C-AACCA-T AC--AAATA- TTACTACT-C TGAA-CTA-C -TACAA-C-- TTTATTTT-C
CHPn            -CCAAACA-T TC-AAAATAA TTCCTCCTCC TGAA-CTAAC TTCCAA-CCT TTTATTTTCC
KC3             ACAAACCA-T AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAACCT TTTATTTTTC
LPoon           ACCAAACAAT TC-AAAATAA TTACTACTAC TGAAACTACC TTACAAAC-T TTTATTTTTC
KPP             ACAAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
LPang           ACCAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAA-C-T TTTATTTTTC
PBoon           ACAAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
PJKRK           ACAAACCAAT ACC-AAATAA TTACTACTAC TGAAACTAAC TTACAA-C-T TTTATTTTTC
P2              ACAAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
SKHT1           ACAAAACAAT AC-AAAATA- TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
PCHBR           ACAAACCA-T AC-AAAATA- TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
KC2             ACAAAACAAT –C--AAATAA TTACTACT-C TGAAACTAAC TTACAAAC-T TTTATTTTTC
SR2             CCAAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
Lobata          ACAAAACAAT AC-AAAATAA TTACTACTAC TGAAACTA-C TTACAA-C-T TTTATTTT-C
P3              CCAAAGCA-T AC-AAAATA- TTACTACTGC TGAAACTAAC TTACAAAC-T TTTATTTTTC
LBR             ACAAAACCAT AC--AAATAA TTACTACTAC TGAAACTAAC TTACAAACCT TTTATTTTCC
SR1             ACAAA-CATT -CC-AAATAT TTTCTACTAC TGAAACTTAC TTACAAAC-T TTTATTTTTC
UTTN            CCAAA-CAAT ACCAAAAATAT TTTCTACTAC TGAAACTAAC TTACAAACTT TTTATTTTTC
SKNK            ACAAA-CA-T AC-AAAATAT TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
CR              ACCAA-CAAT AC--AAATAA TTACTACTAC TGAAACTAAC TTACAA-C-T TTTATTTTTC
Tak             ACAAAACAAT AC-AAAATAT TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
KC1             CCAAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAA-C-T TTTATTTTTC
CM4             ACAAAACAAT AC-AAAATA- TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
Clustal Co       * ** *  *  *  *****  ** ** ** * **** *** *  * *** *   ******** *


                ....|....| .
                     730
P1              GCC-TTTTTT T
SKHT2           GCC-TTTTTT T
SRTN            GCC-TTTTTT T
RB2             GCC-TTTTTT T
RB3             GTC-TTTTTT T
CM1             GTC-TTTTTT T
NKRSM           GTC-TTTTTT T
CHYP            GCC-TTTTTT T
RB1             GCC-TTTTTT T
CM3             GCC-TTTTTT T
CHPn            GCC-TTTTTT T
KC3             GTC-TTTTTT T
LPoon           GCC-TTTTTT T
KPP             G-C-TTTTTT T
LPang           GTC-TTTTTT T
PBoon           GTC-TTTTTT T
PJKRK           GCC-TTTTTT T
P2              GTC-TTTTTT T
SKHT1           GCC-TTTTTT T
PCHBR           GTC-TTTTTT T
KC2             GTC-TTTTTT T
SR2             GTC-TTTTTT T
Lobata          G-CC-TTTTT T
P3              GTCCTTTTTT T
LBR             GTC-TTTTTT T
SR1             GTC-TTTTTT T
UTTN            GTCTTTTTTT T
SKNK            GCC-TTTTTT T
CR              GTC--TTTTT T
Tak             GCC-TTTTTT T
KC1             GCC-TTTTTT T
CM4             GTC-TTTTTT T
Clustal Co      * *  ***** *
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Figure 4.27. (continued)

Table 4.5. Percentages of nitrogenous base composition of ITS sequences of 33 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup.

Cultivar/Base	A	C	G	T
CM1	20.458	31.145	27.786	20.611
CM3	20.336	31.346	27.829	20.489
CM4	20.458	31.298	27.634	20.611
LPang	20.700	31.507	27.245	20.548
MHS	20.611	31.298	27.481	20.611
LPoon	24.159	29.205	27.982	18.654
Nan	20.274	31.250	28.049	20.427
PY	20.611	31.603	27.634	20.153
P1	20.336	31.804	27.676	20.183
P2	23.547	30.122	26.758	19.572
P3	23.206	29.618	27.023	20.153
KPP	23.511	30.687	27.176	18.626
LBR	20.336	31.346	27.982	20.336
PBoon	20.305	31.450	27.786	20.458
PSNL	20.305	31.603	27.786	20.305
SR1	20.611	31.298	27.481	20.611
SR2	20.489	31.346	27.217	20.948
SKHT1	20.611	31.298	27.481	20.611
SKHT2	20.305	31.450	27.786	20.458
UTTN	20.336	31.498	27.676	20.489
KC1	20.458	31.298	27.634	20.611
KC2	22.443	31.298	25.649	20.611
KC3	21.679	30.992	26.718	20.611
PCHBR	20.458	31.298	27.634	20.611
PJKRK	20.763	31.298	27.328	20.611
RB1	22.137	31.145	25.802	20.916
RB2	23.896	31.050	25.723	19.330
RB3	20.611	31.298	27.481	20.611
Tak	20.827	31.394	27.106	20.674
CHYP	20.305	30.992	27.939	20.763
NKRSM	21.069	30.840	27.176	20.916
CHPn	20.763	32.824	23.664	22.748
SRTN	20.458	31.145	27.786	20.611
P. lobata	20.509	31.737	28.892	18.862
Mean	**21.114**	**31.200**	**27.295**	**20.391**

Table 4.6. Percentages of nitrogenous base composition of *trn*L sequences of 39 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup.

Cultivar/Base	**A**	**C**	**G**	**T**
CM1	38.776	16.837	15.561	28.827
CM2	37.245	17.347	15.561	29.847
CM3	38.776	16.837	15.561	28.827
CM4	38.776	16.837	15.561	28.827
CR	38.776	16.837	15.561	28.827
LPang	38.776	16.837	15.561	28.827
MHS	38.265	17.092	15.561	29.082
LPoon	38.776	16.837	15.561	28.827
Nan	37.913	16.794	16.285	29.008
PY	38.677	17.048	15.522	28.753
P1	38.776	16.837	15.561	28.827
P2	38.776	16.837	15.561	28.827
P3	37.659	17.303	16.285	28.753
UTRD	38.776	16.837	15.561	28.827
KPP	38.776	16.837	15.561	28.827
LBR	38.776	16.837	15.561	28.827
NKSW	38.776	16.837	15.561	28.827
PBoon	38.776	16.837	15.561	28.827
PSNL	37.245	17.857	15.816	29.082
SR1	37.340	16.624	17.391	28.645
SR2	38.776	16.837	15.561	28.827
SKHT1	38.776	16.837	15.561	28.827
SKHT2	38.776	16.837	15.561	28.827
UTTN	38.776	16.837	15.561	28.827
KC1	38.520	16.837	15.816	28.827
KC2	38.875	16.368	16.113	28.645
KC3	38.776	16.837	15.561	28.827
PCHBR	38.776	16.837	15.561	28.827
PJKRK	38.776	16.837	15.561	28.827
RB1	38.776	16.837	15.561	28.827
RB2	38.776	16.837	15.561	28.827
RB3	38.776	16.837	15.561	28.827
RB4	38.010	16.837	16.071	29.082
Tak	38.776	16.837	15.561	28.827
CHYP	38.776	16.837	15.561	28.827
NKRSM	38.776	16.837	15.561	28.827
SKNK	38.776	16.837	15.561	28.827
CHPn	38.776	16.837	15.561	28.827
SRTN	38.776	16.837	15.561	28.827
P. lobata	38.776	16.582	16.071	28.571
Mean	**38.575**	**16.874**	**15.694**	**28.857**

Table 4.7. Percentages of nitrogenous base composition of *trn*L-F sequences of 31 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup.

Cultivar/Base	A	C	G	T
CM1	35.883	15.716	12.935	35.466
CM3	34.566	16.501	13.229	35.704
CM4	36.022	15.716	12.796	35.466
CR	35.475	16.201	12.849	35.475
LPang	36.017	15.960	12.853	35.169
LPoon	35.556	16.250	12.778	35.417
P1	35.524	16.084	12.867	35.524
P2	36.061	15.673	12.760	35.506
P3	34.167	16.667	14.028	35.139
KPP	36.299	15.819	12.853	35.028
LBR	35.655	16.156	12.813	35.376
PBoon	36.111	15.694	12.778	35.417
SR1	34.170	15.760	13.808	36.262
SR2	35.972	15.833	12.778	35.417
SKHT1	36.022	15.855	12.796	35.327
SKHT2	35.425	16.039	12.831	35.704
UTTN	35.408	15.906	12.863	35.823
KC1	35.744	15.994	12.935	35.327
KC2	35.894	15.363	13.128	35.615
KC3	35.744	15.994	12.796	35.466
PCHBR	35.794	15.877	12.813	35.515
PJKRK	35.744	16.134	12.796	35.327
RB1	35.744	16.134	12.796	35.327
RB2	35.844	15.900	12.831	35.425
RB3	36.111	15.694	12.778	35.417
Tak	35.972	15.833	12.778	35.417
CHYP	35.972	15.972	12.778	35.278
NKRSM	36.022	15.577	12.935	35.466
SKNK	35.794	15.877	12.813	35.515
CHPn	34.314	17.227	13.025	35.434
SRTN	35.744	16.134	12.796	35.327
P. lobata	36.123	15.621	13.110	35.146
Mean	**35.654**	**15.973**	**12.929**	**35.445**

Table 4.8. The matrix of pairwise similarity (%) of ITS sequences from 33 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup.

Cultivar	CM1	CM2	CM3	CM4	CR	LPang	MHS	LPoon
CM1	-	-	-	-	-	-	-	-
CM3	99	-	-	-	-	-	-	-
CM4	99	99	-	-	-	-	-	-
LPang	98	98	98	-	-	-	-	-
MHS	99	99	99	98	-	-	-	-
LPoon	87	87	87	88	87	-	-	-
Nan	98	98	99	98	98	88	-	-
PY	98	98	98	98	98	88	98	-
P1	99	99	99	98	99	87	99	98
P2	89	89	90	91	89	86	90	90
P3	81	81	81	81	81	81	81	81
KPP	89	89	89	90	89	83	89	89
LBR	99	99	99	99	99	87	99	98
PBoon	99	99	99	99	99	88	99	98
PSNL	99	99	99	99	99	88	99	98
SR1	99	99	99	98	100	87	98	98
SR2	98	97	98	97	98	88	97	97
SKHT1	99	99	99	98	99	88	98	98
SKHT2	99	99	99	99	99	88	99	99
UTTN	99	99	99	99	99	88	99	99
KC1	99	99	100	98	99	87	99	98
KC2	96	96	96	96	96	88	96	96
KC3	97	97	97	96	97	87	96	97
PCHBR	99	99	100	98	99	87	99	98
PJKRK	99	99	99	98	99	87	98	98
RB1	96	96	96	96	96	87	96	95
RB2	87	88	88	88	87	83	87	88
RB3	99	99	99	98	99	87	98	98
Tak	99	99	99	98	99	88	98	98
CHYP	99	99	99	98	99	87	98	98
NKRSM	97	97	98	97	97	87	97	97
CHPn	87	87	87	87	87	80	97	88
SRTN	99	99	99	98	99	87	99	98
P. lobata	90	90	90	89	90	79	89	89

Cultivar	P1	P2	P3	KPP	LBR	PBoon	PSNL	SR1
P1	-	-	-	-	-	-	-	-
P2	90	-	-	-	-	-	-	-
P3	81	80	-	-	-	-	-	-
KPP	89	84	77	-	-	-	-	-
LBR	99	90	81	89	-	-	-	-
PBoon	99	90	81	89	99	-	-	-
PSNL	99	90	81	89	99	99	-	-
SR1	99	89	81	89	99	99	99	-
SR2	97	88	81	88	97	98	98	98
SKHT1	99	90	81	89	99	99	99	99
SKHT2	99	90	81	89	99	99	99	99
UTTN	99	90	81	89	99	99	99	99
KC1	99	90	81	89	99	99	99	99
KC2	96	90	81	88	96	96	96	96
KC3	97	89	81	88	97	97	97	97
PCHBR	99	90	81	89	99	99	99	99
PJKRK	99	90	81	89	99	99	99	99
RB1	96	89	81	88	96	96	96	96
RB2	87	86	79	82	87	88	87	87
RB3	99	89	81	89	99	99	99	99
Tak	98	90	81	89	98	99	99	99
CHYP	99	89	81	89	99	99	99	99
NKRSM	97	89	81	88	98	98	98	97
CHPn	87	83	76	81	87	87	87	87
SRTN	99	90	81	89	99	99	99	99
P. lobata	90	81	72	81	90	90	90	90

Table 4.8. (continued)

Cultivar	SR2	SKHT1	UTTN	KC1	KC2	KC3	PCHBR	PJKRK
SR2	-	-	-	-	-	-	-	-
SKHT1	98	-	-	-	-	-	-	-
SKHT2	98	99	-	-	-	-	-	-
UTTN	98	99	100	-	-	-	-	-
KC1	98	99	99	99	-	-	-	-
KC2	95	96	96	96	96	-	-	-
KC3	96	98	97	97	97	94	-	-
PCHBR	98	99	99	99	100	96	97	-
PJKRK	97	99	99	99	99	96	97	99
RB1	95	97	96	96	96	95	95	96
RB2	88	87	88	88	88	87	87	88
RB3	98	99	99	99	99	96	97	99
Tak	97	99	99	99	99	96	97	99
CHYP	97	99	99	99	99	96	97	99
NKRSM	96	97	98	98	98	95	96	98
CHPn	87	87	87	87	87	90	86	87
SRTN	98	99	99	99	99	96	97	99
P. lobata	88	90	90	90	90	87	88	90

Cultivar	PJKRK	RB1	RB2	RB3	Tak	CHYP	NKRSM	CHPn
PJKRK	-	-	-	-	-	-	-	-
RB1	96	-	-	-	-	-	-	-
RB2	88	86	-	-	-	-	-	-
RB3	99	96	87	-	-	-	-	-
Tak	99	96	88	99	-	-	-	-
CHYP	99	96	87	99	98	-	-	-
NKRSM	98	95	87	97	97	98	-	-
CHPn	87	86	79	87	87	87	86	-
SRTN	99	96	88	99	99	99	98	87
P. lobata	89	87	79	90	89	89	88	79

Cultivar	SRTN	*P. lobata*
SRTN	-	-
P. lobata	89	-

Table 4.9. The matrix of pairwise similarity (%) of *trn*L sequences from 39 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup.

Cultivar	CM1	CM2	CM3	CM4	CR	LPang	MHS	LPoon
CM1	-	-	-	-	-	-	-	-
CM2	97	-	-	-	-	-	-	-
CM3	100	97	-	-	-	-	-	-
CM4	100	97	100	-	-	-	-	-
CR	100	97	100	100	-	-	-	-
LPang	100	97	100	100	100	-	-	-
MHS	99	97	99	99	99	99	-	-
LPoon	100	97	100	100	100	100	99	-
Nan	98	97	98	98	98	98	99	98
PY	100	97	100	100	100	100	99	100
P1	100	97	100	100	100	100	99	100
P2	100	97	100	100	100	100	99	100
P3	99	96	99	99	99	99	98	99
UTRD	100	97	100	100	100	100	99	100
KPP	100	97	100	100	100	100	99	100
LBR	100	97	100	100	100	100	99	100
NKSW	100	97	100	100	100	100	99	100
PBoon	100	97	100	100	100	100	99	100
PSNL	99	96	99	99	99	99	98	99
SR1	97	95	97	97	97	97	97	97
SR2	100	97	100	100	100	100	99	100
SKHT1	100	97	100	100	100	100	99	100
SKHT2	100	97	100	100	100	100	99	100
UTTN	100	97	100	100	100	100	99	100
KC1	99	96	99	99	99	99	99	99
KC2	92	91	92	92	92	92	92	92
KC3	100	97	100	100	100	100	99	100
PCHBR	100	97	100	100	100	100	99	100
PJKRK	100	97	100	100	100	100	99	100
RB1	100	97	100	100	100	100	99	100
RB2	100	97	100	100	100	100	99	100
RB3	100	97	100	100	100	100	99	100
RB4	99	97	99	99	99	99	99	99
Tak	100	97	100	100	100	100	99	100
CHYP	100	97	100	100	100	100	99	100
NKRSM	100	97	100	100	100	100	99	100
SKNK	100	97	100	100	100	100	99	100
CHPn	100	97	100	100	100	100	99	100
SRTN	100	97	100	100	100	100	99	100
P. lobata	98	95	98	98	98	98	97	98

Table 4.9. (continued)

Cultivar	Nan	PY	P1	P2	P3	UTRD	KPP	LBR
Nan	-	-	-	-	-	-	-	-
PY	98	-	-	-	-	-	-	-
P1	98	100	-	-	-	-	-	-
P2	98	100	100	-	-	-	-	-
P3	98	99	99	99	-	-	-	-
UTRD	98	100	100	100	99	-	-	-
KPP	98	100	100	100	99	100	-	-
LBR	98	100	100	100	99	100	100	-
NKSW	98	100	100	100	99	100	100	100
PBoon	98	100	100	100	99	100	100	100
PSNL	98	99	99	99	98	99	99	99
SR1	97	97	97	97	96	97	97	97
SR2	98	100	100	100	99	100	100	100
SKHT1	98	100	100	100	99	100	100	100
SKHT2	98	100	100	100	99	100	100	100
UTTN	98	100	100	100	99	100	100	100
KC1	98	99	99	99	98	99	99	99
KC2	92	92	92	92	91	92	92	92
KC3	98	100	100	100	99	100	100	100
PCHBR	98	100	100	100	99	100	100	100
PJKRK	98	100	100	100	99	100	100	100
RB1	98	100	100	100	99	100	100	100
RB2	98	100	100	100	99	100	100	100
RB3	98	100	100	100	99	100	100	100
RB4	99	99	99	99	98	99	99	99
Tak	98	100	100	100	99	100	100	100
CHYP	98	100	100	100	99	100	100	100
NKRSM	98	100	100	100	99	100	100	100
SKNK	98	100	100	100	99	100	100	100
CHPn	98	100	100	100	99	100	100	100
SRTN	98	100	100	100	99	100	100	100
P. lobata	97	98	98	98	97	98	98	98

Cultivar	NKSW	PBoon	PSNL	SR1	SR2	SKHT1	SKHT2	UTTN
NKSW	-	-	-	-	-	-	-	-
PBoon	100	-	-	-	-	-	-	-
PSNL	99	99	-	-	-	-	-	-
SR1	97	97	97	-	-	-	-	-
SR2	100	100	99	97	-	-	-	-
SKHT1	100	100	99	97	100	-	-	-
SKHT2	100	100	99	97	100	100	-	-
UTTN	100	100	99	97	100	100	100	-
KC1	99	99	99	97	99	99	99	99
KC2	92	92	92	91	92	92	92	92
KC3	100	100	99	97	100	100	100	100
PCHBR	100	100	99	97	100	100	100	100
PJKRK	100	100	99	97	100	100	100	100
RB1	100	100	99	97	100	100	100	100
RB2	100	100	99	97	100	100	100	100
RB3	100	100	99	97	100	100	100	100
RB4	99	99	99	97	99	99	99	99
Tak	100	100	99	97	100	100	100	100
CHYP	100	100	99	97	100	100	100	100
NKRSM	100	100	99	97	100	100	100	100
SKNK	100	100	99	97	100	100	100	100
CHPn	100	100	99	97	100	100	100	100
SRTN	100	100	99	97	100	100	100	100
P. lobata	98	98	97	95	98	98	98	98

Table 4.9. (continued)

Cultivar	KC1	KC2	KC3	PCHBR	PJKRK	RB1	RB2	RB3
KC1	-	-	-	-	-	-	-	-
KC2	92	-	-	-	-	-	-	-
KC3	99	92	-	-	-	-	-	-
PCHBR	99	92	100	-	-	-	-	-
PJKRK	99	92	100	100	-	-	-	-
RB1	99	92	100	100	100	-	-	-
RB2	99	92	100	100	100	100	-	-
RB3	99	92	100	100	100	100	100	-
RB4	99	92	99	99	99	99	99	99
Tak	99	92	100	100	100	100	100	100
CHYP	99	92	100	100	100	100	100	100
NKRSM	99	92	100	100	100	100	100	100
SKNK	99	92	100	100	100	100	100	100
CHPn	99	92	100	100	100	100	100	100
SRTN	99	92	100	100	100	100	100	100
P. lobata	97	90	98	98	98	98	98	98

Cultivar	RB4	Tak	CHYP	NKRSM	SKNK	CHPn	SRTN	*P. lobata*
RB4	-	-	-	-	-	-	-	-
Tak	99	-	-	-	-	-	-	-
CHYP	99	100	-	-	-	-	-	-
NKRSM	99	100	100	-	-	-	-	-
SKNK	99	100	100	100	-	-	-	-
CHPn	99	100	100	100	100	-	-	-
SRTN	99	100	100	100	100	100	-	-
P. lobata	97	98	98	98	98	98	98	-

Table 4.10. The matrix of pairwise similarity (%) of *trn*L-F sequences from 31 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup.

Cultivar	CM1	CM3	CM4	CR	LPang	LPoon	P1	P2
CM1	-	-	-	-	-	-	-	-
CM3	98	-	-	-	-	-	-	-
CM4	99	98	-	-	-	-	-	-
CR	98	97	99	-	-	-	-	-
LPang	99	97	99	98	-	-	-	-
LPoon	99	99	98	98	99	-	-	-
P1	99	99	98	98	99	99	-	-
P2	99	98	99	99	99	99	99	-
P3	97	96	96	96	97	96	97	97
KPP	99	97	99	98	99	99	99	100
LBR	99	99	98	98	99	98	99	99
PBoon	99	98	99	98	99	99	99	100
SR1	96	95	97	97	96	96	96	97
SR2	99	98	99	98	99	99	99	99
SKHT1	99	99	98	98	99	99	99	99
SKHT2	99	98	98	97	98	98	99	99
UTTN	98	98	99	99	98	98	98	98
KC1	98	98	99	99	98	98	98	99
KC2	95	94	94	94	94	94	94	95
KC3	99	99	98	98	99	99	99	99
PCHBR	99	99	99	98	99	98	99	99
PJKRK	99	99	98	98	99	99	99	99
RB1	99	99	98	98	99	99	99	99
RB2	99	98	98	98	98	98	99	99
RB3	99	98	99	98	99	99	99	100
Tak	98	98	99	99	98	98	98	99
CHYP	99	98	99	98	99	99	99	99
NKRSM	99	98	98	98	99	99	99	99
SKNK	98	98	99	98	98	98	98	99
CHPn	97	98	97	96	97	98	97	97
SRTN	99	99	98	98	99	99	99	99
P. lobata	97	97	97	96	97	97	97	98

Cultivar	P3	KPP	LBR	PBoon	SR1	SR2	SKHT1	SKHT2
P3	-	-	-	-	-	-	-	-
KPP	97	-	-	-	-	-	-	-
LBR	97	99	-	-	-	-	-	-
PBoon	97	100	99	-	-	-	-	-
SR1	94	96	96	97	-	-	-	-
SR2	97	99	99	99	96	-	-	-
SKHT1	97	99	99	99	96	99	-	-
SKHT2	96	98	98	99	96	99	99	-
UTTN	96	98	98	98	97	99	98	98
KC1	96	98	98	98	97	99	98	98
KC2	93	95	94	95	93	95	95	94
KC3	97	99	99	99	96	99	99	98
PCHBR	97	99	98	99	97	99	99	98
PJKRK	97	99	99	99	96	99	99	98
RB1	97	99	99	99	96	99	99	99
RB2	96	99	98	99	96	99	99	98
RB3	97	100	99	100	97	99	99	99
Tak	96	99	98	99	97	98	99	98
CHYP	96	99	99	99	96	99	99	99
NKRSM	97	99	99	99	96	99	99	99
SKNK	96	98	98	99	97	98	99	98
CHPn	95	97	97	97	95	97	97	97
SRTN	96	99	99	99	96	99	99	98
P. lobata	95	97	97	98	94	98	97	97

Table 4.10. (continued)

Cultivar	UTTN	KC1	KC2	KC3	PCHBR	PJKRK	RB1	RB2
UTTN	-	-	-	-	-	-	-	-
KC1	99	-	-	-	-	-	-	-
KC2	94	94	-	-	-	-	-	-
KC3	98	98	95	-	-	-	-	-
PCHBR	99	98	95	99	-	-	-	-
PJKRK	98	98	95	99	99	-	-	-
RB1	98	98	95	99	99	99	-	-
RB2	98	98	94	98	99	99	99	-
RB3	98	98	95	99	99	99	99	99
Tak	99	99	94	98	99	99	98	98
CHYP	98	98	95	99	99	99	99	99
NKRSM	98	98	95	99	99	99	99	98
SKNK	99	98	94	98	98	98	98	98
CHPn	97	97	93	97	97	97	97	97
SRTN	98	98	95	99	99	99	99	99
P. lobata	97	97	93	97	97	97	97	97

Cultivar	RB3	Tak	CHYP	NKRSM	SKNK	CHPn	SRTN	*P. lobata*
RB3	-	-	-	-	-	-	-	-
Tak	99	-	-	-	-	-	-	-
CHYP	99	99	-	-	-	-	-	-
NKRSM	99	98	99	-	-	-	-	-
SKNK	99	99	99	98	-	-	-	-
CHPn	97	97	97	97	96	-	-	-
SRTN	99	98	99	99	98	98	-	-
P. lobata	98	97	97	98	97	96	98	-

Table 4.11. The pairwise sequence divergence (%) of ITS sequences from 33 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup.

Cultivar	CM1	CM3	CM4	LPang	MHS	LPoon	Nan	PY
CM1	-	-	-	-	-	-	-	-
CM3	0.305	-	-	-	-	-	-	-
CM4	0.153	0.153	-	-	-	-	-	-
LPang	1.220	1.219	1.067	-	-	-	-	-
MHS	0.305	0.305	0.153	1.219	-	-	-	-
LPoon	12.236	12.112	12.082	11.478	12.235	-	-	-
Nan	1.070	1.071	0.917	1.219	1.070	12.088	-	-
PY	1.374	1.374	1.221	1.678	1.374	11.929	1.529	-
P1	0.764	0.764	0.611	1.069	0.763	12.080	0.918	1.223
P2	9.972	9.977	9.819	8.745	9.971	13.315	09.677	9.053
P3	18.961	18.989	18.807	18.466	18.962	19.285	18.502	18.652
KPP	10.840	10.850	10.687	9.928	10.840	16.975	10.540	10.840
LBR	0.611	0.611	0.458	0.916	0.611	11.938	0.459	1.070
PBoon	0.458	0.458	0.306	0.762	0.458	11.797	0.763	0.917
PSNL	0.611	0.611	0.458	0.915	0.611	11.929	0.764	1.069
SR1	0.305	0.305	0.153	1.219	0.000	12.235	1.070	1.374
SR2	1.990	1.994	1.836	2.598	1.989	12.538	2.297	2.754
SKHT1	0.305	0.305	0.153	1.219	0.305	11.929	1.070	1.374
SKHT2	0.458	0.458	0.305	0.762	0.458	11.777	0.611	0.916
UTTN	0.459	0.459	0.305	0.763	0.458	11.774	0.613	0.918
KC1	0.153	0.153	0.000	1.067	0.153	12.082	0.917	1.221
KC2	3.511	3.514	3.359	3.355	3.511	12.687	3.823	3.664
KC3	2.290	2.292	2.137	3.203	2.290	12.845	3.058	2.748
PCHBR	0.153	0.153	0.000	1.067	0.153	12.082	0.917	1.221
PJKRK	0.458	0.458	0.305	1.067	0.458	12.082	1.224	1.527
RB1	3.206	3.211	3.053	3.811	3.206	13.608	3.825	4.122

Cultivar	P1	P2	P3	KPP	LBR	PBoon	PSNL	SR1
P1	-	-	-	-	-	-	-	-
P2	9.810	-	-	-	-	-	-	-
P3	19.106	20.205	-	-	-	-	-	-
KPP	10.094	15.165	23.141	-	-	-	-	-
LBR	0.460	9.517	18.670	10.544	-	-	-	-
PBoon	0.307	9.375	18.836	10.399	0.154	-	-	-
PSNL	0.458	9.667	18.959	10.534	0.306	0.154	-	-
SR1	0.763	9.971	18.962	10.840	0.611	0.458	0.611	-
SR2	2.141	10.880	18.485	11.469	1.991	1.841	1.989	1.989
SKHT1	0.763	9.665	18.652	10.534	0.611	0.458	0.611	0.305
SKHT2	0.305	9.514	18.805	10.382	0.153	0.000	0.153	0.458
UTTN	0.306	9.504	18.798	10.400	0.154	0.000	0.153	0.458
KC1	0.611	9.819	18.807	10.687	0.458	0.306	0.458	0.153
KC2	3.664	9.807	19.427	11.603	3.515	3.359	3.511	3.511
KC3	2.750	10.577	19.724	11.756	2.597	2.445	2.595	2.290
PCHBR	0.611	9.819	18.807	10.687	0.458	0.306	0.458	0.153
PJKRK	0.916	9.817	18.498	10.687	0.763	0.611	0.763	0.458
RB1	3.667	10.727	18.806	11.603	3.513	3.362	3.511	3.206
RB2	12.065	13.454	21.878	17.581	11.932	11.623	11.914	11.907
RB3	0.763	9.971	18.652	10.840	0.611	0.458	0.611	0.305
Tak	1.072	9.701	18.409	10.430	0.920	0.767	0.919	0.614
CHYP	0.917	10.125	18.961	10.382	0.763	0.612	0.763	0.763
NKRSM	2.139	10.427	19.736	11.603	1.985	1.833	1.985	2.137
CHPn	12.528	16.242	24.958	18.168	12.386	12.226	12.366	12.672
SRTN	0.611	9.819	18.805	10.382	0.458	0.306	0.458	0.458
P. lobata	8.251	17.145	25.243	17.110	8.219	8.208	8.077	8.387

Table 4.11. (continued)

Cultivar	SR2	SKHT1	UTTN	KC1	KC2	KC3	PCHBR	PJKRK
SR2	-	-	-	-	-	-	-	-
SKHT1	1.989	-	-	-	-	-	-	-
SKHT2	1.836	0.458	-	-	-	-	-	-
UTTN	1.835	0.458	0.000	-	-	-	-	-
KC1	1.836	0.153	0.305	0.305	-	-	-	-
KC2	4.584	3.511	3.359	3.359	3.359	-	-	-
KC3	3.669	1.985	2.443	2.445	2.137	5.191	-	-
PCHBR	1.836	0.153	0.305	0.305	0.000	3.359	2.137	-
PJKRK	2.142	0.458	0.611	0.611	0.305	3.511	2.443	0.305
RB1	4.587	2.901	3.359	3.362	3.053	4.885	4.885	3.053
RB2	11.749	11.907	11.759	11.753	11.755	12.511	12.670	11.755
RB3	1.989	0.305	0.458	0.458	0.153	3.511	2.290	0.153
Tak	2.303	0.614	0.767	0.767	0.461	3.223	2.301	0.461
CHYP	2.142	0.763	0.611	0.612	0.611	3.664	2.748	0.611
NKRSM	3.517	2.137	1.832	1.834	1.985	4.580	3.664	1.985
CHPn	12.528	12.672	12.214	12.223	12.519	9.771	13.588	12.519
SRTN	1.836	0.458	0.305	0.305	0.305	3.359	2.443	0.305
P. lobata	9.651	8.387	8.230	8.242	8.231	11.496	10.411	8.231

Cultivar	PJKRK	RB1	RB2	RB3	Tak	CHYP	NKRSM	CHPn
PJKRK	-	-	-	-	-	-	-	-
RB1	3.359	-	-	-	-	-	-	-
RB2	11.755	12.972	-	-	-	-	-	-
RB3	0.153	3.206	11.907	-	-	-	-	-
Tak	0.154	3.374	11.654	0.307	-	-	-	-
CHYP	0.916	3.664	12.060	0.763	1.074	-	-	-
NKRSM	1.985	4.885	12.361	2.137	2.147	1.985	-	-
CHPn	12.672	13.435	19.983	12.672	12.409	12.824	13.740	-
SRTN	0.611	3.359	11.755	0.458	0.768	0.305	1.985	12.519
P. lobata	8.544	10.895	18.384	8.387	8.724	8.836	10.079	19.592

Cultivar	SRTN	*P. lobata*
SRTN	-	-
P. lobata	8.531	-

Table 4.12. The pairwise sequence divergence (%) of *trn*L sequences from 39 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup.

Cultivar	CM1	CM2	CM3	CM4	CR	LPang	MHS	LPoon
CM1	-	-	-	-	-	-	-	-
CM2	2.551	-	-	-	-	-	-	-
CM3	0.000	2.551	-	-	-	-	-	-
CM4	0.000	2.551	0.000	-	-	-	-	-
CR	0.000	2.551	0.000	0.000	-	-	-	-
LPang	0.000	2.551	0.000	0.000	0.000	-	-	-
MHS	0.51	2.041	0.51	0.51	0.51	0.51	-	-
LPoon	0.000	2.551	0.000	0.000	0.000	0.000	0.510	-
Nan	1.026	2.044	1.026	1.026	1.026	1.026	0.517	1.026
PY	0.000	2.556	0.000	0.000	0.000	0.000	0.509	0.000
P1	0.000	2.551	0.000	0.000	0.000	0.000	0.510	0.000
P2	0.000	2.551	0.000	0.000	0.000	0.000	0.510	0.000
P3	1.272	3.817	1.272	1.272	1.272	1.272	1.781	1.272
UTRD	0.000	2.551	0.000	0.000	0.000	0.000	0.510	0.000
KPP	0.000	2.551	0.000	0.000	0.000	0.000	0.510	0.000
LBR	0.000	2.551	0.000	0.000	0.000	0.000	0.510	0.000
NKSW	0.000	2.551	0.000	0.000	0.000	0.000	0.510	0.000
PBoon	0.000	2.551	0.000	0.000	0.000	0.000	0.510	0.000
PSNL	1.786	4.337	1.786	1.786	1.786	1.786	2.296	1.786
SR1	1.794	3.832	1.794	1.794	1.794	1.794	1.791	1.794
SR2	0.000	2.551	0.000	0.000	0.000	0.000	0.510	0.000
SKHT1	0.000	2.551	0.000	0.000	0.000	0.000	0.510	0.000
SKHT2	0.000	2.551	0.000	0.000	0.000	0.000	0.510	0.000
UTTN	0.000	2.551	0.000	0.000	0.000	0.000	0.510	0.000
KC1	0.255	2.806	0.255	0.255	0.255	0.255	0.765	0.255
KC2	6.388	6.126	6.388	6.388	6.388	6.388	6.129	6.388

Cultivar	Nan	PY	P1	P2	P3	UTRD	KPP	LBR
Nan	-	-	-	-	-	-	-	-
PY	1.027	-	-	-	-	-	-	-
P1	1.026	0.000	-	-	-	-	-	-
P2	1.026	0.000	0.000	-	-	-	-	-
P3	2.292	1.269	1.272	1.272	-	-	-	-
UTRD	1.026	0.000	0.000	0.000	1.272	-	-	-
KPP	1.026	0.000	0.000	0.000	1.272	0.000	-	-
LBR	1.026	0.000	0.000	0.000	1.272	0.000	0.000	-
NKSW	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
PBoon	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
PSNL	2.811	1.781	1.786	1.786	3.053	1.786	1.786	1.786
SR1	2.303	1.793	1.794	1.794	3.064	1.794	1.794	1.794
SR2	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
SKHT1	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
SKHT2	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
UTTN	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
KC1	1.280	0.254	0.255	0.255	1.527	0.255	0.255	0.255
KC2	6.651	6.398	6.388	6.388	7.660	6.388	6.388	6.388
KC3	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
PCHBR	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
PJKRK	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
RB1	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
RB2	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
RB3	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
RB4	1.535	1.018	1.020	1.020	2.292	1.020	1.020	1.020
Tak	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
CHYP	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
NKRSM	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
SKNK	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
CHPn	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
SRTN	1.026	0.000	0.000	0.000	1.272	0.000	0.000	0.000
P. lobata	1.549	0.520	0.517	0.517	1.798	0.517	0.517	0.517

Table 4.12. (continued)

Cultivar	NKSW	PBoon	PSNL	SR1	SR2	SKHT1	SKHT2	UTTN
NKSW	-	-	-	-	-	-	-	-
PBoon	0.000	-	-	-	-	-	-	-
PSNL	1.786	1.786	-	-	-	-	-	-
SR1	1.794	1.794	3.580	-	-	-	-	-
SR2	0.000	0.000	1.786	1.794	-	-	-	-
SKHT1	0.000	0.000	1.786	1.794	0.000	-	-	-
SKHT2	0.000	0.000	1.786	1.794	0.000	0.000	-	-
UTTN	0.000	0.000	1.786	1.794	0.000	0.000	0.000	-
KC1	0.255	0.255	2.041	2.049	0.255	0.255	0.255	0.255
KC2	6.388	6.388	7.919	6.398	6.388	6.388	6.388	6.388
KC3	0.000	0.000	1.786	1.794	0.000	0.000	0.000	0.000
PCHBR	0.000	0.000	1.786	1.794	0.000	0.000	0.000	0.000
PJKRK	0.000	0.000	1.786	1.794	0.000	0.000	0.000	0.000
RB1	0.000	0.000	1.786	1.794	0.000	0.000	0.000	0.000
RB2	0.000	0.000	1.786	1.794	0.000	0.000	0.000	0.000
RB3	0.000	0.000	1.786	1.794	0.000	0.000	0.000	0.000
RB4	1.020	1.020	2.806	2.558	1.020	1.020	1.020	1.020
Tak	0.000	0.000	1.786	1.794	0.000	0.000	0.000	0.000
CHYP	0.000	0.000	1.786	1.794	0.000	0.000	0.000	0.000
NKRSM	0.000	0.000	1.786	1.794	0.000	0.000	0.000	0.000
SKNK	0.000	0.000	1.786	1.794	0.000	0.000	0.000	0.000
CHPn	0.000	0.000	1.786	1.794	0.000	0.000	0.000	0.000
SRTN	0.000	0.000	1.786	1.794	0.000	0.000	0.000	0.000
P. lobata	0.517	0.517	2.321	2.314	0.517	0.517	0.517	0.517

Cultivar	KC1	KC2	KC3	PCHBR	PJKRK	RB1	RB2	RB3
KC1	-	-	-	-	-	-	-	-
KC2	6.643	-	-	-	-	-	-	-
KC3	0.255	6.388	-	-	-	-	-	-
PCHBR	0.255	6.388	0.000	-	-	-	-	-
PJKRK	0.255	6.388	0.000	0.000	-	-	-	-
RB1	0.255	6.388	0.000	0.000	0.000	-	-	-
RB2	0.255	6.388	0.000	0.000	0.000	0.000	-	-
RB3	0.255	6.388	0.000	0.000	0.000	0.000	0.000	-
RB4	1.276	6.896	1.020	1.020	1.020	1.020	1.020	1.020
Tak	0.255	6.388	0.000	0.000	0.000	0.000	0.000	0.000
CHYP	0.255	6.388	0.000	0.000	0.000	0.000	0.000	0.000
NKRSM	0.255	6.388	0.000	0.000	0.000	0.000	0.000	0.000
SKNK	0.255	6.388	0.000	0.000	0.000	0.000	0.000	0.000
CHPn	0.255	6.388	0.000	0.000	0.000	0.000	0.000	0.000
SRTN	0.255	6.388	0.000	0.000	0.000	0.000	0.000	0.000
P. lobata	0.773	6.980	0.517	0.517	0.517	0.517	0.517	0.517

Cultivar	RB4	Tak	CHYP	NKRSM	SKNK	CHPn	SRTN	Lobata
RB4	-	-	-	-	-	-	-	-
Tak	1.020	-	-	-	-	-	-	-
CHYP	1.020	0.000	-	-	-	-	-	-
NKRSM	1.020	0.000	0.000	-	-	-	-	-
SKNK	1.020	0.000	0.000	0.000	-	-	-	-
CHPn	1.020	0.000	0.000	0.000	0.000	-	-	-
SRTN	1.020	0.000	0.000	0.000	0.000	0.000	-	-
P. lobata	1.542	0.517	0.517	0.517	0.517	0.517	0.517	-

Table 4.13. The pairwise sequence divergence (%) of *trn*L-F sequences from 31 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup.

Cultivar	CM1	CM3	CM4	CR	LPang	LPoon	P1	P2
CM1	-	-	-	-	-	-	-	-
CM3	1.144	-	-	-	-	-	-	-
CM4	0.279	1.302	-	-	-	-	-	-
CR	0.706	1.303	0.422	-	-	-	-	-
LPang	0.282	1.157	0.283	0.428	-	-	-	-
LPoon	0.834	0.998	0.838	0.984	0.562	-	-	-
P1	0.560	0.856	0.566	0.708	0.285	0.558	-	-
P2	0.139	1.148	0.139	0.566	0.141	0.694	0.422	-
P3	2.388	3.121	2.519	2.535	2.547	3.077	2.665	2.382
KPP	0.141	1.026	0.141	0.571	0.142	0.566	0.288	0.000
LBR	0.840	1.720	0.987	1.275	0.993	1.255	1.267	0.841
PBoon	0.139	1.148	0.139	0.566	0.141	0.694	0.422	0.000
SR1	2.255	3.004	1.826	2.379	2.291	2.673	2.405	2.112
SR2	0.139	1.145	0.279	0.706	0.281	0.833	0.420	0.139
SKHT1	0.418	1.007	0.419	0.847	0.424	0.695	0.281	0.278
SKHT2	0.559	1.141	0.844	1.271	0.852	1.116	0.560	0.700
UTTN	0.976	1.856	0.280	0.838	1.133	1.670	1.261	0.974
KC1	0.559	1.295	0.418	0.838	0.707	0.979	0.562	0.560
KC2	3.769	4.712	3.648	4.085	3.823	4.321	4.064	3.624
KC3	0.140	0.999	0.421	0.706	0.283	0.834	0.561	0.141
PCHBR	0.977	1.431	0.842	1.126	0.991	1.252	0.980	0.835
PJKRK	0.557	1.004	0.423	0.705	0.562	0.834	0.560	0.418
RB1	0.556	1.004	0.561	0.848	0.562	0.834	0.560	0.418
RB2	0.838	1.143	0.701	0.989	0.709	1.114	0.420	0.698
RB3	0.139	1.148	0.139	0.566	0.141	0.694	0.422	0.000
Tak	0.559	1.302	0.139	0.700	0.568	0.838	0.566	0.419
CHYP	0.418	1.151	0.419	0.706	0.422	0.696	0.422	0.278
NKRSM	0.139	1.287	0.417	0.844	0.422	0.972	0.699	0.277
SKNK	1.123	1.597	0.697	1.257	1.141	1.261	0.990	0.981
CHPn	1.844	2.004	1.994	1.989	1.723	1.701	1.702	1.851
SRTN	0.696	1.007	0.701	1.126	0.704	0.973	0.559	0.557
P. lobata	1.124	1.598	1.131	1.421	1.135	1.412	1.273	0.982

Cultivar	P3	KPP	LBR	PBoon	SR1	SR2	SKHT1	SKHT2
P3	-	-	-	-	-	-	-	-
KPP	2.406	-	-	-	-	-	-	-
LBR	3.226	0.852	-	-	-	-	-	-
PBoon	2.384	0.000	0.841	-	-	-	-	-
SR1	4.075	2.151	2.814	2.115	-	-	-	-
SR2	2.242	0.140	0.979	0.139	2.255	-	-	-
SKHT1	2.661	0.142	1.119	0.278	2.398	0.417	-	-
SKHT2	2.944	0.571	1.400	0.700	2.538	0.698	0.560	-
UTTN	2.798	0.993	1.673	0.976	1.812	0.836	1.256	1.256
KC1	2.659	0.426	1.406	0.560	2.382	0.419	0.559	0.840
KC2	5.897	3.682	3.925	3.626	4.509	3.765	3.908	4.341
KC3	2.381	0.142	0.977	0.141	2.111	0.280	0.419	0.699
PCHBR	3.078	0.850	0.701	0.836	2.390	0.975	0.697	1.260
PJKRK	2.661	0.284	0.978	0.418	2.248	0.557	0.418	0.839
RB1	2.525	0.284	1.259	0.418	2.394	0.417	0.418	0.838
RB2	3.083	0.569	1.543	0.698	2.686	0.837	0.558	0.839
RB3	2.384	0.000	0.841	0.000	2.115	0.139	0.278	0.700
Tak	2.655	0.285	1.265	0.419	1.962	0.559	0.419	0.843
CHYP	2.664	0.142	0.838	0.278	2.395	0.417	0.278	0.701
NKRSM	2.677	0.281	0.980	0.278	2.403	0.417	0.556	0.698
SKNK	2.943	0.857	1.126	0.982	1.956	1.122	0.983	1.410
CHPn	3.943	1.739	2.114	1.851	3.534	1.846	1.850	1.978
SRTN	2.795	0.426	1.119	0.557	2.395	0.696	0.557	0.979
P. lobata	3.394	0.992	1.557	0.982	3.126	1.122	1.123	1.553

Table 4.13. (continued)

Cultivar	UTTN	KC1	KC2	KC3	PCHBR	PJKRK	RB1	RB2
UTTN	-	-	-	-	-	-	-	-
KC1	0.557	-	-	-	-	-	-	-
KC2	4.329	4.068	-	-	-	-	-	-
KC3	1.114	0.842	3.776	-	-	-	-	-
PCHBR	1.395	1.262	4.048	0.697	-	-	-	-
PJKRK	0.976	0.560	3.767	0.278	0.697	-	-	-
RB1	1.116	0.559	4.047	0.278	0.976	0.279	-	-
RB2	1.677	0.700	4.338	0.840	1.258	0.696	0.838	-
RB3	0.976	0.560	3.626	0.141	0.836	0.418	0.418	0.698
Tak	0.419	0.418	3.925	0.701	1.121	0.421	0.561	0.701
CHYP	1.254	0.560	3.906	0.419	1.116	0.419	0.419	0.698
NKRSM	1.255	0.839	3.914	0.282	1.114	0.696	0.696	0.975
SKNK	0.975	0.978	4.075	0.985	0.841	0.843	0.985	1.268
CHPn	2.390	1.985	5.378	1.975	2.409	1.986	1.986	2.128
SRTN	1.533	0.839	4.046	0.557	1.253	0.695	0.697	0.836
P. lobata	1.970	1.417	4.656	1.128	1.829	1.268	1.268	1.411

Cultivar	RB3	Tak	CHYP	NKRSM	SKNK	CHPn	SRTN	Lobata
RB3	-	-	-	-	-	-	-	-
Tak	0.419	-	-	-	-	-	-	-
CHYP	0.278	0.419	-	-	-	-	-	-
NKRSM	0.278	0.697	0.556	-	-	-	-	-
SKNK	0.982	0.558	0.982	1.260	-	-	-	-
CHPn	1.851	1.993	1.850	1.989	2.278	-	-	-
SRTN	0.557	0.701	0.557	0.834	0.984	1.422	-	-
P. lobata	0.982	1.273	1.125	0.985	1.847	2.447	1.126	-

4.2.4 Random Amplified Polymorphic DNA (RAPD) patterns

Five RAPD primers i.e. OPA-07, OPA-12, OPD-02, OPD-16 and OPE-01, were randomly chosen for this study. RAPD patterns showed that there were polymorphic in collected cultivars. DNA of 6 cultivars could not be amplified DNA by any primer. They are CM2 (Chiang Dao, Chiang Mai), Nan, P3 (Wang Chin, Phrae), LBR (Lopburi), NKSW (Nakhon Sawan), and PSNL (Phitsanulok). Each of 5 RAPD primers was used to amplify all cultivars. Patterns of amplification were displayed by agarose gel electrophoresis. Size of amplified bands was estimated by comparing to 100 bp ladder Marker (Figure 4.28-4.32). The pattern of each primer performance was rather clearly different. This indicates the polymorphic in samples. Most of results indicated that DNA of *P. lobata* and some cultivars of *P. mirifica* was hardly been amplified by the selected RAPD primers. The size range of amplified bands by each primer was between 250 bp and 1.5 kb. In each cultivar, a result was recorded and analyzed whether a band was present or absent. If a band was present, it was recorded as 1. If it was absent, it was noted as 0. The binary information was used for further analysis. Summary of RAPD results from all 5 RAPD primers on 39 *P. mirifica* cultivars and *P. lobata* (outgroup) were shown (Table 4.14).

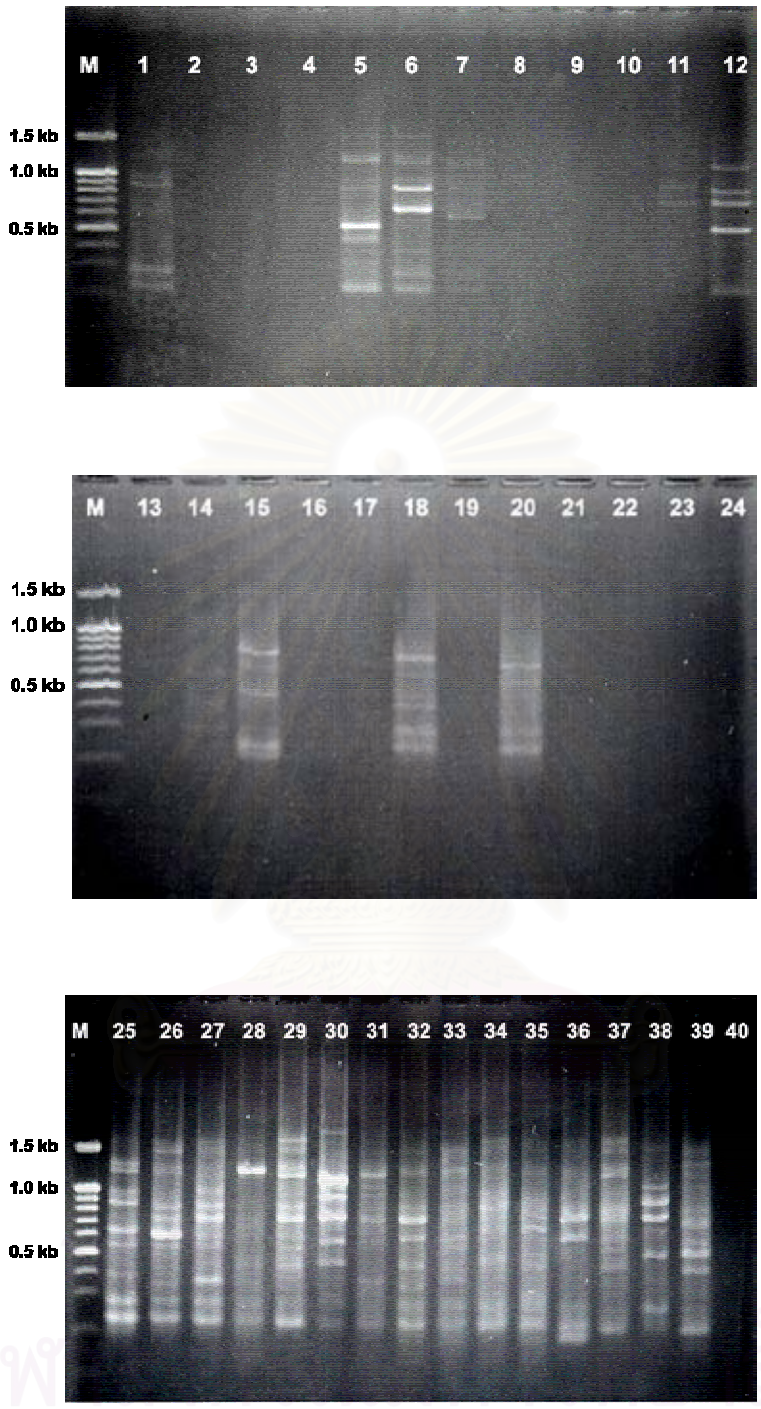

Figure 4.28. RAPD patterns amplified by OPA-07 primer on 2.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-39 contain RAPD products of 39 cultivars of *P. mirifica* in Thailand and lane 40 represents RAPD product of *P. lobata* as an outgroup.

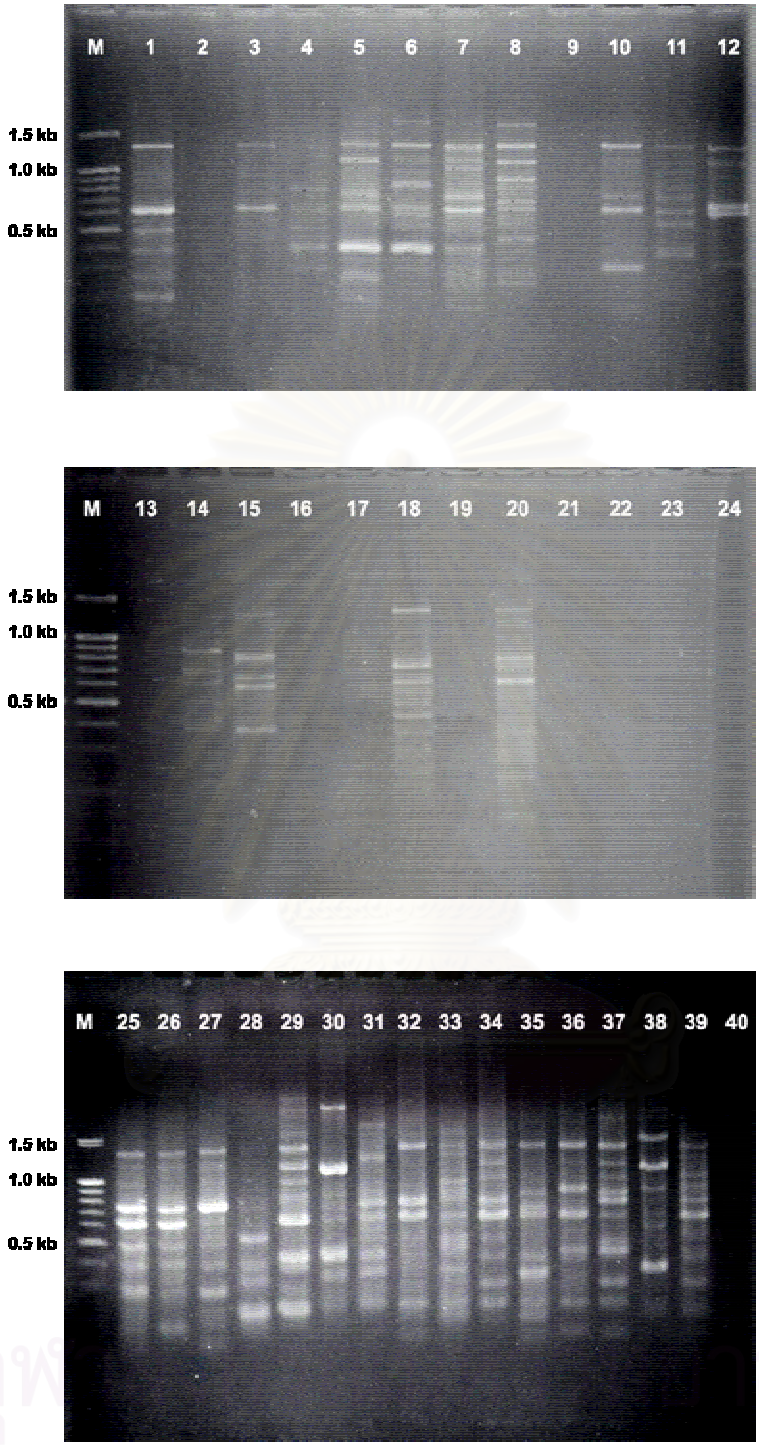

Figure 4.29. RAPD patterns amplified by OPA-12 primer on 2.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-39 contain RAPD products of 39 cultivars of *P. mirifica* in Thailand and lane 40 represents RAPD product of *P. lobata* as an outgroup.

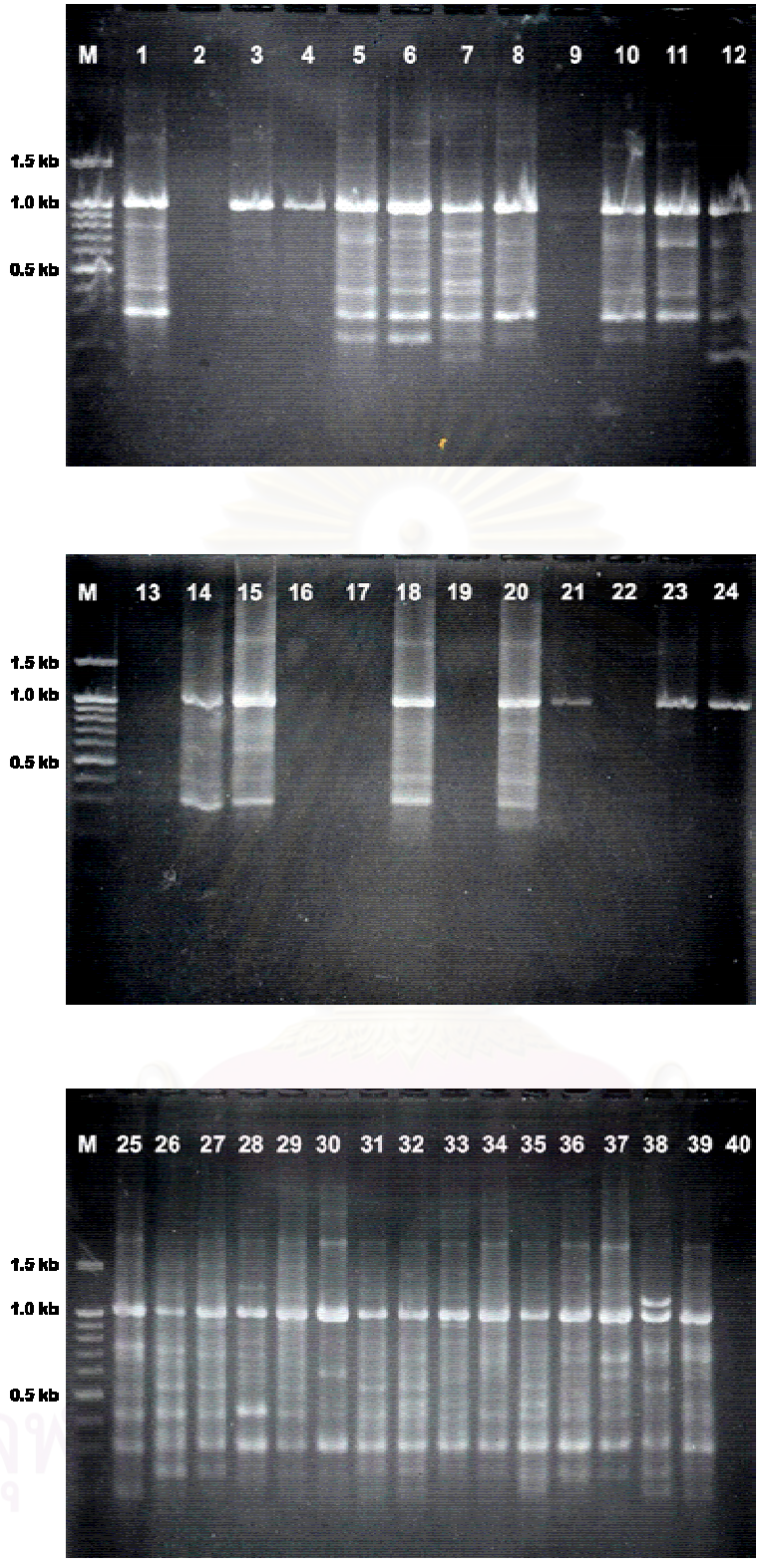

Figure 4.30. RAPD patterns amplified by OPD-02 primer on 2.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-39 contain RAPD products of 39 cultivars of *P. mirifica* in Thailand and lane 40 represents RAPD product of *P. lobata* as an outgroup.

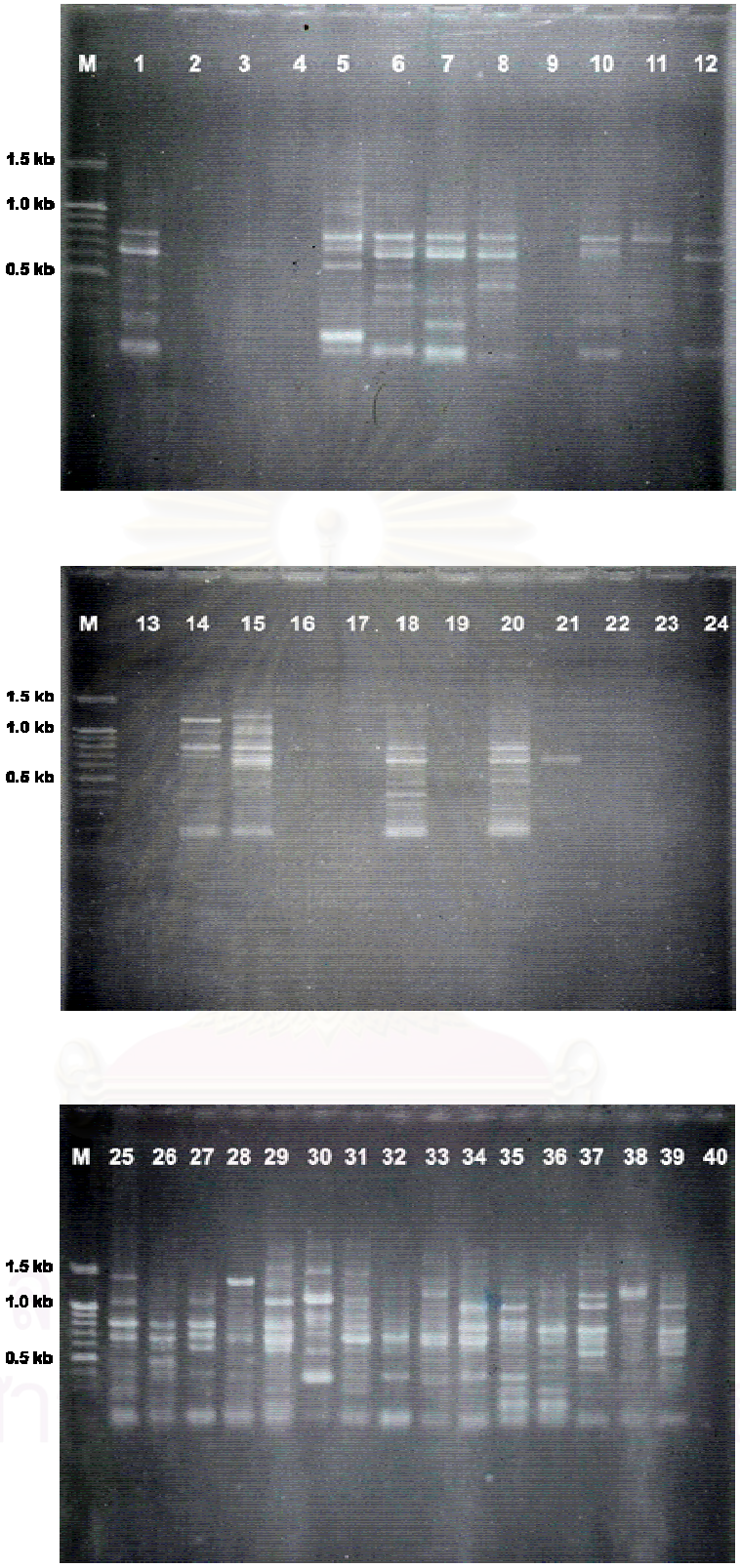

Figure 4.31. RAPD patterns amplified by OPD-16 primer on 2.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-39 contain RAPD products of 39 cultivars of *P. mirifica* in Thailand and lane 40 represents RAPD product of *P. lobata* as an outgroup.

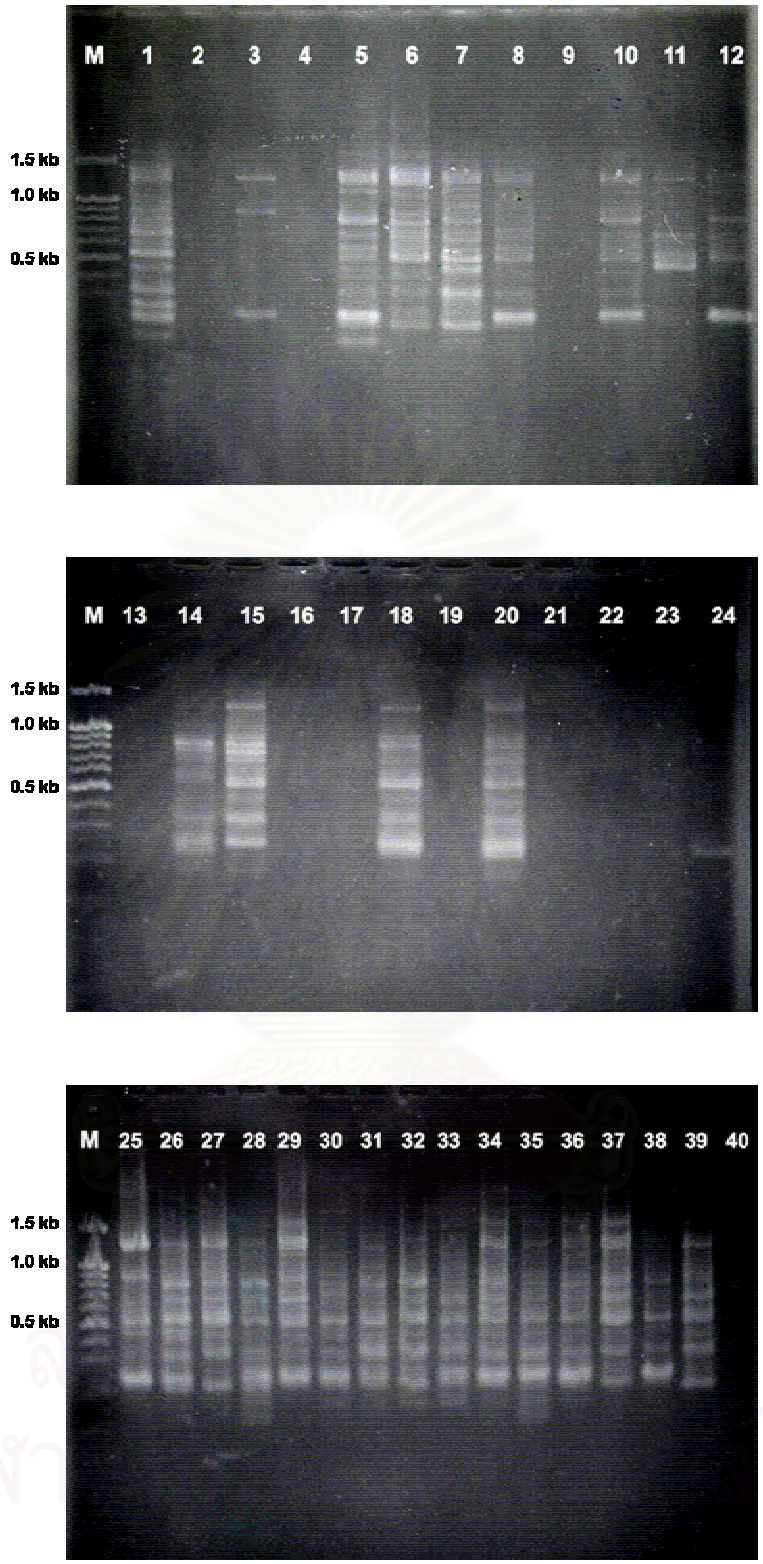

Figure 4.32. RAPD patterns amplified by OPE-01 primer on 2.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-39 contain RAPD products of 39 cultivars of *P. mirifica* in Thailand and lane 40 represents RAPD product of *P. lobata* as an outgroup.

Table 4.14. Summary of RAPD results of *P. mirifica* in Thailand and *P. lobata* as outgroup by all 5 RAPD primers. The √ symbol represents to a sample that was generated band by agarose gel electrophoresis and was used for RAPD analysis.

Primer / Cultivar	OPA-07	OPA-12	OPD-02	OPD-16	OPE-01
CM1	√	√	√	√	√
CM2					
CM3		√	√	√	√
CM4		√	√		
CR	√	√	√	√	√
LPang	√	√	√	√	√
MHS	√	√	√	√	√
LPoon		√	√	√	√
Nan					
PY		√	√	√	√
P1	√	√	√	√	√
P2	√	√	√	√	√
P3					
UTRD		√	√	√	√
KPP	√	√	√	√	√
LBR					
NKSW					
PBoon	√	√	√	√	√
PSNL					
SR1	√	√	√	√	√
SR2			√	√	√
SKHT1			√		
SKHT2			√		
UTTN		√	√		
KC1	√	√	√	√	√
KC2	√	√	√	√	√
KC3	√	√	√	√	√
PCHBR	√	√	√	√	√
PJKRK	√	√	√	√	√
RB1	√	√	√	√	√
RB2	√	√	√	√	√
RB3	√	√	√	√	√
RB4	√	√	√	√	√
Tak	√	√	√	√	√
CHYP	√	√	√	√	√
NKRSM	√	√	√	√	√
SKNK	√	√	√	√	√
CHPn	√	√	√	√	√
SRTN	√	√	√	√	√
P. lobata			√		
Total	**24**	**30**	**34**	**29**	**29**

Total of 93 RAPD fragments or bands by all 5 primers (OPA-07, OPA-12, OPD-02, OPD-16 and OPE-01) were consistently generated. Both monomorphic and polymorphic fragments were displayed in Table 4.15. The OPA-07 primer presented RAPD bands ranging from 250 bp to 1.5 kb. It provided total of 21 consistent and reproducible bands. Also those bands are polymorphic. The OPA-12 primer generated bands ranging from 250 bp to 1.5 kb. Also, it presented total of 20 reproducible bands that composed of 20 polymorphic bands. The OPD-02 primer produced 17 RAPD bands ranging from 250 bp to 1.3 kb and also provided 17 polymorphic bands. The OPD-16 primer generated 20 bands ranging from 250 bp to 1.5 kb and presented 20 polymorphic bands. The OPE-01 primer displayed 15 bands ranging from 250 to 1.3 kb and also provided 15 polymorphic bands. Considering amplified products from all 5 primers, all obtained bands were polymorphic. It indicated that the selected primers were useful in determining the genetic variation and the relationships among cultivars.

Table 4.15. Total numbers of RAPD bands or fragments by 5 RAPD primers. Monomorphic and polymorphic bands of 39 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup.

RAPD primer	Number of total band	Number of monomorphic band	Number of polymorphic band
OPA-07	21	0 (0%)	21 (100%)
OPA-12	20	0 (0%)	20 (100%)
OPD-02	17	0 (0%)	17 (100%)
OPD-16	20	0 (0%)	20 (100%)
OPE-01	15	0 (0%)	15 (100%)
Total	93	0 (0%)	93 (100%)

4.2.5 RAPD analysis

All RAPD products of 39 cultivars of *P. mirifica* in Thailand and 1 cultivar of *P. lobata* as an outgroup were analyzed. Genetic distances (pairwise distances based on Nei-Li distance in PAUP program) which were further used in NJ phylogenetic construction were listed in Table 4.16. The distances were varied from 0 to 0.4381 (Mean = 0.1861). They reveal that the 5 primers used in this study could provide moderate information in the genetic variation of *P. mirifica*.

Table 4.16. The average genetic distances (pairwise distances between taxa) of 39 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup. The data was revealed by RAPD and then calculated by PAUP.

Cultivar	CM1	CM2	CM3	CM4	CR	LPang	MHS	LPoon	Nan
CM1	-	-	-	-	-	-	-	-	-
CM2	0	-	-	-	-	-	-	-	-
CM3	0.2143	0	-	-	-	-	-	-	-
CM4	0.1429	0	0.0952	-	-	-	-	-	-
CR	0.2571	0	0.2619	0.2619	-	-	-	-	-
LPang	0.1905	0	0.2024	0.1429	0.2	-	-	-	-
MHS	0.1714	0	0.2619	0.2619	0.2381	0.2095	-	-	-
LPoon	0.1786	0	0.2024	0.2381	0.25	0.1905	0.1786	-	-
Nan	0	0	0	0	0	0	0	0	-
PY	0.2024	0	0.131	0.1667	0.2262	0.1429	0.1786	0.1429	0
P1	0.2286	0	0.1548	0.1905	0.2191	0.2476	0.2286	0.1905	0
P2	0.2286	0	0.1429	0.2143	0.2	0.1714	0.2286	0.131	0
P3	0	0	0	0	0	0	0	0	0
UTRD	0.2738	0	0.2976	0.4048	0.2976	0.381	0.3214	0.2857	0
KPP	0.2762	0	0.2738	0.3095	0.3238	0.2952	0.3524	0.2857	0
LBR	0	0	0	0	0	0	0	0	0
NKSW	0	0	0	0	0	0	0	0	0
PBoon	0.2	0	0.2143	0.2381	0.3048	0.2571	0.2381	0.1786	0
PSNL	0	0	0	0	0	0	0	0	0
SR1	0.2476	0	0.2024	0.2857	0.2571	0.2667	0.1905	0.1905	0
SR2	0.2381	0	0.0635	0	0.3175	0.2698	0.254	0.1746	0
SKHT1	0.0476	0	0	0	0.2381	0.0952	0.2381	0.0952	0
SKHT2	0.0476	0	0	0	0.2381	0.0952	0.2381	0.0952	0
UTTN	0.1191	0	0.0714	0.1191	0.2381	0.2143	0.2857	0.2143	0
KC1	0.2667	0	0.25	0.2619	0.3143	0.2667	0.3048	0.3333	0
KC2	0.3143	0	0.2857	0.3095	0.3429	0.2381	0.2952	0.2976	0
KC3	0.3429	0	0.2738	0.2619	0.2952	0.2857	0.3238	0.3095	0
PCHBR	0.3143	0	0.2857	0.381	0.2857	0.3143	0.3333	0.3452	0
PJKRK	0.3429	0	0.2738	0.3333	0.2952	0.2857	0.3048	0.2619	0
RB1	0.3619	0	0.2976	0.3095	0.3333	0.3619	0.3238	0.2619	0
RB2	0.3905	0	0.3214	0.4286	0.3238	0.3524	0.3143	0.3333	0
RB3	0.3238	0	0.2381	0.4048	0.2191	0.2667	0.2857	0.25	0
RB4	0.4	0	0.2619	0.381	0.3143	0.3048	0.3238	0.3214	0
Tak	0.3048	0	0.25	0.3333	0.2762	0.2667	0.2476	0.2143	0
CHYP	0.3048	0	0.2857	0.4286	0.2571	0.2857	0.3238	0.25	0
NKRSM	0.2762	0	0.2857	0.4286	0.2286	0.2381	0.3143	0.2262	0
SKNK	0.3905	0	0.3214	0.4048	0.3238	0.2952	0.3333	0.2381	0
CHPn	0.3143	0	0.2857	0.2619	0.2857	0.2762	0.3714	0.2976	0
SRTN	0.4	0	0.2619	0.4048	0.3333	0.3048	0.3429	0.25	0
P. lobata	0.0476	0	0	0	0.2381	0.0952	0.2381	0.0952	0

Table 4.16. (continued)

Cultivar	PY	P1	P2	P3	UTRD	KPP	LBR	NKSW	PBoon
CM1	-	-	-	-	-	-	-	-	-
CM2	-	-	-	-	-	-	-	-	-
CM3	-	-	-	-	-	-	-	-	-
CM4	-	-	-	-	-	-	-	-	-
CR	-	-	-	-	-	-	-	-	-
LPang	-	-	-	-	-	-	-	-	-
MHS	-	-	-	-	-	-	-	-	-
LPoon	-	-	-	-	-	-	-	-	-
Nan	-	-	-	-	-	-	-	-	-
PY	-	-	-	-	-	-	-	-	-
P1	0.1667	-	-	-	-	-	-	-	-
P2	0.0833	0.2095	-	-	-	-	-	-	-
P3	0	0	0	-	-	-	-	-	-
UTRD	0.2619	0.2857	0.2976	0	-	-	-	-	-
KPP	0.3095	0.2762	0.3333	0	0.2143	-	-	-	-
LBR	0	0	0	0	0	0	-	-	-
NKSW	0	0	0	0	0	0	0	-	-
PBoon	0.2262	0.2	0.2762	0	0.2738	0.2286	0	0	-
PSNL	0	0	0	0	0	0	0	0	0
SR1	0.2143	0.1905	0.2286	0	0.2381	0.2381	0	0	0.1619
SR2	0.1905	0.1587	0.2064	0	0.2222	0.2857	0	0	0.1905
SKHT1	0.0952	0.1429	0.1905	0	0.1905	0.1905	0	0	0.0952
SKHT2	0.0952	0.1429	0.1905	0	0.1905	0.1905	0	0	0.0952
UTTN	0.1905	0.2143	0.2381	0	0.3333	0.2381	0	0	0.2143
KC1	0.2619	0.3048	0.2667	0	0.3571	0.3524	0	0	0.2952
KC2	0.25	0.2952	0.2952	0	0.3214	0.3429	0	0	0.2857
KC3	0.2381	0.2476	0.2857	0	0.2857	0.2762	0	0	0.2952
PCHBR	0.3452	0.2571	0.3524	0	0.3214	0.2476	0	0	0.2857
PJKRK	0.2619	0.3238	0.3048	0	0.3333	0.2952	0	0	0.3333
RB1	0.3095	0.3238	0.3429	0	0.3095	0.2952	0	0	0.2762
RB2	0.3333	0.2762	0.3333	0	0.3095	0.2476	0	0	0.2667
RB3	0.2262	0.2667	0.2476	0	0.2738	0.2762	0	0	0.2381
RB4	0.2976	0.3048	0.3048	0	0.3452	0.3524	0	0	0.2571
Tak	0.1905	0.2286	0.2857	0	0.2619	0.2571	0	0	0.2571
CHYP	0.2738	0.2667	0.2667	0	0.25	0.2571	0	0	0.2571
NKRSM	0.25	0.2571	0.2191	0	0.2976	0.2667	0	0	0.2476
SKNK	0.2857	0.2952	0.3143	0	0.3095	0.3048	0	0	0.3238
CHPn	0.2738	0.2571	0.2571	0	0.3214	0.3238	0	0	0.3048
SRTN	0.2262	0.2667	0.2857	0	0.2976	0.3143	0	0	0.3143
P. lobata	0.0952	0.1429	0.1905	0	0.1905	0.1905	0	0	0.0952

Table 4.16. (continued)

Cultivar	PSNL	SR1	SR2	SKHT1	SKHT2	UTTN	KC1	KC2	KC3
CM1	-	-	-	-	-	-	-	-	-
CM2	-	-	-	-	-	-	-	-	-
CM3	-	-	-	-	-	-	-	-	-
CM4	-	-	-	-	-	-	-	-	-
CR	-	-	-	-	-	-	-	-	-
LPang	-	-	-	-	-	-	-	-	-
MHS	-	-	-	-	-	-	-	-	-
LPoon	-	-	-	-	-	-	-	-	-
Nan	-	-	-	-	-	-	-	-	-
PY	-	-	-	-	-	-	-	-	-
P1	-	-	-	-	-	-	-	-	-
P2	-	-	-	-	-	-	-	-	-
P3	-	-	-	-	-	-	-	-	-
UTRD	-	-	-	-	-	-	-	-	-
KPP	-	-	-	-	-	-	-	-	-
LBR	-	-	-	-	-	-	-	-	-
NKSW	-	-	-	-	-	-	-	-	-
PBoon	-	-	-	-	-	-	-	-	-
PSNL	-	-	-	-	-	-	-	-	-
SR1	0	-	-	-	-	-	-	-	-
SR2	0	0.1905	-	-	-	-	-	-	-
SKHT1	0	0.2381	0	-	-	-	-	-	-
SKHT2	0	0.2381	0	0	-	-	-	-	-
UTTN	0	0.2619	0	0	0	-	-	-	-
KC1	0	0.3048	0.3175	0.2857	0.2857	0.3333	-	-	-
KC2	0	0.2952	0.3175	0.3333	0.3333	0.381	0.2571	-	-
KC3	0	0.2857	0.3333	0.3333	0.3333	0.3333	0.2667	0.1619	-
PCHBR	0	0.2571	0.3175	0.381	0.381	0.3095	0.3333	0.3238	0.2571
PJKRK	0	0.3429	0.2857	0.2381	0.2381	0.3095	0.2857	0.2952	0.2667
RB1	0	0.2857	0.254	0.1905	0.1905	0.1905	0.4381	0.3905	0.3429
RB2	0	0.2381	0.3333	0.381	0.381	0.3095	0.3714	0.3048	0.2762
RB3	0	0.2286	0.254	0.3333	0.3333	0.2857	0.3619	0.2191	0.2286
RB4	0	0.2667	0.254	0.2857	0.2857	0.3571	0.3429	0.3333	0.3619
Tak	0	0.2286	0.2222	0.1429	0.1429	0.2619	0.3429	0.3143	0.2857
CHYP	0	0.2286	0.3016	0.3333	0.3333	0.3095	0.3429	0.2762	0.2857
NKRSM	0	0.2381	0.3175	0.381	0.381	0.3095	0.3143	0.2476	0.2762
SKNK	0	0.2381	0.2698	0.2381	0.2381	0.381	0.3524	0.3048	0.2952
CHPn	0	0.2762	0.3016	0.2857	0.2857	0.2857	0.3524	0.3048	0.2762
SRTN	0	0.2286	0.2698	0.2857	0.2857	0.381	0.3429	0.3143	0.3048
P. lobata	0	0.2381	0	0	0	0	0.2857	0.3333	0.3333

Table 4.16. (continued)

Cultivar	PCHBR	PJKRK	RB1	RB2	RB3	RB4	Tak	CHYP	NKRSM
CM1	-	-	-	-	-	-	-	-	-
CM2	-	-	-	-	-	-	-	-	-
CM3	-	-	-	-	-	-	-	-	-
CM4	-	-	-	-	-	-	-	-	-
CR	-	-	-	-	-	-	-	-	-
LPang	-	-	-	-	-	-	-	-	-
MHS	-	-	-	-	-	-	-	-	-
LPoon	-	-	-	-	-	-	-	-	-
Nan	-	-	-	-	-	-	-	-	-
PY	-	-	-	-	-	-	-	-	-
P1	-	-	-	-	-	-	-	-	-
P2	-	-	-	-	-	-	-	-	-
P3	-	-	-	-	-	-	-	-	-
UTRD	-	-	-	-	-	-	-	-	-
KPP	-	-	-	-	-	-	-	-	-
LBR	-	-	-	-	-	-	-	-	-
NKSW	-	-	-	-	-	-	-	-	-
PBoon	-	-	-	-	-	-	-	-	-
PSNL	-	-	-	-	-	-	-	-	-
SR1	-	-	-	-	-	-	-	-	-
SR2	-	-	-	-	-	-	-	-	-
SKHT1	-	-	-	-	-	-	-	-	-
SKHT2	-	-	-	-	-	-	-	-	-
UTTN	-	-	-	-	-	-	-	-	-
KC1	-	-	-	-	-	-	-	-	-
KC2	-	-	-	-	-	-	-	-	-
KC3	-	-	-	-	-	-	-	-	-
PCHBR	-	-	-	-	-	-	-	-	-
PJKRK	0.2762	-	-	-	-	-	-	-	-
RB1	0.2762	0.2667	-	-	-	-	-	-	-
RB2	0.2476	0.2762	0.2381	-	-	-	-	-	-
RB3	0.2571	0.2476	0.2667	0.181	-	-	-	-	-
RB4	0.2571	0.2857	0.2286	0.2571	0.2286	-	-	-	-
Tak	0.2381	0.2286	0.2476	0.2571	0.2095	0.2286	-	-	-
CHYP	0.2191	0.2667	0.2857	0.2	0.1714	0.2286	0.2095	-	-
NKRSM	0.2476	0.2191	0.2571	0.2095	0.1619	0.2381	0.2	0.0857	-
SKNK	0.2857	0.2762	0.3524	0.2476	0.2762	0.2571	0.2	0.2191	0.2286
CHPn	0.3238	0.3714	0.3143	0.3048	0.3524	0.3333	0.3333	0.2571	0.2095
SRTN	0.2762	0.2286	0.3429	0.2571	0.2476	0.2857	0.1905	0.2476	0.2381
P. lobata	0.381	0.2381	0.1905	0.381	0.3333	0.2857	0.1429	0.3333	0.381

Table 4.16. (continued)

Cultivar	SKNK	CHPn	SRTN	*P. lobata*
CM1	-	-	-	-
CM2	-	-	-	-
CM3	-	-	-	-
CM4	-	-	-	-
CR	-	-	-	-
LPang	-	-	-	-
MHS	-	-	-	-
LPoon	-	-	-	-
Nan	-	-	-	-
PY	-	-	-	-
P1	-	-	-	-
P2	-	-	-	-
P3	-	-	-	-
UTRD	-	-	-	-
KPP	-	-	-	-
LBR	-	-	-	-
NKSW	-	-	-	-
PBoon	-	-	-	-
PSNL	-	-	-	-
SR1	-	-	-	-
SR2	-	-	-	-
SKHT1	-	-	-	-
SKHT2	-	-	-	-
UTTN	-	-	-	-
KC1	-	-	-	-
KC2	-	-	-	-
KC3	-	-	-	-
PCHBR	-	-	-	-
PJKRK	-	-	-	-
RB1	-	-	-	-
RB2	-	-	-	-
RB3	-	-	-	-
RB4	-	-	-	-
Tak	-	-	-	-
CHYP	-	-	-	-
NKRSM	-	-	-	-
SKNK	-	-	-	-
CHPn	0.3238	-	-	-
SRTN	0.1619	0.3333	-	-
P. lobata	0.2381	0.2857	0.2857	-

4.2.6 Phylogenetic analysis

The sequences of PCR products of nrDNA ITS, cpDNA *trn*L and *trn*L-F of *P. mirifica* from many collected sites in Thailand and *P. lobata* from Japan were used for phylogenetic analysis. Genetic distances were calculated and created in PAUP. Phylogenetic trees were constructed by neighbor-joining method (NJ in PAUP). The Bootstrap supporting-values (in PAUP) are computated and displayed on nodes of the tree for confirming the strength of branch. Figure 4.33-4.36 presented 4 phylogenetic trees from 3 sequence regions and RAPD. *P. lobata* was used as an outgroup for all generated trees. According to all phylogenetic trees, their topologies were so different. Each group had many inconsistent branches (without Bootstrap values) and some strong branches (with Bootstrap values) that would be presented below and interpreted further.

Considering on an ITS tree, there are many minor groups of cultivars that were classified and had the bootstrap supports on their nodes. Subgroup 1, Lopburi and Nan cultivars were clustered by low bootstrap value (51). Subgroup 2, Mae Hong Son and Saraburi 1 (Phra Phutthabat district) cultivars were also paired (65). Subgroup 3, Chaiyaphum and Surat Thani cultivars were unfirmly paired (53). Cultivars of Lamphun and Phrae 3 (Wang Chin district) were firmly paired (93) and both cultivars were also grouped together with Phrae 2 (Song district) cultivar (52) in subgroup 4. At last, Kanchanaburi 2 (Sai Yok district) and Chumphon cultivars were grouped together (91) in subgroup 5.

A *trn*L phylogeny was divided into many minor groups. There was only one subgroup that had the bootstrap support on the node. In subgroup 1, two cultivars of Kanchanaburi 2 (Sai Yok district) and Chiang Mai 2 (Chiang Dao district) were packed with low bootstrap value (57).

A *trn*L-F tree was further illustrated by some supported branches. There was only one subgroup that had the bootstrap support on the node. In the subgroup 1, Saraburi 1 (Phra Phutthabat district) and Kanchanaburi 2 (Sai Yok district) cultivars were aggregated together with the high value (90).

RAPD phylogram was separated into many inconsistent branches but there are some supported branches. First, Chiang Mai 1 (Chai Prakan district) and Mae Hong Son cultivars were contained in subgroup 1 with low bootstrap value (50). Second, Kanchanaburi 2 (Sai Yok district) and Kanchanaburi 3 (Sai Yok district) cultivars were grouped (80) in subgroup 2. Considering subgroup 3, Sakon Nakhon and Surat

Thani cultivars were formed a pair (75). In subgroup 4, Chaiyaphum and Nakhon Ratchasima cultivars were also firmly clustered (85).

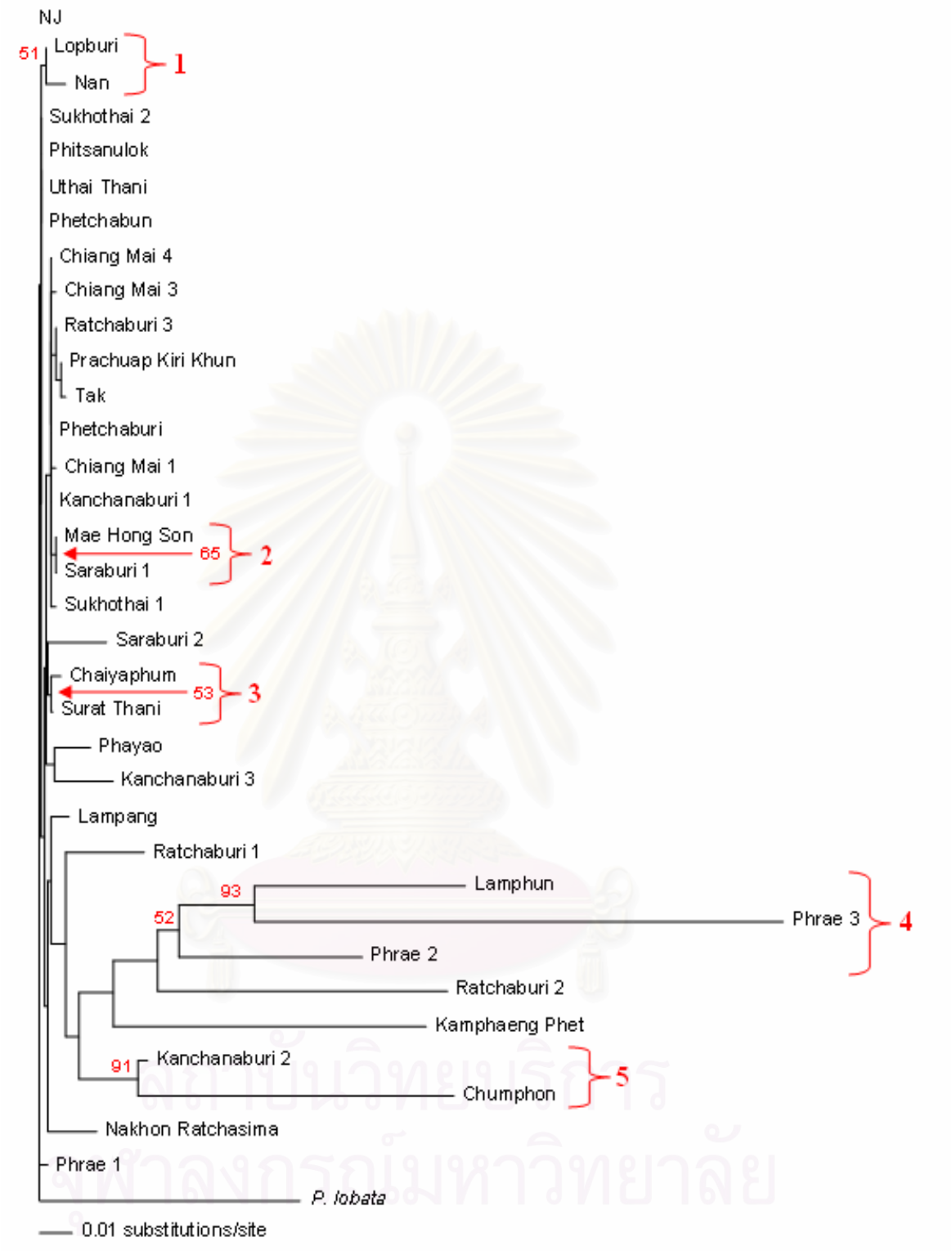

Figure 4.33. A rooted NJ phylogenetic tree or phylogram of ITS region (33 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup) based on K2P genetic distance. The Bootstrap supporting-values are shown on the branches of the phylogenetic tree.

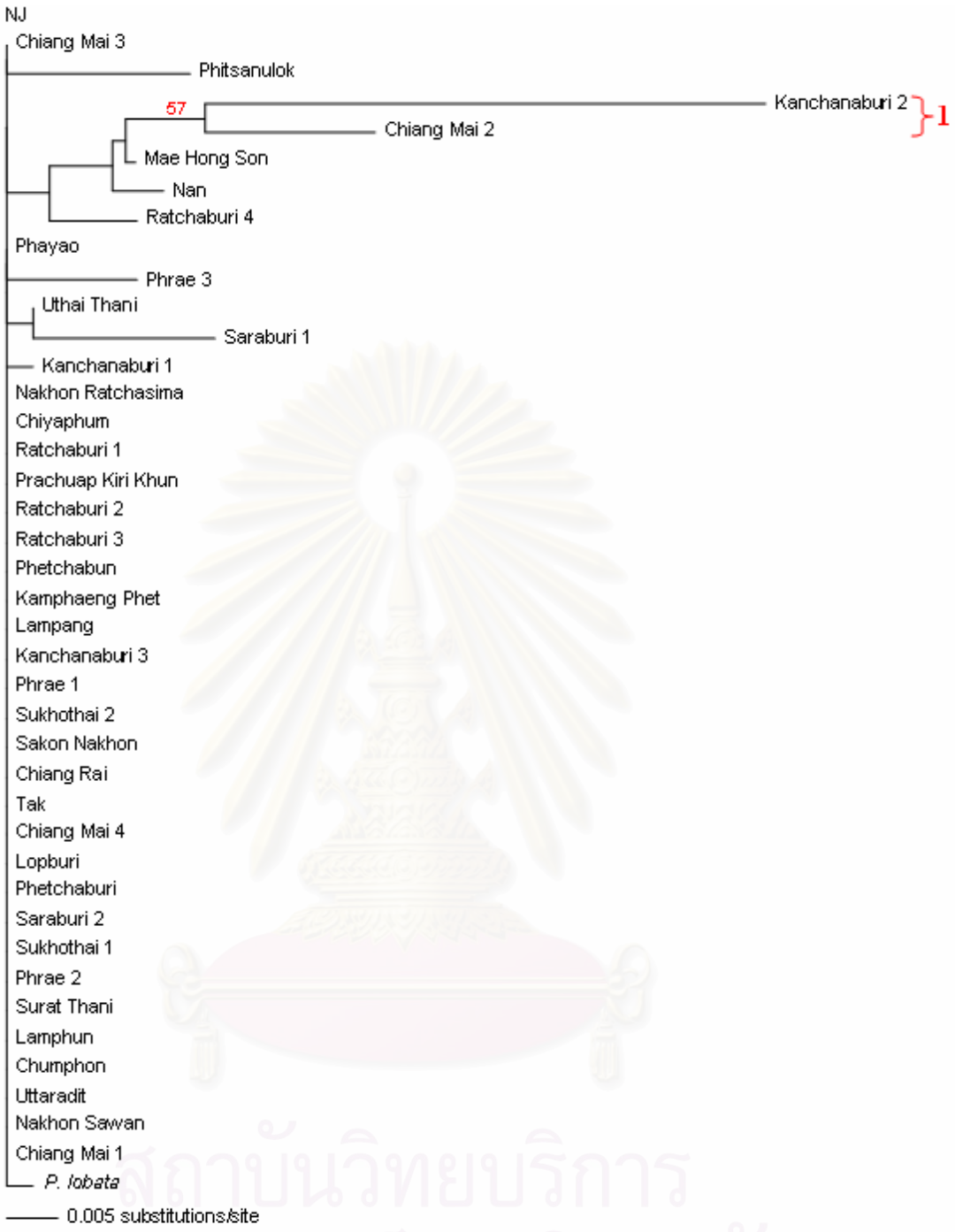

Figure 4.34. A rooted NJ phylogram of *trn*L region (39 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup) based on K2P genetic distance. The Bootstrap supporting-values are illustrated on the branches of tree.

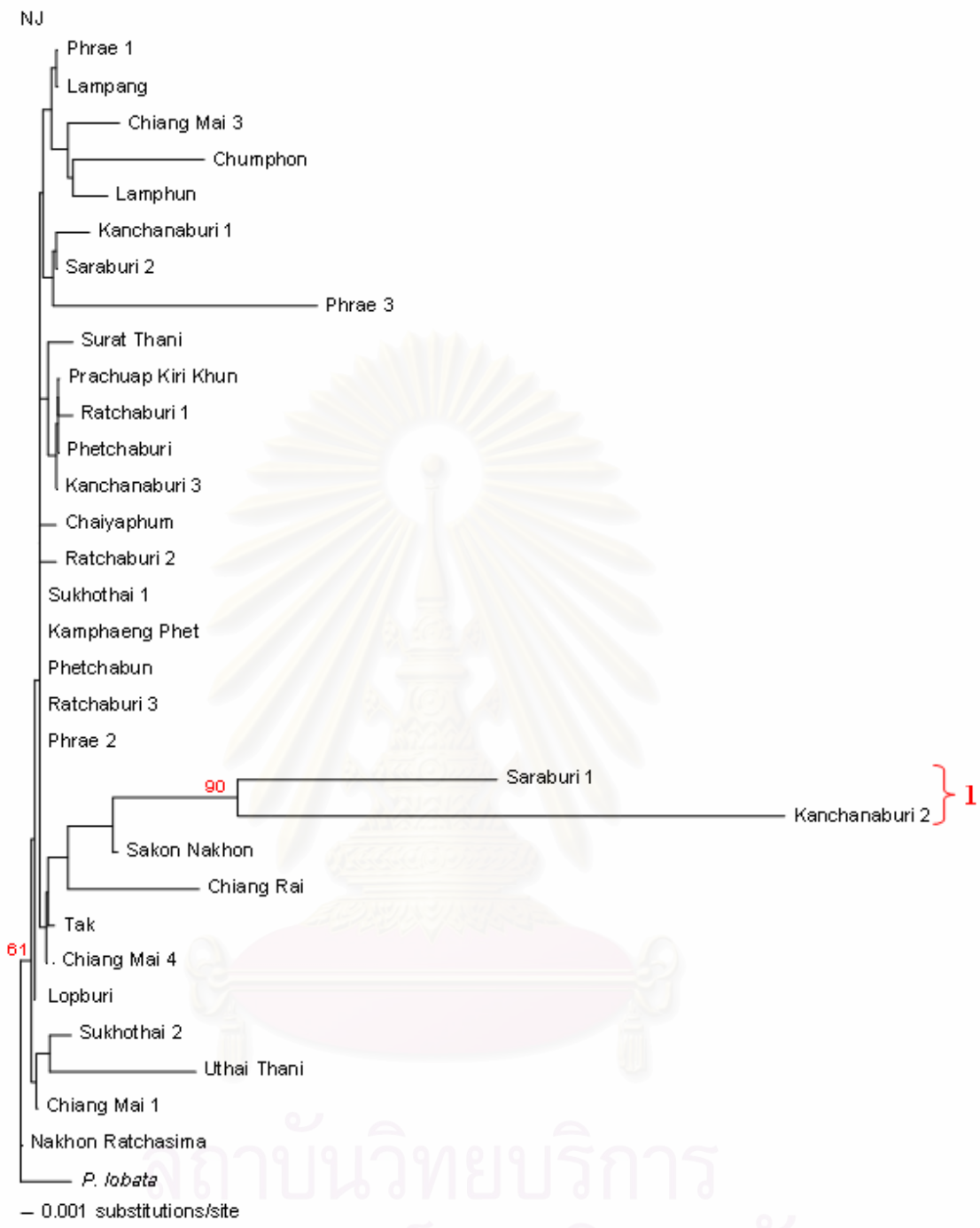

Figure 4.35. A rooted NJ phylogenetic tree of *trn*L-F region (31 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup) based on K2P genetic distance. The Bootstrap supporting-values are noted on the branches of tree.

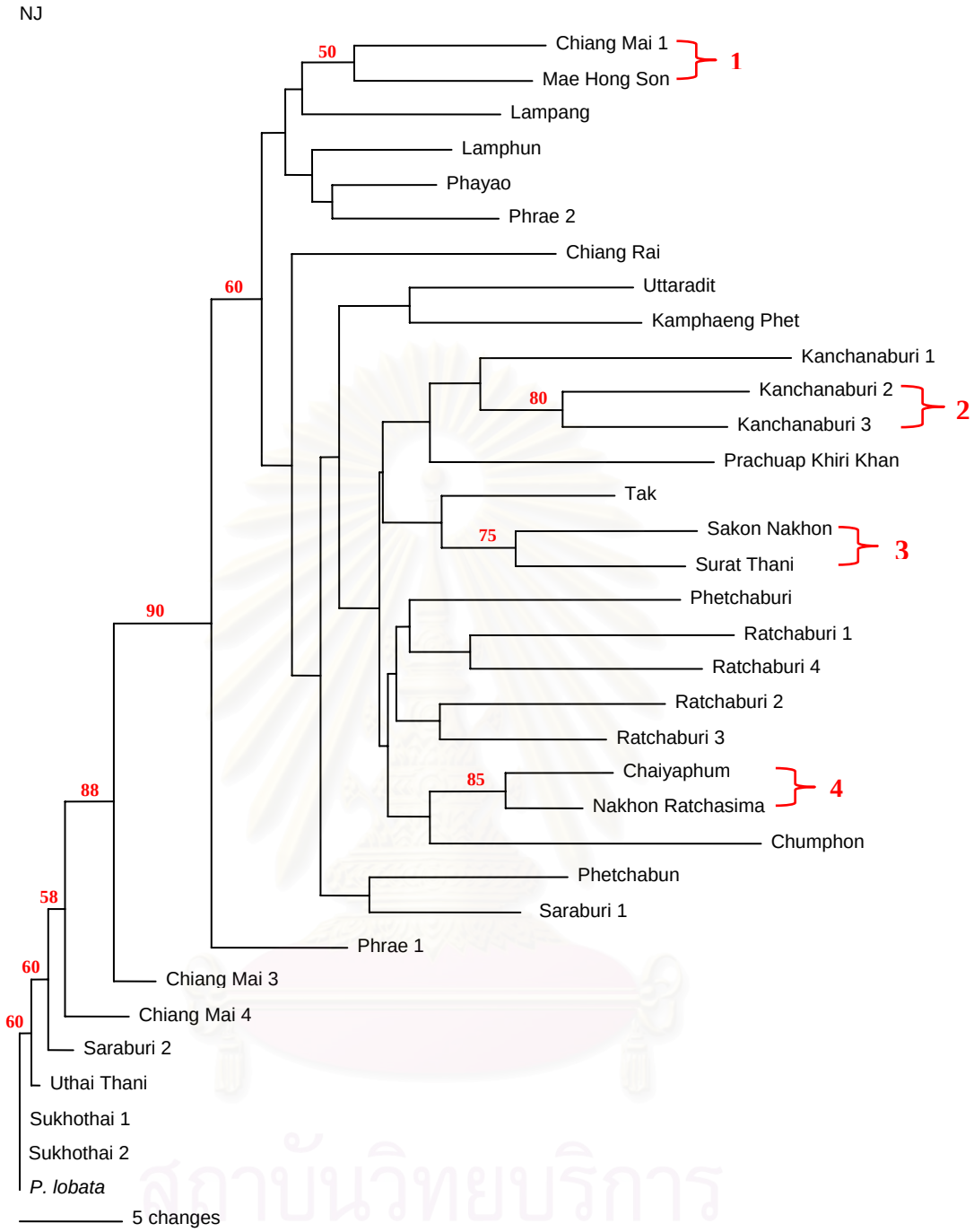

Figure 4.36. A rooted neighbor-joining (NJ) phylogenetic tree based on Nei-Li genetic distances among 33 cultivars of *P. mirifica* in Thailand and 1 cultivar of *P. lobata* as an outgroup derived from RAPD patterns. The Bootstrap supporting-values are above the braches.

4.3 Chemical content analysis

Root or tuber of *P. mirifica* is a storage organ of metabolic compound. Chemical constituents vary due to genetic and external factors. Recently, Cherdshewasart *et al.* (2007) presented the isoflavonoid contents in extracted powders of *P. mirifica* tuberous roots from many locations in Thailand and of *P. lobata* as an outgroup as listed in Table 4.17.

From these statistically analyzed data, we used them in our study in order to calculate and classify *P. mirifica* cultivars based on the chemical contents by using the PCA method of Factor analysis (for data reduction). Later, Cluster analysis (for grouping or classification) was used in order to analyze and compare to the morphometric and genetic results.

Table 4.17. Means of isoflavonoid and total contents (mg/100 g) of *P. mirifica* tuberous powders collected from 29 locations in Thailand in comparison with *P. lobata* (Cherdshewasart *et al.*, 2007).

No.	Abbrev.	Province	Puerarin	Daidzin	Genistin	Daidzein	Genistein	Total
1	CM1	Chiang Mai	16.13	8.01	13.92	3.42	0.79	42.26
2	CM2	Chiang Mai	10.96	12.37	20.99	4.33	1.34	49.98
3	CM3	Chiang Mai	28.36	28.7	58.38	4.05	1.1	120.6
4	CR	Chiang Rai	20.02	8.61	29.58	2.16	0.5	60.87
5	LPang	Lampang	20.85	15.28	29.03	6.27	2.54	73.97
6	MHS	Mae Hong Son	36.99	17.63	55.44	7.52	1.54	119.12
7	LPoon	Lamphun	33.18	28.35	84.13	8.59	0.76	155
8	Nan	Nan	5.32	2.36	7.62	3.31	0	18.61
9	PY	Phayao	12.91	8.46	32.43	3.03	0.73	57.56
10	P3	Phrae	25.2	10.55	30.61	5.45	1.34	73.16
11	UTRD	Uttaradit	30.25	13.69	10.27	7.88	0	62.96
12	KPP	Kamphaeng Phet	15.44	7.01	18.5	2.31	0.46	43.71
13	LBR	Lopburi	19.5	6.84	39.47	2.42	0.98	69.21
14	NKSW	Nakhon Sawan	13.34	16.28	27.71	4.7	0.72	62.75
15	PBoon	Phetchabun	9.4	10.48	15.54	8.11	1.29	44.83
16	PSNL	Phitsanulok	35.24	12.26	26.53	8.36	1.63	84.02
17	SR1	Saraburi	10.87	13.11	29.03	2.74	0.83	56.59
18	SR2	Saraburi	23.42	17.92	37.94	4.86	0.87	85.01
19	SKHT1	Sukhothai	14.12	25.09	51.43	11.16	0.73	102.52
20	UTTN	Uthai Thani	10.85	21.7	50.17	16.48	3.66	102.86
21	KC1	Kanchanaburi	8.33	4.01	13.69	4.01	0.62	30.67
22	PCHBR	Phetchaburi	13.19	20.82	37.56	6	1.13	78.71
23	PJKRK	Prachuap Khiri Khan	10.42	9.62	30.31	2.11	0.59	53.05
24	RB1	Ratchaburi	8.85	15.39	51.15	6.84	2.54	84.77
25	Tak	Tak	29.06	8.97	43.86	4.56	1.15	87.6
26	CHYP	Chaiyaphum	15.83	12.91	29.48	7.02	1.89	67.13
27	NKRSM	Nakhon Ratchasima	13.09	5.61	24.15	1.2	0.21	44.27
28	SKNK	Sakon Nakhon	87.05	11.48	14.83	4.78	1.42	119.57
29	CHPn	Chumphon	8.45	7.38	34.17	2.64	0.07	52.7
Total mean			**20.23**	**13.13**	**32.69**	**5.39**	**1.08**	**72.55**
30	*P. lobata*		32.85	21.9	25.63	10.34	0.81	91.57

4.3.1 Factor analysis of isoflavonoid contents

Factor analysis was conducted and showed the output. All 5 parameters contained Eigen values higher than 1.0. Those parameters were puerarin, daidzin, genistin, daidzein, and genistein. Factor analysis with the above 5 chemical parameters was performed. All were classified into 1 factor or group. The factor also contained parameters with Eigen values higher than 1.0 and counted for 51.6 % of total variance. A scatter plot of a factor against a factor could not be created because there was only one factor.

4.3.2 Cluster analysis of isoflavonoid contents

Only 1 new factor or group was used to computerize by Cluster analysis. By considering isoflavonoid contents, it could generate a dendrogram in order to classify *P. mirifica* cultivars in Thailand (Figure 4.37). The dendrogram suggested that all cultivars were classified into 2 main groups: the minor group containing 6 cultivars of Chiang Mai 3, Mae Hong Son, Ratchaburi 1, Sukhothai 1, Lamphun, and Uthai Thani; and the major group containing other 23 cultivars.

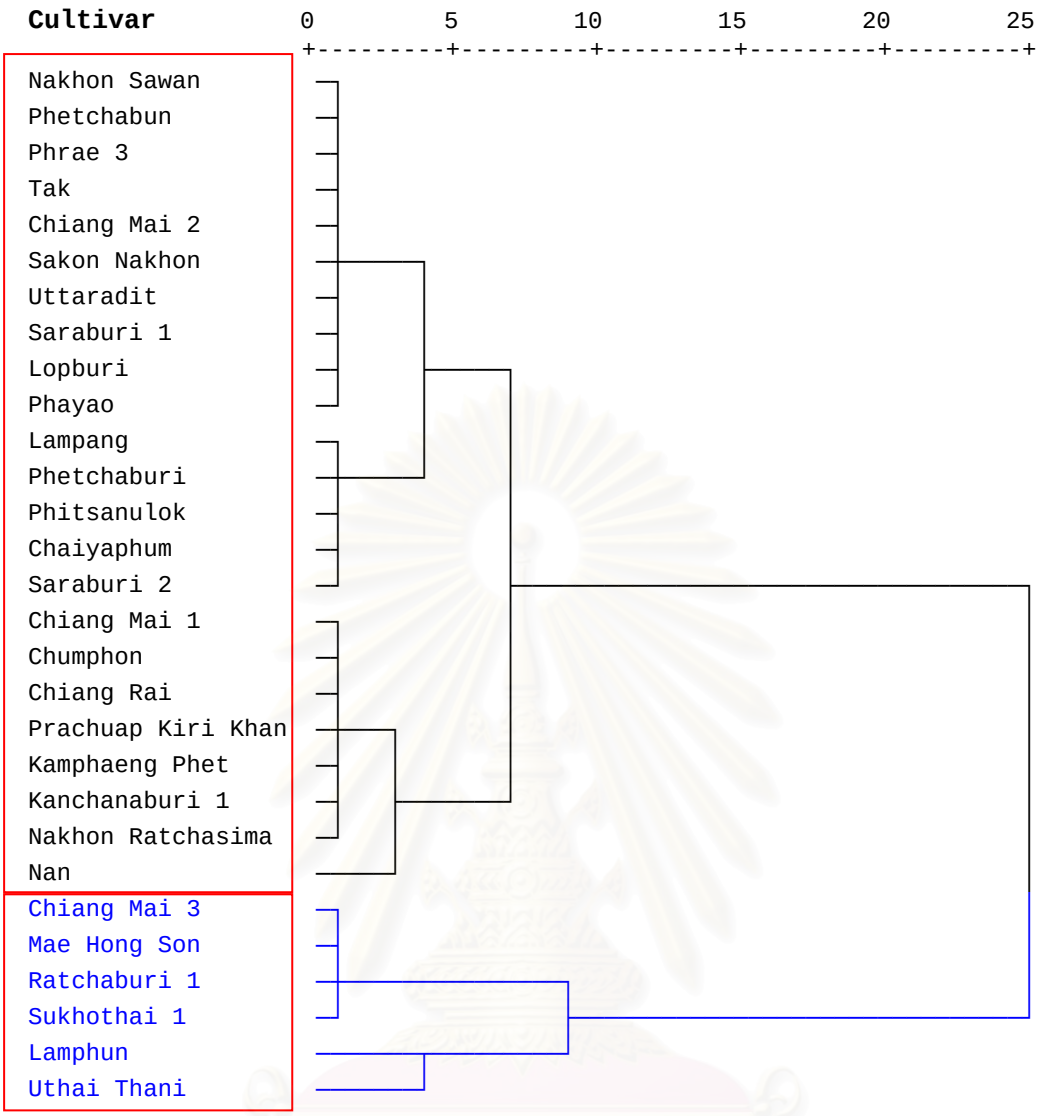

Figure 4.37. A dendrogram of isoflavonoid contents created by Between-groups linkage method of Cluster analysis. *P. mirifica* is classified by collected locations or cultivars.

4.3.3 The chemical characterization of *P. mirifica* in Thailand.

Isoflavonoid contents of *P. mirifica* cultivars in Thailand were conducted. For exploring the clinal patterns in the isoflavonoid-contented characterization of *P. mirifica*, the factor score (only factor 1) was plotted against latitude and longitude. The transitions of characters from the South to the North and the West to the East are indicated in the scatter plots (Figure 4.38-4.39).

Results of Correlation analysis of factor scores against latitude and longitude are summarized in Table 4.18. There was no statistically significant correlation ($P \geq 0.05$) between factor 1 (puerarin, daidzin, genistin, daidzein, and genistein parameters) and latitude & longitude. The result revealed that these chemicals were not significantly correlated to latitude and longitude.

Table 4.18. Correlation analysis of geographic trends in isoflavonoid contents of *P. mirifica* in Thailand derived by the PCA of Factor analysis.

Independent variable or predictor	Dependent variable	R value	P significance
Latitude	Factor 1	0.066	0.732
Longitude	Factor 1	- 0.179	0.353

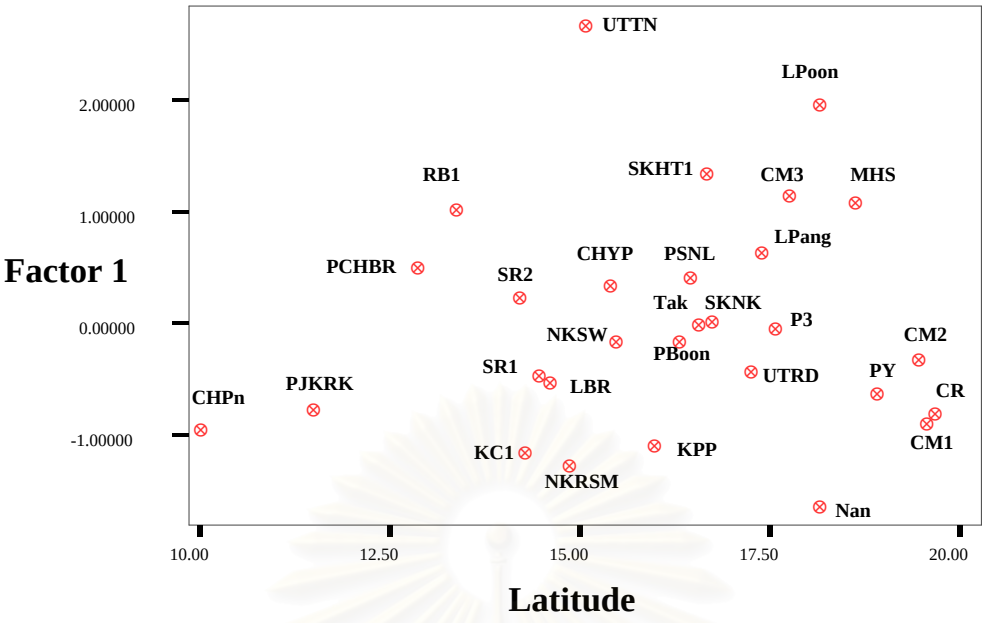

Figure 4.38. Geographic trends in isoflavonoid contents (factor 1) of *P. mirifica* in Thailand: abscissa and latitude. Factor score 1 was derived by PCA. Value labels refer to sampling sites or cultivars.

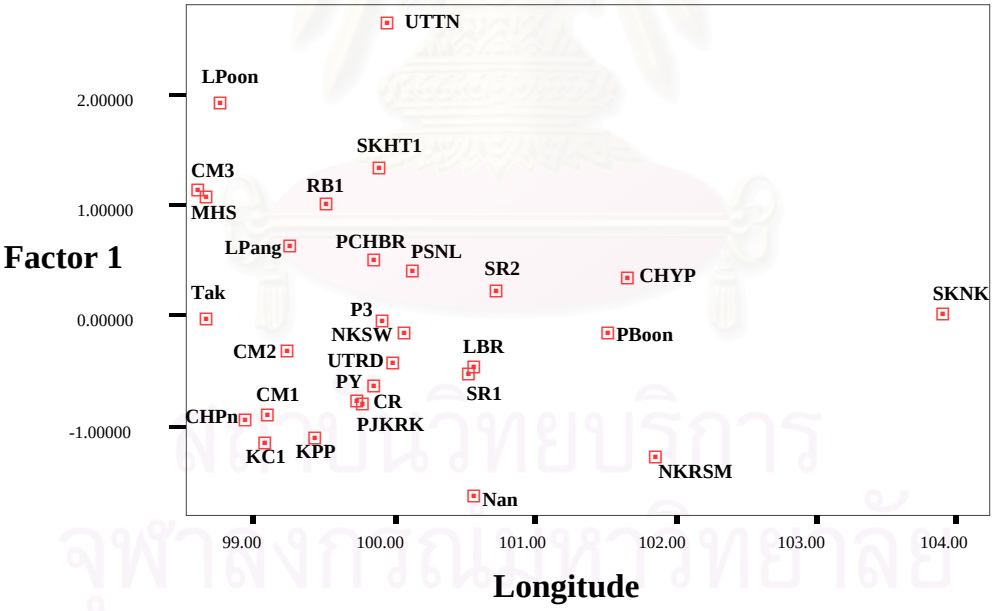

Figure 4.39. Geographic trends in isoflavonoid contents of *P. mirifica* in Thailand: abscissa and longitude. Factor score 1 was derived by PCA. Value labels refer to sampling sites or cultivars.

CHAPTER V
DISCUSSIONS

Considering collection localities, *Pueraria mirifica* are more widely distributed in the North, the Center, and the West than in other parts of Thailand. It is possible that those 3 areas are mainly the deciduous or dry forests and mountainous areas. It should be preferably suitable habitats for this species. *P. mirifica* was rarely found in the East and the South. This may be related to the character of areas since those areas are mainly evergreen forests. Due to our work, 39 cultivars were collected within 27 provinces (Figure 3.1-3.3). Mature leaf, pod, and flowers were collected for morphometric analysis. It was easier to collect mature leaves when compared to collect pods and flowers. It is because leaves have been found throughout the year whereas the flowers and pods have been only found during February to April of the year (Panriansaen, 2000). Although it was harder to collect flowers, it always confirmed us that we got the correct samples since flowers of *P. mirifica* are very distinguishing from other plants. In case of being uncertain in collecting a sample, tuberous root would be obtained and cut to investigate the species. The amount of collected pods and flowers may not be sufficient in analysis. That brings to the possibility that they could not be good representatives. Furthermore, the surveys in this research usually took place in May to October, 2006. In the future, field surveys should be conducted more often and more localities should be visited. In addition, more parts of plants including tuberous roots should be collected. Due to Figure 2.5, the distribution of *P. mirifica* in our research was as same as that was reported in Subtaeng (2002). Although *P. lobata* (Kudzu) was used as an outgroup in order to additionally determine inter-specific differentiation, its flowers were not collected during the survey period because of not blooming season.

Morphometric parameters were selected in order to investigate size and shape of plant organs (Figure 3.4-3.6). The used parameters such as leaf length, leaf width, and pedicel length were partly similar to the research of Creed (1997) that studied about the morphological variation in sea grass and the research of Agustin (2006) that conducted the morphometric study of cherimoya (*Annona cherimola* Mill.). Due to scatter plots in Figure 4.1-4.3, Factor analyses of leaf, pod, and flower morphometries

revealed that there was no grouping structure among cultivars of *P. mirifica* in Thailand. Then, cluster analyses and dendrograms of 3 morphometries presented that *P. mirifica* could be clustered differently (Figure 4.4-4.6). Considering the leaf morphometric dendrogram in Figure 4.4, 39 *P. mirifica* cultivars were distinctly classified into 5 groups. Remarkably, Uthai Thani cultivar was separated clearly from the others. It might be that the size of its leaf was the smallest to be clustered with others. The Uthai Thani cultivar was separated outstandingly from the others. It might be that leaves in the smallest size of Uthai Thani cultivar were related to the local climate which is rather high in humidity. *P. mirifica* propagation usually takes place in low humidity. The climate of Uthai Thani province may affect the growth of this plant which brings to the smallest size of leaves. In pod cluster analysis, the dendrogram was classified 14 cultivars into 2 groups. Both clusters could not separate the cultivars depending on geographic distribution. It could imply that the pod shape and size of *P. mirifica* in Thailand is not significantly different or 3 pod parameters used in this study could not show morphometric variation. In addition, the flower morphometric dendrogram could classify 11 cultivars into 3 clusters and remarkably could separate Kanchanaburi 1 cultivar (Thongphaphum district) from other *P. mirifica* cultivars. Its flower size was the largest. It may be that the climate and geography might be very appropriate to its growth. Other 2 groups were not considerably different in flower morphological traits. It can summarize that *P. mirifica* cultivars have low morphometric variation.

The correlation analyses of the morphometric factor scores against latitude and longitude displayed clinal patterns of characterization of *P. mirifica* in Thailand (Figure 4.7-4.20). From the leaf morphometry, 7 leaf parameters (PD, TLB, PL, RL, TLL, PLL, and AˆB) trended to increase in size from the South to the North of Thailand. It probably depends on the cold weather and mountainous geography in the North which is more suitable for the growth of *P. mirifica*. On the other hand, the trend of NPV and SPL parameters decreased in size from the South to the North. It showed that number of pair of veins (NPV) and stipule length (SPL) of *P. mirifica* leaves in the southern region was likely larger than those in the northern region. Both parameters do not statistically correspond to the above 7 parameters. Moreover, the trend of all 9 parameters decreased in leaf size from the West to the East of Thailand. The dry weather and deciduous forest of the West may be suitable to the growth of *P. mirifica*. Also, higher altitude and mountainous areas in the West may be preferable

for the growth which brings to larger stems and larger leaves. Considering the pod morphometry, it increased in pod length (PodL parameter) from the South to the North. In contrast, it decreased in PodL from the West to the East or it depended on longitude. The dry, cold climate as well as deciduous, mountainous forest of the North and the West may lead to the higher metabolic storage in seeds of pods and more suitable for the growth. Also, this probably leads to larger in size of seeds and in length of pods. At last, the flower morphometric correlation analysis showed that the size in 6 flower parameters (PetL, StmL, PisL, ClxL, PetW, and OvrD) trended to decrease from the West to the East of Thailand. The dry weather and deciduous, mountainous area of the West is suitable to the growth of *P. mirifica*. It may lead to the larger size of the flower. In this research, it can be summarized that latitude and longitude changes have an influence to the leaf, pod, and flower morphologies.

For genetic analysis, a fresh young leaf was used for DNA extraction since its cells are easier to be broken or lysed than the cells of mature leaf and other parts of plants. Large amount of genomic DNA was obtained at high molecular weight and clearly visible as a single band on 0.8% agarose gel (Figure 4.21). After that, nrDNA ITS, cpDNA *trn*L, and *trn*L-F regions were amplified by PCR. Universal primers used in this research were based on Taberlet *et al.* (1991) and White *et al*. (1990). Taberlet *et al.* (1991) designed universal primers from the cpDNA, especially in *trn*L-F region in order to study phylogeny. Also, White *et al*. (1990) presented some universal primers on ribosomal nuclear DNA, including ITS. Under optimum PCR conditions, expected single bands of *P. mirifica* cultivars were obtained (Figure 4.22-4.24). Failed amplification of PCR of some cultivars may be from some contaminants such as phenol, polysaccharides, etc. and degraded DNA.

Considering the sequences of amplified PCR products, ITS sequences of each cultivar were more variable than other 2 regions because of lower similarity percentages (72-100%) and more sequence divergence (0-25%). The results coincided to the location of the genes in the genome. The ITS region is a ribosomal nuclear DNA sequence that is highly varied while the chloroplast region is less variable and more conserved. These cpDNA *trn*L and *trn*L-F regions indicated low levels of intra-specific genetic variation (sequence divergence of 0-7% and 0-4.7%). Although it presented low polymorphism, it was still capable for studying in genetic diversity. However, other high variable non-coding regions which can illustrate high

polymorphic characteristics should be applied to determine the intra-specific variation in the future.

For an additional genetic experiment, RAPD method was conducted via PCR amplification by 5 random primers based on Mienie *et al.* (1995) who studied about identification of South African soybean cultivars. These selected primers designed from soy bean could be successfully amplified *P. mirifica* DNA. It may be that soybean is closely related to *P. mirifica*. Figure 4.28-4.32 showed band patterns of RAPD from all *P. mirifica* cultivars and an outgroup (*P. lobata*). Each primer can identify an individual among cultivars. In the future, more RAPD primers and the combinations of those 5 primers should be performed since, in our work, there were 6 cultivars those their DNA could be not amplified at all. In overall, 93 polymorphic bands could be generated and be sufficient enough to detect genetic variation. The average genetic distances based on Nei-Li distances that were used in NJ phylogenetic construction is displayed in Table 4.16. The distances were varied from 0 to 0.4381 and the mean value was 0.1861. They reveal that those 5 RAPD primers can make moderately clear for evaluating the genetic polymorphism of *P. mirifica* in Thailand. In different species, Heider *et al.* (2004) studied genetic diversity of *P. montana* by using RAPD. It was found that high genetic variation was observed. It could be concluded that RAPD was an effective technique for the intra-specific phylogenetic study.

According to phylogenetic analyses, Figure 4.33 - 4.36 presented 4 phylogenetic trees created by NJ method in PAUP. The sequences from those 3 regions and RAPD patterns were used. The genetic distance calculation based on K2P distance (for sequencing) and Nei-Li distances (for RAPD) was performed before generating phylogeny. Bootstrap supporting-values were calculated and showed on nodes of branches in order to show branch strength on the tree. The bootstrap supporting values could display on a branch if the value was in the range of 50 to 100. If it was equal to 100 (maximum value), the relation of taxa in the branch was ultimately confirmed. According to the 4 NJ phylograms, they were different in topology. Many branches of each tree which were evaluated by bootstraps were not strong. Moreover, all trees showed low genetic polymorphism among *P. mirifica* cultivars, especially *trn*L tree.

From the ITS tree, it could apparently classify some cultivars in the northern region of Thailand (Lamphun, Phrae 2, and 3) into subgroup 4. It could imply that

these cultivars in the North are similar in the ITS sequences so they were located closely on their branches. However, they were distinctly different from the others. The rest of cultivars such as subgroup 2 and 3 were located closely on the top of the tree. It showed that many cultivars were rather similar in the sequences.

The *trn*L phylogeny was divided into many short branches but it could show only one supported subgroup. Most of cultivars could not be distinguished because the sequences were similar especially in several cultivars on the bottom of this tree. It is probably that this cpDNA region is highly conserved. On the other hand, some cultivars were different in sequences and clearly separated from the others. For example, Chiang Mai 2 and Kanchanaburi 2 (from the North and the West) were formed together in subgroup 1. It might be that both cultivars trend to become into a new subspecies.

The *trn*L-F phylogram illustrated one supported branch. These sequences showed more polymorphism than *trn*L sequences because the former sequences are longer. Many members in the tree presented low polymorphic and located closely on the branches. From subgroup 1, Saraburi 1 and Kanchanaburi 2 cultivars (from the Center and the West) were aggregated together with distinct variation. From both CpDNA trees revealed that Kanchanaburi 2 cultivar might be a new subspecies.

The RAPD dendrogram presents many inconsistent branches but there are some unambiguous branches. Moderate genetic polymorphism is found. Cultivars from the same provinces and parts of Thailand [such as (1) Kanchanaburi 1-3 and (2) Ratchaburi 1-4] are clustered closely or in the same minor branch. Although RAPD results present moderate genetic differentiation, they are efficient enough for genetic classification among cultivars.

From all 4 phylogenetic trees, it could be summarized that low molecular genetic differentiation among the cultivars is probably a result of low differences in their heredity but some cultivars clearly differ from the others e.g. Kanchanaburi 2. It indicated that most of cultivars were not isolated by distance or geographic border. However, RAPD marker could be practical in some genetic differentiations and in cultivar classification depending to provinces and regions.

About analysis of chemical content variation, the data was mainly based on Cherdshewasart *et al.* (2007). They presented the comparison of isoflavonoid contents of *P. mirifica* root extracted powder from many localities in Thailand and of *P. lobata* as an outgroup as in Table 4.17. The data was used for Factor analysis and Cluster

analysis in order to resolve whether isoflavonoid contents are related to morphological and genetic data or not. Five types of isoflavonoids (puerarin, daidzin, genistin, daidzein, and genistein) were used and 29 cultivars of *P. mirifica* were applied. Scatter plots of factor scores could not be constructed because factor score produced only 1 factor. The dendrogram was created and the result indicated that there were 2 classified groups. The minor group contained 6 cultivars of Chiang Mai 3, Mae Hong Son, Ratchaburi 1, Sukhothai 1, Lamphun, and Uthai Thani which were separated from the others (in major group). These cultivars had high amount of some isoflavonoids, especially genistin. It is probably caused by a good condition of soils in their areas and it is probably involved in the role of genetics. Furthermore, the characterization was also analyzed by using correlation analysis. The result revealed that the contents of puerarin, daidzin, genistin, daidzein, and genistein did not correlate significantly to latitude and longitude (Figure 4.38 - 4.39). It indicated that the chemical characterization was classified deliberately across latitude and longitude changes. Alternatively, this tuber chemical quantity did not depend on latitude and longitude levels but perhaps depended strongly on local environments and genetic traits.

From the comparison of morphometric and genetic analyses, the details of each analysis were different but the genetic analysis could show the better variation and inter-cultivar classification than other analysis. Four NJ phylogenetic trees could indicate the evolutionary relation. RAPD technique could amplify genomic DNA randomly and present the variation and classification more apparently. However, both analyses detected low level of variation in *P. mirifica*.

From the comparison of morphometric and chemical analyses, cultivars can be classified differently. Lower variation was also detected in the chemical analysis because its dendrogram could classify samples into 2 groups only. The leaf morphometric and chemical-contented (isoflavonoid) dendrograms shared some similar result. For example, Uthai Thani cultivar was distinctly separated into the bottom group which was different from the others. Although leaves of Uthai Thani cultivar were small or the growth was not good, some isoflavonoid contents in the tuber were high. It can explain that the local environmental factors probably enhance the chemical constituents. Consequently, not only genetics play the important role in the plant phenotypes but other factors also do.

From the comparison of genetic and chemical analyses, both did not show the relationship or similarity to each other. The genetic part displayed higher polymorphism than other. Many cultivars show low diversity in chemical constituents. This probably results from low intra-specific genetic polymorphism of *P. mirifica* in Thailand.

CHAPTER VI
CONCLUSIONS

1. From the Factor and Cluster analyses of morphometry, *P. mirifica* cultivars from various localities in Thailand can be classified and clustered. First, leaf morphometry can separate *P. mirifica* cultivars into 5 groups. Second, pod morphometry could completely classify *P. mirifica* cultivars into 2 clusters. Third, flower morphometry could distinguish those cultivars into 3 groups. Briefly, it could summarize that *P. mirifica* cultivars have low morphometric variation.

2. In correlation analysis of morphometry, leaf morphometry presented that 7 leaf parameters (PD, TLB, PL, RL, TLL, PLL, and AˆB) trended to increase from the South to the North of Thailand. On the other hand, NPV and SPL parameters trended to decrease from the South to the North. Moreover, all 9 leaf parameters trended to decrease in size from the West to the East of Thailand. From pod morphometry, it presented that it increased in pod length (PodL parameter) from the South to the North. In contrast, it decreased in PodL length from the West to the East. From flower morphometry, the size in 6 parameters (PetL, StmL, PisL, ClxL, PetW, and OvrD) trended to decrease from the West to the East of Thailand.

3. The sequences of PCR products of ITS, *trn*L, and *trn*L-F regions indicated low level of genetic variation among cultivars of *P. mirifica* in Thailand. However, the sequence divergence of ITS (0-25.2%) is the highest to the sequence divergence of other 2 regions.

4. Due to RAPD analysis, total of 93 RAPD fragments generated by 5 RAPD primers were all reproducibly polymorphic bands. The average genetic distances of 39 *P. mirifica* cultivars and 1 cultivar of *P. lobata* (outgroup) varied from 0 to 0.4381.

5. According to 4 NJ phylogenetic trees (3 trees from sequencing and 1 tree from RAPD), their topologies were different. The genetic diversity could be partially observed from the trees even though low genetic diversity was obtained. Briefly,

RAPD phylogeny showed better result in cultivar classification according to provinces and regions of Thailand.

6. Considering the chemical (isoflavonoids) content analysis, the result also showed 2 groups. The minor group contains 6 cultivars of Chiang Mai 3, Mae Hong Son, Ratchaburi 1, Sukhothai 1, Lamphun, and Uthai Thani. The major group composed of the rest. Remarkably, variation of these chemical contents among cultivars was low. Moreover, there was no significant correlation between the isoflavonoid contents and angular distances on latitude and longitude.

7. From all analyses, morphometric and chemical content analyses could not well determine the variation of *P. mirifica* cultivars in Thailand. On the other hand, the genetic analysis was more practical to analyze the variation and classification of *P. mirifica* cultivars in Thailand, especially RAPD.

8. These 3 analyses did not relate to each other except some results of leaf morphometry and chemical content analysis. They could separate Uthai Thani cultivar from the others.

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APPENDICES

Appendix I

Leaf collection of 39 cultivars of *P. mirifica* named after provinces in Thailand and 1 cultivar of *P. lobata* from Japan as an outgroup.

No.	Cultivar name (District or Amphoe)	Region	Code name	Latitude	Longitude
1	Chiang Mai (Chai Prakan)	Upper North	CM1	19.77836075	99.21592127
2	Chiang Mai (Chiang Dao)	Upper North	CM2	19.67760402	99.35225743
3	Chiang Mai (Doi Tao)	Upper North	CM3	17.96186458	98.72826792
4	Chiang Mai (Mae Rim)	Upper North	CM4	19.27717846	99.25072582
5	Chiang Rai (Mae Suai)	Upper North	CR	19.90144316	99.89506227
6	Lampang (Thoen)	Lower North	LPang	17.6307864	99.36539475
7	Mae Hong Son (Khun Yuam)	Upper North	MHS	18.85139782	98.77673184
8	Lamphun (Ban Hong)	Lower North	LPoon	18.36070438	98.87164331
9	Nan (Wiang Sa)	Lower North	Nan	18.36142298	100.6870832
10	Phayao (Chiang Muan)	Upper North	PY	19.14100835	99.96437119
11	Phrae (Den Chai)	Lower North	P1	18.06727241	100.0358257
12	Phrae (Song)	Lower North	P2	18.06727241	100.0358257
13	Phrae (Wang Chin)	Lower North	P3	17.78747373	100.0355448
14	Uttaradit (Mueang)	Lower North	UTRD	17.46098692	100.0968728
15	Kamphaeng Phet (Khlong Lan)	Upper Center	KPP	16.20503292	99.5428352
16	Lopburi (Mueang)	Lower Center	LBR	14.81690937	100.6455167
17	Nakhon Sawan (Tak Fa)	Lower Center	NKSW	15.67911881	100.1826022
18	Phetchabun (Lom Kao)	Upper Center	PBoon	16.53098045	101.634185
19	Phitsanulok (Wang Thong)	Upper Center	PSNL	16.67563786	100.2390678
20	Saraburi (Phra Phutthabat)	Lower Center	SR1	14.66788586	100.6796166
21	Saraburi (Muak Lek)	Lower Center	SR2	14.41052332	100.8329079
22	Sukhothai (Mueang)	Upper Center	SKHT1	16.88383483	100.0043662
23	Sukhothai (Si Satchanalai)	Upper Center	SKHT2	17.50889576	99.72903387
24	Uthai Thani (Thap Than)	Lower Center	UTTN	15.28094893	100.0645104

No.	Cultivar name (District or Amphoe)	Region	Code name	Latitude	Longitude
25	Kanchanaburi (Thong Pha Phum)	Lower West	KC1	14.51041877	99.203658
26	Kanchanaburi (Sai Yok 1)	Lower West	KC2	14.51041877	99.203658
27	Kanchanaburi (Sai Yok 2)	Lower West	KC3	14.51041877	99.203658
28	Phetchaburi (Nong Ya Plong)	Lower West	PCHBR	13.09554768	99.96776581
29	Prachuap Khiri Khan (Mueang)	Lower West	PJKRK	11.70129703	99.85350351
30	Ratchaburi (Pak Tho 1)	Lower West	RB1	13.58179855	99.63931274
31	Ratchaburi (Pak Tho 2)	Lower West	RB2	13.58179855	99.63931274
32	Ratchaburi (Pak Tho 3)	Lower West	RB3	13.58179855	99.63931274
33	Ratchaburi (Pak Tho 4)	Lower West	RB4	13.58179855	99.63931274
34	Tak (Mae Ramat)	Upper West	Tak	16.76589489	98.78510624
35	Chaiyaphum (Chatturat)	Upper North-east	CHYP	15.61229749	101.7635475
36	Nakhon Ratchasima (Pak Chong)	Lower North-east	NKRSM	15.08416653	101.9700012
37	Sakon Nakhon (Kut Bak)	Upper North-east	SKNK	16.95847364	104.0141878
38	Chumphon (Tha Sae)	Upper South	CHPn	10.22179855	99.06516782
39	Surat Thani (Ban Ta Khun)	Lower South	SRTN	9.104019165	99.38684082
40	*P. lobata* from Hiroshima in Japan (Miyoshi city)	West	Lobata	34.80500000	132.8600000

Pod collection of 14 cultivars of *P. mirifica* named after provinces in Thailand and 1 cultivar of *P. lobata* from Japan as an outgroup.

No.	Cultivar name (District or Amphoe)	Region	Code name	Latitude	Longitude
1	Chiang Mai (Chai Prakan)	North	CM1	19.77836075	99.21592127
2	Chiang Mai (Doi Tao)	North	CM3	17.96186458	98.72826792
3	Chiang Mai (Mae Rim)	North	CM4	19.27717846	99.25072582
4	Lampang (Thoen)	North	LPang	17.6307864	99.36539475
5	Phayao (Chiang Muan)	North	PY	19.14100835	99.96437119
6	Phrae (Wang Chin)	North	P3	17.78747373	100.0355448
7	Kamphaeng Phet (Khlong Lan)	Center	KPP	16.20503292	99.5428352
8	Lopburi (Mueang)	Center	LBR	14.81690937	100.6455167
9	Saraburi (Phra Phutthabat)	Center	SR1	14.66788586	100.6796166
10	Kanchanaburi (Thong Pha Phum)	West	KC1	14.51041877	99.203658
11	Kanchanaburi (Sai Yok 1)	West	KC2	14.51041877	99.203658
12	Phetchaburi (Nong Ya Plong)	West	PCHBR	13.09554768	99.96776581
13	Prachuap Khiri Khan (Mueang)	West	PJKRK	11.70129703	99.85350351
14	Tak (Mae Ramat)	West	Tak	16.76589489	98.78510624
15	*P. lobata* from Hiroshima in Japan (Miyoshi city)	West	Lobata	34.80500000	132.86000000

Flower collection of 11 cultivars of *P. mirifica* named after provinces in Thailand (exclude *P. lobata*).

No.	Cultivar name (District or Amphoe)	Region	Code name	Latitude	Longitude
1	Chiang Mai (Doi Tao)	North	CM3	17.96186458	98.72826792
2	Chiang Mai (Mae Rim)	North	CM4	19.27717846	99.25072582
3	Saraburi (Muak Lek)	Center	SR2	14.41052332	100.8329079
4	Kanchanaburi (Thong Pha Phum)	West	KC1	14.51041877	99.203658
5	Kanchanaburi (Sai Yok 1)	West	KC2	14.51041877	99.203658
6	Phetchaburi (Nong Ya Plong)	West	PCHBR	13.09554768	99.96776581
7	Prachuap Khiri Khan (Mueang)	West	PJKRK	11.70129703	99.85350351
8	Ratchaburi (Pak Tho 1)	West	RB1	13.58179855	99.63931274
9	Ratchaburi (Pak Tho 2)	West	RB2	13.58179855	99.63931274
10	Chaiyaphum (Chatturat)	North-east	CHYP	15.61229749	101.7635475
11	Chumphon (Tha Sae)	South	CHPn	10.22179855	99.06516782

Appendix II

Descriptive statistics from 9 leaf parameters of 39 *P. mirifica* cultivars in Thailand and *P. lobata* as an outgroup. (Mean ± Standard error)

No.	Source	PL	PD	RL	PLL	TLL	TLB	SPL	AB	NPV
1	CM1	35.27± 1.06	0.45± 0.01	5.47± 0.14	1.15± 0.01	22.59± 0.28	19.27± 0.31	0.58± 0.02	23.16± 0.74	5.67± 0.10
2	CM2	25.75± 1.16	0.34± 0.01	5.45± 0.19	0.99± 0.03	21.53± 0.36	16.00± 0.37	0.37± 0.01	29.83± 1.13	6.81± 0.13
3	CM3	32.44± 0.80	0.44± 0.01	7.68± 0.15	0.93± 0.02	24.34± 0.39	20.59± 0.41	0.42± 0.01	23.76± 0.86	7.84± 0.12
4	CM4	21.14± 1.01	0.27± 0.01	5.00± 0.15	0.96± 0.02	18.62± 0.52	14.08± 0.49	0.31± 0.01	36.50± 0.79	5.22± 0.10
5	CR	23.02± 1.03	0.32± 0.01	4.47± 0.24	0.80± 0.01	20.24± 0.33	15.54± 0.31	0.36± 0.01	21.50± 0.76	7.89± 0.17
6	LPang	31.44± 0.85	0.37± 0.01	5.30± 0.14	0.84± 0.02	23.17± 0.24	15.80± 0.18	0.52± 0.02	33.84± 0.53	5.06± 0.08
7	MHS	21.24± 0.90	0.31± 0.01	4.74± 0.15	0.84± 0.02	20.28± 0.57	15.81± 0.35	0.34± 0.01	19.06± 0.60	5.93± 0.10
8	LPoon	27.92± 0.78	0.40± 0.01	5.92± 0.14	0.75± 0.01	22.76± 0.41	19.16± 0.35	0.47± 0.02	17.02± 0.64	5.90± 0.11
9	Nan	24.14± 1.01	0.32± 0.01	4.71± 0.16	0.94± 0.02	19.47± 0.39	14.46± 0.43	0.35± 0.01	22.54± 0.83	7.35± 0.08
10	PY	21.92± 0.77	0.26± 0.01	5.62± 0.17	0.77± 0.01	17.24± 0.33	14.48± 0.36	0.34± 0.01	24.91± 1.02	6.44± 0.08
11	P1	18.52± 0.38	0.22± 0.01	6.28± 0.13	0.64± 0.01	16.51± 0.26	14.63± 0.21	0.48± 0.01	26.98± 0.51	7.85± 0.12
12	P2	20.06± 0.58	0.28± 0.01	5.91± 0.15	0.67± 0.01	20.97± 0.39	16.99± 0.33	0.39± 0.01	24.50± 0.75	5.78± 0.11
13	P3	23.71± 0.43	0.35± 0.01	6.78± 0.12	0.68± 0.01	19.94± 0.26	18.84± 0.33	0.18± 0.01	24.16± 1.03	7.96± 0.13
14	UTRD	28.58± 0.76	0.39± 0.01	5.62± 0.15	0.92± 0.01	23.22± 0.39	18.67± 0.29	0.49± 0.01	25.46± 0.90	6.68± 0.16
15	KPP	28.12± 0.70	0.39± 0.01	4.97± 0.12	0.98± 0.02	23.29± 0.34	17.95± 0.26	0.41± 0.02	30.30± 0.83	7.14± 0.14
16	LBR	16.31± 0.48	0.28± 0.01	4.65± 0.15	0.96± 0.02	15.65± 0.28	14.17± 0.25	0.29± 0.01	26.72± 0.68	8.41± 0.14
17	NKSW	23.77± 0.64	0.27± 0.01	5.29± 0.16	0.56± 0.01	14.65± 0.17	13.93± 0.15	0.31± 0.01	35.90± 0.72	8.00± 0.11
18	PBoon	26.23± 0.88	0.22± 0.01	6.54± 0.19	0.72± 0.01	16.24± 0.37	12.42± 0.29	0.27± 0.01	37.28± 0.26	6.80± 0.08
19	PSNL	22.38± 1.13	0.18± 0.01	4.91± 0.16	0.73± 0.01	16.70± 0.24	12.26± 0.23	0.26± 0.01	38.82± 0.32	6.19± 0.06
20	SR1	21.20± 0.51	0.33± 0.01	6.24± 0.09	0.91± 0.02	22.54± 0.31	18.29± 0.26	0.37± 0.01	38.96± 0.60	6.94± 0.07
21	SR2	19.53± 0.61	0.33± 0.01	5.50± 0.13	0.89± 0.03	21.03± 0.43	17.95± 0.41	0.20± 0.01	20.04± 0.68	6.60± 0.10
22	SKHT1	35.76± 0.86	0.40± 0.01	5.31± 0.18	0.94± 0.02	23.58± 0.34	18.03± 0.31	0.56± 0.02	26.62± 0.78	6.46± 0.09
23	SKHT2	23.93± 0.62	0.31± 0.01	5.75± 0.11	0.67± 0.01	22.89± 0.43	18.00± 0.34	0.28± 0.01	30.12± 0.58	6.88± 0.11
24	UTTN	18.21± 0.44	0.19± 0.00	4.35± 0.11	0.48± 0.01	14.40± 0.24	10.07± 0.20	0.44± 0.01	29.02± 0.56	5.39± 0.07
25	KC1	32.59± 1.13	0.44± 0.01	6.58± 0.13	0.98± 0.01	21.67± 0.34	20.71± 0.56	0.73± 0.01	29.60± 0.98	6.05± 0.06
26	KC2	28.52± 0.81	0.31± 0.00	4.02± 0.12	0.81± 0.01	17.83± 0.28	15.40± 0.20	0.61± 0.01	25.22± 0.62	6.72± 0.13
27	KC3	21.18± 0.66	0.26± 0.00	5.00± 0.13	0.75± 0.01	20.00± 0.27	14.43± 0.20	0.48± 0.01	29.10± 0.85	6.96± 0.11
28	PCHBR	22.54± 0.69	0.30± 0.01	5.64± 0.13	0.72± 0.01	20.80± 0.28	14.30± 0.21	0.25± 0.01	31.70± 0.49	5.56± 0.06

No.	Source	PL	PD	RL	PLL	TLL	TLB	SPL	AB	NPV
29	PJKRK	18.23± 0.62	0.31± 0.00	4.84± 0.13	0.70± 0.01	23.05± 0.31	14.51± 0.22	0.36± 0.01	38.17± 0.57	6.33± 0.11
30	RB1	16.02± 0.38	0.20± 0.00	5.01± 0.09	0.54± 0.01	18.72± 0.23	10.66± 0.14	0.48± 0.01	32.78± 0.48	7.47± 0.09
31	RB2	11.85± 0.47	0.19± 0.01	2.99± 0.09	0.41± 0.01	12.36± 0.25	7.78± 0.21	0.35± 0.01	35.90± 0.77	7.93± 0.15
32	RB3	29.96± 0.91	0.34± 0.01	7.03± 0.12	0.91± 0.01	18.68± 0.29	15.04± 0.25	0.36± 0.01	6.26± 0.75	7.03± 0.12
33	RB4	33.82± 0.78	0.47± 0.01	7.98± 0.13	1.02± 0.01	24.61± 0.32	18.81± 0.21	0.79± 0.02	27.00± 0.59	4.78± 0.07
34	Tak	25.67± 0.91	0.37± 0.00	5.87± 0.17	0.88± 0.01	19.71± 0.47	18.61± 0.36	0.61± 0.01	37.98± 0.66	5.83± 0.08
35	CHYP	31.45± 0.90	0.40± 0.01	6.04± 0.15	0.77± 0.01	20.59± 0.27	17.03± 0.25	0.47± 0.03	28.89± 0.49	7.55± 0.18
36	NKRSM	17.64± 0.33	0.28± 0.00	4.32± 0.07	0.61± 0.01	15.39± 0.19	14.82± 0.21	0.13± 0.01	21.18± 0.59	6.80± 0.07
37	SKNK	27.53± 0.95	0.30± 0.01	4.64± 0.14	1.00± 0.03	19.77± 0.45	15.10± 0.37	0.37± 0.01	28.86± 0.76	5.43± 0.07
38	CHPn	22.69± 0.87	0.37± 0.01	5.57± 0.16	1.03± 0.02	20.58± 0.35	16.72± 0.32	0.60± 0.03	25.34± 0.70	6.30± 0.08
39	SRTN	19.99± 0.55	0.26± 0.01	5.48± 0.14	0.78± 0.02	21.22± 0.38	14.71± 0.36	0.39± 0.01	30.42± 0.63	7.09± 0.12
40	Lobata	15.27± 0.51	0.22± 0.00	4.39± 0.10	0.54± 0.01	12.36± 0.21	11.46± 0.22	0.27± 0.01	23.64± 0.34	5.23± 0.06
Total mean		24.37	0.32	5.47	0.81	19.92	15.79	0.41	28.09	6.64
Total SD.		5.73	0.08	0.97	0.17	3.02	2.83	0.14	6.84	0.92
Total SE.		0.92	0.01	0.16	0.03	0.48	0.45	0.02	1.10	0.15
Max.		35.76	0.47	7.98	1.15	24.61	20.71	0.79	38.96	8.41
Min.		11.85	0.18	2.99	0.41	12.36	7.78	0.13	6.26	4.78

Descriptive statistics from 3 pod parameters of 14 *P. mirifica* cultivars in Thailand and *P. lobata* as an outgroup (Mean ± Standard error).

No.	Source	PodL	PodW	SNP
1	CM1	7.45±0.21	1±0.02	3.33±0.20
2	CM3	2.95±0.08	0.78±0.01	1.75±0.10
3	CM4	4.78±0.35	0.79±0.01	2±0.28
4	LPang	6.01±0.15	0.74±0.01	4.33±0.20
5	PY	4.49±0.31	0.93±0.01	2±0.19
6	P3	7.27±0.31	0.99±0.01	3.8±0.25
7	KPP	4.87±0.16	0.73±0.01	3.34±0.17
8	LBR	5.17±0.43	1.02±0.02	2.1±0.23
9	SR1	3.42±0.12	0.77±0.01	2.34±0.15
10	KC1	6.78±0.26	1±0.02	3.55±0.25
11	KC2	3.59±0.29	0.9±0.02	1.5±0.19
12	PCHBR	5.61±0.13	0.95±0.01	3.54±0.16
13	PJKRK	4.88±0.26	0.77±0.01	2.87±0.34
14	Tak	5.55±0.12	0.9±0.01	2.74±0.18
15	Lobata	8.57±0.10	1.61±0.04	1.94±0.11
Total mean		5.43	0.93	2.74
Total SD.		1.58	0.22	0.87
Total SE.		0.41	0.06	0.22
Max.		8.57	1.61	4.33
Min.		2.95	0.73	1.5

Descriptive statistics from 7 flower parameters of 11 cultivars of *P. mirifica* in Thailand. (Mean ± Standard error)

No.	Cultivar	StmL	PisL	OvrD	ClxL	PdcL	PetW	PetL
1	CM 3	1.18±0.01	1.14±0.03	0.06±0.00	0.63±0.01	0.35±0.01	1.16±0.01	1.37±0.02
2	CM 4	1.41±0.05	1.34±0.05	0.12±0.01	0.89±0.01	0.41±0.01	1.24±0.02	1.6±0.02
3	SR 2	1.05±0.02	1.09±0.14	0.04±0.00	0.63±0.01	0.44±0.02	0.93±0.02	1.17±0.01
4	KC 1	1.23±0.01	1.16±0.02	0.06±0.00	0.78±0.01	0.7±0.04	1.06±0.02	1.49±0.02
5	KC 2	1.4±0.02	1.25±0.03	0.04±0.00	0.97±0.01	0.24±0.01	1.19±0.05	1.42±0.03
6	PCHBR	1.16±0.05	1.14±0.07	0.04±0.00	0.87±0.03	0.43±0.01	1.04±0.01	1.33±0.03
7	PJKRK	1.39±0.01	1.55±0.03	0.07±0.00	0.97±0.01	0.37±0.01	1.24±0.01	1.5±0.02
8	RB 1	1.11±0.02	1.12±0.03	0.04±0.00	0.84±0.01	0.51±0.01	1.1±0.02	1.4±0.04
9	RB 2	1.54±0.02	1.47±0.04	0.06±0.00	0.94±0.03	0.36±0.01	1.12±0.02	1.51±0.02
10	CHYP	1.05±0.01	1.09±0.01	0.05±0.00	0.51±0.01	0.36±0.02	0.99±0.02	1.05±0.02
11	CHPn	1.07±0.01	1.12±0.01	0.04±0.00	0.63±0.01	0.45±0.01	1.09±0.01	1.16±0.02
Total mean		1.24	1.22	0.06	0.79	0.42	1.11	1.36
Total SD.		0.17	0.16	0.02	0.16	0.12	0.10	0.17
Total SE.		0.05	0.05	0.01	0.05	0.04	0.03	0.05
Max.		1.54	1.55	0.12	0.97	0.7	1.24	1.6
Min.		1.05	1.09	0.04	0.51	0.24	0.93	1.05

Appendix III

Factor analysis

Factor analysis from 9 leaf parameters of 39 cultivars of *P. mirifica*.

KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.832
Bartlett's Test of Sphericity	Approx. Chi-Square	6276.634
	df	36
	Sig.	.000

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.815	42.393	42.393	3.815	42.393	42.393	3.654	40.603	40.603
2	1.312	14.573	56.966	1.312	14.573	56.966	1.473	16.363	56.966
3	.831	9.228	66.194						
4	.812	9.023	75.217						
5	.709	7.882	83.099						
6	.576	6.396	89.495						
7	.413	4.592	94.086						
8	.300	3.331	97.418						
9	.232	2.582	100.000						

Extraction Method: Principal Component Analysis.

Component Matrix[a]

	Component	
	1	2
Zscore(pd)	.879	
Zscore(tlb)	.835	-.172
Zscore(tll)	.753	
Zscore(pl)	.719	
Zscore(pll)	.695	.180
Zscore(rl)	.617	-.328
Zscore(npv)	-.194	-.739
Zscore(spl)	.462	.563
Zscore(ab)	-.386	.520

Extraction Method: Principal Component Analysis.

a. 2 components extracted.

Rotated Component Matrix[a]

	Component	
	1	2
Zscore(pd)	.852	.213
Zscore(tlb)	.851	
Zscore(tll)	.708	.267
Zscore(pl)	.706	.139
Zscore(rl)	.680	-.161
Zscore(pll)	.626	.350
Zscore(ab)	-.506	.406
Zscore(npv)		-.764
Zscore(spl)	.304	.662

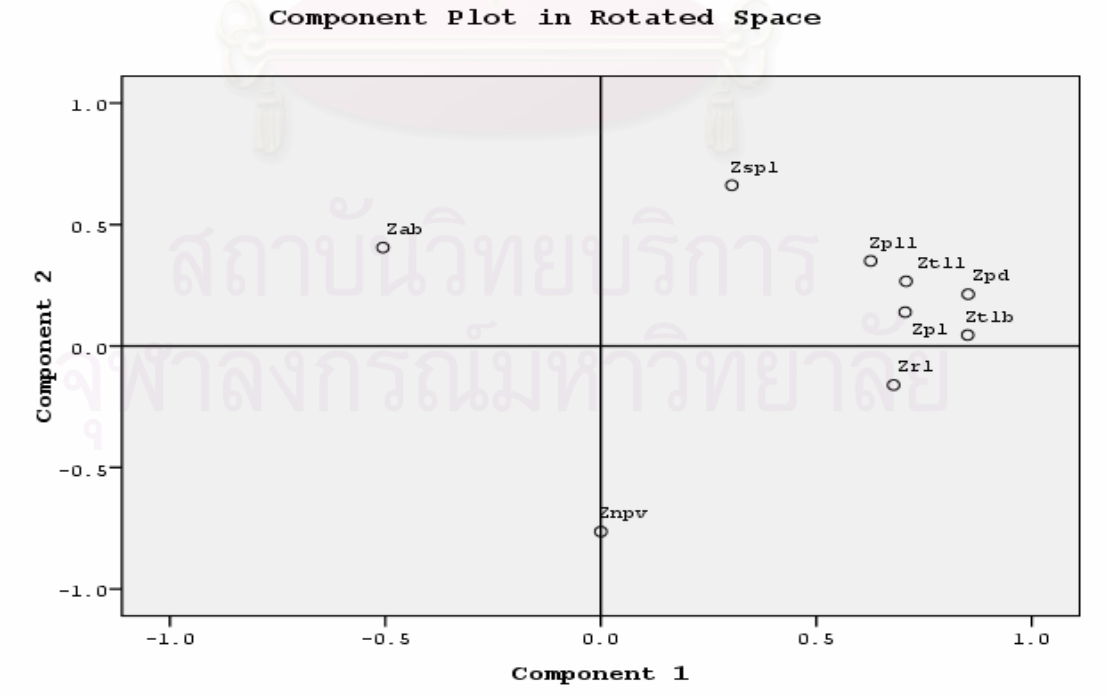
Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 3 iterations.

Component Transformation Matrix

Component	1	2
1	.967	.254
2	-.254	.967

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.



Factor analysis from 7 flower parameters of 11 cultivars of *P. mirifica*

KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.717
Bartlett's Test of Sphericity	Approx. Chi-Square	345.226
	df	21
	Sig.	.000

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.494	49.910	49.910	3.494	49.910	49.910	3.389	48.421	48.421
2	1.107	15.814	65.724	1.107	15.814	65.724	1.211	17.304	65.724
3	.939	13.408	79.132						
4	.613	8.757	87.889						
5	.426	6.087	93.976						
6	.274	3.920	97.896						
7	.147	2.104	100.000						

Extraction Method: Principal Component Analysis.

Component Matrix[a]

	Component	
	1	2
Zscore(stml)	.869	
Zscore(petl)	.851	.377
Zscore(cll)	.816	
Zscore(petw)	.759	-.195
Zscore(pistl)	.628	
Zscore(ovrd)	.561	.159
Zscore(pdcl)	-.252	.941

Extraction Method: Principal Component Analysis.

a. 2 components extracted.

Rotated Component Matrix[a]

	Component	
	1	2
Zscore(petl)	.911	.191
Zscore(stml)	.829	-.276
Zscore(cll)	.814	
Zscore(petw)	.702	-.350
Zscore(pistl)	.612	-.142
Zscore(ovrd)	.582	
Zscore(pdcl)		.973

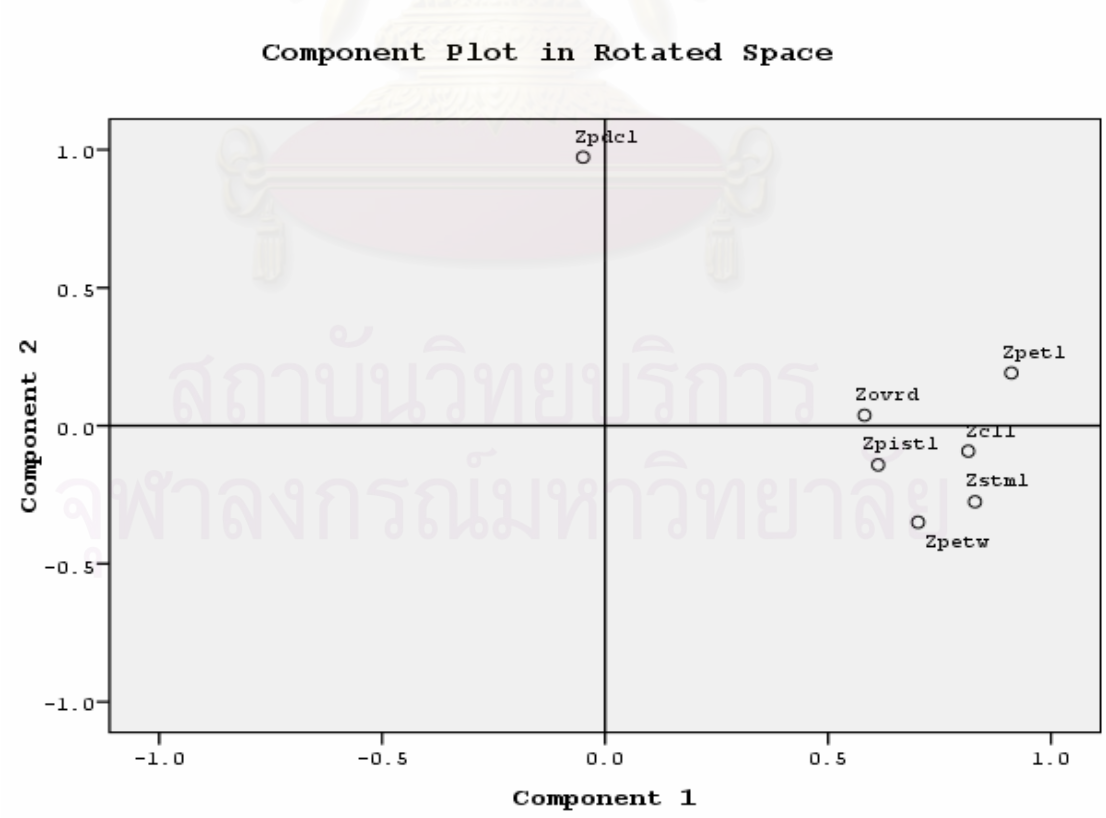
Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 3 iterations.

Component Transformation Matrix

Component	1	2
1	.978	-.209
2	.209	.978

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.



Factor analysis from 5 chemical parameters of 29 cultivars of *P. mirifica*

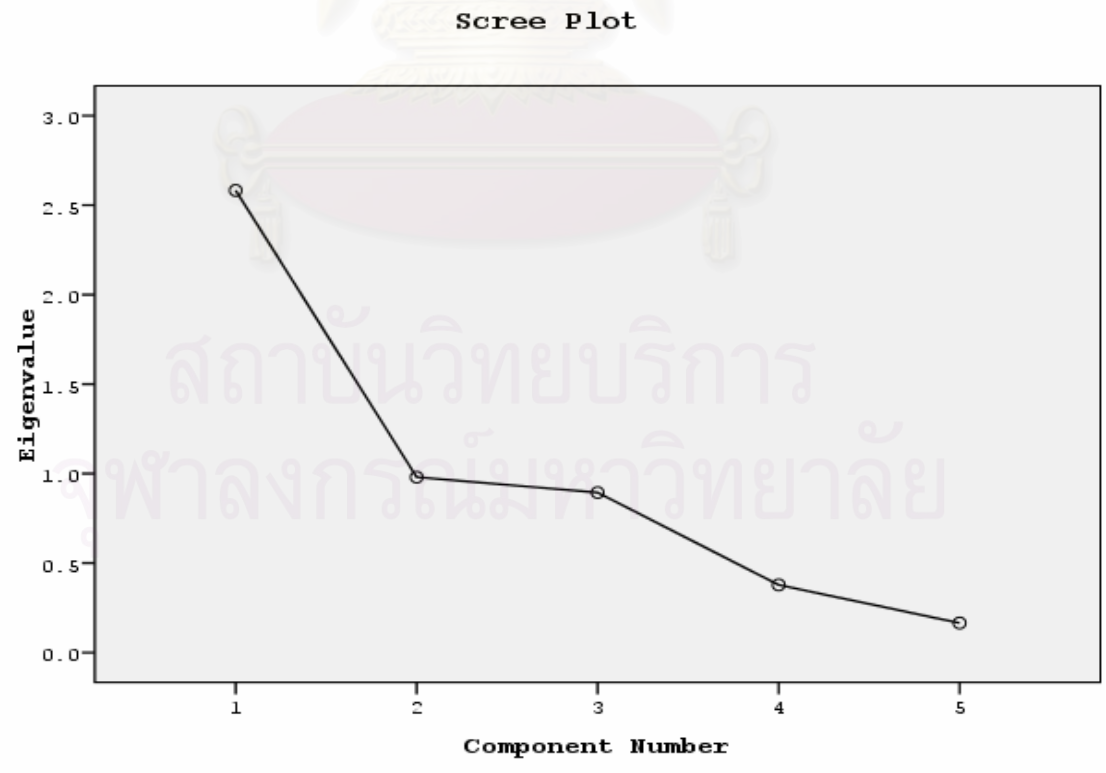
KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.579
Bartlett's Test of Sphericity	Approx. Chi-Square	49.891
	df	10
	Sig.	.000

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.582	51.647	51.647	2.582	51.647	51.647
2	.980	19.604	71.252			
3	.894	17.883	89.135			
4	.378	7.562	96.696			
5	.165	3.304	100.000			

Extraction Method: Principal Component Analysis.



Component Matrix[a]

	Component
	1
Daidzin	.872
Daidzein	.824
Genistin	.769
Genistein	.709
Puerarin	.221

Extraction Method: Principal Component Analysis.

a. 1 components extracted.

Rotated Component Matrix[a]

a. Only one component was extracted. The solution cannot be rotated.

Appendix IV

A. Reagent preparation

Agarose gel electrophoresis

1) 1% (w/v) agarose gel

- agarose 0.25 g
- 1x TBE buffer 25 ml

2) 10x Tris Boric EDTA buffer (TBE buffer), pH 8.0

- Tris aminomethane (50 mM) 108 g
- Boric acid (50 mM) 50.4 g
- EDTA (0.65 mM) 7.44 g

Adjust pH to 8.0 and make a total volume to 1,000 ml by d-H_2O. Then, the buffer will be stirred by a magnetic stirrer. Finally, this buffer will be diluted to be 1x TBE before use. For example, 100 ml of 10x TBE must be diluted by adding 900 ml of d-H_2O.

BIOGRAPHY

Mr. Trin Suwanvijitr was born on October 12th, 1981 in Mueang district, Songkhla province, Thailand. He finished his high school level from Triam Udom Suksa School in 2000, Bangkok. He graduated the Bachelor Degree of Science in Biology from Department of Biology, Faculty of Science, Chulalongkorn University in 2004. Then, He has been a graduate student in the Master's Degree in Biotechnology program, Faculty of Science, Chulalongkorn University since 2004.

Research publications:

Suwanvijitr, T., Chanchao, C., and Cherdshewasart, W. (2005) Leaf morphometry, genetic variation, and phylogeny of white Kwao Krua *Pueraria mirifica* in Thailand. Abstract. *The 10th Biological Sciences Graduate Congress*, National University of Singapore, Singapore. 43.

Suwanvijitr, T., Chanchao, C., and Cherdshewasart, W. (2006) Leaf morphometry, genetic variation, and phylogeny of white Kwao Krua *Pueraria mirifica* in Thailand. Abstract. *The KMITL International Conference on Science and Applied Science 2006*, Bangkok, Thailand. 33.

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