

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

Mr. Trin Suwanvijitr

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Biotechnology

Faculty of Science

Chulalongkorn University

Academic Year 2006

Copyright of Chulalongkorn University

มอร์โฟเมตริกของใบ ความแปรผันทางพันธุกรรม และวงศ์วานทางวิวัฒนาการของ
กวาวเครือขาว *Pueraria mirifica* ในประเทศไทย

นายตฤณ สุวรรณวิจิตร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเทคโนโลยีชีวภาพ

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2549

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

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

Mr. Trin Suwanvijitr

**A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Biotechnology**

Faculty of Science

Chulalongkorn University

Academic Year 2006

Copyright of Chulalongkorn University

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

By Mr. Trin Suwanvijitr

Field of study Biotechnology

Thesis Advisor Assistant Professor Chanpen Chanchao, Ph.D.

Thesis Co-advisor Associate Professor Wichai Cherdshewasart, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial
Fulfillment of the Requirements for the Master's Degree



.....Dean of the Faculty of Science
(Professor Piamsak Menasveta, Ph.D.)

THESIS COMMITTEE



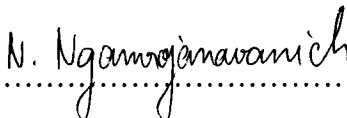
.....Chairman
(Associate Professor Somsak Panha, Ph.D.)



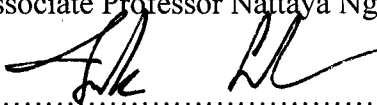
.....Thesis Advisor
(Assistant Professor Chanpen Chanchao, Ph.D.)



.....Thesis Co - advisor
(Associate Professor Wichai Cherdshewasart, Ph.D.)



.....Member
(Associate Professor Nattaya Ngamrojanavanich, Ph.D.)



.....Member
(Tosak Seelanan, Ph.D.)

ศฤงคาร วิจิตร: มอร์โฟเมตริกของใบ ความแปรผันทางพันธุกรรม และวงศ์วานทางวิวัฒนาการของ
 กวาวเครือขาว *Pueraria mirifica* ในประเทศไทย (LEAF MORPHOMETRY, GENETIC
 VARIATION, AND PHYLOGENY OF WHITE KWAO KRUA *Pueraria mirifica* IN THAILAND)
 อ.ที่ปรึกษา: ผศ. ดร. จันทรเพ็ญ จันทรเจ้า, อ.ที่ปรึกษาร่วม: รศ. ดร. วิชัย เชิดชูวิทยาศาสตร์,
 156 หน้า

เก็บตัวอย่างของกวาวเครือขาว โดยเก็บใบ ฝัก และดอก จากแหล่งต่างๆ ในประเทศไทยมาใช้ในการ
 วิเคราะห์ลักษณะทางมอร์โฟเมตริกและทางพันธุกรรมเพื่อหาความสัมพันธ์ว่าสอดคล้องกับข้อมูลของสารเคมีใน
 หัวกวาวเครือขาวหรือไม่ ผลของการวิเคราะห์ทางมอร์โฟเมตริก พบว่าสามารถแบ่งกลุ่มกวาวเครือขาวได้ โดยมีมอร์
 โฟเมตริกของใบสามารถแยกสายพันธุ์ออกเป็น 5 กลุ่ม ส่วนมอร์โฟเมตริกของฝักแสดงให้เห็นว่าสามารถแบ่งได้เป็น
 2 กลุ่ม และมอร์โฟเมตริกของดอกสามารถแยกสายพันธุ์ได้เป็น 3 กลุ่ม ทั้งนี้สามารถสรุปได้ว่าผลของมอร์โฟเมตริก
 สามารถบ่งบอกความแตกต่างแต่ละสายพันธุ์ได้ค่อนข้างต่ำ หลังจากนั้นนำข้อมูลมาวิเคราะห์สหสัมพันธ์ต่อค่า
 ละติจูด และลองจิจูด เพื่อดูถึงความสัมพันธ์ และแนวโน้มการเปลี่ยนแปลงทางมอร์โฟเมตริกของพืชชนิดนี้ใน
 ประเทศไทย พบว่าทั้งมอร์โฟเมตริกของใบ ฝัก และดอก ล้วนมีความสัมพันธ์ และแนวโน้มต่อค่าละติจูด และ
 ลองจิจูดที่แตกต่างกัน

ในส่วนการวิเคราะห์ทางพันธุกรรม พิจารณาผลจากลำดับเบสและอาร์เอพีดี ในส่วนของลำดับเบส ทำ
 การเพิ่มขึ้นส่วนและหาลำดับเบสบนบริเวณ ITS, *trnL* และ *trnL-F* พบว่าลำดับเบสทั้งหมดที่ได้จากทุกสายพันธุ์
 แสดงความแปรผันทางพันธุกรรมที่ต่ำ อย่างไรก็ตามค่าไคเอร์เจนท์ของลำดับเบสของ ITS มีค่าอยู่ระหว่าง 0-
 25.2% ซึ่งสูงกว่าค่าไคเอร์เจนท์ของอีก 2 บริเวณ ส่วนผลของอาร์เอพีดีนั้นพบว่า เกิดแบนด์ที่แตกต่างกันทั้งหมด
 93 ชิ้น มีค่าเฉลี่ยของระยะห่างทางพันธุกรรมอยู่ในช่วง 0-0.4381 จากการวิเคราะห์วงศ์วานทางวิวัฒนาการโดย
 เอ็นเจ 4 วงศ์วาน (3 วงศ์วานโดยลำดับเบสและ 1 วงศ์วานโดยอาร์เอพีดี) พบความแตกต่างของแต่ละวงศ์วาน
 ถึงแม้ว่าจะพบคิเอ็นเอโพลิเมอร์เฟอซีซึมที่ต่ำ แต่วงศ์วานของอาร์เอพีดีสามารถนำมาใช้ในการจัดกลุ่มสายพันธุ์
 กวาวเครือขาวได้สอดคล้องตามจังหวัดและภูมิภาคของประเทศไทยได้

ส่วนการวิเคราะห์ทางองค์ประกอบสารเคมีพวกไอโซฟลาโวนอยด์ สามารถแบ่งกลุ่มสายพันธุ์
 กวาวเครือขาวออกเป็น 2 กลุ่ม พบว่าความแปรผันทางองค์ประกอบของสารเคมีของแต่ละสายพันธุ์มีค่าต่ำ และยัง
 พบอีกว่าความแปรผันดังกล่าวไม่มีความสัมพันธ์ และแนวโน้มใดๆ ต่อค่าละติจูด และลองจิจูด กล่าวโดยสรุปได้
 ว่าผลจากการวิเคราะห์ทั้ง 3 รูปแบบแสดงให้เห็นถึงความแปรผันทางสายพันธุ์ของกวาวเครือขาวในประเทศไทย
 ที่ต่ำ

สาขาวิชา...เทคโนโลยีชีวภาพ.....

ปีการศึกษา...2549.....

ลายมือชื่อนิสิต..... ศฤงคาร วิจิตร.....
 ลายมือชื่ออาจารย์ที่ปรึกษา..... จันทรเพ็ญ จันทรเจ้า.....
 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4772302123: MAJOR BIOTECHNOLOGY
KEY WORD: *Pueraria mirifica* WHITE KWAO KRUA, MORPHOMETRY,
GENETIC VARIATION, AND PHYLOGENY

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 all 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, *trnL*, and *trnL-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 Cherdshewasart

ACKNOWLEDGEMENTS

I am especially grateful to my advisor, Assistant Professor Dr. Chanpen Chanchao, for her suggestions. She always provides me a lot of knowledge and also supports me to do researches.

I would like to thank my co-advisor, Associate Professor Dr. Wichai Cherdshewasart for his advices, comments, and all supports.

My appreciation is also passed to Associate Professor Dr. Somsak Panha, Associate Professor Dr. Nattaya Ngamrojanavanich, and Dr. Tosak Seelanan for serving as thesis committee.

My special thanks is to Assistant Professor Dr. Patchanee Singha-asa, Assistant Professor Dr. Orawan Sattayalai, Assistant Professor Dr. Jessada Denduangboripant, and members of CBCB for laboratory services and other hospitality.

I also thank Mr. Atsalek Rattanawanee and Ms. Ratana Panriansaen for some suggestions, molecular technical supports, and information.

I also thank Department of Biology, Faculty of Science, Chulalongkorn University for the laboratory facilities.

I wish to send my sincere thanks to lab-mates and friends in Biology Department and Biotechnology Program for their friendship, kind assistance, and helpfulness.

Financial supports were from (1) Thailand Research Fund, grants # MRG4780007, (2) the Center of Excellence in Biodiversity (CEB) under the Research Program on Conservation and Utilization of Biodiversity of Faculty of Science, Chulalongkorn University, (3) Graduate School of Chulalongkorn University, and (4) Biodiversity Research and Training Program (BRT).

Finally, the greatest gratitude is expressed to my family for their encouragement, love, and all supports throughout my life.

CONTENTS

	Page
THAI ABSTRACT.....	iv
ENGLISH ABSTRACT.....	v
ACKNOWLEDGEMENTS.....	vi
CONTENTS.....	vii
LIST OF FIGURES.....	ix
LIST OF TABLES.....	xii
ABBREVIATIONS.....	xiv
CHAPTER I: INTRODUCTION.....	1
CHAPTER II: LITERATURE REVIEWS.....	4
Botanical characteristics, ethnobotany and applications of <i>P. mirifica</i>	7
Chemical constituents and bioassay of root extract from <i>P. mirifica</i>	8
Distribution and propagation of <i>P. mirifica</i>	12
Morphometric study	14
Genetic variation in plants.....	16
CHAPTER III: MATERIALS AND METHODS.....	24
Morphometric study equipments.....	24
Genetic study materials	24
Equipments.....	24
Chemicals.....	25
Plant materials and sampling collection.....	27
Morphometric measurement and data analysis.....	34
Molecular and phylogenetic analyses.....	34
DNA extraction.....	34
Agarose gel electrophoresis.....	35
PCR Amplification.....	36
Purification of the PCR products.....	37
Direct DNA sequencing.....	38
Random Amplified Polymorphic DNA technique (RAPD).....	38
Phylogenetic analysis.....	39

	Page
CHAPTER IV: RESULTS.....	40
Morphometric variation.....	40
Factor analysis.....	40
Factor analysis of leaf morphometry.....	40
Factor analysis of pod morphometry.....	42
Factor analysis of flower morphometry.....	43
Cluster analysis.....	45
Cluster analysis of leaf morphometry.....	45
Cluster analysis of pod morphometry.....	47
Cluster analysis of flower morphometry.....	48
Characterization of <i>P. mirifica</i> in Thailand.....	49
Leaf morphometry.....	49
Pod morphometry.....	52
Flower morphometry.....	55
Genetic variation.....	58
DNA extraction.....	58
PCR amplification.....	58
Sequence analysis	62
Random Amplified Polymorphic DNA (RAPD) patterns	100
RAPD analysis.....	108
Phylogenetic analysis.....	113
Chemical content analysis.....	119
Factor analysis of isoflavonoid contents.....	121
Cluster analysis of isoflavonoid contents.....	121
The chemical characterization of <i>P. mirifica</i> in Thailand.....	123
CHAPTER V: DISCUSSIONS.....	125
CHAPTER VI: CONCLUSIONS.....	132
REFERENCES.....	134
APPENDICES.....	140
BIOGRAPHY.....	156

LIST OF FIGURES

	Page
Figure 2.1. <i>P. mirifica</i> plants in the field.....	5
Figure 2.2. Parts of <i>P. mirifica</i> plant.....	6
Figure 2.3. The structures of chemical constituents in <i>P. mirifica</i> root extract.....	10
Figure 2.4. The comparison of the structures of isoflavonoid nucleus and Estrogen.....	11
Figure 2.5. Locations that Subtaeng (2002) surveyed and collected <i>P. mirifica</i> tubers.....	13
Figure 2.6. Map of nuclear ribosomal DNA internal transcribed spacer (ITS) region in eukaryotes	19
Figure 2.7. Map of chloroplast DNA <i>trnL</i> -F region in plants. Universal primers (a-f) were designed in phylogenetic analysis	19
Figure 3.1. Map for leaf collection of 39 cultivars of <i>P. mirifica</i> among 27 provinces in Thailand.....	29
Figure 3.2. Map for pod collection of 14 cultivars of <i>P. mirifica</i> among 11 provinces in Thailand.....	30
Figure 3.3. Map for flower collection of 11 cultivars of <i>P. mirifica</i> among 8 provinces in Thailand.....	31
Figure 3.4. Nine morphometric parameters for measurement of collected <i>P. mirifica</i> leaf.....	32
Figure 3.5. Three morphometric parameters for measurement of collected <i>P. mirifica</i> pod	33
Figure 3.6. Seven morphometric parameters for measurement of collected <i>P. mirifica</i> flower.....	33
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.....	41
Figure 4.2. Position of each <i>P. mirifica</i> cultivar in Thailand of pod morphometric analysis. The 3-D scatter plot constructed on the axes of X-axis (PodL), Y-axis (PodW) and Z-axis (SNP).....	42

Figure 4.3. Position of each *P. mirifica* cultivar in Thailand. Factor axes of 1 and 2 were derived from Factor analysis of flower morphometric analysis: ordinate; factor 1 and abscissa; factor 2.....44

Figure 4.4. Leaf morphometric dendrogram created by Between-groups linkage method of Cluster analysis.46

Figure 4.5. Pod morphometric dendrogram created by Between-groups linkage method of Cluster analysis.....47

Figure 4.6. Flower morphometric dendrogram created by Between-groups linkage method of Cluster analysis.....48

Figure 4.7. Geographic trends in leaf morphometry of *P. mirifica* in Thailand: abscissa; latitude; the factor score 1 derived from PCA.....50

Figure 4.8. Geographic trends in leaf morphometry of *P. mirifica* in Thailand: abscissa; longitude; the factor score 1 derived from PCA50

Figure 4.9. Geographic trends in leaf morphometry of *P. mirifica* in Thailand: abscissa; latitude; the factor score 2 derived from PCA51

Figure 4.10. Geographic trends in leaf morphometry of *P. mirifica* in Thailand: abscissa; longitude; the factor score 2 derived from PCA51

Figure 4.11. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; latitude; the Zscore of PodL53

Figure 4.12. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; latitude; the Zscore of PodW53

Figure 4.13. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; latitude; the Zscore of SNP.....53

Figure 4.14. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; longitude; the Zscore of PodL54

Figure 4.15. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; longitude; the Zscore of PodW.....54

Figure 4.16. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; longitude; the Zscore of SNP.....54

Figure 4.17. Geographic trends in flower morphometry of *P. mirifica* in Thailand: abscissa; latitude; the factor score 1 derived from PCA56

Figure 4.18. Geographic trends in flower morphometry of *P. mirifica* in Thailand: abscissa; longitude; the factor score 1 derived from PCA56

Figure 4.19. Geographic trends in flower morphometry of <i>P. mirifica</i> in Thailand: abscissa; latitude; the factor score 2 derived from PCA	57
Figure 4.20. Geographic trends in flower morphometry of <i>P. mirifica</i> in Thailand: abscissa; longitude; the factor score 2 derived from PCA	57
Figure 4.21. Genomic DNA on 0.8% agarose gel	58
Figure 4.22. PCR products of ITS on 1.0% agarose gel	59
Figure 4.23. PCR products of <i>trnL</i> on 1.0% agarose gel.....	59
Figure 4.24. PCR products of <i>trnL</i> -F on 1.0% agarose gel.	60
Figure 4.25. A 687 bp character matrix of 33 cultivars of <i>P. mirifica</i> and 1 cultivar of <i>P. lobata</i> based on nrDNA ITS sequences	63
Figure 4.26. A 397 bp character matrix of 39 cultivars of <i>P. mirifica</i> and 1 cultivar of <i>P. lobata</i> based on <i>trnL</i> sequences	70
Figure 4.27. A 731 bp character matrix of 31 cultivars of <i>P. mirifica</i> and 1 cultivar of <i>P. lobata</i> based on <i>trnL</i> -F sequences	77
Figure 4.28. RAPD patterns amplified by OPA-07 primer on 2.0% agarose gel	101
Figure 4.29. RAPD patterns amplified by OPA-12 primer on 2.0% agarose gel	102
Figure 4.30. RAPD patterns amplified by OPD-02 primer on 2.0% agarose gel	103
Figure 4.31. RAPD patterns amplified by OPD-16 primer on 2.0% agarose gel	104
Figure 4.32. RAPD patterns amplified by OPE-01 primer on 2.0% agarose gel	105
Figure 4.33. A rooted NJ phylogenetic tree or phylogram of ITS region (33 cultivars of <i>P. mirifica</i> in Thailand and 1 cultivar of <i>P. lobata</i> as an outgroup).....	115
Figure 4.34. A rooted NJ phylogram of <i>trnL</i> region (39 cultivars of <i>P. mirifica</i> in Thailand and 1 cultivar of <i>P. lobata</i> as an outgroup)	116
Figure 4.35. A rooted NJ phylogenetic tree of <i>trnL</i> -F region (31 cultivars of <i>P. mirifica</i> in Thailand and 1 cultivar of <i>P. lobata</i> as an outgroup).....	117
Figure 4.36. A rooted neighbor-joining (NJ) phylogenetic tree based on Nei-Li genetic distances among 33 cultivars of <i>P. mirifica</i> in Thailand and 1 cultivar of <i>P. lobata</i> as an outgroup derived from RAPD patterns.....	118
Figure 4.37. A dendrogram of isoflavonoid contents created by Between-groups linkage method of Cluster analysis.....	122
Figure 4.38. Geographic trends in isoflavonoid contents (factor 1) of <i>P. mirifica</i> in Thailand: abscissa and latitude. Factor score 1 was derived by PCA.....	124
Figure 4.39. Geographic trends in isoflavonoid contents of <i>P. mirifica</i> in Thailand: abscissa and longitude. Factor score 1 was derived by PCA.....	124

LIST OF TABLES

Page

Table 2.1. The important chemical constituents in the root extract of *P. mirifica*9

Table 3.1. List of sampling collection of *P. mirifica* in Thailand and *P. lobata*.
The √ symbol represents a collected and analyzed sample.....28

Table 3.2. Lists of 5 primers used for PCR amplification and direct sequencing37

Table 3.3. Lists of 5 arbitrary primers for RAPD38

Table 4.1. Correlation analysis of geographic trends in leaf morphometric factors of *P. mirifica* in Thailand derived from Principal Component Analysis49

Table 4.2. Correlation analysis of geographic trends in pod morphometric factors of *P. mirifica* in Thailand derived from Principal Component Analysis.....52

Table 4.3. Correlation analysis of geographic trends in flower morphometric factors of *P. mirifica* in Thailand derived from Principal Component Analysis.....55

Table 4.4. Summary of PCR amplification of ITS (ITS_1 and ITS_4 primers), *trnL* (c and d primers) and *trnL*-F (c and f primers) regions of all *P. mirifica* samples in Thailand and *P. lobata* from Japan as outgroup.....61

Table 4.5. Percentages of nitrogenous base composition of ITS sequences of 33 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup84

Table 4.6. Percentages of nitrogenous base composition of *trnL* sequences of 39 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup85

Table 4.7. Percentages of nitrogenous base composition of *trnL*-F sequences of 31 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup86

Table 4.8. The matrix of pairwise similarity (%) of ITS sequences from 33 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup87

Table 4.9. The matrix of pairwise similarity (%) of *trnL* sequences from 39 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup89

Table 4.10. The matrix of pairwise similarity (%) of *trnL*-F sequences from 31 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup92

Table 4.11. The pairwise sequence divergence (%) of ITS sequences from 33 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup.....94

Table 4.12. The pairwise sequence divergence (%) of *trnL* sequences from 39 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup96

Table 4.13. The pairwise sequence divergence (%) of *trnL-F* sequences from 31 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup.....98

Table 4.14. Summary of RAPD results of *P. mirifica* in Thailand and *P. lobata* as outgroup by all 5 RAPD primers106

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.....107

Table 4.16. The average genetic distances (pairwise distances between taxa) of 39 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup108

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*.....120

Table 4.18. Correlation analysis of geographic trends in isoflavonoid contents of *P. mirifica* in Thailand derived by the PCA of Factor analysis123

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
<i>trnL-F</i>	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 (*trnL* and *trnL-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	<i>Pueraria</i>
Species	<i>P. mirifica</i>



(A)

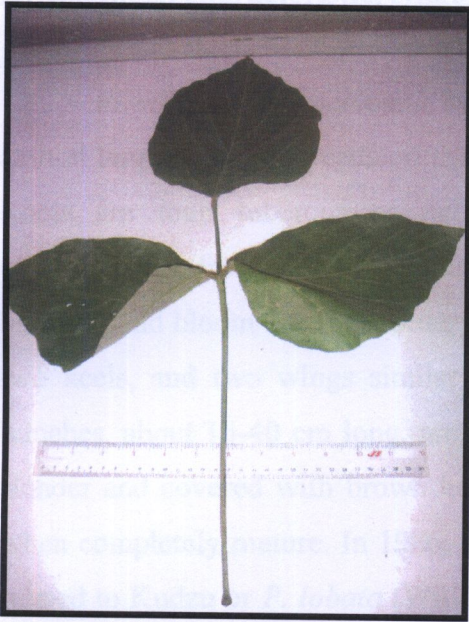


(B)

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-foliolate 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 <i>et al.</i> , 1986
Coumestans	Coumestrol Mirificoumestan Mirificoumestan glycol Mirificoumestan hydrate	Ingham <i>et al.</i> , 1986; 1988
Isoflavones	Daidzin Daidzein Genistin Genistein Kwakhurin Kwakhurin hydrate Mirificin Puerarin Puerarin 6' - monoacetate	Ingham <i>et al.</i> , 1986; 1989
Steroids	β -sitosterol Stigmatosterol	Hayodom, 1971

Isoflavone and Isoflavone glycosides

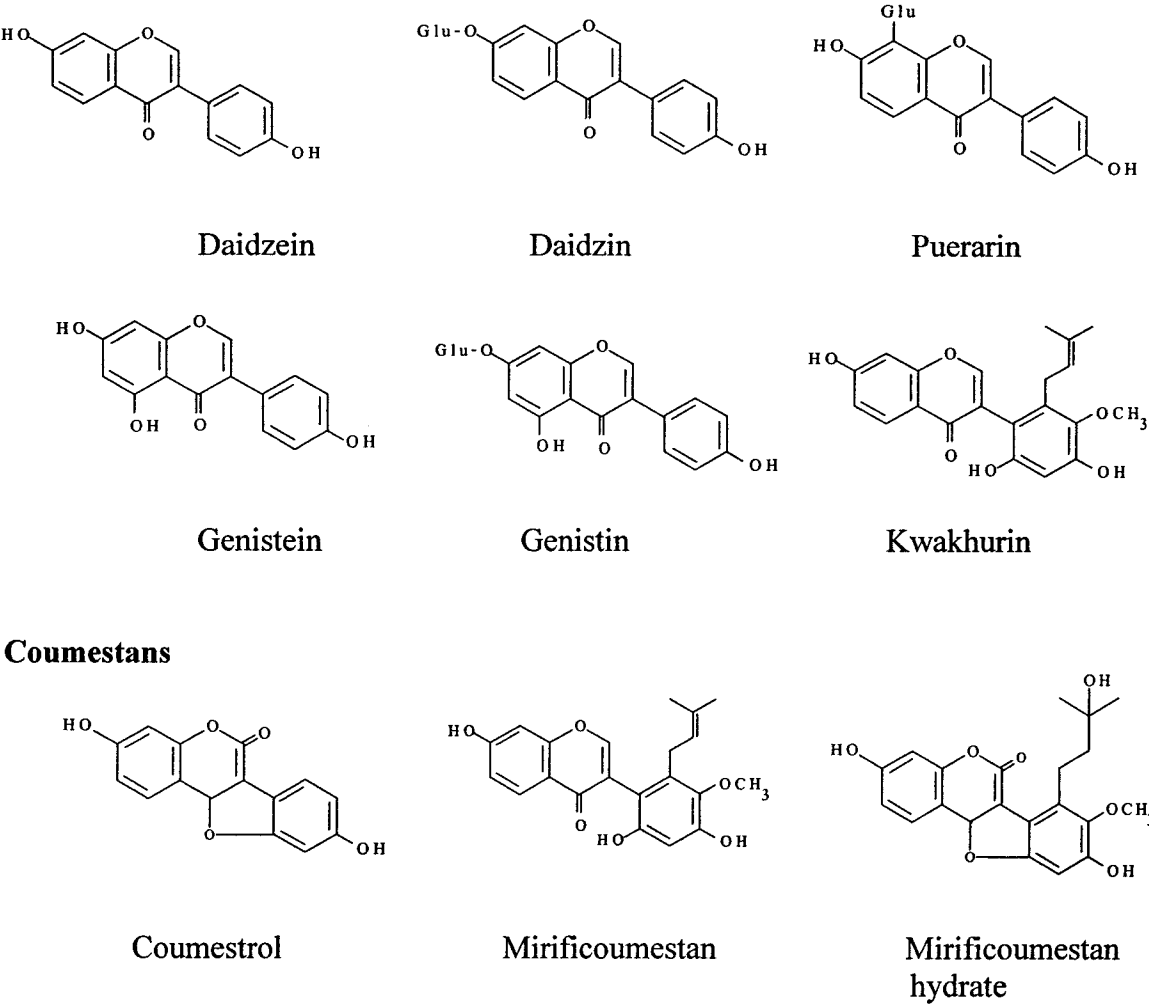
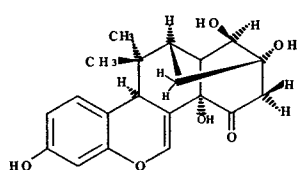
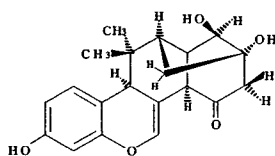


Figure 2.3. The structures of chemical constituents in *P. mirifica* root extract.
(Ingham *et al.*, 1986)

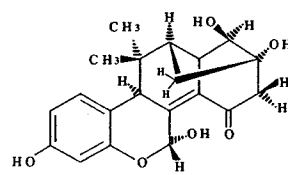
Chromenes



Miroestrol

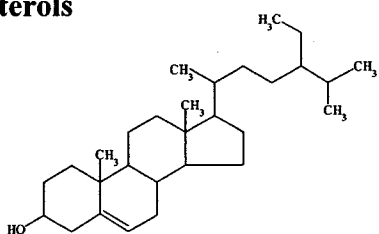
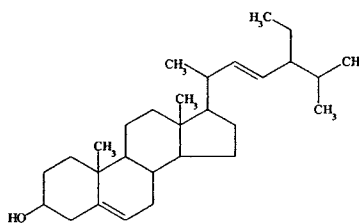


Deoxymiroestrol



Isomiroestrol

Sterols

 β -sitosterol

Stigmasterol

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

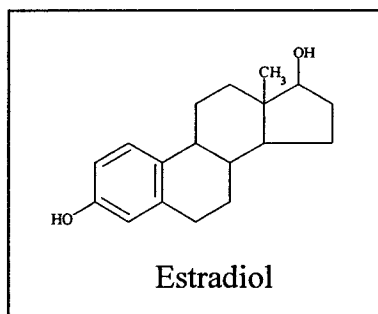
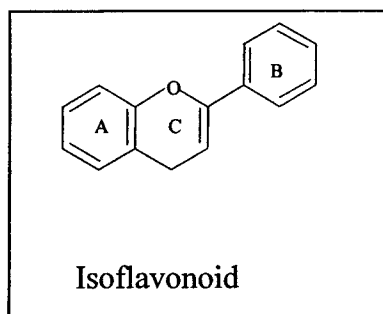
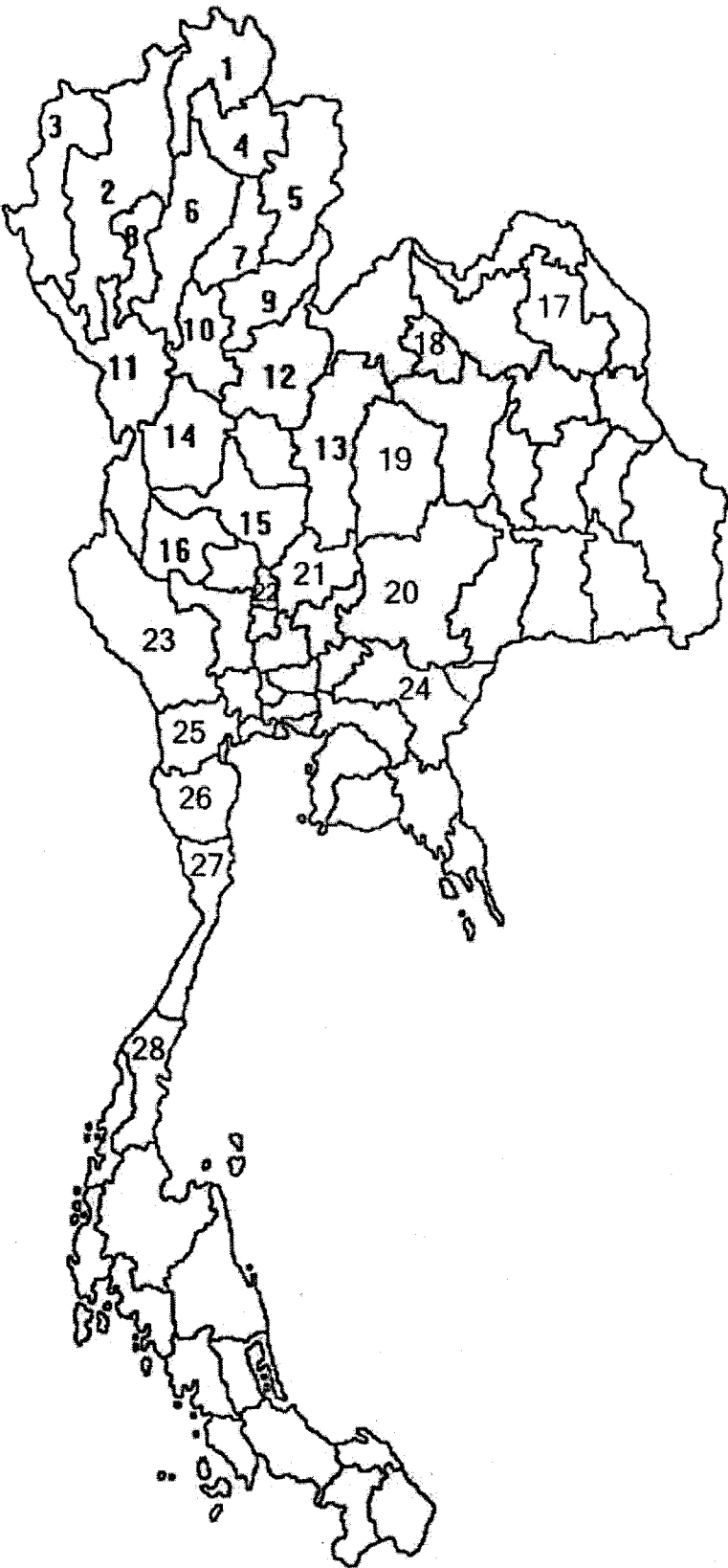


Figure 2.4. The comparison of the structures of isoflavonoid nucleus and estrogen.

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 *trnL*-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 (*trnL*-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 interpret the genetic relationship. In addition, Taberlet *et al.* (1991) designed primers from the cpDNA, especially in *trnL*-F region in order to study phylogeny. The maps of ITS, *trnL* and *trnL*-F regions are displayed in the figure 2.6 and 2.7.

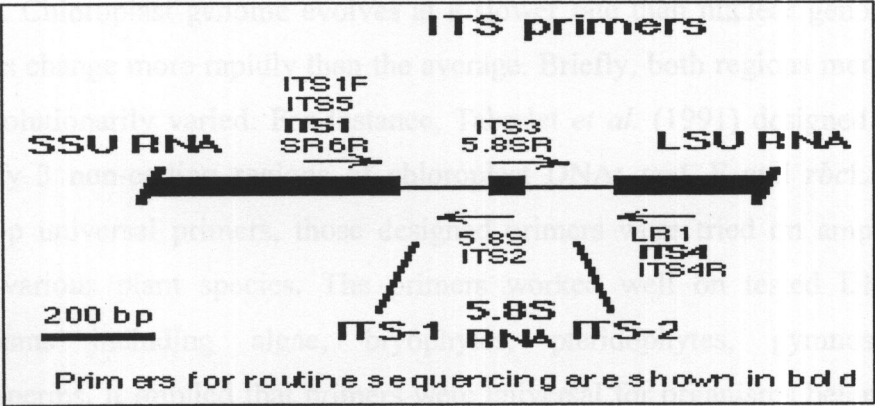


Figure 2.6. Map of nuclear ribosomal DNA internal transcribed spacer (ITS) region in eukaryotes (White *et al.*, 1990).

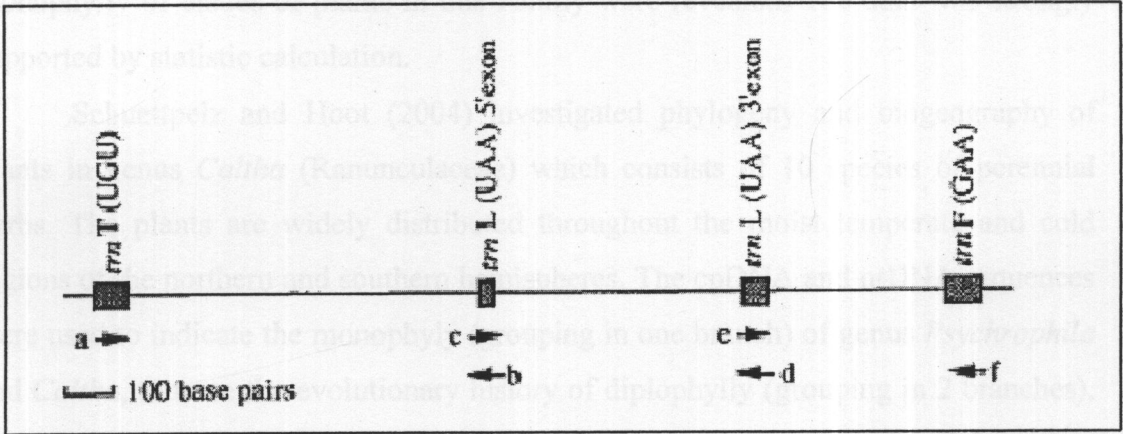


Figure 2.7. Map of chloroplast DNA *trnL*-F region in plants. Universal primers (a-f) were designed in phylogenetic analysis (Taberlet *et al.*, 1991).

In early 1990s, molecular phylogenetic researches have been mostly relied on cpDNA *rbcL* sequence but nowadays they have been relied on nrDNA internal transcribed spacer (ITS), cpDNA *trnL*-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. *trnL*-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: *trnL*-F and *rbcL*. 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, monophyly of clades of plants in this Family were revealed. The data was strongly supported by statistic calculation.

Schuettelpelz 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 diplophyly (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 *trnL*-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 Viciae) 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 *trnL-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 *trnT-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 *trnL* 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 (Mettler, 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 *HindIII* 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 (*i*NtRON BIOTECHNOLOGY, Korea)
- Primers or oligonucleotides (Bioservice unit, Thailand.)
- QIAquick® PCR purification kit, catalog# 28104 (QIAGEN GmbH, Germany)
- Tris-(Hydroxymethyl)-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°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	<i>P. Lobata</i>	√	√	
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

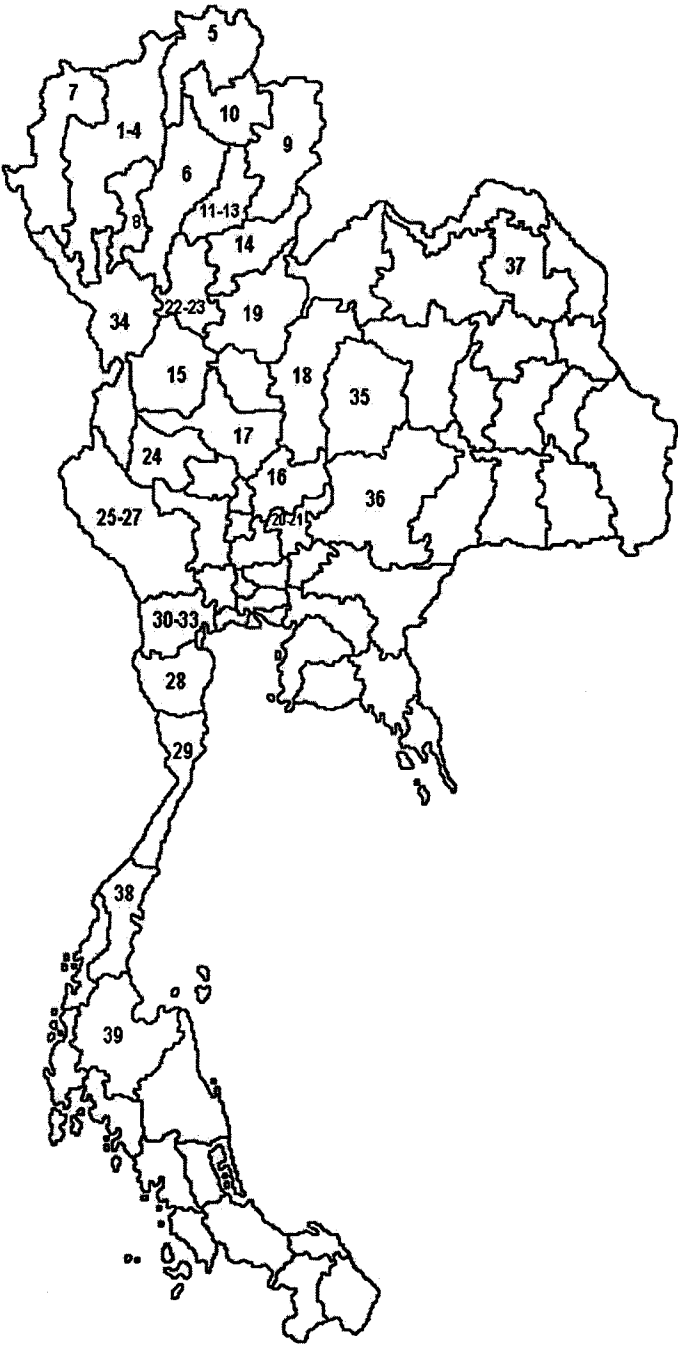


Figure 3.1. Map for leaf collection of 39 cultivars of *P. mirifica* among 27 provinces in Thailand.

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

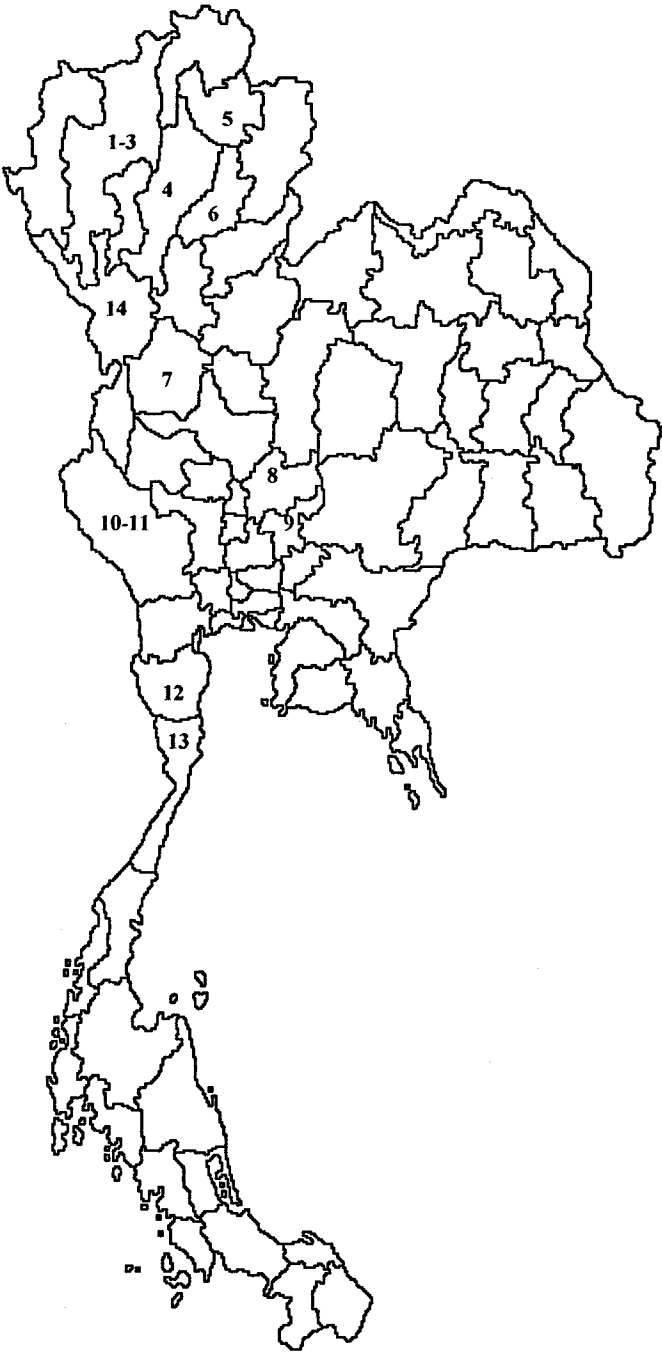


Figure 3.2. Map for pod collection of 14 cultivars of *P. mirifica* among 11 provinces in Thailand.

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

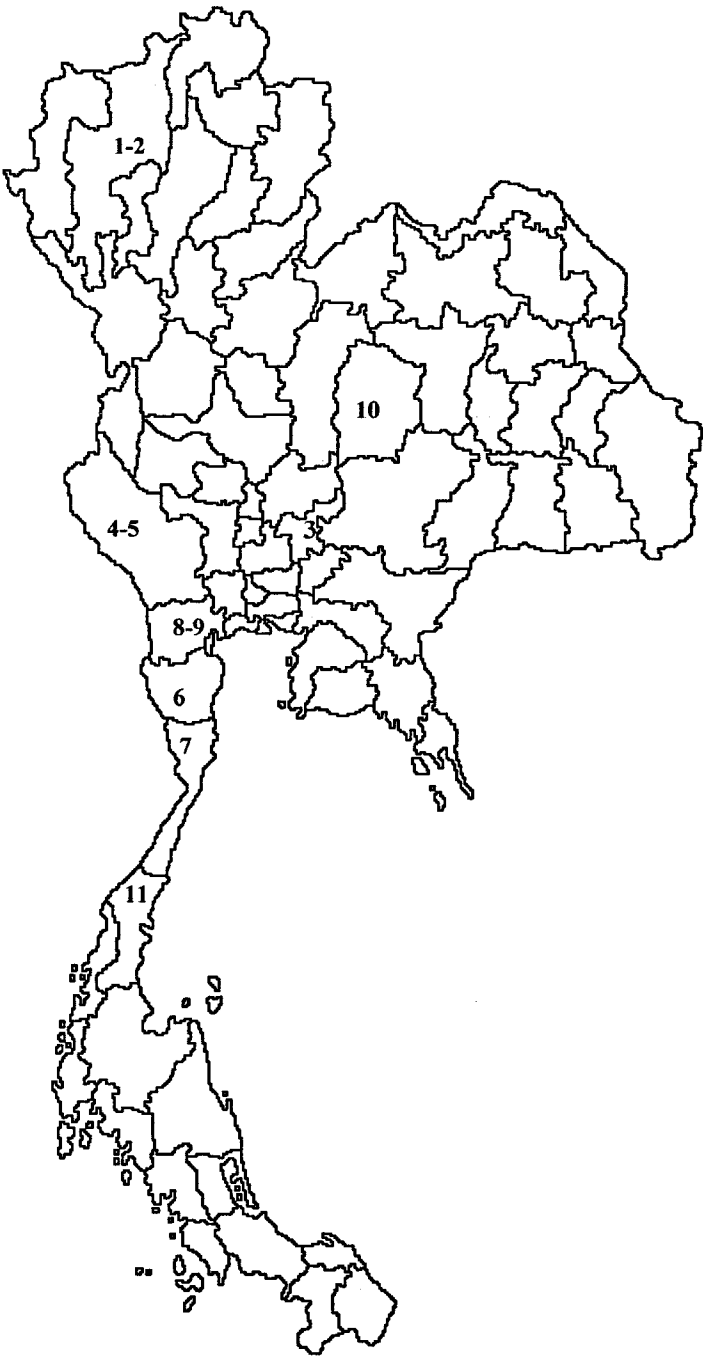


Figure 3.3. Map for flower collection of 11 cultivars of *P. mirifica* among 8 provinces in Thailand.

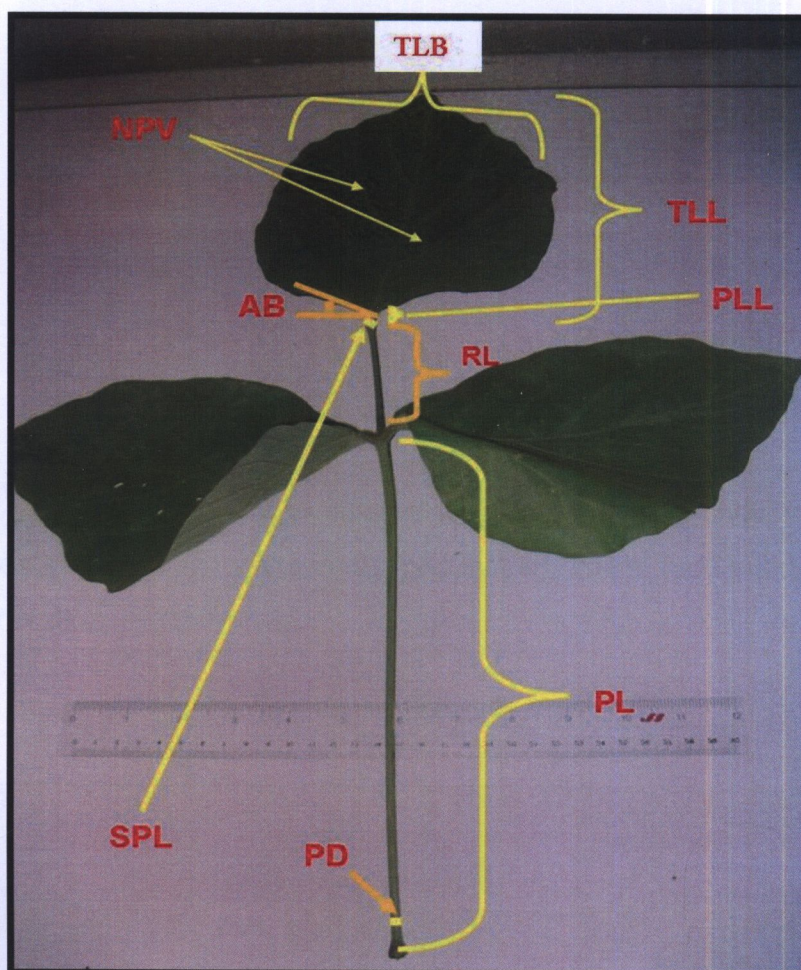


Figure 3.4. Nine morphometric parameters for measurement of collected *P. mirifica* leaf. Abbreviations used above are PL (petiole length), PD (petiole diameter), RL (rachis length), PLL (petiolet length), TLL (terminal leaflet length), TLB (terminal leaflet breadth), SPL (stipule length), $(A^{\wedge}B)^{\circ}$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

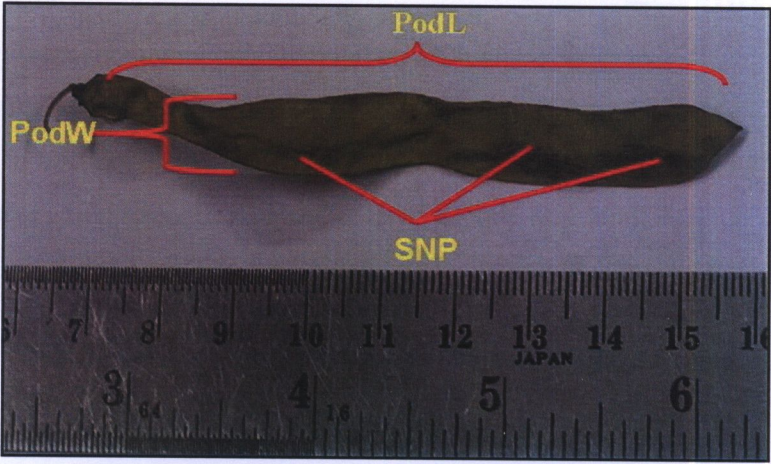


Figure 3.5. Three morphometric parameters for measurement of collected *P. mirifica* pod. Abbreviations used above are PodL (pod length), PodW (pod width), SNP (seed numbers per pod).

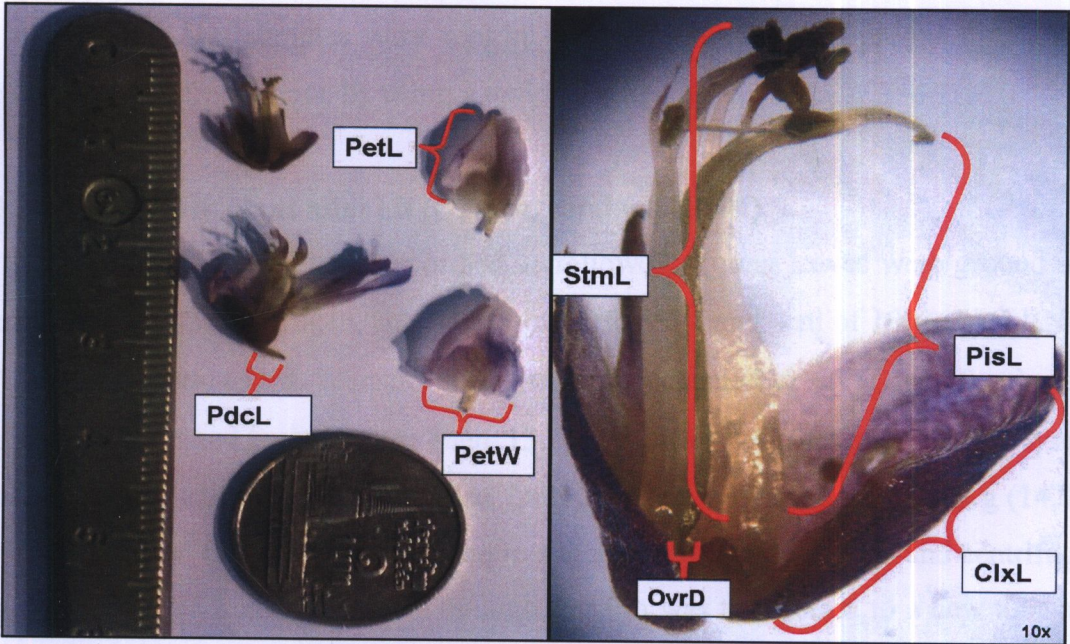


Figure 3.6. Seven morphometric parameters for measurement of collected *P. mirifica* flower (A and B). Abbreviations above are PdcL (pedicel length), PetW (petal width), PetL (petal length), StmL (stamen length), PisL (pistil length), OvrD (ovary diameter), and ClxL (calyx length). Figure 3.6 (B) was 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 °C 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 mortar. 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°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°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. Lambda Hind 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 1x TBE running buffer). After that, the gel was stained by 10 µg/ml Ethidium bromide (EtBr) solution for 5-10 min

and destained in d-H₂O 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 (*trnL-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-H₂O 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-H₂O 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 <i>et al.</i> , 1990
ITS_4	reverse	TCCTCCGCTTATTGATATGC	White <i>et al.</i> , 1990
<i>trnL</i> (UAA)5'exon primer_c	forward	CGAAATCGGTAGACGCTACG	Taberlet <i>et al.</i> , 1991
<i>trnL</i> (UAA)3'exon primer_d	reverse	GGGGATAGAGGGACTTGAAC	Taberlet <i>et al.</i> , 1991
<i>trnF</i> (GAA) primer_f	reverse	ATTTGAACTGGTGACACGAG	Taberlet <i>et al.</i> , 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°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 <i>et al.</i> (1995)
OPA-12	TCGGCGATAG	Mienie <i>et al.</i> (1995)
OPD-02	GGACCCAACC	Mienie <i>et al.</i> (1995)
OPD-16	AGGGCGTAAG	Mienie <i>et al.</i> (1995)
OPE-01	CCCAAGGTCC	Mienie <i>et al.</i> (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-H₂O 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-H₂O 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 - ($A^{\wedge}B$)°

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 A^B). 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.

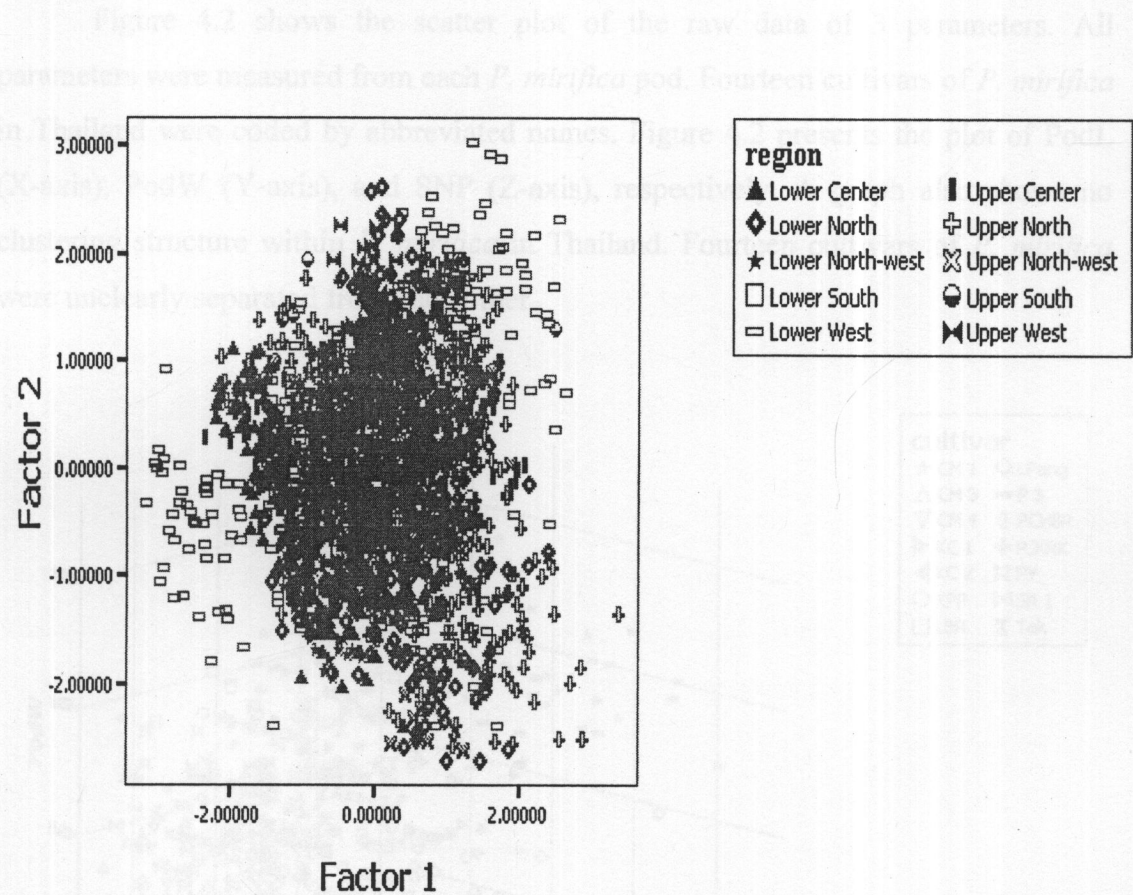


Figure 4.1. Position of each region in Thailand. Factor axes of 1 and 2 were derived from Factor analysis of leaf morphometric analysis: ordinate; factor 1 and abscissa; factor 2.

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.

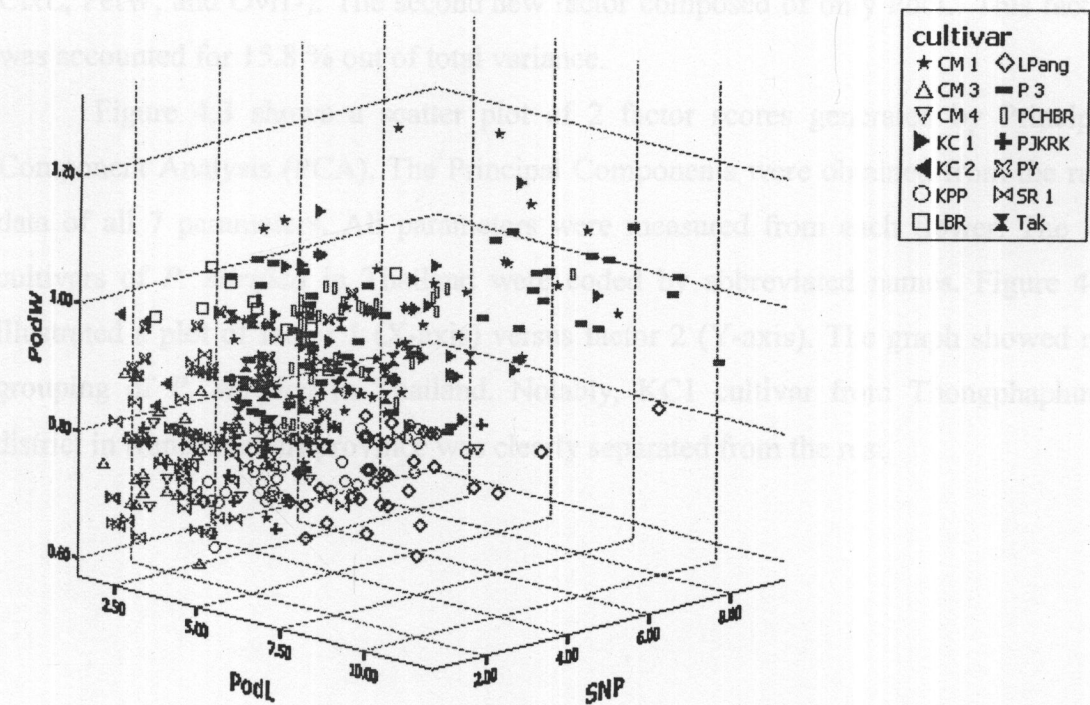


Figure 4.2. Position of each *P. mirifica* cultivar in Thailand for pod morphometric analysis. The 3-dimensional scatter plot was constructed on the axes of X-axis (PodL), Y-axis (PodW), and Z-axis (SNP).

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.

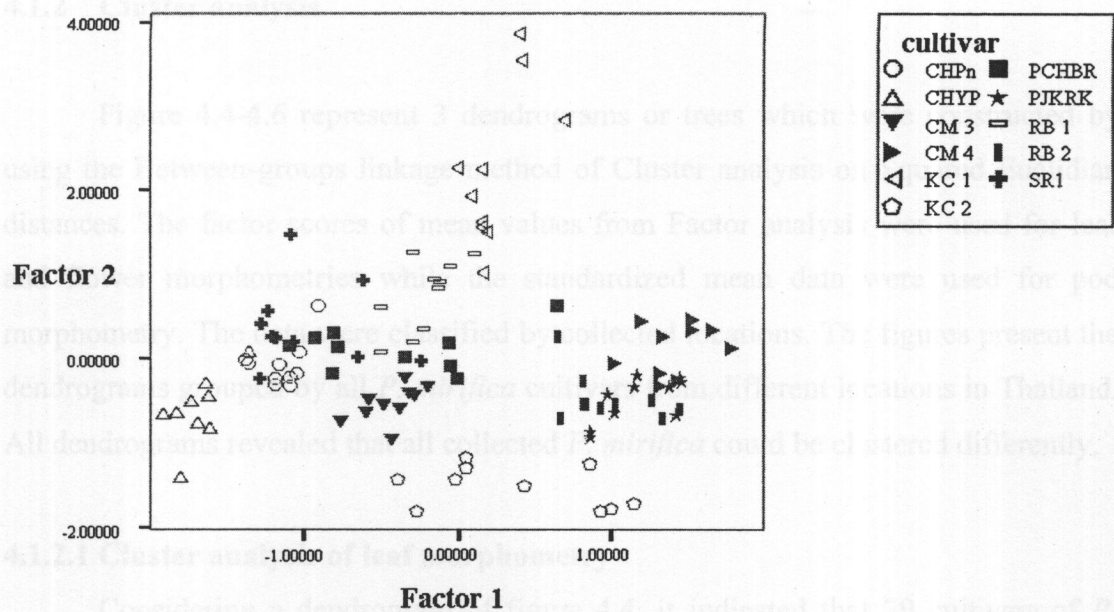


Figure 4.3. Position of each *P. mirifica* cultivar in Thailand for flower morphometric analysis. Factor axes of 1 and 2 were derived from Factor analysis: ordinate; factor 1 and abscissa; factor 2.

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.

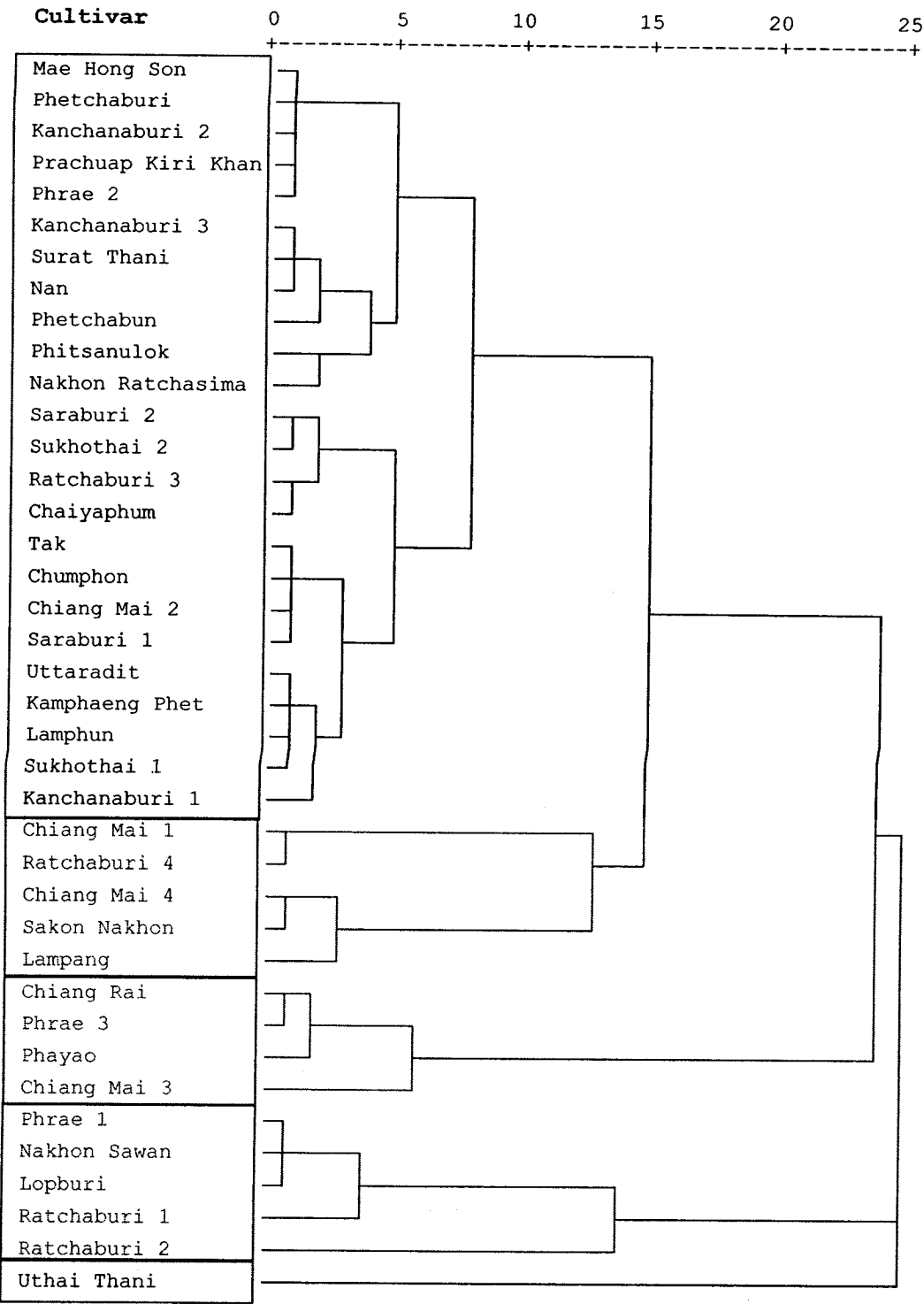


Figure 4.4. Leaf morphometric dendrogram created by Between-groups linkage method of Cluster analysis. *P. mirifica* is classified by collected location names.

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.

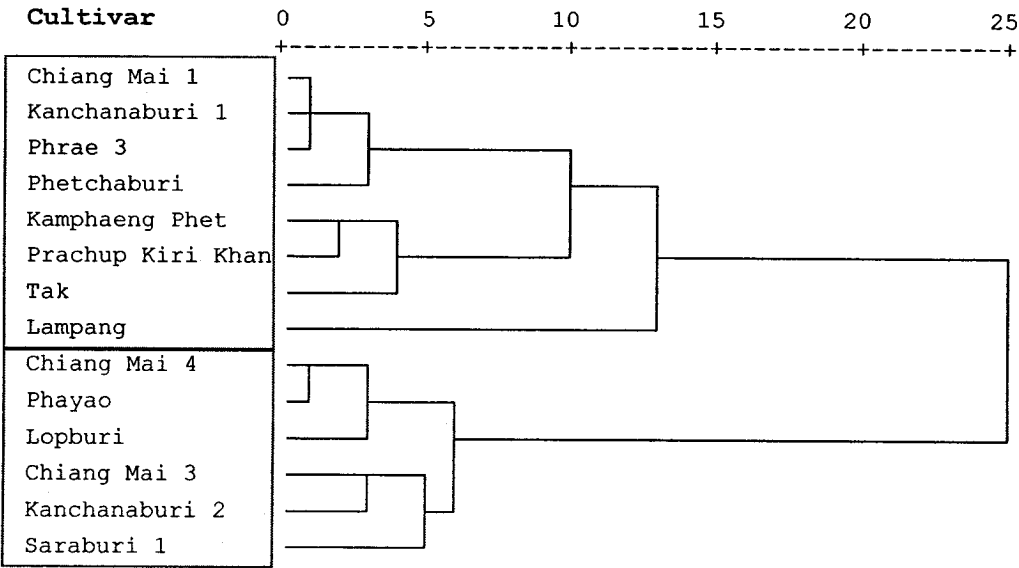


Figure 4.5. Pod morphometric dendrogram created by Between-groups linkage method of Cluster analysis. *P. mirifica* is classified by collected location names.

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.

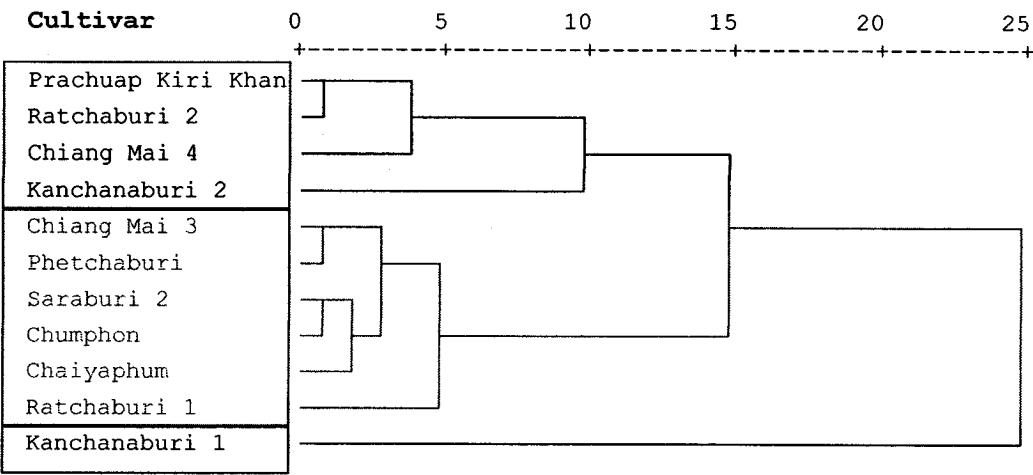


Figure 4.6. Flower morphometric dendrogram created by Between-groups linkage method of Cluster analysis. *P. mirifica* is classified by collected location names.

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 A^B 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 A^B 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) decrease 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).

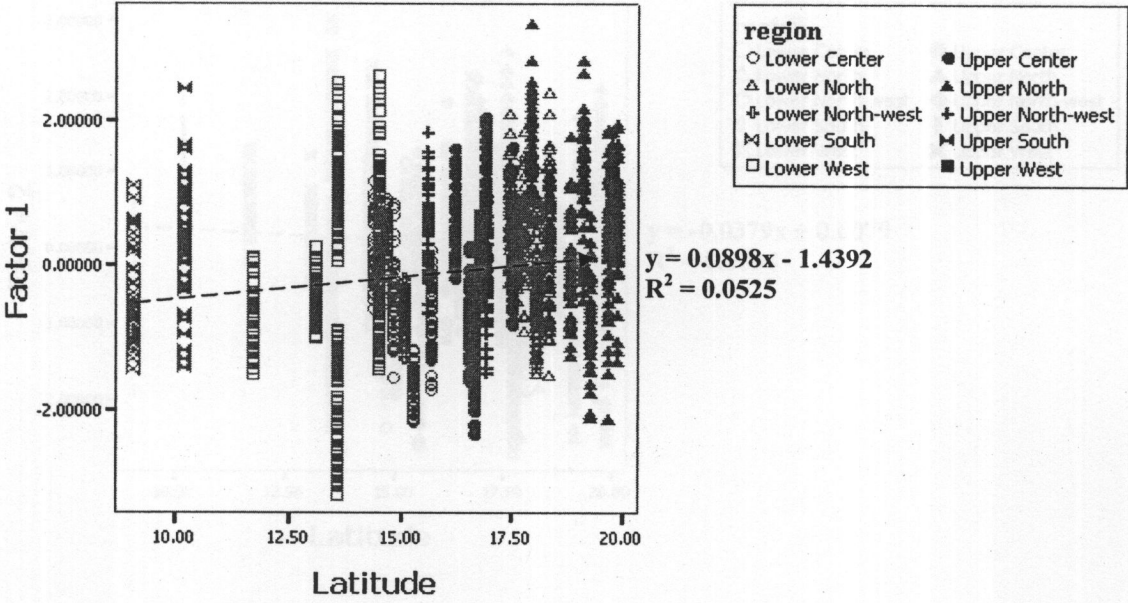


Figure 4.7. Geographic trends in leaf morphometry of *P. mirifica* in Thailand: abscissa; latitude; the factor score 1 derived from PCA. Value labels refer to regions.

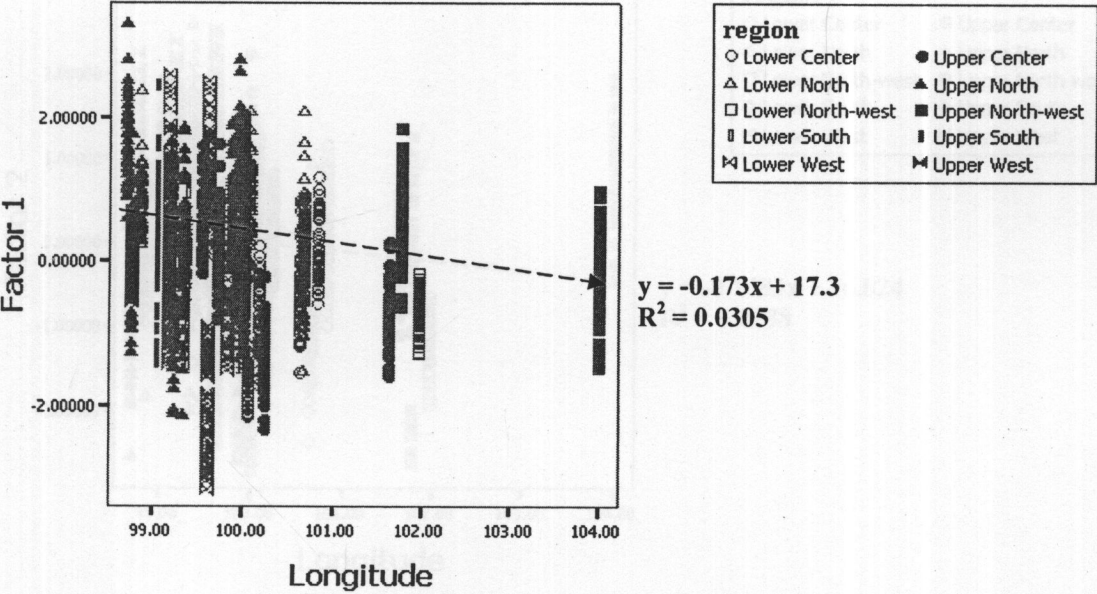


Figure 4.8. Geographic trends in leaf morphometry of *P. mirifica* in Thailand: abscissa; longitude; the factor score 1 derived from PCA. Value labels refer to regions.

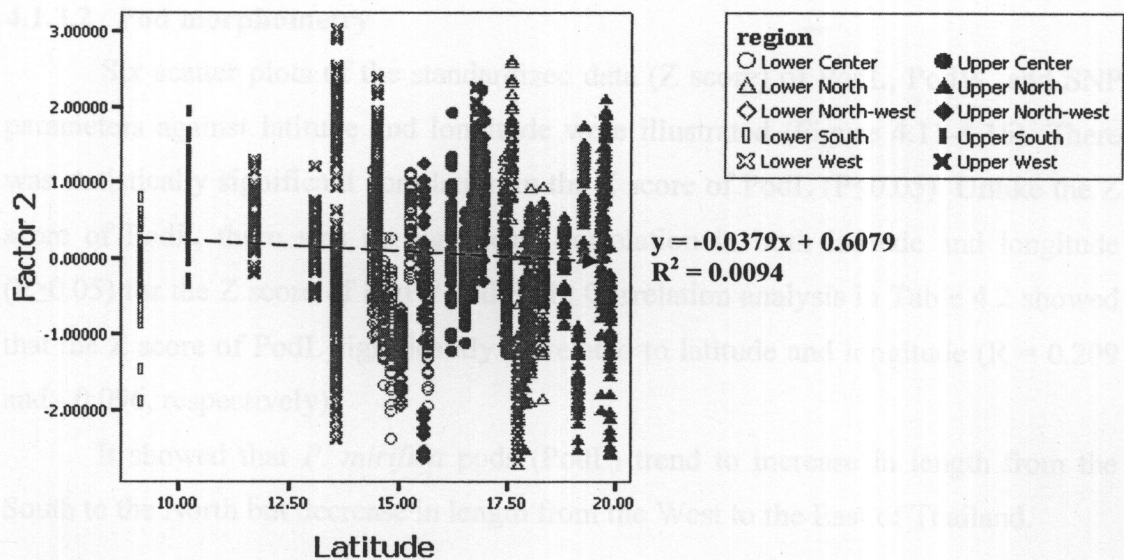


Figure 4.9. Geographic trends in leaf morphometry of *P. mirifica* in Thailand: abscissa; latitude; the factor score 2 derived from PCA. Value labels refer to regions.

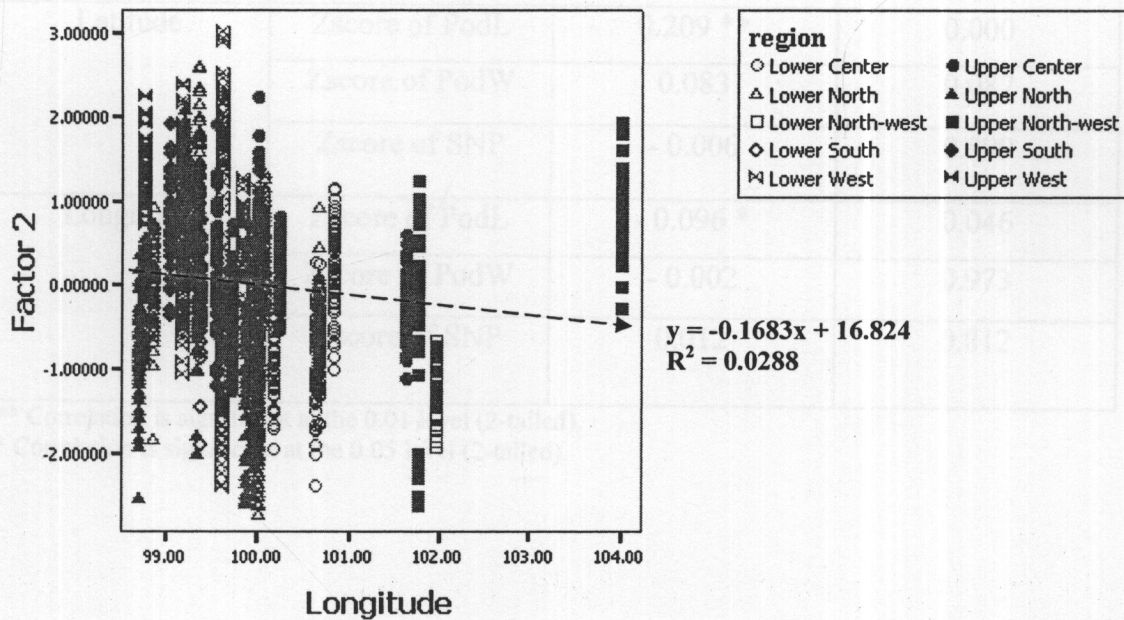


Figure 4.10. Geographic trends in leaf morphometry of *P. mirifica* in Thailand: abscissa; longitude; the factor score 2 derived from PCA. Value labels refer to regions.

4.1.3.2 Pod morphometry

Six scatter plots of the standardized data (Z score) 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).

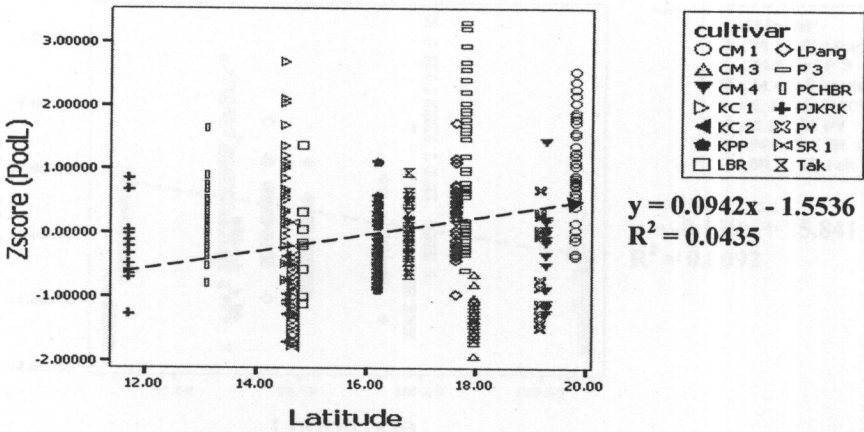


Figure 4.11. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; latitude; the Zscore of PodL. Value labels refer to cultivars.

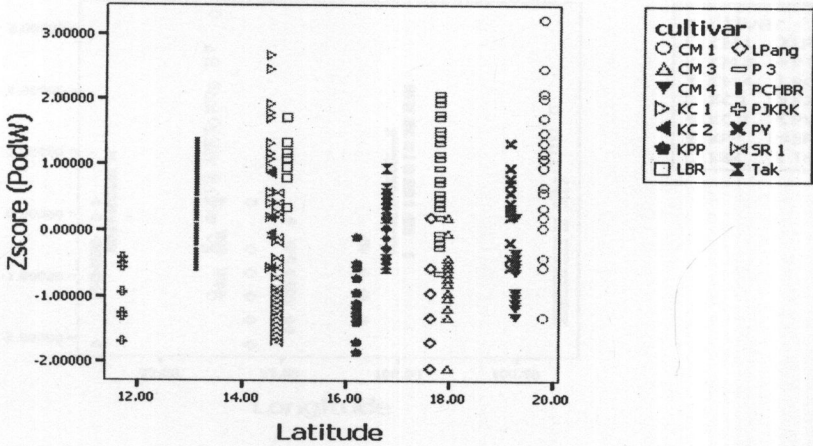


Figure 4.12. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; latitude; the Zscore of PodW. Value labels refer to cultivars.

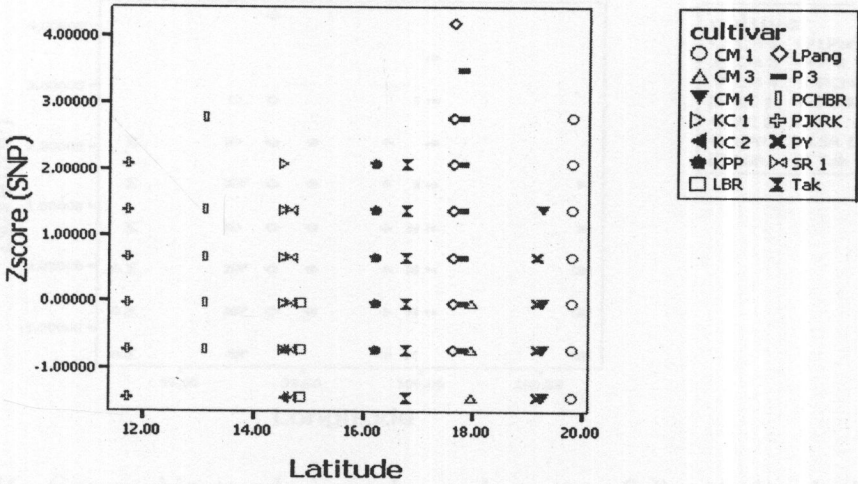


Figure 4.13. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; latitude; the Zscore of SNP. Value labels refer to cultivars.

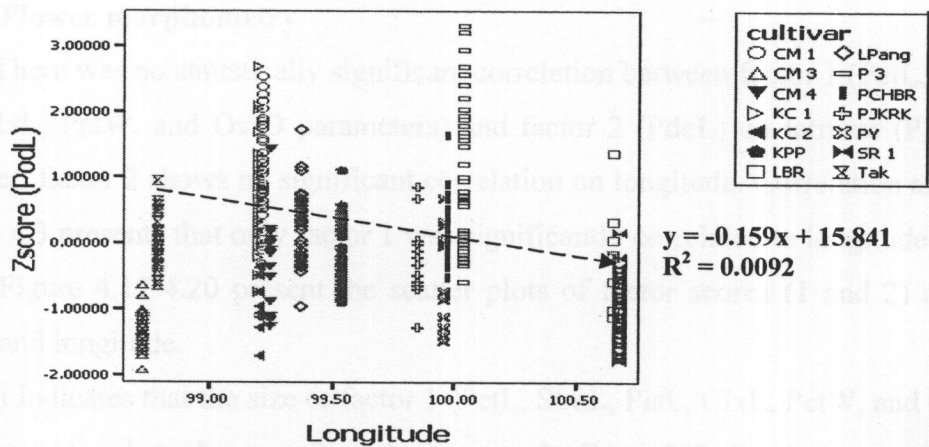


Figure 4.14. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; longitude; the Zscore of PodL. Value labels refer to cultivars.

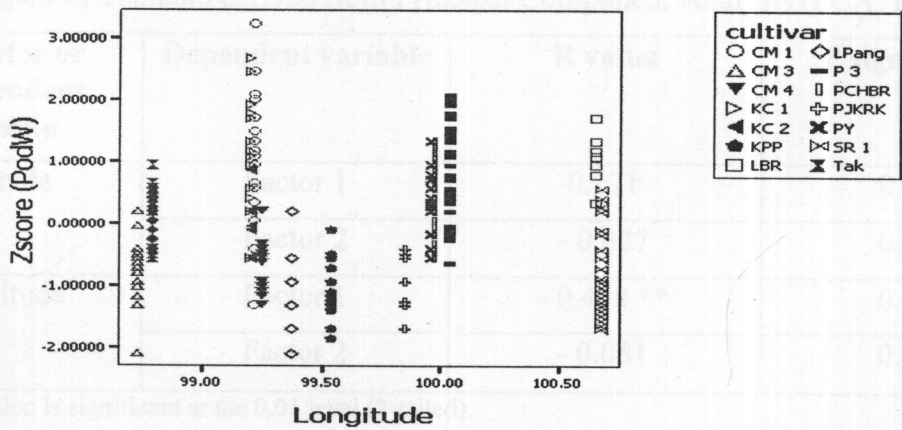


Figure 4.15. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; longitude; the Zscore of PodW. Value labels refer to cultivars.

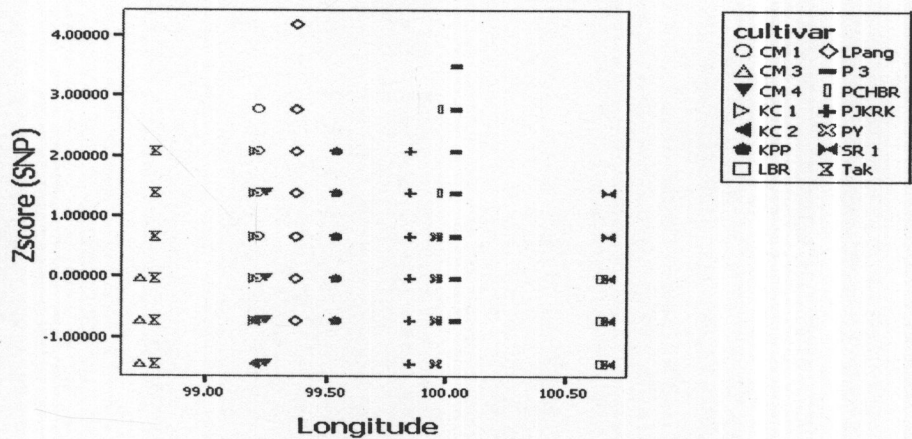


Figure 4.16. Geographic trends in pod morphometry of *P. mirifica* in Thailand: abscissa; longitude; the Zscore of SNP. Value labels refer to cultivars.

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).

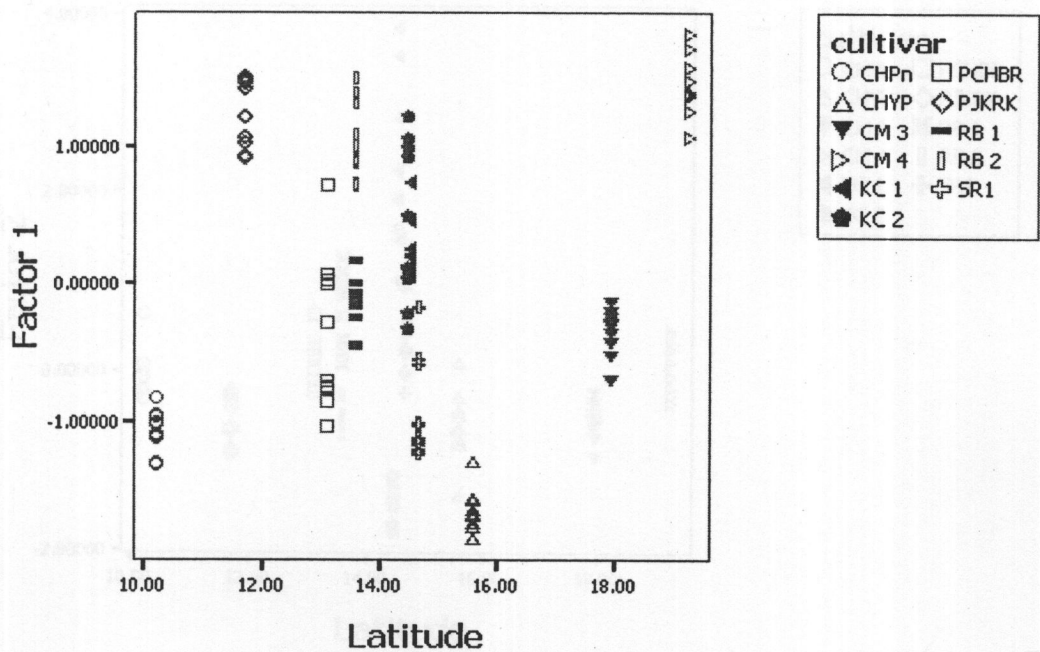


Figure 4.17. Geographic trends in flower morphometry of *P. mirifica* in Thailand: abscissa; latitude; the factor score 1 derived from PCA. Value labels refer to cultivars.

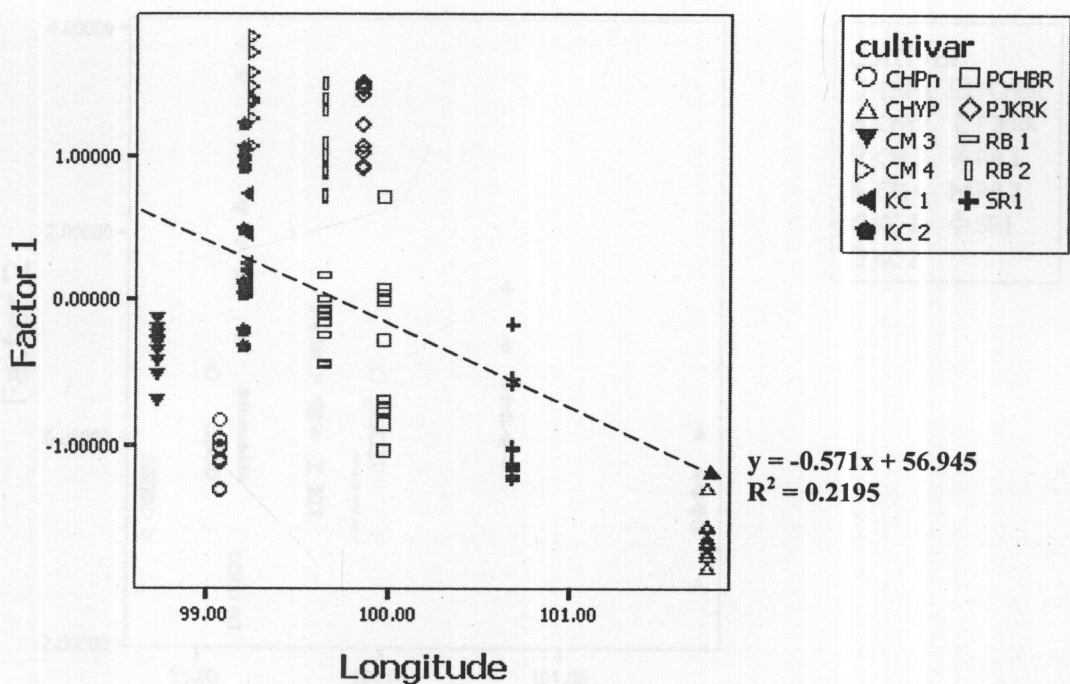


Figure 4.18. Geographic trends in flower morphometry of *P. mirifica* in Thailand: abscissa; longitude; the factor score 1 derived from PCA. Value labels refer to cultivars.

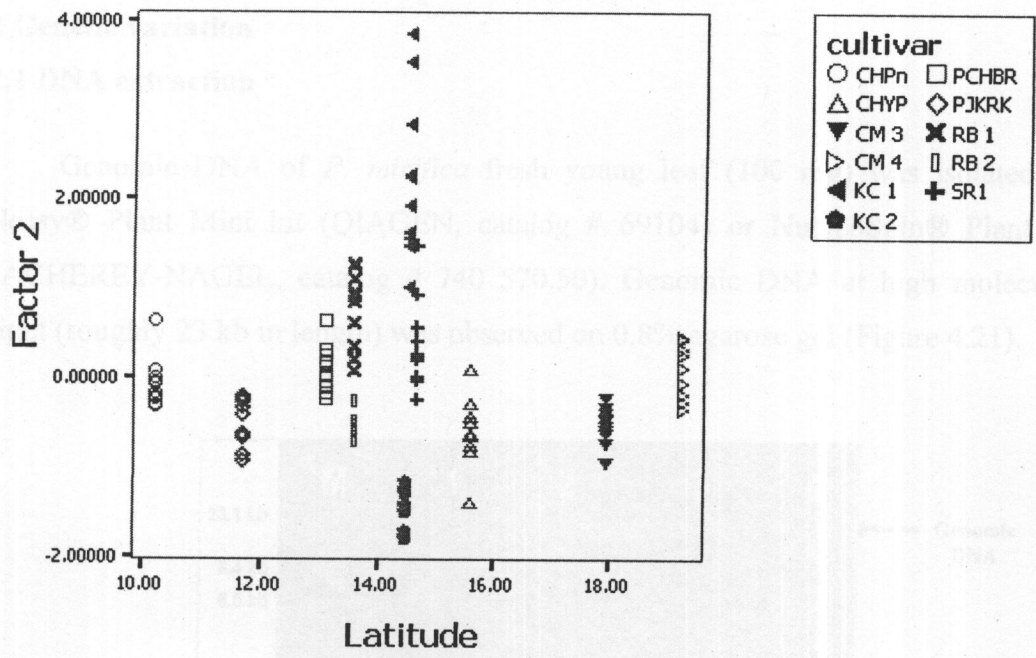


Figure 4.19. Geographic trends in flower morphometry of *P. mirifica* in Thailand: abscissa; latitude; the factor score 2 derived from PCA. Value labels refer to cultivars.

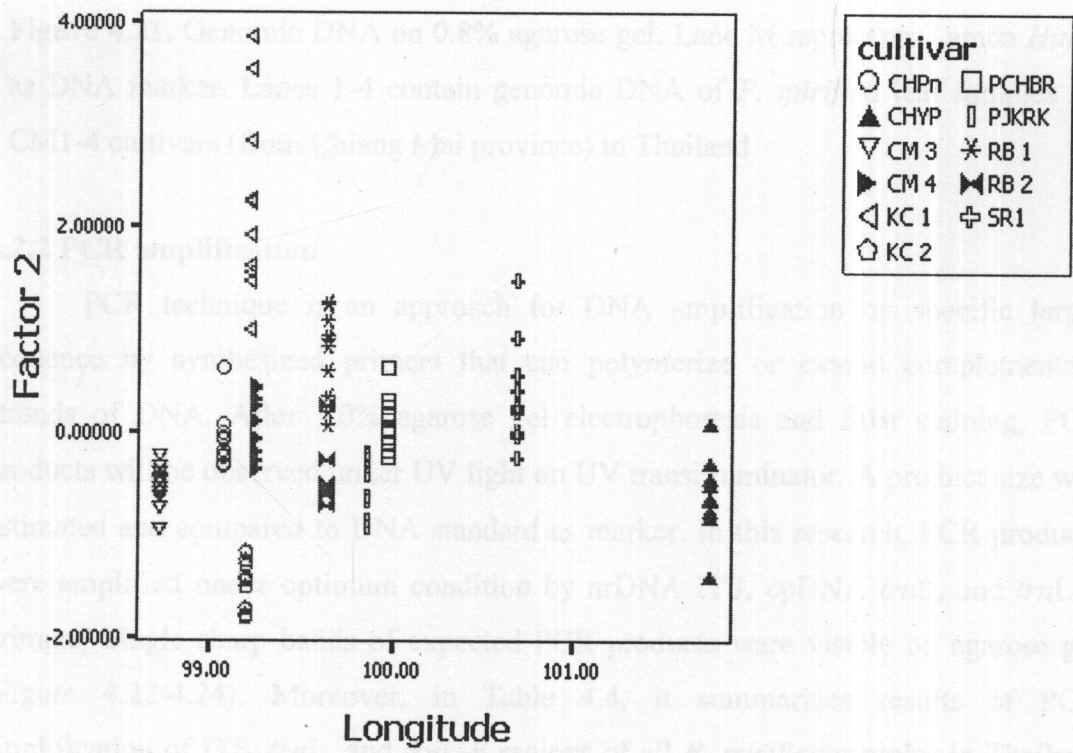


Figure 4.20. Geographic trends in flower morphometry of *P. mirifica* in Thailand: abscissa; longitude; the factor score 2 derived from PCA. Value labels refer to cultivars.

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).

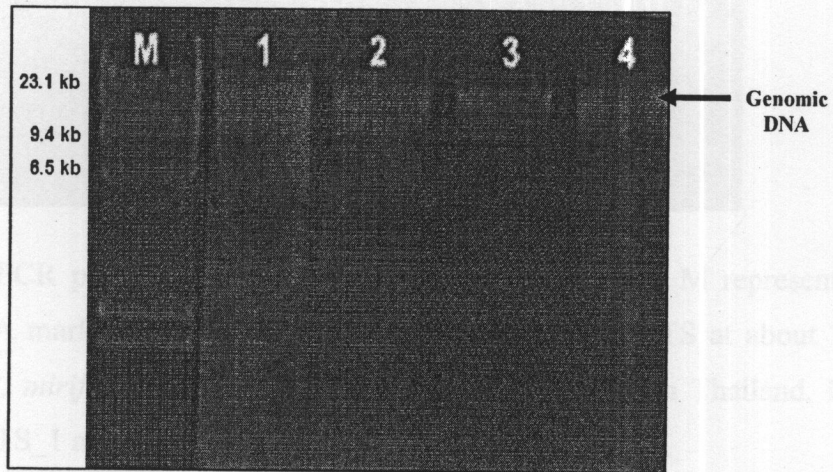


Figure 4.21. Genomic DNA on 0.8% agarose gel. Lane M represents Lamda *Hind* III as DNA marker. Lanes 1-4 contain genomic DNA of *P. mirifica* leaf samples from CM1-4 cultivars (from Chiang Mai province) in Thailand.

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 *trnL*, and *trnL*-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, *trnL*, and *trnL*-F regions of all *P. mirifica* samples in Thailand and *P. lobata* as outgroup.

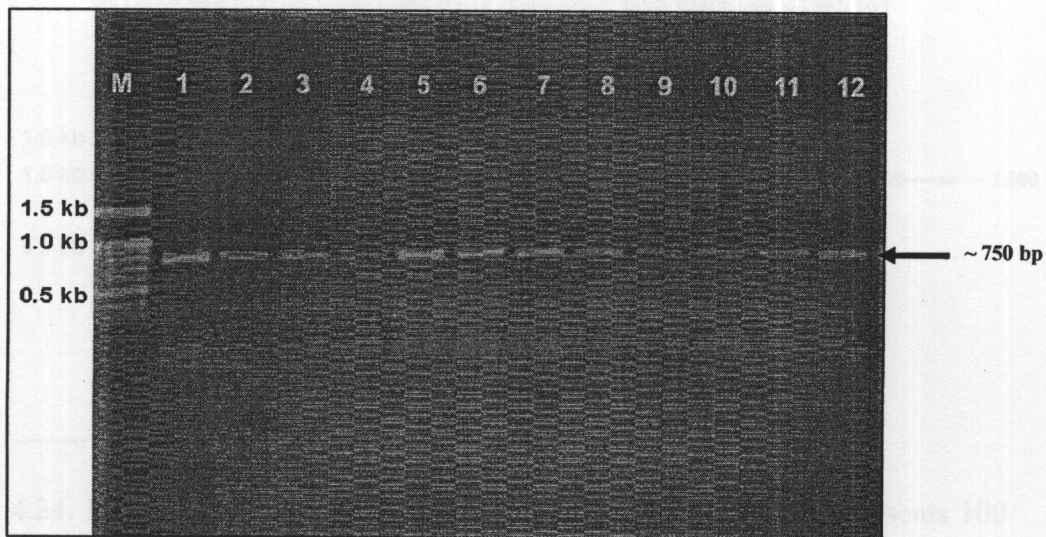


Figure 4.22. PCR products of ITS on 1.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-12 contain PCR products of ITS at about 750 bp in length from *P. mirifica* leaf samples from different locations in Thailand. DNA was amplified by ITS_1 and ITS_4 primers.

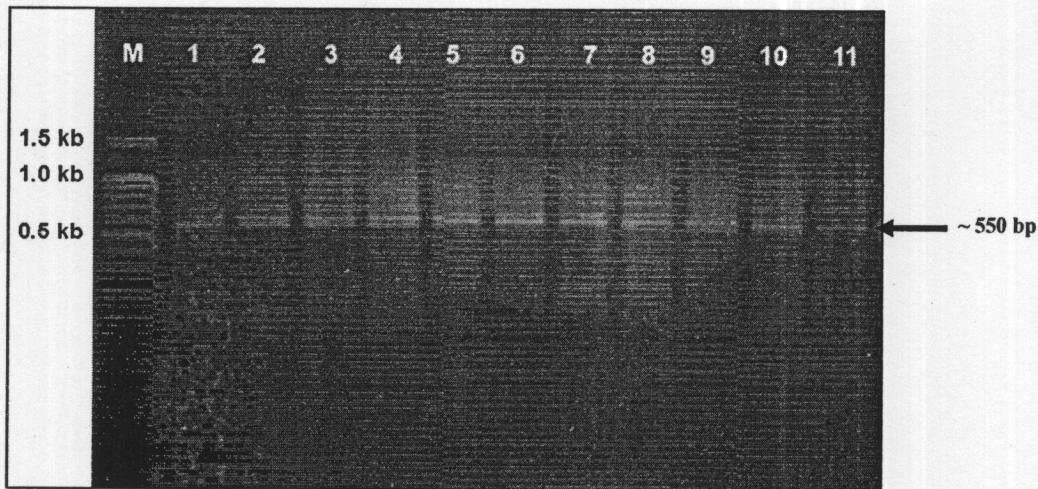


Figure 4.23. PCR products of *trnL* on 1.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-11 contain PCR products of *trnL* at about 550 bp in length from *P. mirifica* leaf samples from different locations in Thailand. DNA was amplified by c and d primers.

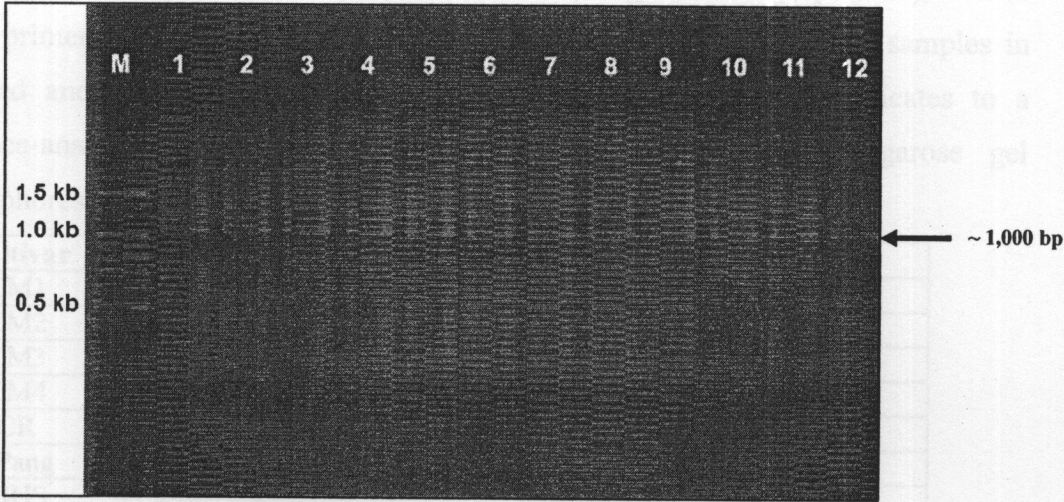


Figure 4.24. PCR products of *trnL-F* on 1.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-12 contain PCR products of *trnL-F* at about 1,000 bp in length from *P. mirifica* leaf samples from different locations in Thailand. DNA was amplified by c and f primers.

Table 4.4. Summary of PCR amplification of ITS (ITS_1 and ITS_4 primers), *trnL* (c and d primers) and *trnL*-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	<i>trnL</i>	<i>trnL</i> -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	✓	✓	✓
<i>P. lobata</i>	✓	✓	✓
Total	34	40	32

4.2.3 Sequence analysis

All PCR products of nrDNA ITS, cpDNA *trnL* and *trnL*-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, *trnL* region at 397 bp in length, and *trnL*-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, *trnL* and *trnL*-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 occurred. 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 *trnL* and *trnL*-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	AAGCAAGCAT	CGACT-TGCC	TGGCCTCCTC	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	GGTC-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	GTCTTCGCGC	CGACCTTCCTC	TCCTCCTTCC
RB1	GGGGTGG---	-----ACACA	AAACAAGCAT	CGTCT-TGCC	TGGCCTCCTC	TCCTCCTTCC
KPP	GGGGTGG---	-----ACACG	AAACAAGCAT	CGGC-CTGCC	TGGCCTCCTC	TCCTCCTTCC
P2	GGGGTGG---	-----ACACA	AAGCAAGCAT	CGTCT-TGCC	TGGCCTCCTC	TCCTCCTTCC
RB2	GGGGTGG---	-----ACACA	AAGCAAGCAT	CGTCT-TGCC	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	TCCATCTGT	TAAGT---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	CAACCCCCGG	CGCTTCGTGC	GCCAAGGAAC	TCCAATCTGT	TAAGT---GC
SR1	C-AACAAACA	CAACCCCCGG	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	TAAGT---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	CAACCCCCGG	CGCTTCGTGC	GCCAAGGAAC	TCCAATCTGT	CAAGTTGTGC
RB1	C-AACAAACA	CAACCCCCGG	CGCTTCGTGC	GCCAAGGAAC	TCCAATCTGT	TAAGT---GC
KPP	C-AACTAACA	CAACCCCCGG	CGCTTCGGGC	GCCAAGGAAC	TCCAATCTGT	CAAGG---GC
P2	C-AACAAAAA	CAACCCCCGG	GGCTTCCTGG	GCCAAGGAAC	TCCAATCTGT	TAAGG---GG
RB2	C-AACAAAAA	CAACCCCCGG	GGCTTCCTGG	GCCAAGGAAC	TCCAATCTGT	TAAGG---GG
LPoon	C-AACAAACA	CAACCCCCGG	GGCTTCGGGG	GCCAAGGAAC	TCCAATCTGT	TAAGG---GG
P3	C-AACAAACA	CAACCCCCGG	CGCTTCGTGC	GCCAAGGAAC	TCCAATCTGT	TAAGT---GC
Clustal Co	*	* * * *	**** *	** *	*****	**** * * *

Figure 4.25. (continued)

	190	200	210	220	230	240
KC2	GACTCCCAAC	GGCCCCGAGA	CAGAGCCCCG	CGGGCGTTGT	CACGACCCAC	ATTATT-ATA
CHPn	GACTCCCTCC	GGCCCCGAGA	CAGAGCCCCA	CCCgcGTTGT	CTCGACCCAC	ATTATT-TTA
CM3	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
PCHBR	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
KC1	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
MHS	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
SR1	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
CM1	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
CM4	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
PJKRK	AACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
Tak	AACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
RB3	AACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
SKHTH1	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
KC3	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
CHYP	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
SRTN	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
LBR	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
Nan	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
P1	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
UTTN	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
SKHTH2	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
PSNL	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
PBoon	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
PY	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
LPang	GACTCCCAAC	GGCCCCGAAA	CGGTGCCCCG	CGGGCGTTGT	CACGACCCAC	ATTATT-ATA
NKRSM	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
SR2	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACGCAC	ATTATT-ATA
Lobata	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTCGT	CACGACACAC	ACGATT-TATA
RB1	GACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGCGTTGT	CACGACACAC	ATTATT-ATA
KPP	GACTCCCAAC	GGCCCCGAGA	CGGAGACCCG	CGGCGTTGT	CACTACCCAC	ATTATT-ATA
P2	GACTCCCAAC	GGGCCGAAA	CGGGGGCCCG	CGGGCGTTGT	CACCACCCAC	ATTATT-ATA
RB2	GACTCCCAAC	GGCCCCGAAA	CGGGGGCCCG	CGGGCGTTGT	CACGACCCAC	ATTATT-ATC
LPool	GACTCCCAAC	GGGCCGAAA	CGGGGGCCCG	CGGGGGTTGT	CCCGACCCAC	ATTATT-TTA
P3	AACTCCCAAC	GGCCCCGAGA	CGGTGCCCCG	CGGGGGTTGT	CCCCACCAAC	ATTATT-TTT
Clustal Co	*****	* ** *****	* * * * *	* * * * *	* * * * *	* * * * *
	250	260	270	280	290	300
KC2	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTTGCATC	GATGAAGAAC	GTACCGAAAT
CHPn	CATAATGACT	CTCGCCACG	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	GATGAAGAAC	GTAGCGAAAT
KPP	CAAAATGACT	CTCGGTCACG	GATATCTCGG	TTCTTGCATC	GGTGAAGAAC	ATAACGAAAT
P2	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTTGGATC	CATGAAAAAC	GTAACGAAAT
RB2	CAAAATGACT	CTCGGCAACG	GATATCTCGG	CTCTTGGATC	GATGAAGAAC	GTAACGAAAT
LPool	CAAAATGACT	CTCGGCAACG	GAAATCTCGG	TTCTTGCATC	AATAAAAAAC	GTACCGAAAT
P3	CAAAATGACT	CTCGGCAACG	GAAATTTTCG	CTCTTGGATC	CATGAAAAAC	GGACGAAAT
Clustal Co	** *****	****	** * * * *	*****	* * * * *	* * * * *

Figure 4.25. (continued)

	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
SKH1	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
SKH2	GCGATACTTG	GTGTGAATTG	CAGAATCCCG	TGAACCATCG	AGTCTTTGAA	CGCAAGTTGC
PSNL	GCGATACTTG	GTGTGAATTG	CAGAATCCCG	TGAACCATCG	AGTCTTTGAA	CGCAAGTTGC
PBoon	GCGATACTTG	GTGTGAATTG	CAGAATCCCG	TGAACCATCG	AGTCTTTGAA	CGCAAGTTGC
PY	GCGATACTTG	GTGTGAATTG	CAGAATCCCG	TGAACCATCG	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	TGAACCATCG	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	CGCAAGTTGC
P3	GGGAAACTTG	GGGGGAATTG	GAAAATCCCG	GGAACCATCC	AGTCTTTGAA	CCCAAGTTGC
Clustal Co	* * * * *	* * * * *	* * * *	* * * * *	* * * * *	* * * * *

	370	380	390	400	410	420
KC2	GCCCCAAGCC	ATT-AAGTGG	AGGGAACGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
CHPn	GTCCGACCCC	ATT-AAGTGG	AGGGAACGCC	TGCCTGGGTG	GCAGACATCG	TTACCCCCAC
CM3	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
PCHBR	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
KC1	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
MHS	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
SR1	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
CM1	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
CM4	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
PJKRK	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
Tak	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
RB3	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
SKH1	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
KC3	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
CHYP	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
SRTN	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
LBR	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
Nan	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
P1	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
UTTN	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
SKH2	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
PSNL	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
PBoon	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
PY	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	GCACACATCG	TTACCCCCAC
LPang	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
NKRSM	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
SR2	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
Lobata	GCCCCAAGCC	ATTAGGCAAG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
RB1	GCCCCAAGCC	ATT-ATGTGG	AGGGGACGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
KPP	GCCCCAAGCC	ATT-ACGTGG	AGGGGAGGCC	TGCCTGGGTG	TGACACATCC	TTACCCCCAC
P2	GCCCCAAGCC	ATT-AAGTGG	AGGGCAGGCC	TGGCTGGGTG	GCACACATCG	TTACCCCCAC
RB2	GCCCCAAGCC	ATT-AGGTGG	AGGGCAGGCC	TGCCTGGGTG	TCACACATCG	TTACCCCCAC
LPoon	GCCCCAAGCC	ATT-AGGGGG	AGGGGAGGCC	TGCCTGGGGG	TCACAAATCG	TTACCCCCAC
P3	GCCCCAAGCC	ATT-AAGGGG	AAGGGACGCC	TGGCTGGGTG	GCACACATCG	TTACCCCCAC
Clustal Co	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *	* * * * *

Figure 4.25. (continued)

						
	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	TGCACGCTGA
LPang	GCAAACAAAT	GT---CTCAC	ACGACAGA--	---CGTTCTG	CGTAGTAGGG	TGCACGCTGA
NKRSM	GCAAACAAAT	GT---CTCGC	ATCACAGA--	---CGTTCTG	CGTAGTAGGG	TGCACGCTGA
SR2	GCAAACAAAT	GT---CTCAC	ACGACAGA--	---CGTTCTG	CGTAGTAGGG	TGCACGCTGA
Lobata	GCAAACAGAC	GT---CCCAC	ACGACGGC--	---CGTT--G	CGTGGTAGGG	TGCACGCTGA
RB1	GCAAACAAAT	GT---CTCAC	ACGACAAA--	---CATTCCTG	CGTAGTAAGG	TGCACGCTGA
KPP	GCAAACAAAT	GT---CTCAG	ACGACAAA--	---CATTCAG	CGTAGTAGGG	TGCACGCTGA
P2	CCAAACAAAT	GG---CTCAC	ACCAACAAA--	---GTTCTG	CGTAATAAGG	TGCACGCTGA
RB2	GCAAACAAAT	GTTTCTCTC	ACAAAAGA--	---TCTTCTG	AGTAGGATGG	AGGACGATCA
LPoon	CCAAACAAAG	GT---CTCAA	ACAAAAAA--	---CGTTCTG	CGAAAAAGGG	GGCAGCTTAA
P3	CCAAAAAAT	GG---CTCAC	ACGAAAAAAG	TTCGTGTTCTG	TTAGGGAGGG	GGCAAACTTA
Clustal Co	**** *	* *	* *	* *	* **	* * *

						
	490	500	510	520	530	540
KC2	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAACAAG	TTCGTGGCCG	AGTGAGCCGT
CHPn	CCTACCTCCA	GCACCGTCTC	GCGGTTGGTT	GAAAAAAAAG	TTCGTGGCCG	AGTGAGCCGT
CM3	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTACGCCGT
PCHBR	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTACGCCGT
KC1	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTACGCCGT
MHS	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTACGCCGT
SR1	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTACGCCGT
CM1	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTACGCCGT
CM4	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTACGCCGT
PJKRK	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTACGCCGT
Tak	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAACAAG	TTCGTGGCCG	AGTACGCCGT
RB3	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTACGCCGT
SKHTH1	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTACGCCGT
KC3	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAACAAC	TTCGTGGCCG	ACTACGCCGT
CHYP	CCTCCCGCGA	GCACCGTCTC	GTGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTGCGCCGT
SRTN	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTGCGCCGT
LBR	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTGCGCCGT
Nan	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTGCGCCGT
P1	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTGCGCCGT
UTTN	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTGCGCCGT
SKHTH2	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTGCGCCGT
PSNL	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTGCGCCGT
PBoon	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTGCGCCGT
PY	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAACAAG	TTCGTGGCCG	AGTGCGCCGT
LPang	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTGCGCCGT
NKRSM	CCTCACGCGA	GCACCGTCTC	GTGGTTGGTT	GAAAAATCGAA	TTCGTGGCCG	AGTGACCCGT
SR2	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTGCGCCGT
Lobata	CCTCCCGCGA	GCGGGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCGTGGCCG	AGTGCGCCGT
RB1	CCTCCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCGAG	TTCATGGCCG	AGAAGGCCGT
KPP	CCTCCCGCGA	GCACCGGCTC	GCGGTTGGTT	GAAAAATCAA	ATCGAGGCCG	AGGGCGACGG
P2	ACTCCGCGA	GCACCGTCTC	GCGGTTGGTT	GAAAAATCCAG	TTCGTGGCCG	AATGCCCCCT
RB2	CCCCCGGGA	GCGGCTCTC	GCGGGTGGAA	AAAAAATATC	TTCGCGGACC	AAAGCGCCCT
LPoon	CCTCCCGGGA	GAACCGTCTC	GGGGTTGGTT	AAAAATCAAT	TTCGGGGCCA	AATGCCCCCG
P3	CCTCCAGCAA	GGACCGGCTG	TGGGTTGAAA	AAAAATTTCG	TGCCCGATCG	AGCGCGCCTA
Clustal Co	*	* *	***	****	* * *	* *

Figure 4.25. (continued)

	550	560	570	580	590	600
KC2	GATAAAATGG	TGGATGAACA	ACGCTCGATA	CCAATCAAGC	GCCGCGACTC	GGTCGGGCTCC
CHPn	GATAAAATGG	TGGATGAACA	ACGCTCGATA	CCAATCACGC	GCCGCTACTC	GGTCGGGCTCC
CM3	GG-AAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
PCHBR	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
KC1	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
MHS	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
SR1	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
CM1	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
CM4	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
PJKRK	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
Tak	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
RB3	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
SKH7H1	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
KC3	GATAAAATGA	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
CHYP	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
SRTN	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
LBR	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
Nan	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
P1	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
UTTN	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
SKH7H2	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
PSNL	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
PBoon	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
PY	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
LPang	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
NKRSM	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
SR2	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
Lobata	GATAAAATGG	TGGATGAGCA	ACGCTCGAGA	CCAATCACGC	GCTTGCAGTC	GGTCGGCGAA
RB1	GATAAAATGG	TGGATGAGAA	ACGCTCGAGA	CCAATCACGC	GCCGCGACTC	GGTCGGGCTCC
KPP	GATAAAATGG	AGGAGGAGCA	ACACTCAAGA	CCAATCACGC	GCCGCGACTC	GGACGGGTCC
P2	GATAAAATGG	GGGAAGAACA	ACGCTCAAGA	ACAATCACGC	GCCGCTACTC	GGTCGGGTCC
RB2	GAAAAATGG	GGGAAGAACA	ACCCACAAGA	AACATCACGC	GCCACGACTC	CGGCTGCTCA
LPoon	GAAAAATGG	GGGATAAGCA	ACGTTCAAGAA	CCAATCACGC	GCCGGGACTC	GGTCGGTTC
P3	AATGGAGGGA	TGAACAAGCA	ACGGTCCAAA	TCAATCACGC	GCCCTGGCTC	GGTTCGGTCC
Clustal Co	** *	* * *	* * *	* * *	* * *	* * *

[illegible]

Figure 4.25. (continued)

	
	670 680
KC2	AGACCTCAGG -TCAGGCGGG GCTACCC
CHPn	AGACCTCAGG -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 GGCGCC
Clustal Co	* * * * * * * * * * * * *

Figure 4.25. (continued)

	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	TTAGGAAAAG
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	TTAGGAAAAG
NKSW	TCAATGGAAG	CTGTTCTAAC	AAACGGAGTT	GACGATTTTT	CCTTTTTGCA	TTAGGAAAAG
Clustal Co	** ** *		* * * *	*****	*****	*** ** *

Figure 4.26. A 397 bp character matrix of 39 cultivars of *P. mirifica* and 1 cultivar of *P. lobata* based on *trnL* sequences. Asterisk symbols (*) show that all samples provide nitrogenous base (A, C, G, and T) consensus or identity.

							
	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	TCAAAATTCC	AGGAATGGAT	CAAAGATAAA	CATATATATA	CTGAAATACT	
UTTN	AATCCTTCCA	TCAAAATTCC	AGGAATGGAT	CAAAGATAAA	CATATATATA	CTGAAATACT	
SRNK	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	AATCCTTCCA	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	CTGAAATACT	
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	
LEPang	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	*** * ****	*****	*****	*****	*****	*****	*****

Figure 4.26. (continued)

							
	130	140	150	160	170	180	
CM3	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
PY	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
PSNL	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
RB2	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
RB3	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
P1	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
SKHT2	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
SRTN	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
SR1	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
UTTN	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
SKNK	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
CR	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
Tak	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
KC1	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
CM4	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
LBR	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
PCHBR	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
KC2	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
SR2	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
Lobata	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATAAAAATA	TTCACAAATG	AAAGATGTGA	
P3	ATTTC AATTG	ATTAATGAGG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
P2	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
SKHT1	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
CM1	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
NKRSM	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
CHYP	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
RB1	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
PBoon	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
PJKRK	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
KPP	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
LPang	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
KC3	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
LPoon	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
CHPn	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
UTRD	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
CM2	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
RB4	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
MHS	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
Nan	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
NKSW	ATTTC AATTG	ATTAATGAAG	ATCCATTGT	GATCAAAATA	TTCACAAATG	AAAGATGTGA	
Clustal Co	*****	*****	*****	*****	*****	*****	*****

Figure 4.26. (continued)

```

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
      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
PC2      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 *****

```

Figure 4.26. (continued)

```

.....|.....| .....|.....| .....|.....| .....|.....| .....|.....|
      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 TTGAAGA--- --TTAATCAG 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
NKS       ATCATAATCT GATAGATCCC TTGAAGA--- --TTAATCAG ACGAGAATAA AGATAGAGTC
Clustal Co *****

```

Figure 4.26. (continued)

							
	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	*****	*****	*****	*****	*****	*****	

Figure 4.26. (continued)

				
	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)

						
	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	** **	* **	*** **	** *****	***** ***	*****

Figure 4.27. A 731 bp character matrix of 31 cultivars of *P. mirifica* and 1 cultivar of *P. lobata* based on *trnL-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     AATCCTTCCA 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 CTGAAATACT
LBR     AATCCTTCCA TCAAAATTCC AGGAATGGAT CAAAGATAAA CATATATATA CTGAAATACT
SR1     AATCCTTCCA TCAAAATTCC GGAATGGAT 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 GATAAAATA 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  ***** ***** * **** ***** ** ***** ***** *****

```

Figure 4.27. (continued)


```

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

```

```

.....|.....|.....|.....|.....|.....|.....|.....|
      370      380      390      400      410      420
P1      TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA ACTTTCTAAT
SKHT2   TTAAGAAATC GTGAGGGTTC AAGTCCCTCT ATCCCCAAAA GGCCTGTTTA 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
NKRS     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 GGCCTGTTTA 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 *****

```

Figure 4.27. (continued)


```

....|....| ....|....| ....|....| ....|....| ....|....|
      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     ACAAACATT AC-AAAATAA TTACTACTAC TGAAACTAAC TTCCAA-C-T TTTATTTTCC
RB2      ACAAACAAT AC--AAATA- TTACTACTAC TGAA-CTAAC TTACAA-CTT TTTATTTTTC
RB3      ACAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
CM1      -CAAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
NKRSM    ACAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
CHYP     ACAAACCAT ACAAATAA 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 TTCCTCTCC TGAA-CTAAC TTCCAA-CCT TTTATTTTCC
KC3      ACAAACCA-T AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAACCT TTTATTTTTC
LPoon    ACCAAACAAT TC-AAAATAA TTACTACTAC TGAAACTACC TTACAAAC-T TTTATTTTTC
KPP      ACAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
LPang    ACCAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAA-C-T TTTATTTTTC
PBoon    ACAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
PJKRK    ACAAACCAAT ACC-AAATAA TTACTACTAC TGAAACTAAC TTACAA-C-T TTTATTTTTC
P2       ACAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
SKHT1    ACAAACAAT AC-AAAATA- TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
PCHBR    ACAAACCA-T AC-AAAATA- TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
KC2      ACAAACAAT -C--AAATAA TTACTACT-C TGAAACTAAC TTACAAAC-T TTTATTTTTC
SR2      CCAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
Lobata   ACAAACAAT AC-AAAATAA TTACTACTAC TGAAACTA-C TTACAA-C-T TTTATTTT-C
P3       CCAAGCA-T AC-AAAATA- TTACTACTGC TGAAACTAAC TTACAAAC-T TTTATTTTTC
LBR      ACAAACCAT AC--AAATAA TTACTACTAC TGAAACTAAC TTACAAACCT TTTATTTTCC
SR1      ACAA-CATT -CC-AAATAT TTTCTACTAC TGAAACTTAC TTACAAAC-T TTTATTTTTC
UTTN     CCAA-CAAT ACCAAAATAT TTTCTACTAC TGAAACTAAC TTACAAACTT TTTATTTTTC
SKNK     ACAA-CA-T AC-AAAATAT TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
CR       ACCAA-CAAT AC--AAATAA TTACTACTAC TGAAACTAAC TTACAA-C-T TTTATTTTTC
Tak      ACAAACAAT AC-AAAATAT TTACTACTAC TGAAACTAAC TTACAAAC-T TTTATTTTTC
KC1      CCAAACAAT AC-AAAATAA TTACTACTAC TGAAACTAAC TTACAA-C-T TTTATTTTTC
CM4      ACAAACAAT 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-TTTTTT T
P3       GTCCTTTTTT T
LBR      GTC-TTTTTT T
SR1      GTC-TTTTTT T
UTTN     GTCCTTTTTT T
SKNK     GCC-TTTTTT T
CR       GTC--TTTTT T
Tak      GCC-TTTTTT T
KC1      GCC-TTTTTT T
CM4      GTC-TTTTTT T
Clustal Co * * * * * *

```

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
<i>P. lobata</i>	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 *trnL* 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
LPool	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
<i>P. lobata</i>	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 *trnL-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
LPool	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
<i>P. lobata</i>	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
<i>P. lobata</i>	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
<i>P. lobata</i>	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
<i>P. lobata</i>	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
<i>P. lobata</i>	89	87	79	90	89	89	88	79

Cultivar	SRTN	<i>P. lobata</i>
SRTN	-	-
<i>P. lobata</i>	89	-

Table 4.9. The matrix of pairwise similarity (%) of *trnL* 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
<i>P. lobata</i>	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
<i>P. lobata</i>	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
<i>P. lobata</i>	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
<i>P. lobata</i>	97	90	98	98	98	98	98	98

Cultivar	RB4	Tak	CHYP	NKRSM	SKNK	CHPn	SRTN	<i>P. lobata</i>
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	-	-
<i>P. lobata</i>	97	98	98	98	98	98	98	-

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

Cultivar	CM1	CM3	CM4	CR	LPang	LPool	P1	P2
CM1	-	-	-	-	-	-	-	-
CM3	98	-	-	-	-	-	-	-
CM4	99	98	-	-	-	-	-	-
CR	98	97	99	-	-	-	-	-
LPang	99	97	99	98	-	-	-	-
LPool	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
<i>P. lobata</i>	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
<i>P. lobata</i>	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
<i>P. lobata</i>	97	97	93	97	97	97	97	97

Cultivar	RB3	Tak	CHYP	NKRSM	SKNK	CHPn	SRTN	<i>P. lobata</i>
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	-	-
<i>P. lobata</i>	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
CHPh	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
<i>P. lobata</i>	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
<i>P. lobata</i>	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
<i>P. lobata</i>	8.544	10.895	18.384	8.387	8.724	8.836	10.079	19.592

Cultivar	SRTN	<i>P. lobata</i>
SRTN	-	-
<i>P. lobata</i>	8.531	-

Table 4.12. The pairwise sequence divergence (%) of *trnL* 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
<i>P. lobata</i>	1.549	0.520	0.517	0.517	1.798	0.517	0.517	0.517

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

Cultivar	CM1	CM3	CM4	CR	LPang	LPool	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	-	-	-	-
LPool	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
<i>P. lobata</i>	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
<i>P. lobata</i>	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
<i>P. lobata</i>	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	-	-
<i>P. lobata</i>	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).

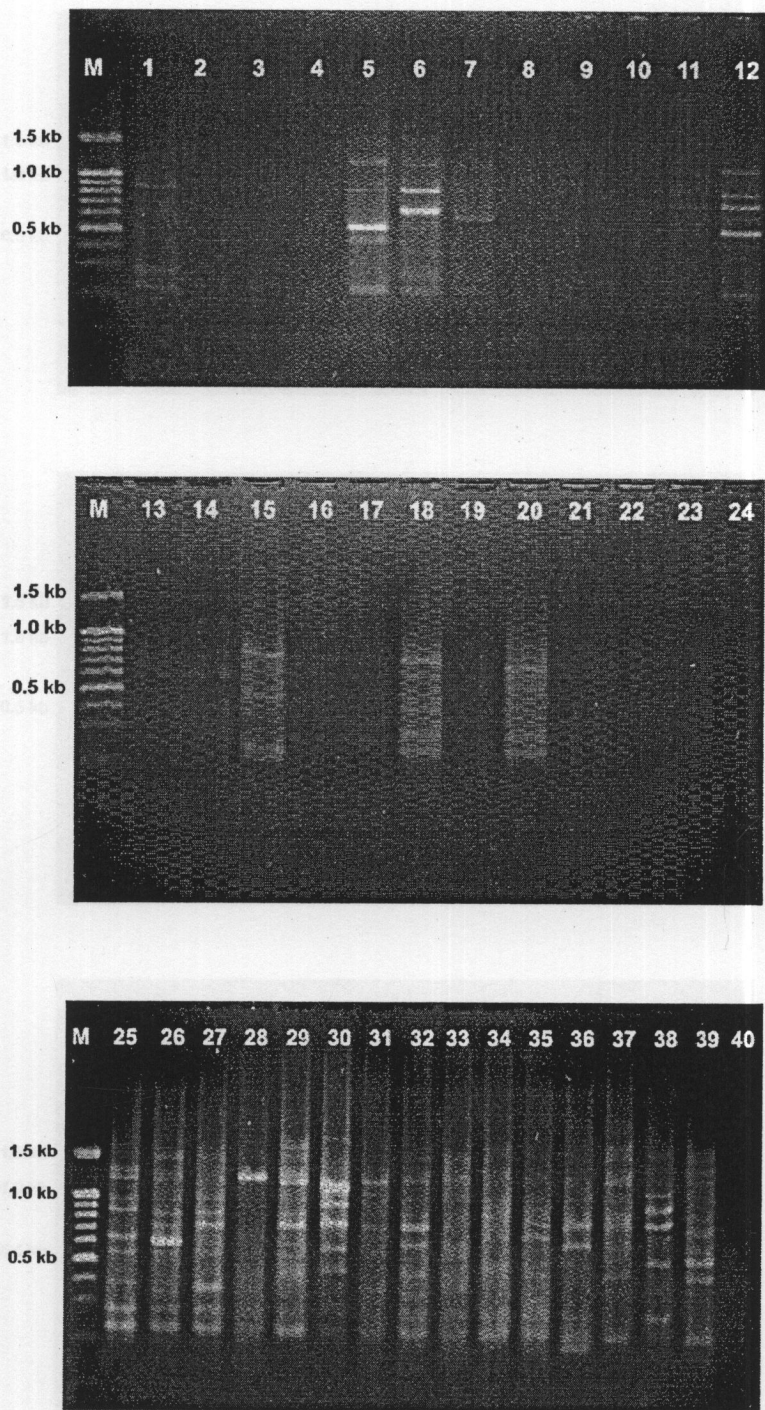


Figure 4.28. RAPD patterns amplified by OPA-07 primer on 2.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-39 contain RAPD products of 39 cultivars of *P. mirifica* in Thailand and lane 40 represents RAPD product of *P. lobata* as an outgroup.

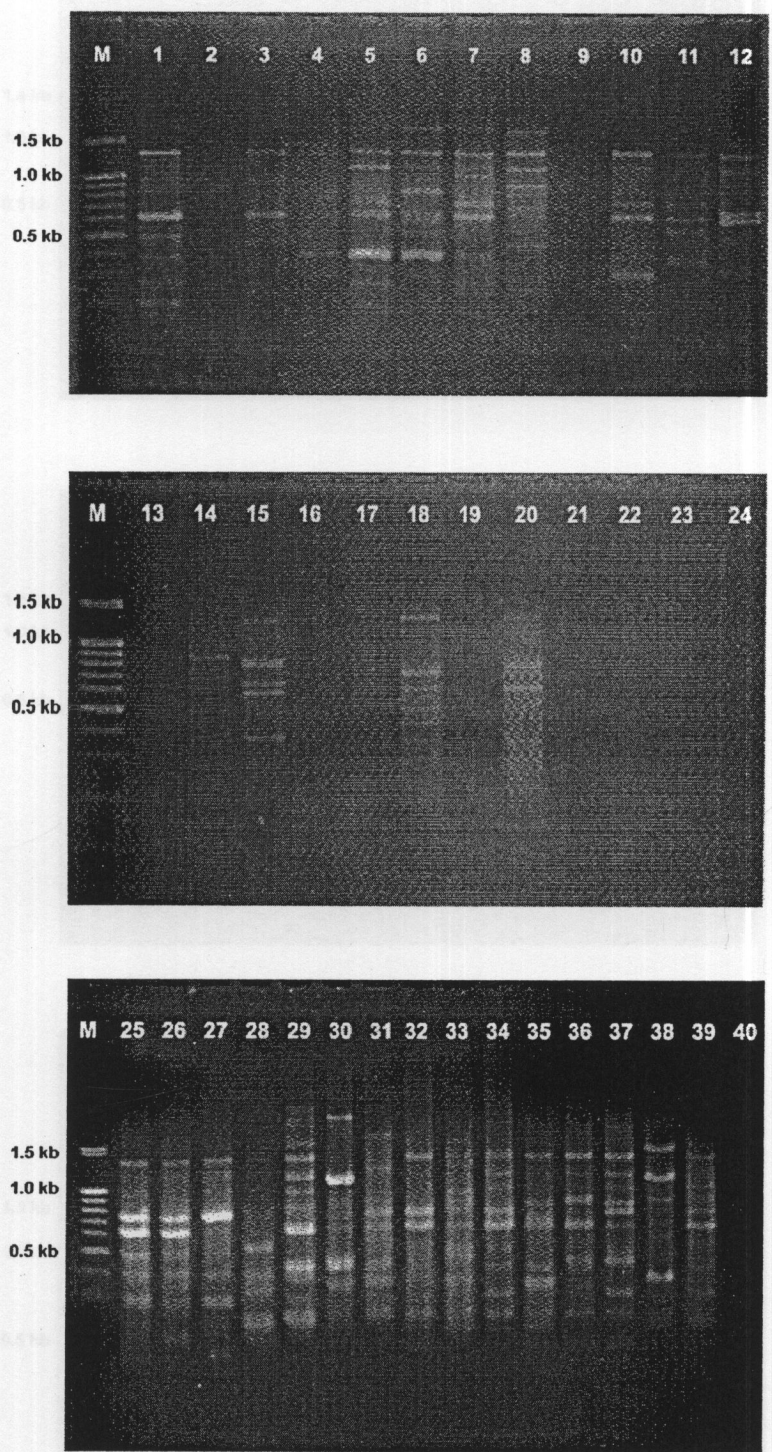


Figure 4.29. RAPD patterns amplified by OPA-12 primer on 2.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-39 contain RAPD products of 39 cultivars of *P. mirifica* in Thailand and lane 40 represents RAPD product of *P. lobata* as an outgroup.

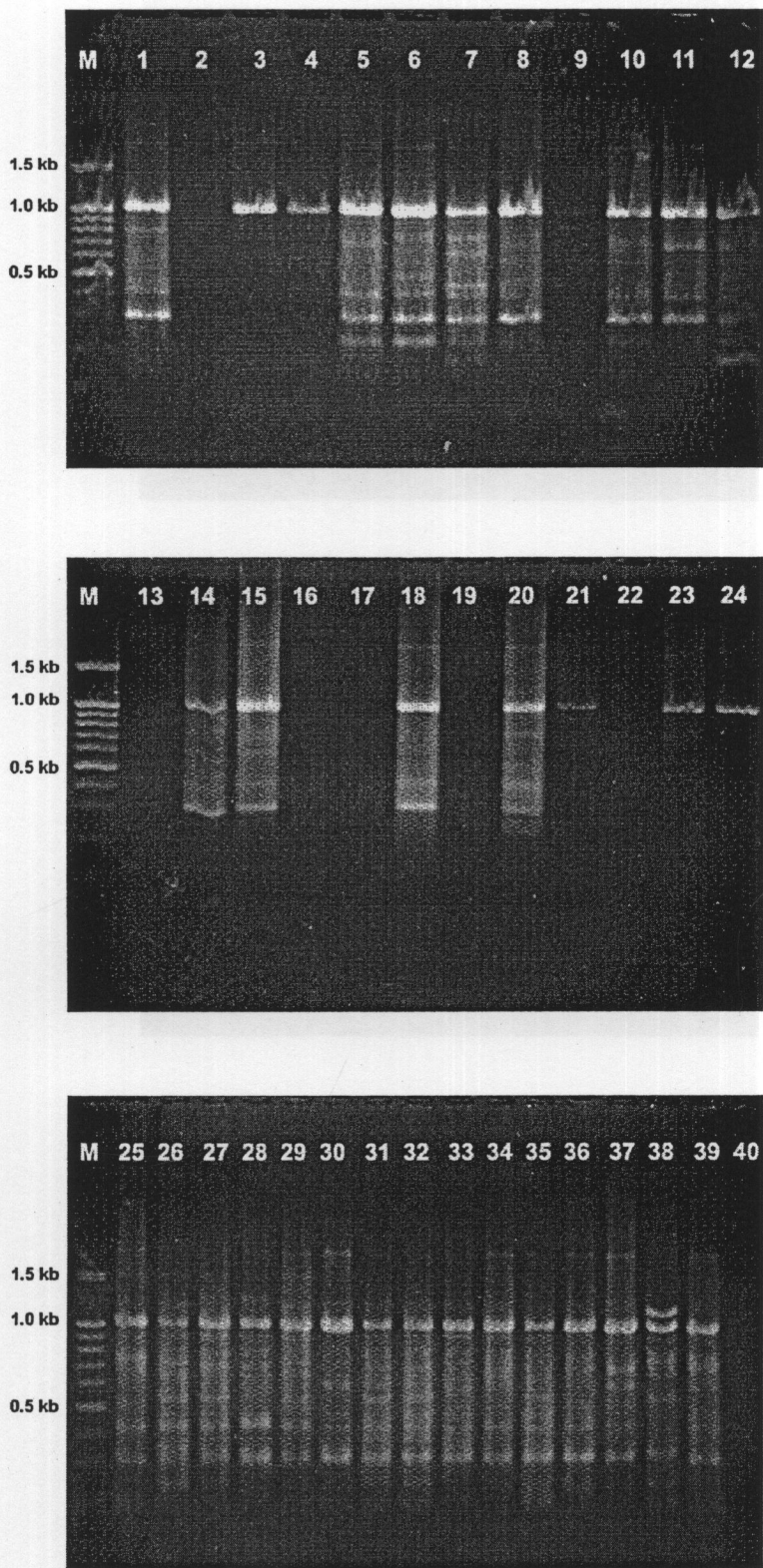


Figure 4.30. RAPD patterns amplified by OPD-02 primer on 2.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-39 contain RAPD products of 39 cultivars of *P. mirifica* in Thailand and lane 40 represents RAPD product of *P. lobata* as an outgroup.

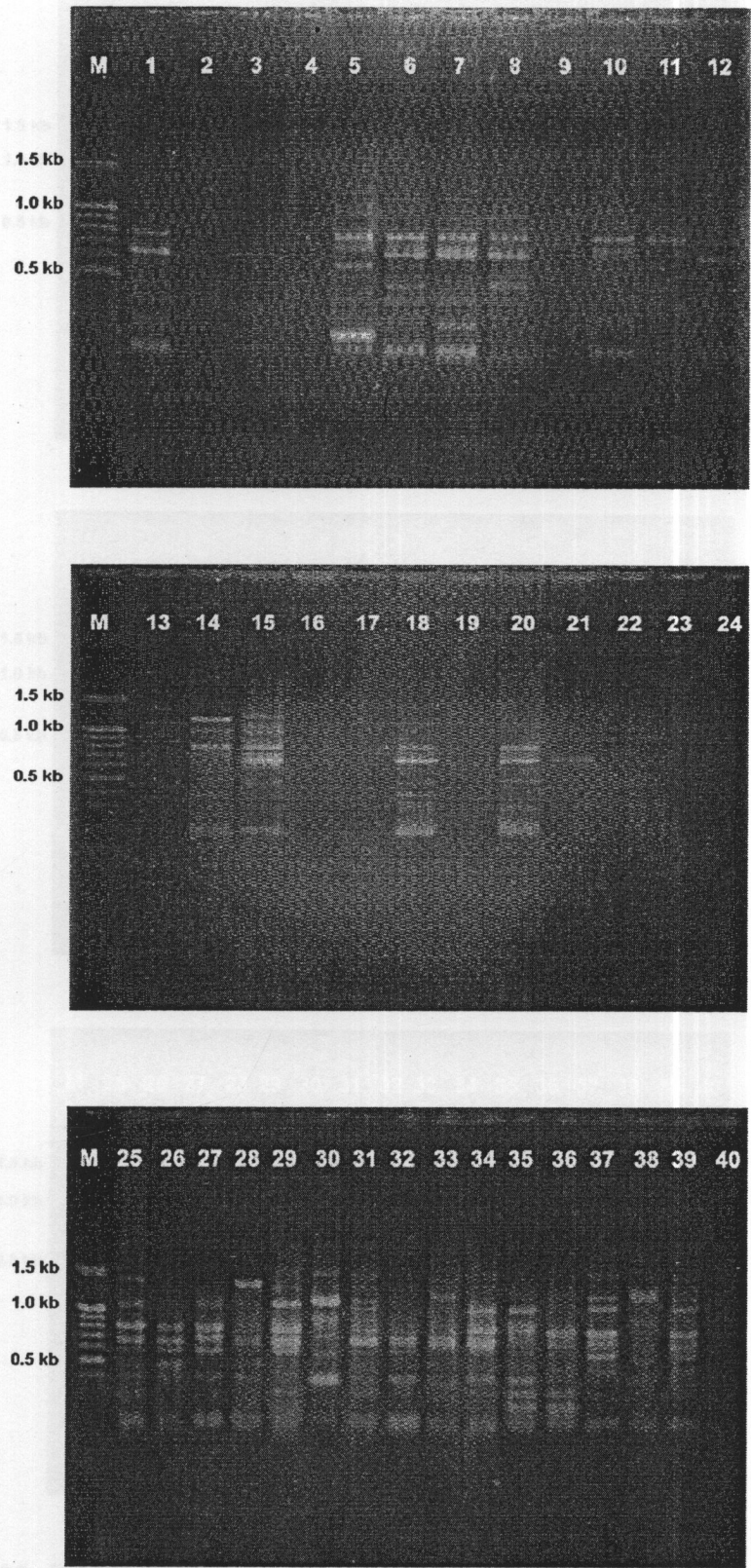


Figure 4.31. RAPD patterns amplified by OPD-16 primer on 2.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-39 contain RAPD products of 39 cultivars of *P. mirifica* in Thailand and lane 40 represents RAPD product of *P. lobata* as an outgroup.

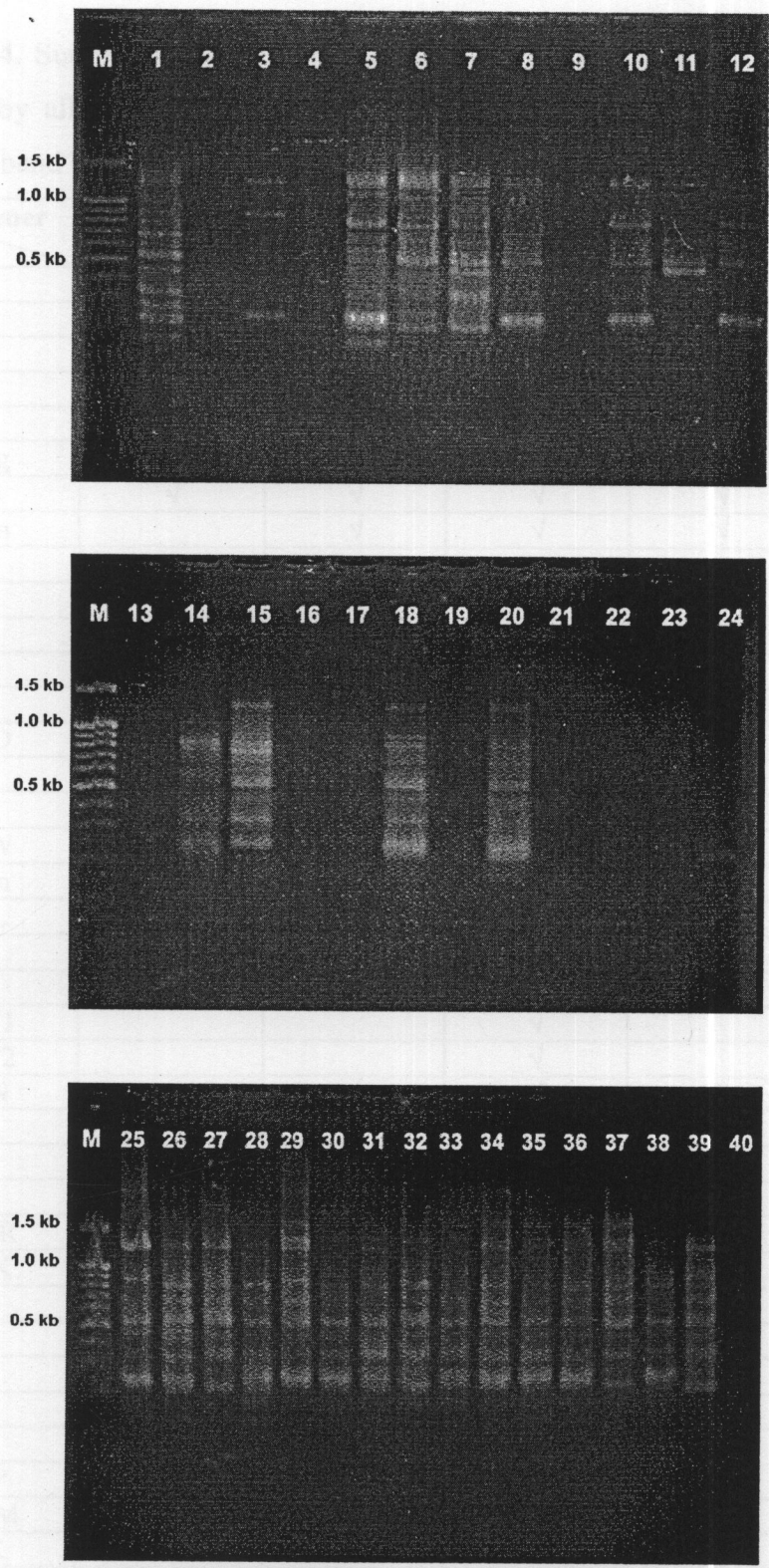


Figure 4.32. RAPD patterns amplified by OPE-01 primer on 2.0% agarose gel. Lane M represents 100 bp ladder as DNA marker. Lanes 1-39 contain RAPD products of 39 cultivars of *P. mirifica* in Thailand and lane 40 represents RAPD product of *P. lobata* as an outgroup.

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	√	√	√	√	√
<i>P. lobata</i>			√		
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	LPool	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	-	-	-
LPool	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
<i>P. lobata</i>	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	-	-	-	-	-	-	-	-	-
LPool	-	-	-	-	-	-	-	-	-
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
<i>P. lobata</i>	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
<i>P. lobata</i>	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
<i>P. lobata</i>	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	<i>P. lobata</i>
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	-	-
<i>P. lobata</i>	0.2381	0.2857	0.2857	-

4.2.6 Phylogenetic analysis

The sequences of PCR products of nrDNA ITS, cpDNA *trnL* and *trnL-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 computed 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 *trnL* 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 *trnL-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).

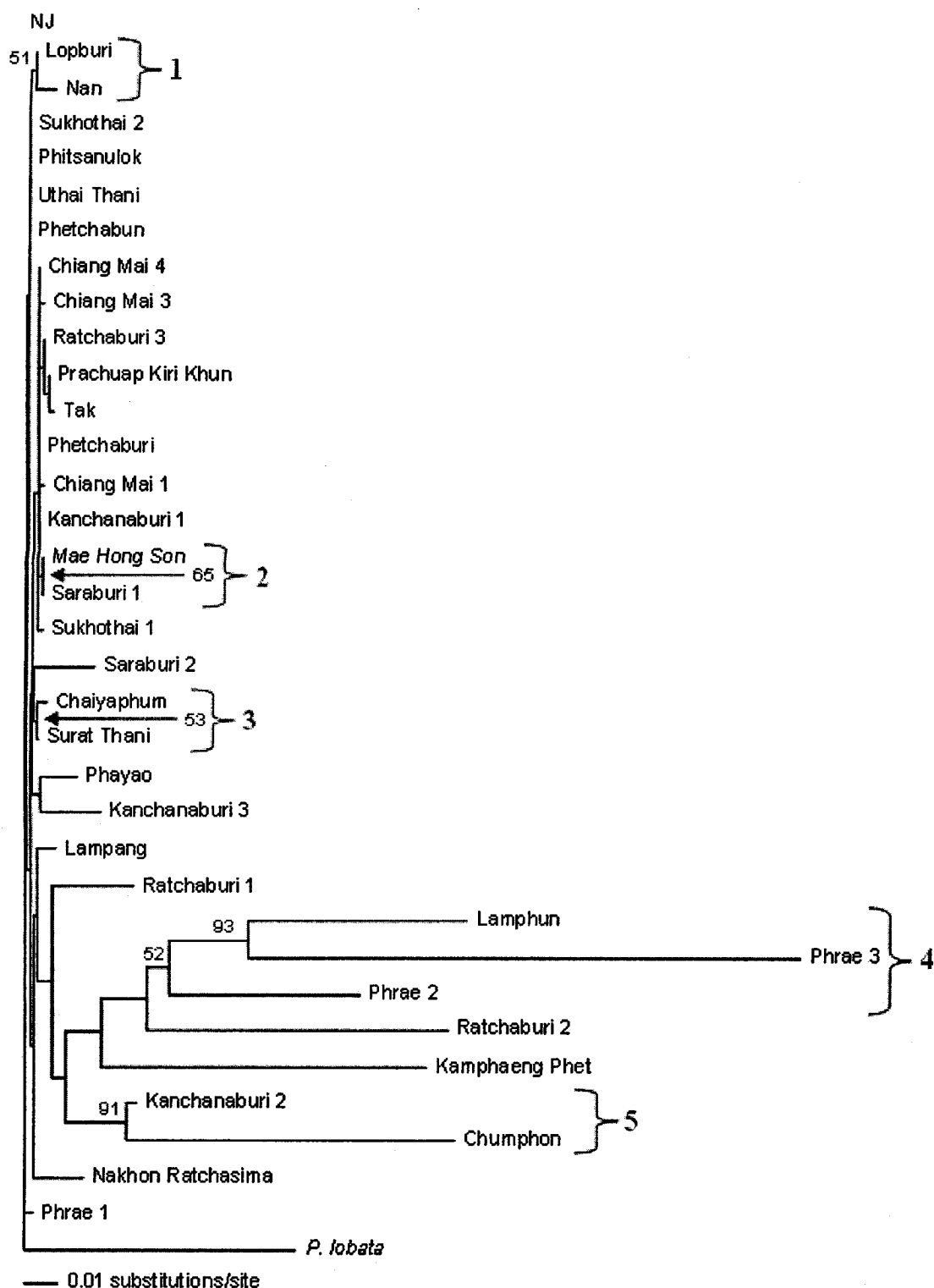


Figure 4.33. A rooted NJ phylogenetic tree or phylogram of ITS region (33 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup) based on K2P genetic distance. The Bootstrap supporting-values are shown on the branches of the phylogenetic tree.

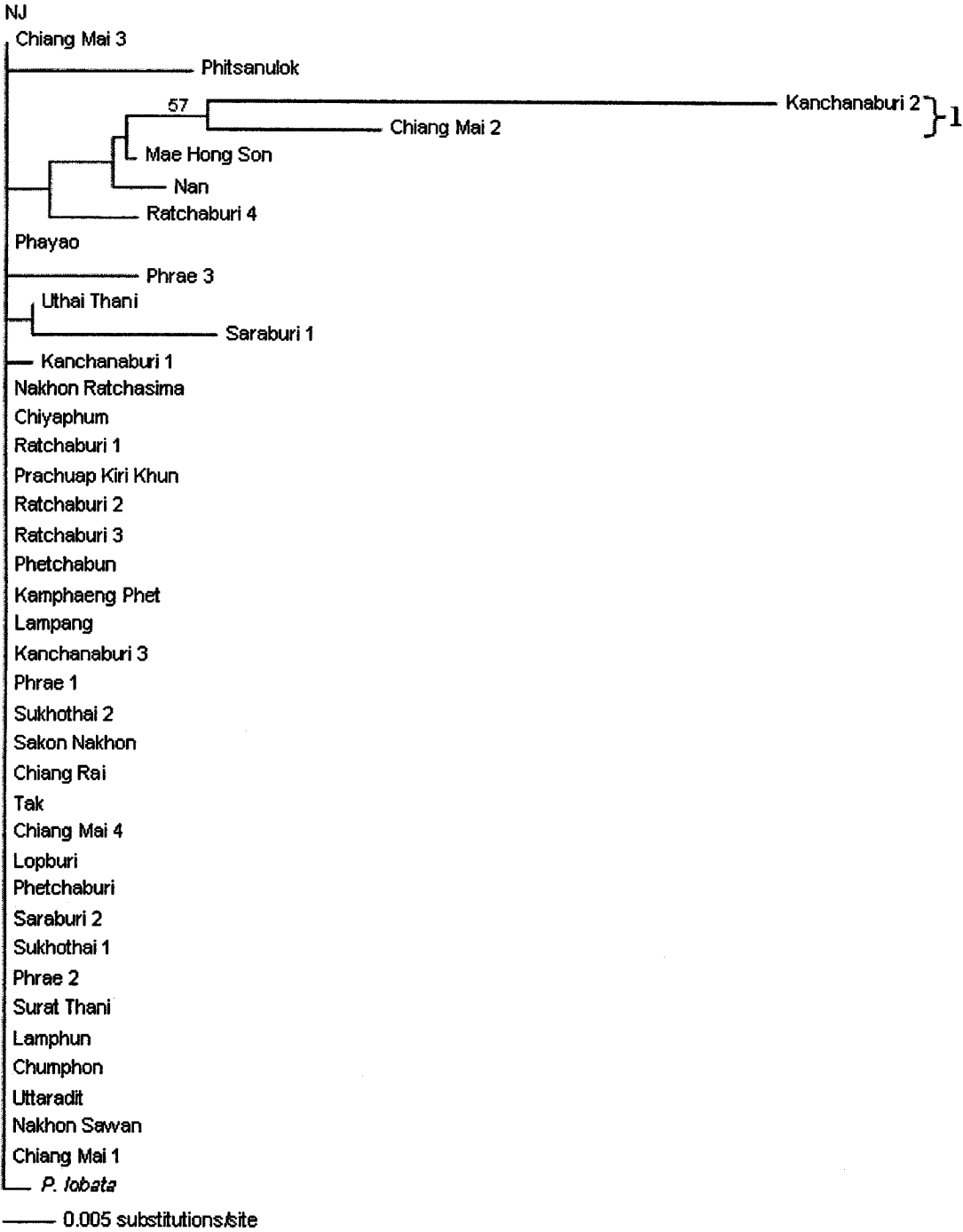


Figure 4.34. A rooted NJ phylogram of *trnL* region (39 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup) based on K2P genetic distance. The Bootstrap supporting-values are illustrated on the branches of tree.

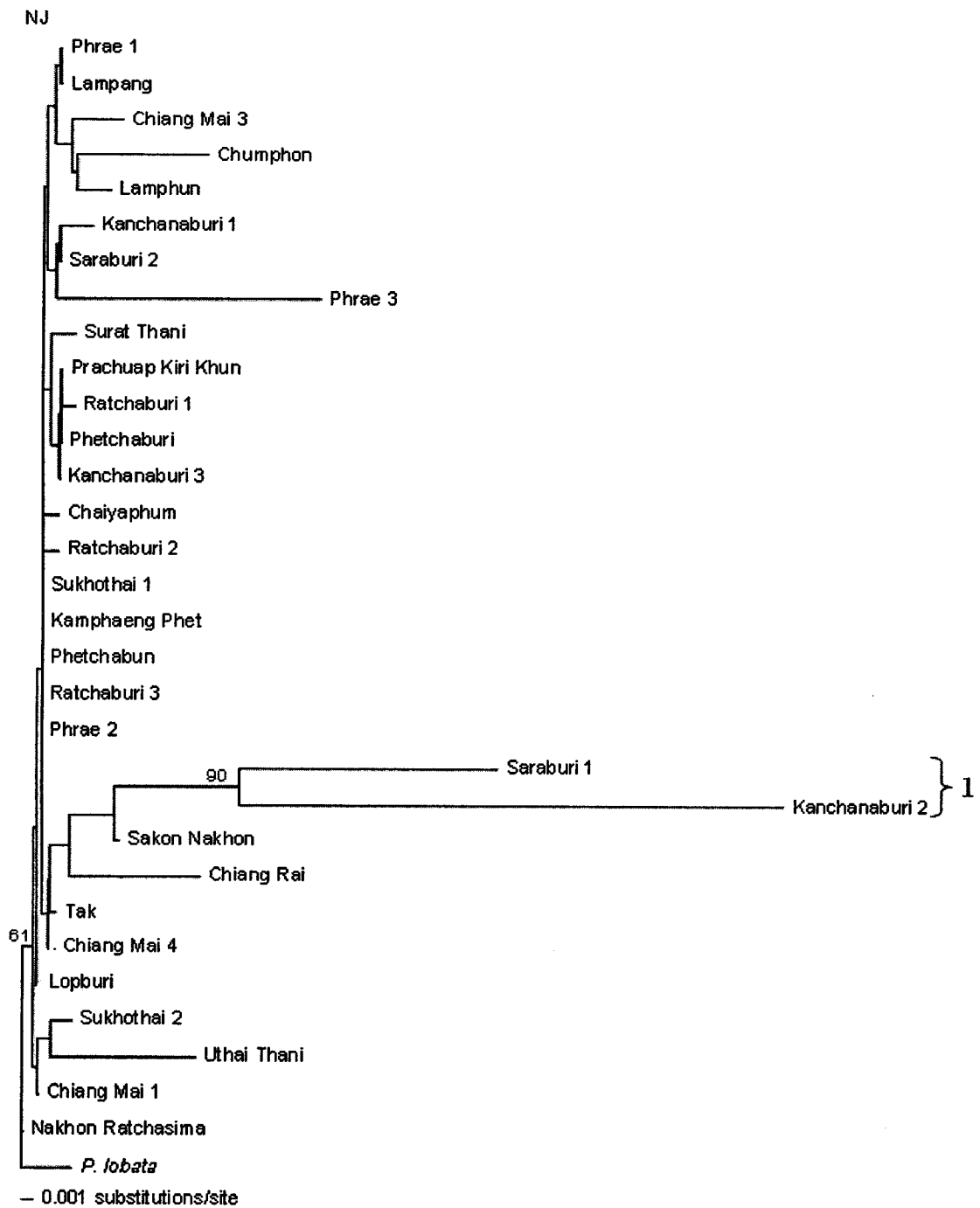


Figure 4.35. A rooted NJ phylogenetic tree of *trnL-F* region (31 cultivars of *P. mirifica* in Thailand and *P. lobata* as an outgroup) based on K2P genetic distance. The Bootstrap supporting-values are noted on the branches of tree.

NJ

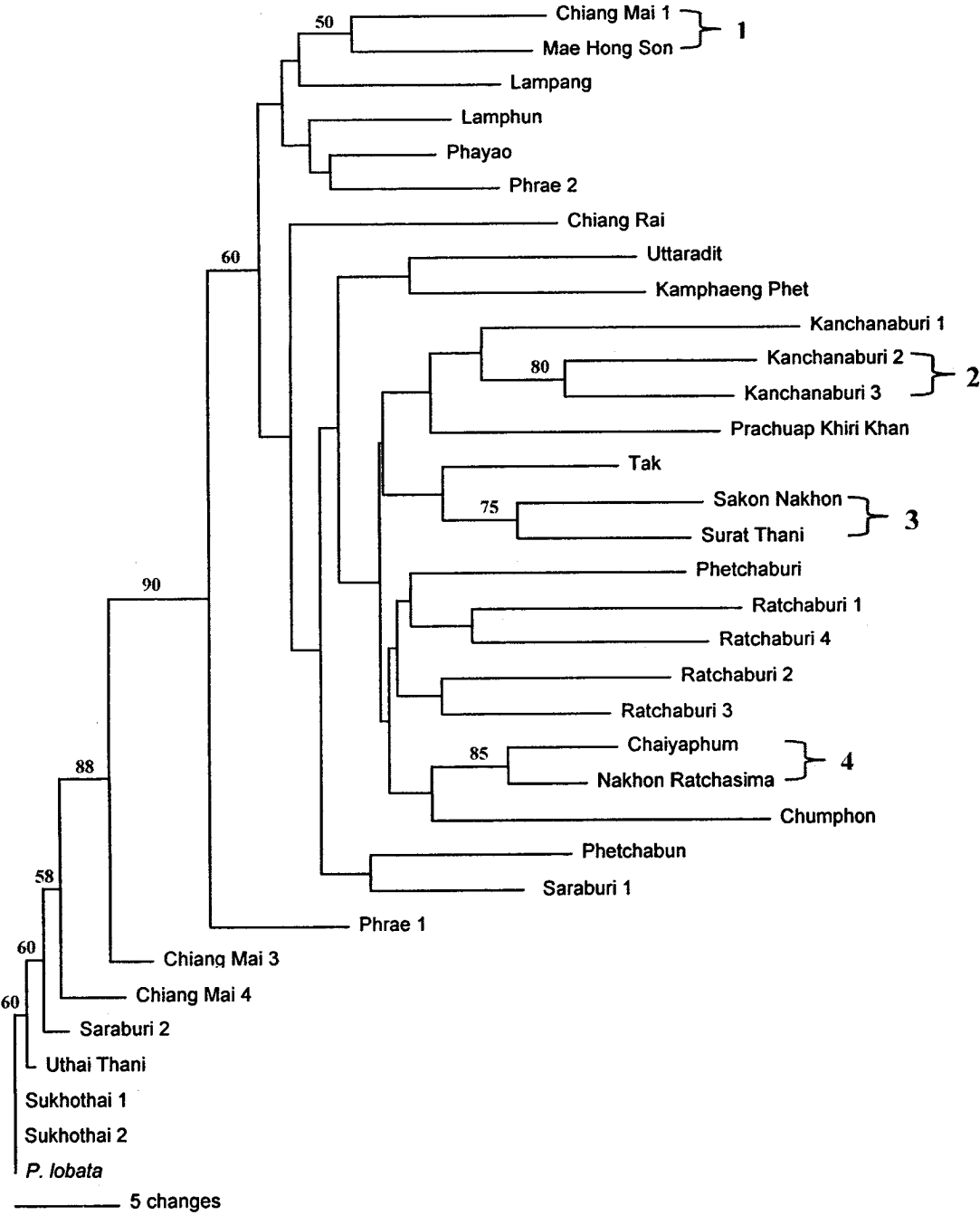


Figure 4.36. A rooted neighbor-joining (NJ) phylogenetic tree based on Nei-Li genetic distances among 33 cultivars of *P. mirifica* in Thailand and 1 cultivar of *P. lobata* as an outgroup derived from RAPD patterns. The Bootstrap supporting-values are above the braches.

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* tuberos 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	<i>P. lobata</i>		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.

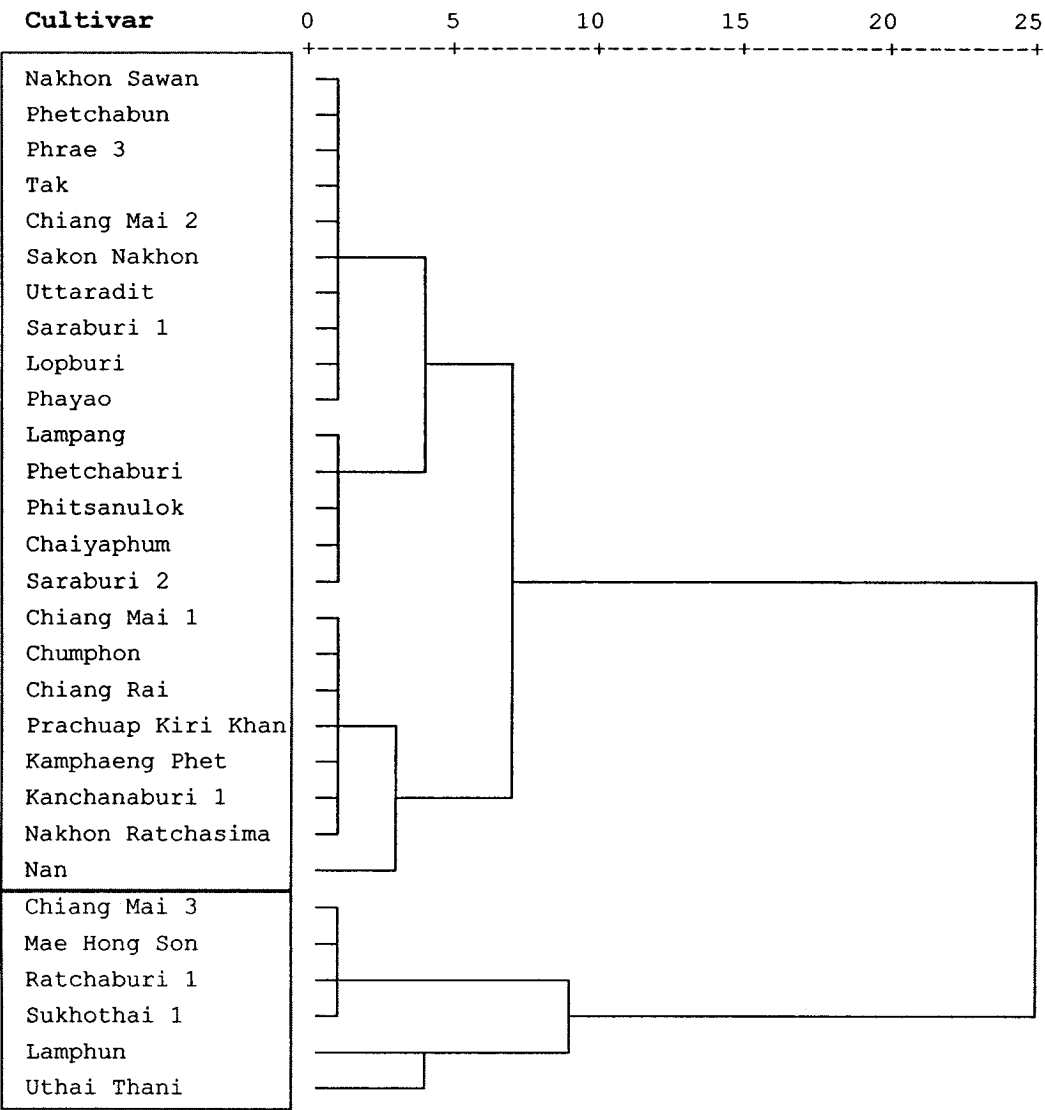


Figure 4.37. A dendrogram of isoflavonoid contents created by Between-groups linkage method of Cluster analysis. *P. mirifica* is classified by collected locations or cultivars.

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

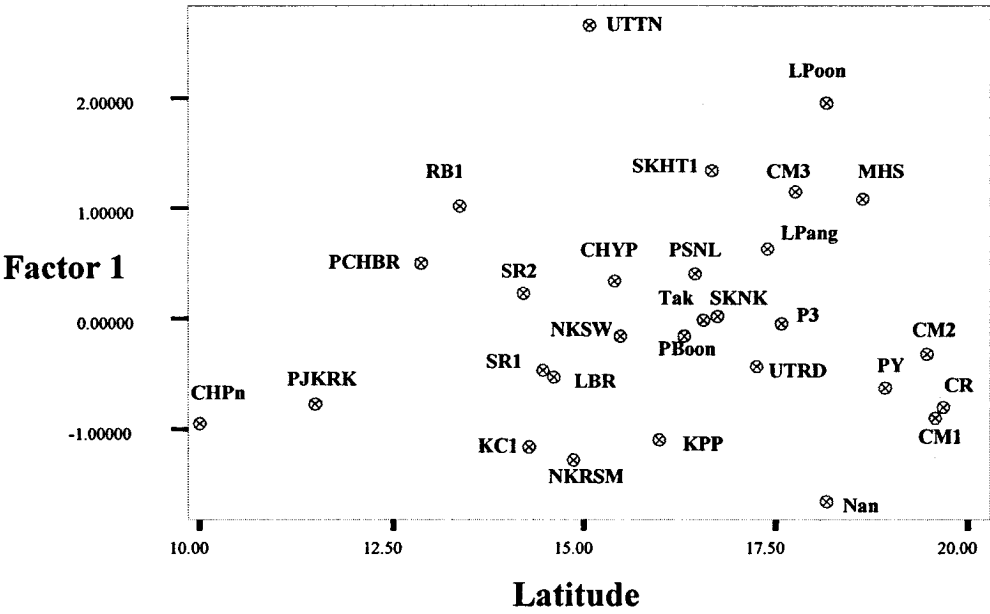


Figure 4.38. Geographic trends in isoflavonoid contents (factor 1) of *P. mirifica* in Thailand: abscissa and latitude. Factor score 1 was derived by PCA. Value labels refer to sampling sites or cultivars.

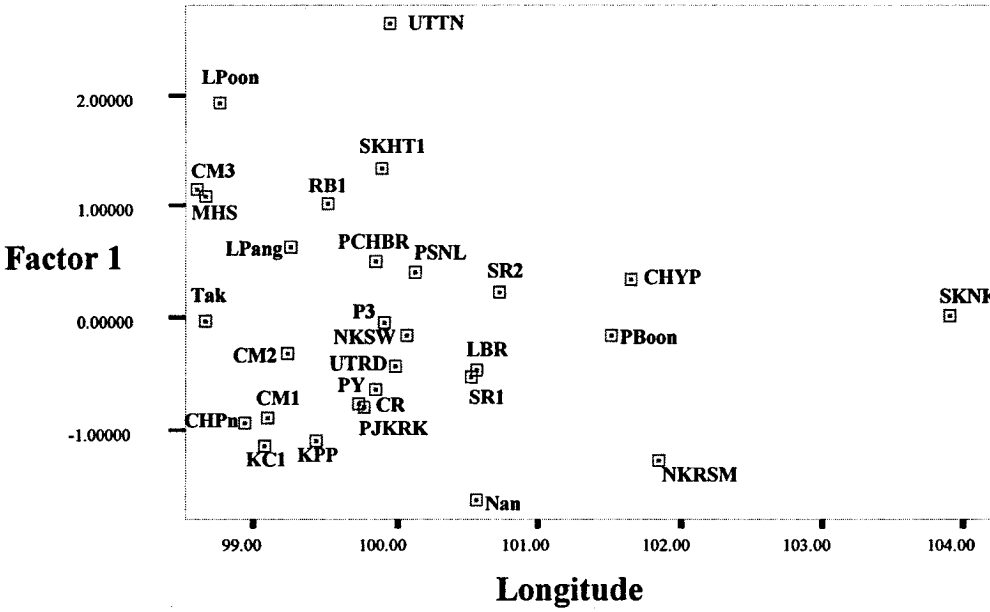


Figure 4.39. Geographic trends in isoflavonoid contents of *P. mirifica* in Thailand: abscissa and longitude. Factor score 1 was derived by PCA. Value labels refer to sampling sites or cultivars.

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 A²B) 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 *trnL*, and *trnL*-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 *trnL*-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 *trnL* and *trnL*-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 *trnL* 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 *trnL* 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 *trnL*-F phylogram illustrated one supported branch. These sequences showed more polymorphism than *trnL* 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 A[^]B) 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, *trnL*, and *trnL*-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.

REFERENCES

- Agustin, J. A., Andres, F. G., Angel, R. N. and Priego, A. F. B. 2006. Morphometry of the organs of cherimoya (*Annona cherimola* Mill.) and analysis of fruit parameters for the characterization of cultivars, and Mexican germplasm selections. *Scientia Horticulturae* 107(4): 337-346.
- Alvarez, I. and Wendel, J.F. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417-434.
- Bailey, C. D., Price, R. A. and Doyle, J. J. 2002. Systematics of the Halimolobine Brassicaceae evidence from three loci and morphology. *Systematic Botany* 27(2): 318-332.
- Baranek, M., Kadlec, M., Raddova, J., Vachun, M. and Pidra, M.. 2002. Evaluation of genetic diversity in 19 *Glycine max* (L.) Merr. accessions included in the Czech National Collection of Soybean Genotypes. *Czech Journal Genetic Plant Breeding* 38(2): 69-74.
- Bautista, N. S., Solis, R., Kamijima, O., and Ishii, T. 2001. RAPD, RFLP, and SSLP analyses of phylogenetic relationships between cultivated and wild rice. *Genes Genetic System.* 76: 71-79.
- Bound, D. G. and Pope, G. S. 1960. Light absorption and chemical properties of miroestrol, the oestrogenic substance of *Pueraria mirifica*. *Journal of the Chemical Society* 3196-3705.
- Cain, J. C. 1960. Miroestrol: an estrogen from the plant *Pueraria mirifica*. *Nature* 158: 774-777.
- Chansakaew, S., Ishikawa, T., Seki, H., Sekine, K., Okada, M. and Chaichantipyuth, C. 2000a. Identification of deoxymiroestrol as the actual rejuvenation principle of Kwao Keur", *Pueraria mirifica*. The known miroestrol may be an artifact. *Journal of Natural Products* 63: 173-175.
- Chansakaew, S., Ishikawa, T., Seki, H., Sekine, K., Okada, M., Higuchi, Y and Chaichantipyuth, C. 2000b. Isoflavonoids from *Pueraria mirifica* and their estrogenic activity. *Planta Medica* 66: 572-575.
- Cherdshewasart, W., Cheewasopit, W. and Picha, P. 2004a. The differential anti-proliferation effect of white (*Pueraria mirifica*), red (*Butea superba*), and

- black (*Mucuna collettii*) Kwao Krua plants on the growth of MCF-7 cells. *Journal of Ethnopharmacology* 93: 255-260.
- Cherdshewasart, W., Cheewasopit, W. and Picha, P. 2004b. Anti-proliferation effects of the white (*Pueraria mirifica*), red (*Butea superba*), and black (*Mucuna collettii*) Kwao Krua plants on the growth of HeLa cells. *Journal of Scientific Research of Chulalongkorn University* 29 (1):27-32.
- Cherdshewasart, W., Subtang, S. and Dahlan, W. 2006. Major isoflavonoid contents of the phytoestrogen rich-herb *Pueraria mirifica* in comparison with *Pueraria lobata*. *Journal of Pharmaceutical and Biomedical Analysis* [Epub ahead of print]
- Cherdshewasart, W., Subtang, S. and Dahlan, W. 2007. Major isoflavonoid contents of the phytoestrogen rich-herb *Pueraria mirifica* in comparison with *Pueraria lobata*. *Journal of Pharmaceutical and Biomedical Analysis* 43: 428–434.
- Chowdhury, A. K., Srinives, P., Tongpamnak, P., Saksoong, P. and Chatwachirawong, P. 2002. Genetic Relationship among Exotic Soybean Introductions in Thailand: Consequence for Varietal Registration. *Science Asia* 28: 227-239.
- Compton, J. A., Clennett, J. C. B. and Culham, A. 2004. Nomenclature in the dock. Overclassification leads to instability: a case study in the horticulturally important genus *Cyclamen* (Myrsinaceae). *Botanical Journal of the Linnean Society* 146: 339–349.
- Creed, J. C. 1997. Morphological variation in the seagrass *Halodule wrightii* near its southern distributional limit. *Aquatic Botany* 59: 163-172.
- Dweck, A. C. 2002. The *Pueraria* family with special interest in *Pueraria mirifica*. *Personal Care Magazine* 3(1):7-10.
- Ellison, N. W., Liston, A., Steiner, J. J., Williams, W. M. and Taylor, N. L. 2006. Molecular phylogenetics of the clover genus (*Trifolium*—Leguminosae). *Molecular Phylogenetics and Evolution* 39: 688–705.
- Hayodom, M. 1971. *Constituents of the tuberous roots of Pueraria mirifica*. Master's Thesis, Chulalongkorn University. 33 pp. (in Thai)
- Heider, B., Fischer, E., Berndt, T. and Kraft, R. S. 2004. *Pueraria montana* — Genetic Diversity of a Neglected Crop in North Vietnam. *Deutscher Tropentag*. October 5-7, 2004. Germany.

- Hoey, B. K., Crowe, K. R., Jones, V. M. and Polans, N. O. 1996. A phylogenetic analysis of *Pisum* based on morphological characters, and allozyme and RAPD markers. *Theoretical and Applied Genetics* 92: 92-100.
- Ingham, J. L., Tahara, S and Dziedzic, S.Z 1986. A chemical investigation of *Pueraria mirifica* root. *Z Naturforsch Ser C* 41: 403-408.
- Ingham, J. L., Tahara, S and Dziedzic, S.Z 1988. Coumestan from the roots of *Pueraria mirifica* root. *Zeitschrift fur Naturforschung Section C: Biosciences* 43: 5-10.
- Ingham, J. L., Tahara, S. and Dziedzic, S. Z. 1989. Minor isoflavones from the root of *Pueraria mirifica* . *Zeitschrift fur Naturforschung Section C: Biosciences* 44 (9/10): 724-726.
- Jones, H. E. H., and Pope, G. S. 1960. A study of the action of miroestrol and other oestrogens on the reproductive tract of the immature female mouse. *Journal of Endocrinology* 20: 229-235.
- Kerr, A., 1932. A reputed rejuvenator. *Journal of Siam Society Natural History* 8: 336-338.
- Lakshmi, M., Parani, M. and Parida, A. 2002. Molecular marker assisted intra-specific variation and species relationships in the Indian mangrove tribe Rhizophoreae. *Aquatic Botany* 74: 201-217.
- Lakshnakara, K. M. C. and Suvatabandhu, K. 1952. A new species of *Pueraria* (leguminosae) from Thailand, yielding and oestrogenic principle. *Kew Bulletin*: 263-266.
- Malaivijitnond, S., Jaroenporn, S., Wattanasemkit, K. and Cherdshewasart, W. 2003a. Biotechnology of phytoestrogen-rich; *Pueraria mirifica*: XII Effects of *Pueraria mirifica* on fertility in mice, *The 29th Congress on Science and Technology of Thailand at Khonkaen University*, 20-22 October 2003. Thailand.s
- Malaivijitnond, S., Jaroenporn, S., Wattanasemkit, K. and Cherdshewasart, W. 2003b. Biotechnology of Phytoestrogen-rich; *Pueraria mirifica*: XIII Sex differences in response of anterior pituitary to *P. mirifica* phytoestrogens in rats. *The 29th Congress on Science and Technology of Thailand at Khonkaen University*, 20-22 October, 2003. Thailand.

- Malaivijitnond, S., Kiatthaipipat, P., Cherdshewasart, W., Watanabe, G. and Taya, K. 2004. Different effects of *Pueraria mirifica*, a herb containing phytoestrogens, on LH and FSH secretion in gonadectomized female and male rats. *Journal of Pharmacological Sciences* 96: 428-435.
- Mienie, C.M.S., Smit, M.A. and Pretorius, P.J. 1995. Use of random amplified polymorphic DNA for identification of South African soybean cultivars. *Field Crops Research* 43: 43-49.
- Muangman, V. and Cherdshewasart, W. 2001. Clinical trail of the phytoestrogen-rich herb, *Pueraria mirifica* as a crude drug in the treatment of syptoms in menopausal women. *Siriraj Hospital Gazette* 53: 300-309.
- Murkies, A. L., Wilcox, G. and Davis, S. R. 1998. Clinical review 92-Phytoestrogen. *The Journal of Clinical Endocrinology & Metabolism* 83: 297-303.
- Nilandihi, T., Kamthong, B., Isarasena, K. and Shiengthong, D. 1957. Constituents of the tuberous roots of *Pueraria mirifica*. *Zeitschrift fur Naturforschung Section C: Biosciences* 5: 41.
- Panriansaen, R. 2000. *Characterization of Pueraria mirifica populations from various parts of Thailand*. Master's Thesis, Chulalongkorn University.
- Panriansaen, R. 2005. *Evaluation of preventive and antitumor activities of Pueraria mirifica and Butea superba in DMBA-induced mammary carcinoma in the rat*. Doctor 's Thesis, Chulalongkorn University. 184 pp.
- Pappert, R. A., Hamrick, J. L., and Donovan, L. A. 2000. Genetic variation in *Pueraria lobata* (Fabaceae), an introduced, clonal, and invasive plant of the Southern United States. *American Journal of Botany*. 87(9): 1240–1245.
- Perez, R., Vargas, P. and Arroyo, J. 2003. Convergent evolution of flower polymorphism in *Narcissus spp.* (Amaryllidaceae). *New Phytologist* 161: 235–252.
- Pisetpakasit, R. 1976. *A Pharmaconostical study of Pueraria mirifica*. Master's thesis, Chulalongkorn University (in Thai).
- Radmann, E. B., Bianchi, V. J., Oliveira, R. P., and Fachinello, J. C. 2006. Characterization and genetic diversity of strawberry cultivars. *Horticultura Brasileira* 26: 84-87.
- Schuettpeitz, E. and Hoot, S. B. 2004. Phylogeny and biogeography of *Caltha* (Ranunculaceae) based on chloroplast and nuclear DNA sequences. *American Journal of Botany* 91(2): 247–253.

- Smitasiri, Y. and Wungjai, C. 1986. Some biological aspects of *Pueraria mirifica* :1) flower, pod and seed. *Journal of the Science Faculty of Chiang Mai University* 14 (1) : 67-74.
- Stuessy, T. F. and Urtubey, E. 2006. Phylogenetic implications of corolla morphology in subfamily Barnadesioideae (Asteraceae) *Flora* 201: 340–352.
- Subtang, S. 2002. *Comparative isoflavone HPLC fingerprints from the extracts of White Kwao Krua Pueraria mirifica in Thailand* Master's Thesis, Chulalongkorn University. 165 pp.
- Sukhavachana, P. 1949. The comparison of the effects from *Pueraria mirifica* extract with estrogenic Hormone. *J Med Assoc Thai* 3: 104-110 (in Thai).
- Sun, J. H., Li, Z. C., Jewett, D. K., Britton, K. O., Ye, W. H. and GE, X. J. 2005. Genetic diversity of *Pueraria lobata* (kudzu) and closely related taxa as revealed by inter-simple sequence repeat analysis. *European Weed Research Society Weed Research* 45: 255–260.
- Suntara, A. 1931. *The remedy pamphlet of Kwao Krua tuber of Luanganusarn-suntarakromkarnphiset* Chiang Mai: Upatipongsa Press Chiang Mai. pp. 1-13 (in Thai).
- Suvatti, C. 1978. *Flora of Thailand* Thailand, Kurusapha Ladpro press: 680.
- Swamy, K. K., Kumar, H. N. K., Ramakrishna, T. M. and Ramaswamy, S. N. 2004. Studies on Seed Morphometry of Epiphytic Orchids from Western Ghats of Karnataka. *Taiwania*, 49(2): 124-140.
- Szczepaniak, M., Cieslak, E. and Bednarek, P. T. 2002. Morphological and AFLP variation of *Elymus repens* (L.) Gould (Poaceae). *Cellular & Molecular Biology Letters* 7: 547 – 558.
- Tham, D. M., Gardner, D. D. and Haskell, W. L. 1998. Clinical review 97-potential health benefits of dietary phytoestrogen-a review of the clinical, epidemiological, and mechanistic evidence. *The Journal of Clinical Endocrinology & Metabolism* 83: 2223-2235.
- Taberlet, P., Gielly, L., Pautou, G. and Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105-1109.

- Thompson, J. A., Nelson, R. L. and Vodkin, L. O. 1998. Identification of diverse soybean germplasm using RAPD markers. *Published in Crop Science* 38:1348–1355.
- Trisomboon, H., Malaivijitnond, S., Watanabe, G. and Taya, Kazuyoshi. 2004. Estrogenic effects of *Pueraria mirifica* on the menstrual cycle and hormone-related ovarian functions in cyclic female monkeys. *Journal of Pharmacological Sciences* 94: 51-59.
- Trisomboon, H., Malaivijitnond, S., Watanabe, G., Cherdshewasart, W. and Taya, K. 2006a. The estrogenic effect of *Pueraria mirifica* on gonadotropin levels in aged monkeys. *Journal of Endocrin* 29: 129-134.
- Trisomboon, H., Malaivijitnond, S., Cherdshewasart, W., Watanabe, G. and Taya, K. 2006b. Effect of *Pueraria mirifica* on the sexual skin coloration of aged menopausal cynomolgus monkeys. *Journal of Reproductive Development* 52: 537-542.
- White, T. J., Bruns, T., Lee, S. and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *Protocols: A Guide to Methods and Applications in PCR*: 315-322.
- Wu, C. J., Cheng, Z. Q., Huang, X. Q., Yin, S. H., Cao, K M. and Suna, C. R. 2004. Genetic diversity among and within populations of *Oryza granulata* from Yunnan of China revealed by RAPD and ISSR markers: implications for conservation of the endangered species *Plant Science* 167: 35–42.
- Xu, D. H. and Gai, J. Y. 2003. Genetic diversity of wild and cultivated soybeans growing in China revealed by RAPD analysis. *Plant Breeding* 122: 503-506.
- Yulita, K. S., Bayer, R. J. and West, J. G. 2005. Molecular phylogenetic study of *Hopea* and *Shorea* (Dipterocarpaceae): Evidence from the *trnL-trnF* and internal transcribed spacer regions. *Plant Species Biology* 20: 167–182.
- Zimmer, E. A., Roalson, E. H., Skog, L. E., Boggan, J. K and Idnurm, A. 2002. Phylogenetic relationships in the Gesnerioideae (Gesneriaceae) based on nrDNA ITS and cpDNA *trnL-F* and *trnE-T* spacer region sequences. *American Journal of Botany* 89(2): 296–311.

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 (Khlung 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	<i>P. lobata</i> 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	<i>P. lobata</i> from Hiroshima (Miyoshi city)	West (Japan)	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 $\pm$ Standard error)

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

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

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	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(rf)	.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

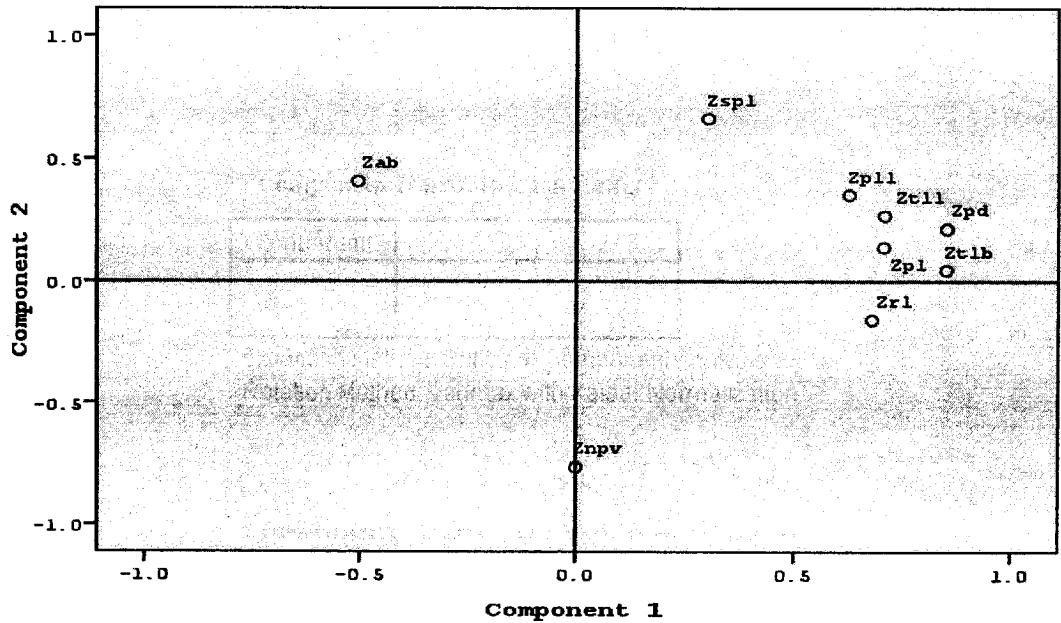
Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 3 iterations.

Component Transformation Matrix

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

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

Component Plot in Rotated Space



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

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	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(cli)	.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(cil)	.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.

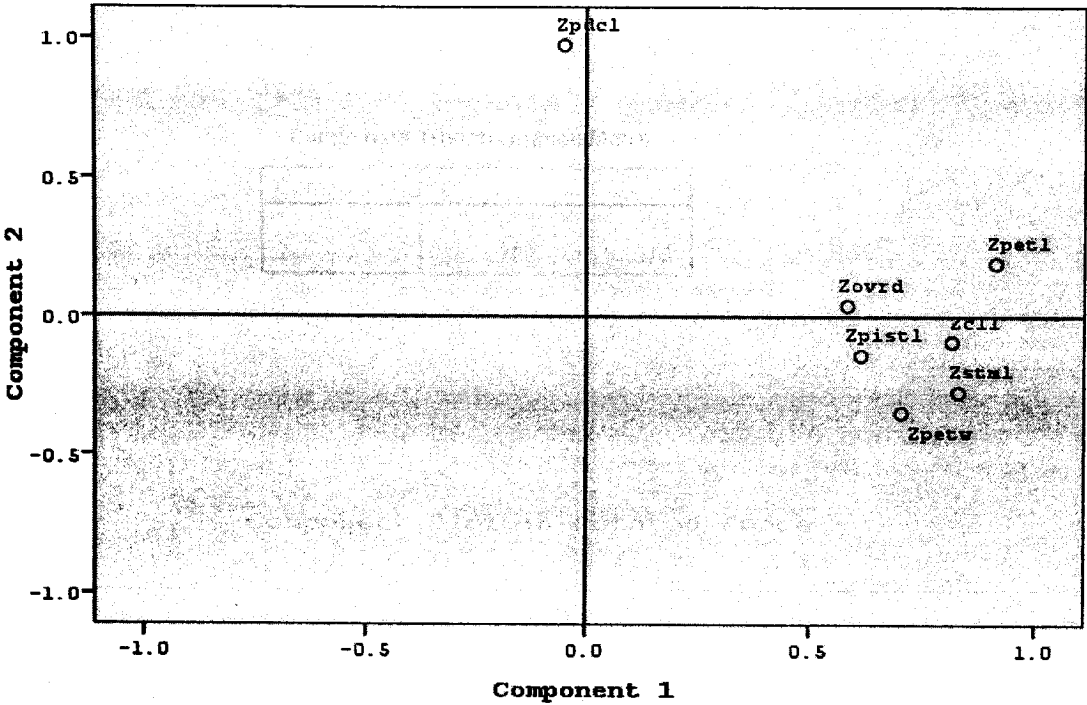
a. Rotation converged in 3 iterations.

Component Transformation Matrix

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

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

Component Plot in Rotated Space



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

KMO and Bartlett's Test

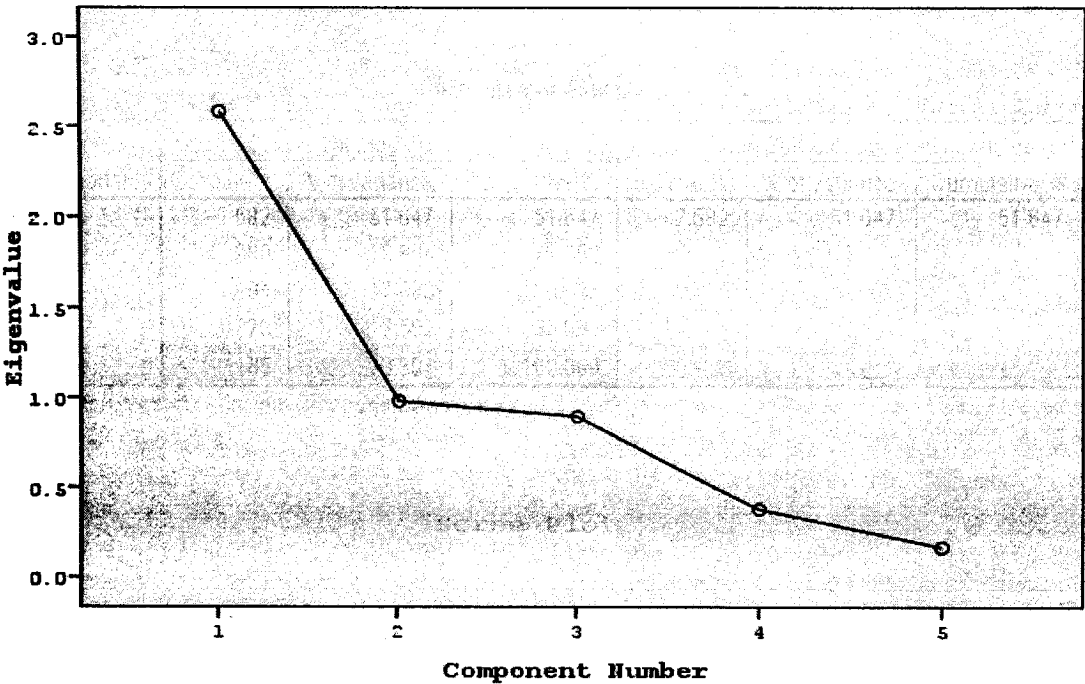
Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.579
Bartlett's Test of Sphericity	Approx. Chi-Square	49.891
	df	10
	Sig.	.000

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	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.

Scree Plot



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-H₂O. 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-H₂O.

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.

Suwanvijitr, T., Chanchao, C., and Cherdshewasart, W. (2006). Leaf morphometry, genetic variation, and phylogeny of white Kwao Krua *Pueraria mirifica* in Thailand. Abstract. *The 14th Faculty of Science Congress*, Chulalongkorn University, Bangkok, Thailand. 116.

Suwanvijitr, T., Chanchao, C., and Cherdshewasart, W. (2006). Leaf morphometry, genetic variation, and phylogeny of white Kwao Krua *Pueraria mirifica* in Thailand. Abstract. *10th BRT Annual Conference*. Krabi, Thailand. 44.