การวิเคราะห์ลายพิมพ์ดีเอ็นเอร่วมกับองค์ประกอบพอลีแซคคาไรด์ในทุเรียนต่างสายพันธุ์

นางสาว อรอนงค์ หนูชูเชื้อ



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาชีวเวชเคมี ภาควิชาชีวเคมี
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ปีการศึกษา 2549

# ANALYSIS OF DNA FINGERPRINT AND POLYSACCHARIDE CONSTITUENT IN DURIAN CULTIVARS

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

Department of Biochemistry
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Chulalongkorn University

Academic Year 2006

| | |
|---|---|
| Thesis Title | ANALYSIS OF DNA FINGERPRINT AND POLYSACCHARIDE CONSTITUENT IN DURIAN CULTIVARS |
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| Field of Study | Biomedicinal Chemistry |
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อรอนงค์ หนูชูเชื้อ : การวิเคราะห์ลายพิมพ์ดีเอ็นเอร่วมกับองค์ประกอบพอลีแซคคาไรด์ในทุเรียนต่างสายพันธุ์. (ANALYSIS OF DNA FINGERPRINT AND POLYSACCHARIDE CONSTITUENT IN DURIAN CULTIVARS) อ. ที่ปรึกษา : รศ. ดร. สุนันท์ พงษ์สามารถ, อ.ที่ปรึกษาร่วม : ผศ. ร.ต.อ. หญิง ดร. สุชาดา สุขหร่อง, 116 หน้า.

สารสกัดเจลพอลีแซคคาไรด์ (PG) จากเปลือกทุเรียน (*Durio zibethinus* Merr.) ที่มีการศึกษามาก่อนแล้วพบว่าเป็นสารประเภทเพคตินมีฤทธิ์ต้านเชื้อแบคทีเรียและสามารถกระตุ้นระบบภูมิคุ้มกันได้ สารสกัด PG สามารถใช้เป็นสารช่วยเตรียมเภสัชภัณฑ์และอาหาร เช่น เยลลี่ ยาเม็ดเคลือบ แผ่นฟิล์มปิดแผล เจลฆ่าเชื้อและผลิตภัณฑ์เจลจุ่มเต้านมวัวป้องกันโรคเต้านมวัวอักเสบ เป็นต้น ผลการทดลองก่อนหน้านี้ที่น่าสนใจคือ PG จากเปลือกทุเรียนพันธุ์ชะนี พื้นเมือง และหมอนทองจากจังหวัดชุมพรมีความแรงในการต้านเชื้อแบคทีเรียแตกต่างกัน ในการศึกษาครั้งนี้มีจุดประสงค์เพื่อศึกษาคุณลักษณะและจำแนกทุเรียนในระดับโมเลกุลของทุเรียนต่างสายพันธุ์และต่างพื้นที่เพาะปลูก ได้แก่ ทุเรียนกระดุมทอง หมอนทอง และชะนี จากจังหวัดจันทบุรี และทุเรียนพื้นเมือง หมอนทองและชะนี จากจังหวัดชุมพร และนำข้อมูลมาเปรียบเทียบกับคุณสมบัติของสาร PG ที่มีฤทธิ์ชีวภาพจากทุเรียนต่างสายพันธุ์และต่างพื้นที่ การศึกษาลำดับเบสดีเอ็นเอของยีน *mat*K ในคลอโรพลาสต์ของทุเรียนต่างสายพันธุ์มีความยาวทั้งหมด 1,509 bp เปรียบเทียบกับลำดับเบสของยีน *mat*K ของทุเรียนที่มีการศึกษาก่อนหน้านี้ในฐานข้อมูล GenBank (AY321188) ในยีน *mat*K ที่ตำแหน่ง 275 ของทุเรียนพันธุ์พื้นเมืองเป็นเบสอะดีนีน (A) หรือ ไซโทซีน (C) ในขณะที่ทุเรียนพันธุ์กระดุมทอง หมอนทอง และชะนีจากทั้ง 2 จังหวัด เป็นเบสไซโทซีนเหมือนข้อมูลใน GenBank ส่วนที่ตำแหน่งของ 860 และ 862 ของทุเรียนทุกสายพันธุ์เป็นเบสไซโทซีน (C) และไธมีน (T) ตามลำดับ จากข้อมูลของ*mat*K มีความแตกต่างน้อยยังไม่เหมาะสมที่จะใช้เป็นเครื่องหมายทางโมเลกุล จึงทำการศึกษาเพิ่มเติมโดยเทคนิค RAPD ในเบื้องต้นระบุได้ว่ามีการแปรผันทางพันธุกรรมของทุเรียนต่างสายพันธุ์และให้รูปแบบดีเอ็นเอของแต่ละสายพันธุ์ที่มีลักษณะเฉพาะ เมื่อวิเคราะห์ด้วยวิธี Unweighted pair group method with arithmetic averages หรือ UPGMA สามารถแบ่งทุเรียนออกได้เป็น 2 กลุ่มหลักคือ ทุเรียนพื้นเมืองที่เป็นพันธุ์ดั้งเดิมและทุเรียนสายพันธุ์เพาะปลูก (กระดุมทอง หมอนทอง และชะนี) การวิเคราะห์คุณสมบัติสารสกัด PG พบว่าเปลือกทุเรียนหมอนทองให้ปริมาณ PG และมีความหนืดของสารละลายมากที่สุดด้วยเช่นกัน ($P<0.05$) สารละลาย PG ของทุเรียนทุกสายพันธุ์มี pH อยู่ในช่วง 2.437-2.526 PG จากเปลือกทุเรียนพันธุ์กระดุมทองมีปริมาณของน้ำตาล Galacturonic acid มากที่สุดอย่างมีนัยสำคัญ ผลการทดลองหาปริมาณของน้ำตาล Galacturonic acid ใน PG ในสายพันธุ์เดียวกันให้ค่าไม่แตกต่างกัน ($P>0.05$) และพบว่ามีปริมาณ Galacturonic acid ใน PG แตกต่างกันอย่างมีนัยสำคัญเมื่อสกัดมาจากต่างสายพันธุ์กัน จากผลการทดลองอาจเสนอแนะได้ว่าเทคนิค RAPD สามารถใช้เป็นเครื่องหมายทางโมเลกุลร่วมกับองค์ประกอบของน้ำตาล Galacturonic acid ของ PG ในการจำแนกทุเรียนต่างสายพันธุ์ได้

ภาควิชา.....ชีวเคมี.....ลายมือชื่อนิสิต.....อรอนงค์ หนูชูเชื้อ.....

สาขาวิชา.....ชีวเวชเคมี.....ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2549.....ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

# # 4776619933 : MAJOR BIOMEDICINAL CHEMISTRY
KEY WORD: *Durio zibethinus* / POLYSACCHARIDE ANALYSIS / DNA FINGERPRINT / *mat*K gene / RAPD

ONANONG NUCHUCHUA : ANALYSIS OF DNA FINGERPRINT AND POLYSACCHARIDE CONSTITUENT IN DURIAN CULTIVARS. THESIS ADVISOR : ASSOC. PROF. SUNANTA PONGSAMART, Ph.D., THESIS COADVISOR : ASST. PROF. SUCHADA SUKRONG, Ph.D., 116 pp.

Polysaccharide Gel (PG) from fruit-rinds of durian (*Durio zibethinus* Merr.) is a pectic polysaccharide, according to prior studied, the polysaccharide exhibits antibacterial activity and immunomodulatory activity. PG have found to be useful for food and pharmaceutical applications such as jelly, tablet coating, film dressing, antiseptic gel, PG teat dip for protecting bovine mastitis, etc. Interestingly, PG from durian cultivars, 'Chani' 'Pauenmuang' (native cultivar) and 'Monthong' from Chumporn province, have the different bactericidal potency. In this study aimed to characterize and identify the difference between durian cultivars and between cultivated areas in molecular level together with bioactive PG in fruit-rinds. The cultivated-durians, 'Kradumthong', 'Monthong' and 'Chani' from Chanthaburi province; 'Pauenmuang', 'Monthong' and 'Chani' from Chumporn province were investigated. The *mat*K gene in chloroplast genome of these durians was 1,509 bp in length. In comparison with the previous reported in GenBank, accession no. AY321188. The *mat*K of 'Pauenmuang' cultivar presented either adenosine or cytosine substitutions at the position 275, whereas 'Monthong' and 'Chani' cultivars from both provinces, and 'Kradumthong' from Chanthaburi province presented the cytosine substitutions at the same position as same as the previous reported in GenBank. The *mat*K sequences of all tested durian cultivars were also found the cytosine and thymidine substitutions at the position 860 and 862, respectively. The results provided not enough information to characterize the variation of durian cultivars, then the *mat*K gene was not suitable to be used as the molecular marker for durian identification in this study. In addition, the preliminary RAPD study indicated that these durian cultivars exhibited genetic variation. The DNA profiles showed the specific patterns of different durian cultivars. The dendrogram was constructed by unweighted pair group method with arithmetic averages, UPGMA. Durian cultivars can be divided into two main groups, native planted 'Pauenmuang' and commercially cultivated ('Monthong', 'Chani' and 'Kradumthong'). The results of PG analysis showed that PG of 'Monthong' fruit-rinds from both provinces gave the highest percentage of the total yield ($P$<0.05) and also the highest viscosity ($P$<0.05). The pH range of PG was 2.437-2.526. The important major sugar, galacturonic acid content, in PG from 'Kradumthong' cultivars was the highest. The results of the galacturonic acid content in PG was not significantly different ($P$>0.05) within the same cultivars, but significantly different ($P$<0.05) from different durian cultivars. The results suggested that the polymorphic band profiles of RAPD could be used as molecular marker for identification durian cultivars together with the galacturonic acid content in PG in durian-rinds.

Department……Biochemistry……Student's signature ONANONG NUCHUCHUA .....
Field of study…..Biomedicinal Chemistry…Advisor's signature… Sunanta Pongsamart .....
Academic year……..2006……Co-advisor's signature… Suchada

## ACKNOWLEDGEMENTS

It is very great pleasure to thanks the many people who made this thesis possible.

I would like to express sincere gratitude to my thesis advisor, Assoc. Prof. Dr. Sunanta Pongsamart, for her guidance, support, technical and non-technical advice. Throughout my thesis-writing period. I thank her also for providing me an opportunity to grow as a good student.

It is difficult to overstate my gratitude to my thesis co-advisor, Assist. Prof. Dr. Suchada Sukrong. With her great efforts to explain things clearly and simply. Throughout my thesis-writing period, she provided encouragement, good teaching, and lots of good ideas.

I also wish express deep appreciation to all members of the thesis committee for their suggestions and comments.

I would like to thank the many people who have supported the specimens: the first is Assoc. Prof. Thitirat Panmaung and her relationship (Mrs. Krung) for their kindness to assist and take care of me all the time in Chumporn province, the special thanks to durian plantation owner, Mr. Boonpaem Chaoungsom, Mrs. Dutsadee Tanthasatian and the friend of Mrs. Krung at Lhangsaun district for providing all of durian specimens and knowledgements in this experiment.

Thanks are also due to Chulalongkorn University and the Ministry of University Affairs for granting partial financial support to fulfill this study.

I wish to thank my best friend in Department of Biochemistry and other person whose names have not been mentioned here for helping me get through the difficult times, and for all the emotional support, comraderie, entertainment, and caring they provided.

Lastly, and most importantly, I wish to thank my family, on whose constant encouragement and love. Their unflinching courage and conviction will always inspire me. I thank them also for providing me to grow as a good human.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| 18s rDNA | 18s ribosomal RNA gene |
| AFLP | Amplified fragment length polymorphism |
| AP-PCR | Arbitrarily primed PCR |
| BSU | BioService Unit |
| cpDNA | Chloroplast DNA |
| DAF | DNA amplification fingerprinting |
| DE | Degree of esterification |
| DNA | Deoxyribonucleic acid |
| ETS | External transcribed spacer |
| FT-IR | Fourier transform-infrared spectrometry |
| HG | Homogalacturonan |
| HM pectins | High-methoxy pectins |
| IGS | Intergenic spacer |
| ITS | Internal transcribed spacer |
| ITS-1 | Internal transcribed spacer 1 |
| ITS-2 | Internal transcribed spacer 2 |
| LM-pectins | Low-methoxy pectins |
| *mat*K gene | Gene encoding maturase K |
| mtDNA | Mitochondial DNA |
| *ndh*F gene | Gene encoding NADH dehydrogenase F |
| nDNA | Nuclear DNA |
| PAUP | Phylogenetic analysis using parsimony |
| PCR | Polymerase chain reaction |
| PCR-RFLP | Polymerase chain reaction- Restriction fragment length polymorphism |
| PG | Polysaccharide gel |
| RAPD | Random amplified polymorphic DNA |
| *rbc*L gene | Gene encoding the large subunit of the ribulose-bisphosphate carboxylase |
| RFLP | Restriction fragment length polymorphism |
| RGI | Rhamnogalacturonan I |

| | |
|---|---|
| RGII | Rhamnogalacturonan II |
| RNA | Ribonucleic acid |
| $tRNA^{Lys}$ | Transfer RNA of Lysine |
| *trn*K gene | Gene encoding $tRNA^{Lys}$ |
| UPGMA | Unweighted pair group method with arithmetic averages |

# CHAPTER I

## GENERAL BACKGROUND

### 1. Introduction

*Durio zibethinus* Merr. (เต็ม สมิตินันทน์, 2544) belongs to the genus *Durio* in the family Bombacaceae, in a revision of durio by Kostermans comprises 27 species, all found within areas covering Sri Lanka and a large part of Southeast Asia. At least six species of *Durio*, *D. zibethinus* Merr., *D. graveolens* Becc., *D. kutejensis* Becc., *D. testudinarum* Becc., *D. dulcis* Becc. and *D. oxleyanus* Griff are considered edible (Somsri and Wangnai, 2006).

Durian (*Durio zibethinus* Merr.) is a true tropical fruit species. In particular, numerous durian cultivars are grown commercially throughout Southeast Asia. Durian is one of the important fruit crops in Thailand. About 200 durian clones are recognized as cultivars. There are many kinds of cultivated durian such as 'Monthong' (หมอนทอง), 'Chani' (ชะนี), 'Kanyao' (ก้านยาว), 'Kradumthong' (กระดุมทอง), 'Kopphikul' (กบพิกุล), 'Phuangmani' (พวงมณี), etc. (Somsri and Wangnai, 2006) including 'Pauenmuang' cultivar, which is the native durian or endemic species. Figure 1 shows fruits of commercially cultivated durian in Thailand. 'Monthong' and 'Chani' are the most commercially cultivated durian. Durian production for commercial is cultivated mainly in the eastern provinces such as Chanthaburi and Rayong, and the southern provinces such as Chumporn. Fruits from both eastern and southern areas are harvested and marketed from April to June and July to September, respectively (Subhadrabandhu and Ketsa, 2001). The durian has an aril fruit with sharp spines on the pericarp. It is ovoid-oblong to round shaped. Fruit weight varies between 1.5 and 4.0 kg for commercial grades. Durian is very popular fruit in Thai people. Because the rind weight is more than half of the total fruit weight, thus, produces several hundred thousand tons of durian-rinds waste every year. Agricultural wastes of durian fruit rinds have found to be used as a source of commercial important plant materials for isolation of antibacterial polysaccharide for pharmaceutical and cosmetic applications.

a)

b)

c)

d)

Figure 1. Commercial durian-fruits (*Durio zibethinus* Merr.); (a) 'Monthong', (b) 'Chani', (c) 'Kradumthong' and (d) 'Pauenmuang'.

'Polysaccharide Gel' or PG was first isolated from durian rinds by Pongsamart and Panmaung (1998). PG has been characterized as a pectic polysaccharide (Hokputsa et al., 2004). The major content of PG is a polygalacturonic acid, (1→4) α galacturonic acid chain of 67.9% as a major component, and other neutral sugar side chains which are arabinose (1.2%), rhamnose (4.8%), xylose (0.4%) and galactose (4.9%). Furthermore, glucose as a starch contaminant is also include. PG exhibits the gelling and film forming property. Application of PG in food and pharmaceuticals such as jelly and tablet have been reported (Pongsamart and Panmaung, 1998; Umprayn et al., 1990). PG has also studied as a film dressing for pharmaceutical purposes (Gerddit, 2002). Dressing films prepared from PG attractively enhancing wound healing in pig, dog and cat skin *in vivo* (Chansiripornchai et al., 2005; Chansiripornchai et al., 2006). Interestingly, PG has antibacterial activity against both gram positive and gram negative bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis*, *Escherichia coli*, *Micrococcus luteus* and *Bacillus subtilis* but it does not inhibit the growth of fungi, *Candida albican* and *Saccharomyces cerevisiae* (Lipipan, et al 2002; Pongsamart et al., 2005). PG can be formulated as the oral-fresh film, antiseptic gel, anti-acne gel, etc. (Pongwiwatana, 2005; Paphattarapong, 2005; Lertchaiporn et al., 2006). Moreover, PG has potential to activate cells of the immune system in bovine mammary gland of non-lactating cows (Maktrirat et al., 2006). The post-milking teat dip has been developed from PG, this product expected to be used to protect bovine mastitis disease in cows (Maktrirat et al., 2007). Toxicity studies of PG have also been reported, no toxic effect have found in oral consumption of PG in acute and subchronic toxicity test (Pongsamart et al., 2001; Pongsamart et al., 2002). Interestingly, the previous study of PG from fruit-rinds of durian cultivars from Chumporn province has been found that PG from 'Chani' and 'Pauenmuang' has bactericidal activity higher than that of 'Monthong' (Phaunfoong, 2005).

The biological difference could be the diversity of different cultivars according to breeding, planted areas or any major active conpounds. Moreover, the quality of PG varies greatly depending on genetic materials and environmental effects such as the origin of the species, planted location, extraction technique, etc. Unlike chemical studies, DNA marker decreases the environmental factors. DNA-based

molecular markers have been widely used for characterization of many organisms in the fields like taxonomy, physiology, embryology, genetics, etc. The markers have been also used as standardization for a production of herbal drugs and correlated to chemical profiles (Joshi et al., 2004). There are many polymorphic molecular markers for identification of related individuals; the *mat*K gene sequences and PCR-RFLP have been used to evaluate the genetic differentiation of cultivated radish (Yamane et al., 2005); RAPD markers have been used for cultivar-identification of apples (Koller et al., 1993), calla lily (Hamada and Hagimori, 1996) and *Dimocarpus longan* subspecies (Yonemoto et al., 2006). The *ndh*F gene and ITS sequences were used in durian identification for the investigation of phylogeny of core Durineae and related family (Nyffeler and Baum, 2000; Nyffeler and Baum, 2001) and the genetic relationships of 56 cultivars of *D. zibethinus* have also characterized by DAF technique (Somsri et al., 2005).

The objectives of this study were to identify different durian cultivars by using the molecular markers together with total polysaccharide analysis. The molecular technique was focused on the *mat*K gene sequencing in chloroplast genome and preliminary studied the RAPD profiles of different cultivated-durians. The durian specimens were ‘Monthong’, ‘Chani’, ‘Kradumthong’ and ‘Pauenmuang’ cultivars, which were collected from the main production areas, Chanthaburi and Chumporn province, Thailand.

## 2. Literature reviews

### 2.1 Molecular markers (Joshi et al., 2004)

The DNA-based molecular markers can be divided into 3 major techniques.

*‘Hybridization-based methods’* including Restriction Fragment Length Polymorphisms (RFLP), DNA is digested and hybridized by restriction enzymes and labelled probes, respectively. Polymorphisms are analyzed after hybridization by observing present or absent bands.

*'PCR-based methods'* are the amplification of DNA fragments *in vitro* using thermostable DNA polymerase and either random or specific primers. For examples, Random amplified polymorphic DNA (RAPD), Arbitrarily primed PCR (AP-PCR), DNA amplification fingerprinting (DAF), Amplified fragment length polymorphism (AFLP) and Polymerase chain reaction-Restriction fragment length polymorphism (PCR-RFLP).

*'Sequencing-based markers'* are DNA sequencing which can efficiently identify single nucleotide polymorphisms (insertion/deletion), depending on organism relationships.

The DNA-based markers have proved their utility in fields like taxonomy, physiology and genetics. As the science of plant genetic progressed, researchers have tried to explore these molecular techniques for their application in commercially important plants such as food crops, horticultural plants and recently in pharmaceutical sciences for the characterization of herbal medicine. According to the identification of species and prediction of the concentration of active phytochemicals are required for quality control of plant materials for pharmaceutical and industrial purposes. For identification of particular plants, the selected-phytochemical markers can correlate with their DNA fingerprint to apply in quality control of raw materials.

### 2.1.1 Hybridization-based methods

Restriction fragment length polymorphism (RFLP) is an example. RFLP are unequal lengths of DNA fragments obtained by cutting genomic DNA with restriction enzymes at specific sites. On an agarose gel, RFLPs can be visualized using radiolabeled complementary DNA sequences. There is no need for PCR amplification of DNA in this method. A routine southern blot experiment is used instead. Normally, RFLPs are used to identify the origins of a particular plant species, setting the stage for mapping its evolution. There are some problems with the RFLP method of DNA fingerprinting. Firstly, the results do not specifically indicate the chance of a match between two organisms. Secondly, the process involves a lot of money and labor, which not many laboratories can afford. Finally, unlike the microsatellites, a few loci in the assay must suffice (Vasudevan, 2007).

### 2.1.2 PCR-based markers

#### (1) Random amplified polymorphic DNA (RAPD)

It is a type of PCR reaction using oligoprimers (8-12 nucleotides). The knowledge of the DNA sequences for the targeted gene is not required. The primers bind somewhere in the sequences as random amplification. The polymorphic bands are performed by agarose gel electrophoresis. RAPD techniques was used as the species-specific markers of five *Derris* species, *D. scandens*, *D. elliptica*, *D. malaccensis*, *D. trifoliata* and *D. reticulata* (Sukrong et al., 2005). Echeverrigaray et al. (2001) were successful to classify the thyme cultivars, Burpee, Blumen, Battle, SEM, Tropical and Isla by RAPD analysis and their essential oil composition. In addition, the chemical content and genomic of Italian garlic and rice were analyzed. The results of Italian garlic found the correlation between its chemicals and genetic materials (Brandolini et al., 2005) but did not found these correlation in Italian rice (Brandolini et al., 2006).

Although, RAPD marker is wildly used by many researchers, because the methods were rapid and inexpensive, and do not need too many genetic information. However, the disadvantage of this marker is to make it reproducible. The specimens should be replicated in the same and suitable condition to ensure the reproducible pattern (Atienzar and Jha, 2006).

#### (2) Amplified fragment length polymorphism (AFLP)

AFLP is a highly sensitive method for detecting polymorphisms in DNA, the method originally described by Vos in 1995**.** Briefly, total DNA is digested with restriction enzymes and ligated by specific adaptors to all restriction fragments. The selective amplification of some of these fragments with primers that have corresponding adaptors and restriction site specific sequences (Figure 2). The band patterns are shown by polyacrylamide gel. AFLP could be successfully used to resolve the correlation of AFLP data with the selected chemicals of *Withania somnifera* (Dhar et al., 2006). AFLP is also capable of determining a large number of polymorphisms.

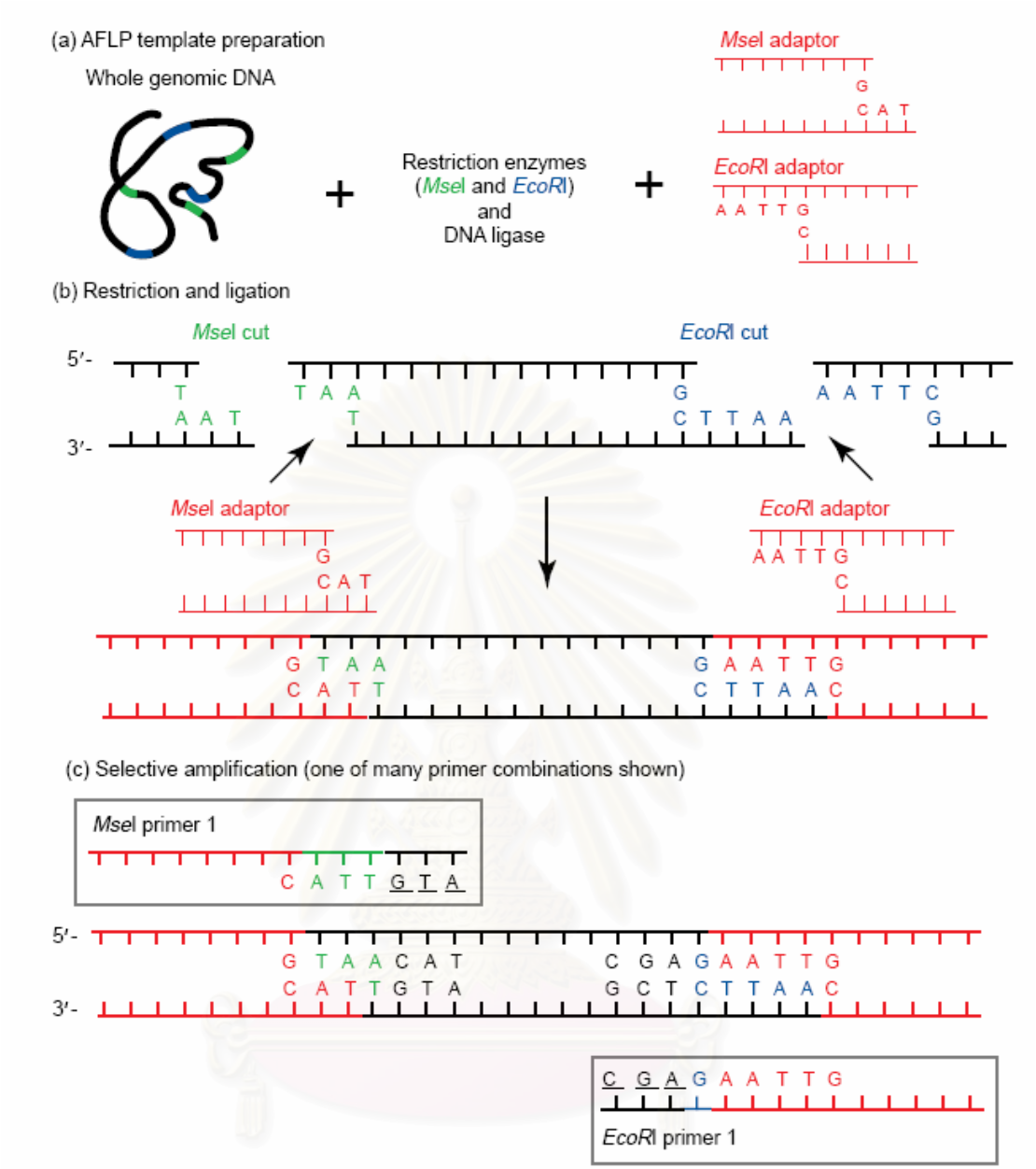



Figure 2. The principle of Amplified fragment length polymorphism. a) The steps of digestion with restriction enzyme, b) Putting the selective adapters and c) The selective PCR amplification (Mueller and Wolfenbarger, 1999).

### 2.1.3 Sequencing-based markers (Soltis et al., 1998)

The nucleotide sequencing is one of the most techniques to utilize the phylogenetic history. DNA sequence data are the power of informative tool for molecular systematics, and comparative analysis of DNA sequences is becoming increasingly important in plant systematics. There are two major reasons why nucleotide sequencing is useful in systematics of plants. First, the nucleotides are the basic units of information encoded in organisms. Second, the potential sizes of data sets are immense. However, the disadvantage of this technique is expensive for repetition. Furthermore, different genes or parts of the genome might evolve at different rates. The selection of genes or any parts of genome depends on the taxonomic levels.

Unlike animals, plants have three kinds of genomes, the chloroplast genomes (cpDNA) in addition to the nuclear (nDNA) and mitochondial (mtDNA) genomes. The mtDNA is rarely used in molecular markers of plants due to its structure, size, and gene order are various depending on plant species. The nDNA and cpDNA are commonly able to investigate in the molecular systematics and taxonomy of plants. The nDNA is more complexity and repetitive properties. On the other hand, the cpDNA is well suitable for evolutionary and phylogenetic studies above the species level because cpDNA; 1) is a relative abundant component of total DNA, 2) contains primarily single copy genes, 3) has a conservative rate of nucleotide substitution. The most common genes in nDNA is nuclear ribosomal gene consists of a transcribed region that comprises an external transcribed spacer (ETS), followed by 18s rDNA, an internal transcribed spacer (ITS-1), the 5.8s rDNA, a second internal transcribed spacer (ITS-2), and finally the 26s rDNA. Each repeat is separated from the next repeat by an intergenic spacer (IGS). For the most common genes in cpDNA are *rbc*L, *ndh*F, *trn*K/*mat*K gene, chloroplast ribosomal gene, etc.

For examples, Zhao et al. (2003) and Xia et al. (2005) studied the sequences of 5s-rRNA spacer domain and assess the chemicals of traditional Chinese medicine, *Angelica* (Danggui) and Curcumae, respectively. The *ndh*F gene and ITS sequences were used in durian identification for the investigation of phylogeny of core Durineae and related family (Nyffeler and Baum, 2000; Nyffeler and Baum,

2001). Locust bean gum and guar gum are neutral polysaccharide products (galactomannans) as food additives from *Ceratinia siliqua* and *Cyamopsis tetragonoloba*, respectively. Both gums are basically the same structures. The sequences of ITS regions, ITS-1 and ITS-2 were used as DNA markers to characterize that species (Urdiain et al., 2004; Urdiain et al., 2005).

### 2.2 The *mat*K gene

The *mat*K gene is named according to its possible maturase function and its location within the *trn*K gene encoding the $tRNA^{Lys}$ (UUU). In Figure 3 illustrates the structure of *trn*K/*mat*K gene. The *mat*K locates within the intron of the *trn*K (Hilu and Liang, 1997). In plant molecular systematics and evolution, the *mat*K gene is emerging as another valuable gene to study because of its reasonable size, high substitution rate, evenly distributed codon position variation, low transition and transversion ratio, and the easiness of amplification due to its two flanking coding *trn*K gene. Since its high substitution rate has a potential of providing more informative sites, most application of *mat*K in phylogenetic reconstruction has been at the family, genus, species and even population (Soltis et al, 1998). There have been several studies using the *mat*K gene sequences in plant systematics, molecular genetics and chemical assessment.

For instance, Ping et al. (2002) suggested that DNA sequence data of *mat*K gene generated the tree cluster of *Pogostemon cablin* cultivars which were identical to 18s rDNA cluster. They could be combined with plant chemotypes as a quality control for production. The preliminary experiment of Valerianaceae family was reconstructed to study the evolution, based on the various plastid gene including *mat*K gene region (Bell, 2004). However, the chemical of *Aristolochia* species could not relate to their molecular evidence from *mat*K sequence and other chloroplast regions (Silva-Brandao, Solferini and Trigo, 2006). Thus, the *mat*K gene may be useful in this investigation. However, the general background information of this gene of *Durio zibethinus* is available on Genbank (AY321188), however specific cultivars of *Durio* species has not been reported. The *mat*K sequence is shown in the Figure 4.


Figure 3. The sketching structure of *trn*K/*mat*K gene in chloroplast genome.

```
   1 ggcttttaag tgcgactagc atcttttaca catttgtatg aagaaaggga ttcgttcata
  61 ccatcggtac agtttgtaag accacgactg atcctgaaag gagtggatgg aaaaaagagc
 121 atgtcgtatc aatggagaat tctaagaatc catttttttt ccggatcagt ccaaaaaaaa
 181 aaatcgtctt tgaatttttg gtgcggaaca aaaaaaatta attgaattca aagttgggtc
 241 gagtgaataa atggatagag ctctacggcc ccaattatag ggaaacaaaa agtaacgagc
 301 ttctgttcgc aatttgaatg attacccgat ctaattaaac gttaaaaata aattagtgcc
 361 taatgcggta aaggtttttc tcatgagtaa attatcgatt tttttatgag tcctaattat
 421 tagttattcc ctttatgggt tagacatgaa tgtgtataag aagcagtata ttgataaaga
 481 aaagatattt ttttttttt tccaaaanca aaagagcgat ngggtngaaa aaataaagga
 541 ttyytancca tyttyttatc ctataacgaa ncataaatca attagatggc aaaagatagg
 601 atagagaatc cgttgatgaa tctacctgtc tccgaggtat ctattatttt cttactataa
 661 taccttgttt tgactgtatc gcactatgta tcatttgata accgaataga tcccctatac
 721 tttggttcaa atcgaatttg aaatggagga atttcaagta tatttagaac taaatagatc
 781 tcgccgacat gatttcctat acccacttat ttttcgggag tatatttatg cacttgctca
 841 tgatcatggt ttaaataaat cgatgatttt tttggaaaat cagggttatg gtaataaatt
 901 cagttcacta attgtgaaac gtttaattat tcgaatggat caacagaatc atttgattat
 961 ttctgctaat gattccaacc aaaatccatt ttttgggcac aacaataatt tatattctca
1021aatgatatcg gtgggatttg cagtcattgt ggaaattcca ttttccttac gattagtatc
1081ttactcacaa ggggaagaag tcgcaaaatc ccataatttc caatcaattc attcaatatt
1141tccttttta gaggacaaat tctcacattt aaattatgtg ttagatgtac taatacctta
1201ccccatccat ctagaaatct tggttcaagc ccttcgctac tggataaaag atgcttcttc
1261tttgcattta ttacggttct ctctctacga gtattgtaat ttgaagagtt ttattactcc
1321aaagaaatct atttctattt ttaatccaag attattcttg ttcctatata attctcatgt
1381atgtgaatac gaatccattt tccttttct ccgtaatcaa tcttcttatt tacgatcaac
1441atcttctgga ttctttcttg aacgaattaa tttctatgga aaaatagagt atcttgtaga
1501agtcttttat aatgattttc agaacaacct atggttgttc aaagaccctt tcatacattt
1561ttttaggtat caaggaaagg caattctggc atcaaaggat aagcctcttc tgatgaataa
1621gtggaaatat tactttgtcg atttatggaa atattatttt tacgtgcggt ctcaatcagg
1681aagcgtccgt ataaatcaat tatctaaata ttctctcgac tttctgggct atctttcaag
1741tgtgcgatta aatacttcag tggtacggag tcaaatgcta gaaaattcat ttataataga
1801taatgctatg aagaagttgg atacaagaat tccaattatt tctctcattg gatcattgtc
1861taaagcgaaa ttttgtaaca cattagggca tcccattagt aagccgacgt ggtccgattc
1921ctccgattct gatattattg accgatttgt gcgtatatgc agaaatcttt ctcattatca
1981cagtggatct tcaaaaaaaa agagtttgta tcgaataaaa tatatacttc ggctttcttg
2041tgttaaaact ttggctcgta aacacaaaag tactgtacgt gctttttga aaagattagg
2101ttcggaattt ttggaagaat tctttacgga agaagaacat gttttttctt tgatcttccc
2161aagagttttt ttgacttcgc gaaagttata tagggtgcga atttggtatt tggatattat
2221ttgtatcaat gctctggtca atcatgaatg attggttatg aaatcatgta aattcaaatt
2281caatataaaa tgggaatttt tcctaaatga tgaagagata acaaaagaat ttattcagtt
2341ctagtattaa atgttcatgc agtaagaata agaggggatt ggctgagtag tccacttttt
2401tgagtcctgt ttagggaata aattggtttt agatgtatac atagagaaag ccgtgtgcaa
2461tgaaaaatgc aagcacggtt tggggaggga tttttt
```

Figure 4. The *trn*K/*mat*K sequences of *Durio zibethinus* are total 2496 bp in length which reports in GenBank, accession number AY321188. The *mat*K gene sequences are 1,509 bp in length as marked in the blue color at the position of 743 to 2251.

## 2.3 Primer design (Dieffenbach and Dveksler, 2003)

Several variables must be taken into consideration when designing PCR primers which are:

### 2.3.1 Primer length

Since both specificity, the temperature and time of annealing are partly dependent on primer length, this parameter is critical for successful PCR amplification. In general, the length of primers between 18 and 24 bases provide a specificity. The annealing temperature is also optimal. The longer primer is more inefficient for annealing. As the results, the yield of PCR products is decrease.

### 2.3.2 Specificity

The primer specificity depends on the primer length. Primers have a unique sequence within the template DNA that is to be amplified. A primer with highly repetitive sequence will result in a smear when amplifying genomic DNA. However, primer extension will occur at the lower temperature of annealing. If the temperature is too low. Non specific priming may occur which can be extended by the polymerase if there is a short homology at the 3' end. In general, a melting temperature of 55°C -72°C gives the best results.

### 2.3.3 Complementary primer sequences

Primers need to be designed with absolutely no intra-primer homology such as self-homology, partially double-stranded structures, etc. Inter-primer homology is also important, two primer anneals together, primer dimer formation. Both of them will interfere with annealing to the template.

### 2.3.4 GC content, polypyrimidine and polypurine stretches

The base composition of primers should be between 45%-55% of GC content. The primer sequence must be chosen such that there is no polyG or polyC that can promote non-specific annealing. PolyA and polyT streches are also to be avoided these will breath and open-up streches of the primer template complex. This can lower the efficiency of amplification. Both polypyrimidine (T, C) and polypurine (A, G) stretches should also be avoided.

### 2.3.5 3' end

It is well established that the 3' terminal position in PCR primers is essential for the control of mis-priming. A G or C residue at the 3' end is *'GC clamp'*, helps to ensure correct binding at the 3' end due to the stronger hydrogen bonding of G/C residue. It also helps to improve the efficacy of the reaction by minimizing any breathing that might occur. At present, there are several online free software programs. For examples, Fast PCR, Primers3 and Primo Pro 3.4. They can calculate many parameters as mentioned above so primer design is so easily. Fast PCR progam was used in this investigation which can be downloaded from http://www.biocenter.helsinki.fi/bi/ Programs/fastpcr.htm.

## 2.4 Sequence alignment

In bioinformatics, a sequence alignment is a way of arranging the primary sequences of DNA, RNA, or protein to identify regions of similarity that may be a consequence of functional, structural, or evolutionary relationships between the sequences. Aligned sequences of nucleotide or amino acid residues are typically represented as rows within a matrix. Alignments are commonly represented both graphically and in text format. In almost all sequence alignment representations, sequences are written in rows arranged so that aligned residues appear in successive columns. In text formats, aligned columns containing identical or similar characters are indicated with a system of conservation symbols. Many sequence visualization programs also use color to display information about the properties of the individual sequence elements; in DNA and RNA sequences, this equates to assigning each

nucleotide its own color. Sequence alignments can be stored in a wide variety of text-based file formats, many of which were originally developed in conjunction with a specific alignment program or implementation. Most web-based tools allow a number of input and output formats, such as FASTA format and GenBank format (Corpet, 1988).

## 2.5 Pectin (Dumitriu, 1998)

Naturally, pectin is found in the primary cell wall and espectially in the middle lamella, pectins are responsible for the structural properties of fruits and vegetables. Pectin helps to bind cells together and regulates water in the plant. The amount and composition of pectin in plant material vary from one variety of plant to another. Mainly citrus fruits and apples are used as raw materials for manufacturing of commercial pectins.

### 2.5.1 Molecular structure

Pectin is an important polysaccharide with applications in food, cosmetic and pharmaceutical industries. Pectin is a heterogeneous complex polysaccharide that isolates from plants. The main types of pectic matrix are homogalacturonan (HG), rhamnogalacturonan I (RGI), and rhamnogalacturonan II (RGII). Briefly, the major constituent is linear sequences of 1, 4 linked α-D-galactopyranosyluronic acid that forms the pectin-backbone, a homogalacturonan (HG). There are regions where galacturonic acid is replaced by (1-2)-linked L-rhamnose in this backbone. From rhamnose, sidechains of various neutral sugars branch off. This type of pectin is called rhamnogalacturonan I. The stretches consist of alternating galacturonic acid and rhamnose called “hairy regions” and others with lower density of rhamnose called “smooth regions”. Furthermore, the neutral sugar side chain are also present such as galactose, xylose and arabinose. The last type of pectin is Rhamnogalacturonan II, The backbone of RG-II contains 1,4-linked α-D-Gal*p*A residues which is a highly branched polysaccharide. Their structure are proposed in Figure 5, 6 and 7 (Ridley et al., 2001).

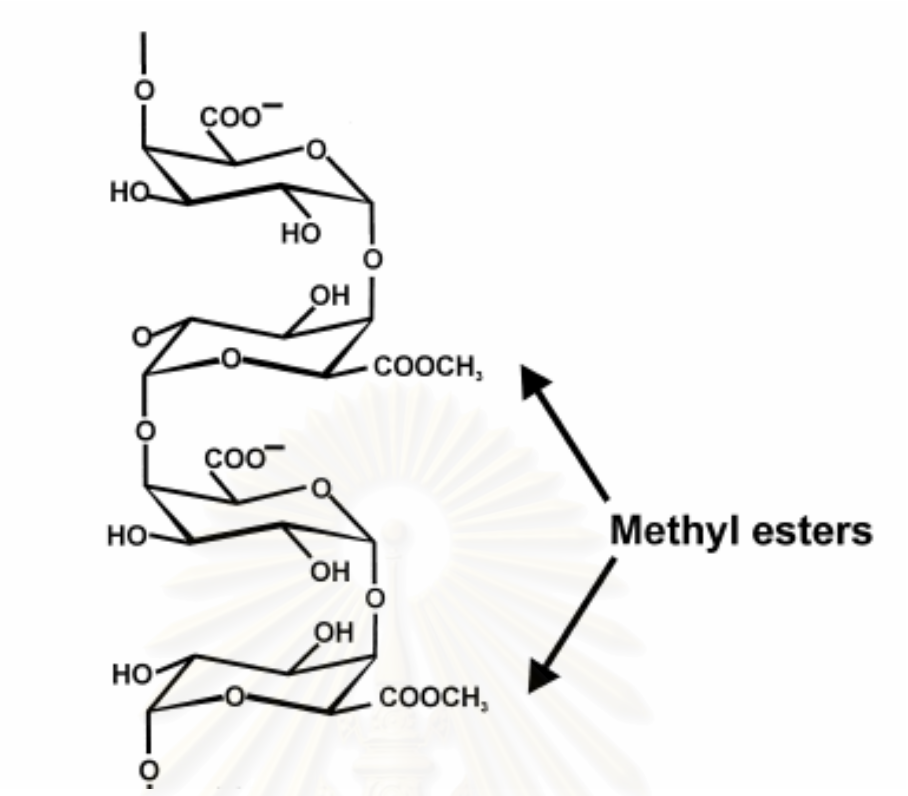



Figure 5. Homogalacturonan structure (HG). HG is a linear polymer of 1 → 4 linked α-D-Gal*p*A residues. Some of the carboxylates of the Gal*p*A residues are esterified with methanol (Ridley et al., 2001).

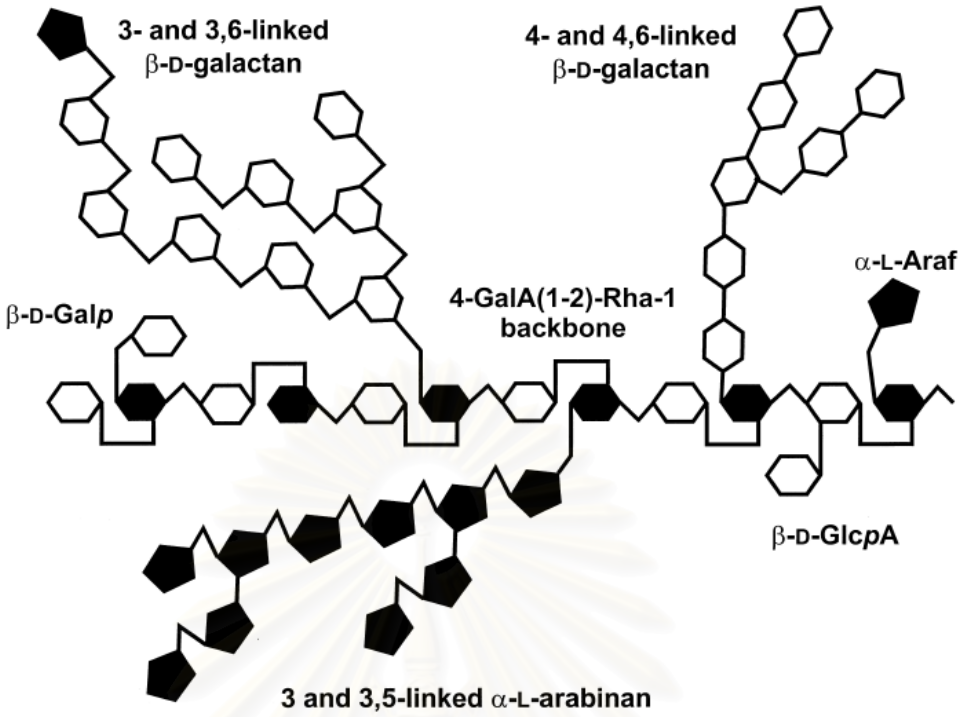



Figure 6. Rhamnogalacturonan I (RGI). The backbone is composed of the disaccharide repeating, galacturonic acid (white) and rhamnose (black), [→4-α-D-Gal*p*A-(→2)-α-L-Rha*p*-(1→)]. Branched and linear oligosaccharides composed predominantly of α-L Ara*f* and β-D-Gal*p* residues are linked to C4 of some of the Rha*p* residues (Ridley et al., 2001).

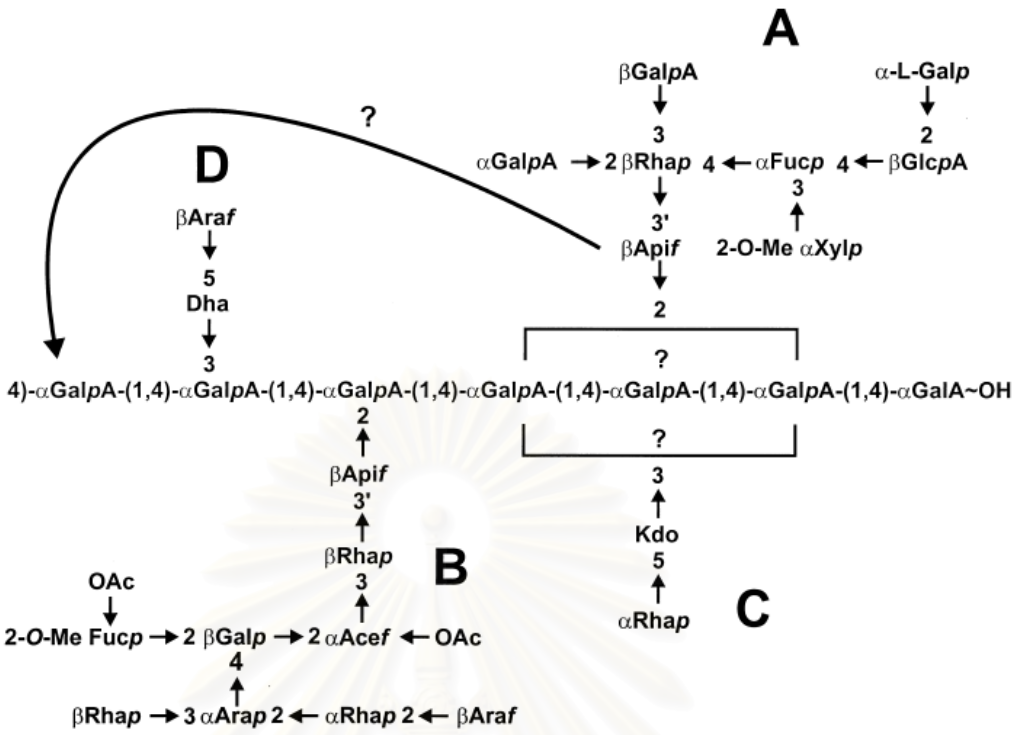



Figure 7. Rhamnogalacturonan II (RG II). Four structurally different oligosaccharide side chains (A–D) are linked to the RG-II backbone (Ridley et al., 2001).

### 2.5.2 Degree of esterification

In nature, around 80% of carboxyl groups of galacturonic acid are esterified with methanol. This proportion is decreased more or less during pectin extraction. The ratio of esterified to non-esterified galacturonic acid which is the degree of esterification (DE). Pectins are classified as high or low-ester pectins, in short termed HM or LM-pectins, with more or less than half of all the galacturonic acid esterified, respectively. It can determine the behavior of pectin in food applications, especially the solubility and the gel forming characteristics.

### 2.5.3 The physical properties of pectins

#### (a) Solubility

Pectin must be completely dissolve to ensure full utilization and to avoid heterogeneous gel formation. Complete dissolution requires dispersion without lumping; if pectin lumps are allowed to form they are extremely difficult to dissolve. Pectin, like any other gelling agent, will not dissolve in media where gelling conditions exist. It is recommended that HM-pectin is dissolved at solids below 20% and preferably in water.

#### (b) Gelling property

The most important factors which influence the gel formation are temperature, degree of esterification, pH, sugar and other solutes, and calcium ions. HM-pectins require a minimum amount of soluble solids and a pH within a pretty narrow range, around 3.0, in order to form gels. LM-pectins require the presence of a controlled amount of calcium or other divalent cations for gelation and do not require sugar and/or acid.. The degree of esterification of a high ester pectin influences the gelling properties. This difference is reflected in terms of rapid set, medium set and slow set. Furthermore, the gel formation depens on the temperature. Gels form on cooling and melt when heating.

**(c) Viscosity**

Pectin solutions usually show relatively low viscosities compared to other plant gums and thickeners. Pectin with a high degree of esterification is more viscous in solution than otherwise comparable pectin of lower degree of esterification so the degree of esterification is important for gel application. Viscosity of a pectin solution may be determined for the purpose of obtaining a measure of the molecular weight of the pectin or for evaluating the thickening effect of the pectin. Calcium or other polyvalent ions increase the viscosity of pectin solutions and low ester pectin solutions may even gel if the calcium content exceeds a certain limit. Moreover, the viscosity of pectin solution is also a function of the temperature and pectin concentration as shown in the Figure 8. The viscosity increases exponentially with pectin concentration. However, pH also influences the viscosity of pectin solutions. In a calcium-free solution the viscosity drops when pH is increased.

**(d) pH**

The pK-value of pectin is approximately 3.5. LM-pectins are higher pH-values than high-ester pectins. At low pH-values and elevated temperatures degradation due to hydrolysis of glycosidic links is observed. De-esterification is also favoured by low pH. As the results, pectin becomes slower setting or gradually adapts low ester pectin characteristics. At near to neutral pH (5-6), HM-pectin is stable at room temperature only. As the temperature (or pH) increases, the polysaccharide chains are cleavage, so-called The β-elimination. It is very rapid loss of viscosity and gelling properties.

### 2.5.4 Characterization of polysaccharide gel (PG) from durian fruit-rinds

Polysaccharide gel (PG) from fruit-rinds of *Durio zibethinus* is a pectic polysaccharide. The molecular weight of crude PG is approximately 100-1300 kDa. The sugar compositions of PG are 67.9% of galacturonic acid, 1.2% of arabinose, 0.4% of xylose, 4.9% of galactose and 4.8% of rhamnose. PG is seperated

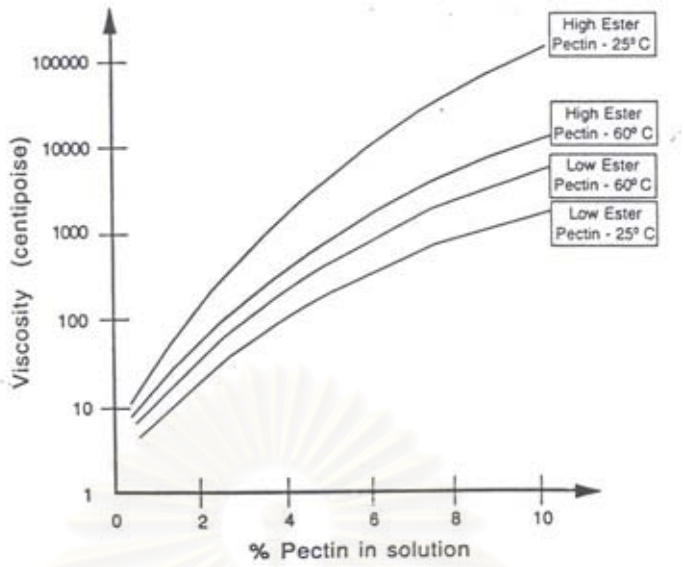



Figure 8. Correlation between viscosity and concentration of pectin.

into two main fractions, ‘acidic chain fraction’ and ‘neutral chain fraction’ by DEAE-Sepharose column. The main sugar in acidic chain is 86.2% of galacturonic acid which is 1, 4 linked polygalacturonic acid. Neutral chain consists of 34.9% of galacturonic acid containing other side chain neutral sugar contents more than acidic chain (Hokputsa et al., 2004). In addition, PG has also exhibited the effect on the immune system, the polysaccharide inhibits the heamolysis by complement fixation test. Bioactivity of PG has been studied, promissing antibacterial and immunostimulating activity are elucidated (Lipipan, et al 2002; Pongsamart et al., 2005; Phaunfoong, 2005; Maktrirat et al., 2006). Pharmaceutical applications of PG are established, the following PG products have been prepared: teat dip for protecting bovine mastitis, film dressing for healing wound, antiseptic hand-gel, anti-acne gel (Paphattarapong, 2005; Pongwiwatana, 2005; Chansiripornchai et al., 2006; Lertchaiporn et al. 2006; Maktrirat et al., 2007). Like PG, other pectic substances from medicinal plants has been characterized. For instance, Inngjerdingen, et al. (2005) identified polysaccharide from the aerial parts of *Glinus oppositifolius* as the pectic polysaccharide, a rhamnogalacturonan backbone, with arabinose and galactose side chains. They also exhibit the complement fixation activities and induced chemotaxis of macrophages, T cells and NK cells. Crude water soluble polysaccharide has been isolated from *Angelica sinensis* (Oliv.) Diels. Its pectic polysaccharide is fractionated into neutral and acidic polysaccharide by anion-exchang chromatography (Sun et al., 2005) like PG of durian rinds.

However, the amount and composition of pectin in plant material vary from one variety of plant to another. Mayworm et al. (2000) examined the polysaccharide contents in seed cell wall of Vochysiaceae family, genus *Callisthene*, *Qualea*, *Salvertia* and *Vochysia* as the phytochemical markers. The neutral sugars, arabinose, galactose, glucose mannose and rhamnose are existed in those pectin. Arabinose is always the predominant component that can be used as a chemical markers of Vochysiaceae family. All of four genus could be divided into 2 main clusters which were *Callisthene* and *Qualea*, and *Salvertia* and *Vochysia* by ANOVA statistics and Chemotaxonomic analysis. Nevertheless, the chemotaxonomic study has limitations because the quality of chemicals varies greatly depending on genetic materials and environmental effects such as the origin of the species, planted location, extraction technique, etc (Joshi et al., 2004).

Thus, the properties of a botanical raw material are not constant. In this experiment PG was isolated from different cultivated durian fruit-rinds, and from different cultivated areas. Determination for PG content and the major sugar composition, galacturonic acid, in PG were carried out. Physical properties of PGs were also investigated such as pH and viscosity. As the results, PG contents were analyzed in combination with DNA profiles, by RAPD technique and *mat*K sequence analysis.

# CHAPTER II

# MATERIALS AND METHODS

## Materials

### 1. Chemicals

-DNeasy Plant Mini Kits and Geneclean II Kit were obtained from QIAGEN (Germany) and Q-Biogene (USA).

-*Taq* DNA polymerase, deoxynucleotide triphosphates (dNTPs), agarose powder, blue/orange 6X loading dye and 1 Kb DNA ladder, were obtained from Promega (USA).

-50X TAE buffer was obtained from Bio-Rad (Italy).

-Sulfamic acid and Citric acid were analytical reagent grade obtained from Fisher Scienific (UK).

-m-Hydroxydiphenyl was analytical reagent grade obtained from Aldrich (Germany).

-D-(+)-Galacturonic acid monohydrate, hydrochloric acid, sodium hydrogen carbonate, sulfuric acid (96.4% assay), potassium hydroxide and potassium bromide were analytical reagent grade obtained from Merck (Germany).

-Pectin from citrus peel (P9135, galacturonic acid ≥74.0 %) were obtained from Sigma (Germany).

-Sodium tetraborate was analytical reagent grade obtained from Ajax Finechem (Australia).

-Sodium hydroxide was analytical reagent grade obtained from APS Finechem (Australia).

-Potassium bromide was IR spectroscopic grade obtained from Merck (Germany).

**2. Equipments**

-Balance, XT 620M, Precisa Instruments Ltd. (Switzerland)

-PCR cycler, Eppendorf Master Cycler, Perkin-Elmer, Co. (USA)

-Gel documentation, Quantity One 1-D Analysis software, Gel Doc XR, Bio-Rad Laboratories, Inc. (Canada)

-Spectrophotometer, model Educator, Thermo Electron, Co. (USA)

-Rheometer, Rheowin-RV1 software, HAAKE Rheowin (Germany)

-Oven, Mammert (Germany)

-Magnetic stirrer, Model SP 46920-26, Barnstead/Hermolyne (USA)

-Suction apparatus, Buchner Funnel, Aspirator, SIBATA circulating aspirator WJ-20 (Japan)

-Rotary evaporator, Buchi Rotavapor R-200 (Switzerland)

-DNA electrophoresis, Mini-sub cell electrophoresis chambers with 7x10 cm trays, Bio-Rad Laboratories, Inc. (Canada)

-pH meter, MP 230, Mettler Toledo, LE413, ME 51340 251 (Switzerland)

-FT-IR spectrometer, Spectrum 2000, Model Spectrum GX FT-IR, Perkin-Elmer, Co. (USA)

**3. Plant specimens**

Leaves and fruits of 29 durian specimens were collected from Chantaburi and Chumporn province, Thailand. Pauenmuang is a native cultivar in Chumporn province. Details are in Table 1 and 2, respectively. Herbarium leaf samples were preserved at the Museum of Natural Medicines, Faculty of Pharmaceutical Sciences, Chulalongkorn University. The specimens were *Durio zibethinus* Merr., which identity to TH. Wongprasert, No. 021-3 (BKF No. 139367) by The Office of Forest and Plant Conservation Research National Park, Wildlife and Plant Conservation Department, Thailand.

Table 1. Durian specimens used in this study are ‘Kradumthong’, ‘Monthong’ and ‘Chani’ from Chanthaburi province.

| **Scientific Name*** | **Common name** | **Voucher specimens** | **Location**** | **Date of collection** |
|---|---|---|---|---|
| *Durio zibethinus* Merr. | Kradumthong | DZ - KDJ 1<br>DZ - KDJ 2<br>DZ - KDJ 3<br>DZ - KDJ 4<br>DZ - KDJ 5 | Dutsadee Manthasatian,<br>Amphoe Klung,<br>Chanthaburi province | Jan 28, 2005 |
| *Durio zibethinus* Merr. | Monthong | DZ - MTJ 3<br>DZ - MTJ 4<br>DZ - MTJ 5 | Dutsadee Manthasatian,<br>Amphoe Klung,<br>Chanthaburi province | Jan 28, 2005 |
| *Durio zibethinus* Merr. | Chani | DZ - CNJ 1<br>DZ - CNJ 2<br>DZ - CNJ 3<br>DZ - CNJ 4<br>DZ - CNJ 5 | Dutsadee Manthasatian,<br>Amphoe Klung,<br>Chanthaburi province | Jan 28, 2005 |

*เต็ม สมิตินันทน์, 2544

**Appendix A

Table 2. Durian specimens used in this study are 'Pauenmuang', 'Monthong' and 'Chani' from Chumporn province.

| **Scientific Name*** | **Common name** | **Voucher specimens** | **Location**** | **Date of collection** |
|---|---|---|---|---|
| *Durio zibethinus* Merr. | Pauenmuang | DZ - PMC O<br>DZ - PMC 1<br>DZ - PMC 2<br>DZ - PMC 3<br>DZ - PMC 4<br>DZ - PMC 5 | Amphoe Lhangsaun,<br>Chumporn province | July 25, 2005 |
| *Durio zibethinus* Merr. | Monthong | DZ - MTC 1<br>DZ - MTC 2<br>DZ - MTC 3<br>DZ - MTC 4<br>DZ - MTC 5 | Boonpaem Chaoungsom<br>Tambon Taamsinhg,<br>Amphoe Muang, Chumporn | July 26, 2005 |
| *Durio zibethinus* Merr. | Chani | DZ - CNC 1<br>DZ - CNC 2<br>DZ - CNC 3<br>DZ - CNC 4<br>DZ - CNC 5 | Amphoe Lhangsaun,<br>Chumporn province | July 25, 2005 |

*เต็ม สมิตินันทน์, 2544

**Appendix A

## Methods

### 1. DNA fingerprint analysis

#### 1.1 Preparation of leaf samples

About 100 mg of fresh leaves of twenty-nine durian specimens were grinded to fine powder in liquid nitrogen before DNA extraction.

#### 1.2 DNA extraction

Total DNA were extracted from fresh leaves by the DNeasy Plant Mini Kit (QIAGEN, Germany). The 50 μl of DNA solution were purified by Genclean II Kit (Q-Biogene, USA) in three basic steps as follows: binding to silica matrix, washing by alcohol and eluting by TE buffer. Then DNA solution was stored at –20°C for further studies. Total genomic DNA were performed on 1% agarose gel electrophoresis to check quality.

#### 1.3 Primer design

In these studies, the focus was on amplifying the *mat*K gene region embeded in the intron of *trn*K gene of the chloroplast genome. The *trn*K/*mat*K sequences of *Durio zibethinus* were retrieved from GenBank, accession no. AY321188. The primers were designed by freeware program, Fast PCR for *mat*K amplification and sequencing. They were synthesized by Sigma (Germany).

The sequence of the primers are:

***mat*K amplification primers**

*mat*KD617F : 5′-tga atc tac ctg tct ccg agg t-3′

*mat*KD2396R : 5′-agt gga cta ctc agc caa tcc-3′

***mat*K sequencing primers**

walkingB/F8 : 5′-gca ttt att acg gtt ctc tc-3′

walking C/F15 : 5′-atg ata tcg gtg gga ttt gc-3′

walking D/R20 : 5′-cat gat tga cca gag cat tg-3′

## 1.4 Polymerase chain reaction (PCR) amplification

### 1.4.1 The *mat*K amplification and sequencing

The *mat*K gene was amplified by PCR techniques. The PCR reaction was carried out in a volume of 50 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25 °C) and 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.5 µM of each specific primers, *mat*KD617F and *mat*KD2396R, 1.5 units of *Taq* DNA polymerase and 100 ng of total genomic DNA. Deionized water was added instead of total DNA in equal volume as a negative control. The thermo cycle profile was 95 °C for 2 minutes; 35 cycles of 95 °C for 40 seconds, 58 °C for 40 seconds, and 72 °C for 2 minutes; final extension for 10 minutes at 72 °C. DNA amplification was performed in an Eppendorf Master Cycler (Perkin-Elmer, Co., USA). The amplified products were further analyzed by agarose gel electrophoresis and sequenced by using sequencing primers, walkingC/F15, walkingB/F8 and walkingD/R20 provided by BioService Unit (BSU), BIOTEC, Thailand. The location of primer are shown in the Figure 9.

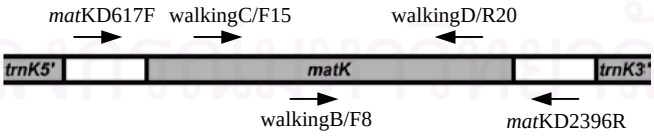



Figure 9. The sketch showing positions on *trn*K/*mat*K gene of amplified primers, *mat*KD617F and *mat*KD2396R, and sequencing primers, walkingC/F15, walkingB/F8 and walkingD/R20.

### 1.4.2 RAPD analysis

PCR was performed in a volume of 20 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25 °C) and 0.1% Triton X-100, 3 mM $MgCl_2$, 0.33 mM of each dNTPs, 2 µM of oligoprimers, SN06 (5′-gag acg cac a-3′), SN20 (5′-ggt gct ccg t-3′) and SO15 (5′-tgg cgt cct t-3′), 2 units of *Taq* DNA polymerase and 100 ng of total genomic DNA. DNA amplification was performed in an Eppendorf Master Cycler (Perkin-Elmer, Co., USA). The PCR profile was 95 °C for 4 minutes; 39 cycles of 95 °C for 1 minute, 50 °C for 1 minute, 74 °C for 1 minute; and following to 95 °C for 1 minute, 50 °C for 1 minute, 74 °C for 10 minutes (Atienzar and Jha, 2006). The amplified products were seperated by agarose gel electrophoresis.

## 1.5 Agarose gel electrophoresis

PCR products were analyzed by electrophoresis in 1.5% agarose gel in 1X TAE buffer (0.04M Tris-acetate, and 1 mM EDTA pH 8.0). The agarose was boiled for 3 minutes and allowed to cool then poured (~60°C) into the assembled tray. The gel was allowed to set for 20 to 30 minutes at room temperature. Loading dye (0.03% bromophenol blue, 0.03% xylene cyanol FF, 0.4%orange G, 15% ficoll® 400, 10 mM Tris-HCl (pH 7.5), and 50 mM EDTA (pH 8.0)) was added to the DNA samples which were then loaded into the wells. The electrophoresis was performed at a constant voltage;100 volt, 20 minutes and 50 volt, 120 minutes for PCR products of *mat*K amplification and RAPD analysis, respectively. The gel was stained with ethidium bromide solution for 30 minutes and destained with deionized water for 20 minutes. Then the gel was determined under ultraviolet (UV) light by Gel documentation (Gel Doc XR, Bio-Rad Laboratories, Inc., Canada).

## 1.6 DNA fingerprint analysis

### 1.6.1 *mat*K sequences analysis

The *mat*K gene sequences of all durian cultivars were aligned using multiple sequence alignment (Corpet, 1988) comparing to durian accession no.

AY321188 from GenBank. Finally, all sequences were submitted to DDBJ/EMBL/GenBank database to provide the accession numbers.

### 1.6.2 RAPD band scoring analysis

The only clear and reproducible bands were scored as “present” or “absent” for each primers and transferred to a binary code with 1 or 0, respectively. Dendrogram was generated using PAUP program package (version 4.0 b4a, Sinauer Assoc.Inc., USA), selecting the unweighted pair-group method with arithmetic averages (UPGMA) algorithym (Sneath and Sokal, 1973 and Sokal and Rohlf, 1981).

## 2. Analysis of Polysaccharide Gel (PG) from durian fruit-rinds

### 2.1 Preparation of dried fruit-rinds of durian

Fresh durian fruit-rinds of each 29 specimens were cleaned and ground. One kilogram of ground fresh fruit- rinds was dried by hot air oven at 50 °C until constant weight, about 200 grams of dried weight was obtained. Dried fruit-rinds were kept in room temperature until used.

### 2.2 Isolation of PG from dried fruit-rinds of durian

PG was extracted from dried durian fruit-rinds of each specimens by hot water extraction. The procedure was carried out using the method modified previously by Pongsamart and Panmaung (1998). Briefly, PG was extracted in boiling water about 30-40 minutes and filtrated. The aqueous extract was concentrated and precipitated by acid-alcohol, filtered and dried at 50°C in hot-air oven and then ground to powder. The percentage yield was calculated. PG was further determined for pH, viscosity, FT-IR spectra and galacturonic acid contents

### 2.3 pH and viscosity of PG

Solution at 3% w/v PG in distilled water was measured the pH by pH meter and scanned the viscosity at shear rate from 0 to 6000 1/s by Rheometer (Rheowin-RV1 software, HAAKE Rheowin) using C60/1 Ti as a sensor. The shear rate at 10 1/s was used to determine the viscosity of PG in this sudy.

### 2.4 FT-IR spectra of PG

The infrared spectra of PG were evaluated by Fourier Transform Infrared Spectrometry (FT-IR) (Spectrum 2000, Model Spectrum GX FT-IR). The KBr disc containing PG powder was prepared the ratio of KBr : PG was 75 : 1. The mixture was ground using an agate mortar and pestle to obtain an uniform mixture, speed it in the die of 7 mm diameter and compressed with Qwik Handi-Press. The spectra were scanned in the range of 370-4000 $cm^{-1}$.

### 2.5 Galacturonic acid assay in PG

The galacturonic acid contents were determined by spectrophotometry assay using m-hydroxydiphenyl reagent (Filisetti-Cozzi et al.1991). A volume of 0.4 ml of D-(+)-Galacturonic acid standard at 50-250 nmol concentrations was used, a positive control was pectin at 0.01% concentration and PG sample at 0.0125% solution in distilled water was determined. In each test solution was added 40 μl of 4 M sulfamic acid-potassium sulfamate (pH 1.6) and mixed thoroughly. $H_2SO_4$ (96.4% assay) containing 75 mM sodium tetraborate (2.4 ml) is then added and vortexed vigorously. The solution mixture was heated for 20 minutes in boiling water, the tubes were capped with marbles. Then the tubes were placed in ice bath to quickly cool to room temperature. After that, 80 μl of 0.15% (w/v) m-hydroxydiphenyl in 0.5% (w/v) NaOH was overlaid and mixed by vortex. The pink color developed in about 5-10 minutes and was stable for about 1 hour. Absorbance was read at 525 nm by spectrophotometer (model Educator, Thermo Electron, Co., USA).

### 2.5.1 Standard curve and positive control

D-(+)-Galacturonic acid was dissolved in distilled water containing 50, 100, 150, 200 and 250 nmol as the standard curve.

Pectin (Galacturonic acid ≥74.0 %) was prepared at 0.01% concentration in distilled water as a positive control.

### 2.5.2 Preparation of PG sample solution

PG solution of each specimens at 0.0125% concentration in distilled water was prepared to measure the galacturonic acid contents.

### 2.5.3 Calculation

The absorbance of pectin and PG solution was correlated the galacturonic acid in solution (X, nmol) by standard curve and calculated the percentage of galacturonic acid in PG by the formular below.

$$\% \text{ Galacturonic acid in PG} = (0.42432)X$$

$$\% \text{ Galacturonic acid in Pectin} = (0.53040)X$$

## 2.6 Statistical analysis of PG

The results of PG analysis; yield, pH, viscosity and galacturonic acid, were analyzed statistically using ONE WAY ANOVA, either LSD or Tukey HSD statistic. The values were considered to be significantly different when the *P* value was less than 0.05.

**3. Correlation of DNA fingerprint and PG analysis from fruit-rinds**

The DNA profiles and PG properties of all durian specimens (from Chanthaburi and Chumporn province, Thailand) were compared together.

# CHAPTER III

# RESULTS AND DISCUSSION

## 1. DNA fingerprint analysis

### 1.1 DNA extraction

Total genomic DNA was isolated from leaves of *Durio zibethinus* by using the DNeasy Plant Mini Kit, and then DNA in solution was purified by Geneclean II Kit to remove any polysaccharide molecules, because polysaccharide could be one of PCR inhibitors. Polysaccharides are macromolecules containing long chain polymer of monosaccharide units, the structure similar to nucleic acids. Contaminated polysaccharides in DNA preparations interfere with the activity of enzymes DNA polymerases (Kim, 2000; Peist, 2001). Genomic DNA was examined on 1% agarose gel electrophoresis. The size of isolated durian DNA in each cultivar was more than 12 kb as shown in the Figure 10. The purified DNA was stored at –20°C until used.

### 1.2 Primer design

The *mat*K gene locates within the *trn*K gene. Nyffeler et al. (2005) have previously reported total *trn*K/*mat*K sequences of *Durio zibethinus* in GenBank, accession number AY321188. In this study, the total *trn*K gene was used as the template for primer design. The primers were designed by Fast PCR program for both *mat*K amplification and DNA sequencing. The first was the *mat*K amplification primers which were the *mat*KD617F and *mat*KD2396R. The second was sequencing primers which were walkingB/F8, walkingC/F15 and walkingD/R20. Their sequences are shown below.

***mat*K amplification primers**

*mat*KD617F : 5′-tga atc tac ctg tct ccg agg t-3′

*mat*KD2396R : 5′-agt gga cta ctc agc caa tcc-3′

***mat*K sequencing primers**

walkingB/F8 : 5′-gca ttt att acg gtt ctc tc-3′

walking C/F15 : 5’-atg ata tcg gtg gga ttt gc-3′

walking D/R20 : 5′-cat gat tga cca gag cat tg-3′

However, the *mat*KD617F and *mat*KD2396R were not only to amplify the PCR products but also the sequencing primers. The positions of PCR and sequencing primers on *trn*K/*mat*K gene are illustrated in Figure 11. From the picture, the size of PCR products were estimated containing 1,780 bp in length corresponding to complete *mat*K region and partial *trn*K gene. The optimum annealing temperature for PCR amplification was found in the range of 57°-60°C from the calculation of primer design program.

The forward and reverse primers were abbreviated into F and R, respectively. Thus, the *mat*KD617F, walkingB/F8 and walkingC/F15 were forward primers and the reverse primers were *mat*KD2396R and walkingD/R20.

The genomic DNA or even PCR product is a double stranded DNA. Generally, nucleotide sequences should be presented, only by a single strand, in the 5′ to 3′ direction, from left to right. As the results of primer design, the forward primer sequences were identical to nucleotide sequences of DNA template. According to the fact that DNA replication occurs from 5′ to 3′ direction. The forward primer (5′ to 3′) then hybridized into a complementary DNA (3′ to 5′). On the other hand, The reverse primer at the 3′ end was the reverse complement of the 5′ end of another DNA template. The Figure 12 showed the hybridization between DNA template or complementary DNA to PCR primer, *mat*KD617F and *mat*KD2396R.

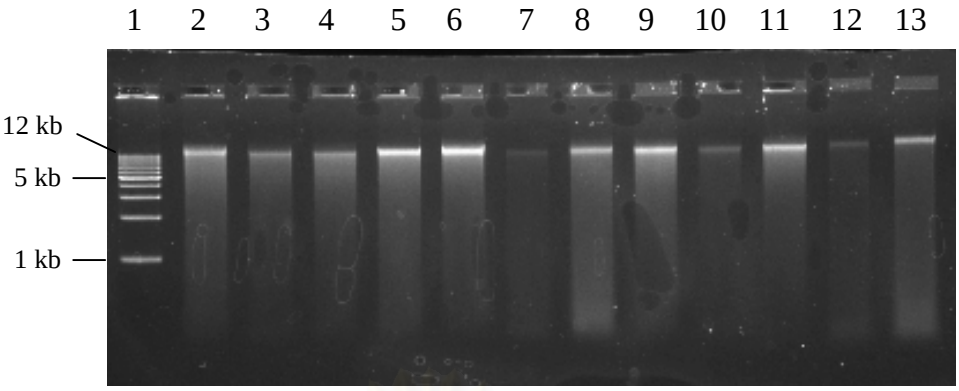



Figure 10. Genomic DNA of durian cultivars performed on 1% agarose gel electrophoresis. The size of DNA of each cultivar was more than 12 kb.

Lane 1: 1 kb DNA Ladder (the sizes are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 kb, respectively.)

Lane 2-3: Kradumthong (Chanthaburi)

Lane 4-5: Monthong (Chanthaburi)

Lane 6-7: Chani (Chanthaburi)

Lane 8-9: Pauenmuang (Chumporn)

Lane 10-11: Monthong (Chumporn)

Lane 12-13: Chani (Chumporn).


Figure 11. The total sequences of *trn*K/*mat*K gene (2,496 bp) have been reported in GenBank, accession no. AY321188. The complete *mat*K gene sequences are 1,509 bp in length as marked between 743 to 2251 in the blue color. The positions and directions of primers on the *trn*K/*mat*K gene are shown in the red color. The underline sequences are start and stop codon, respectively.

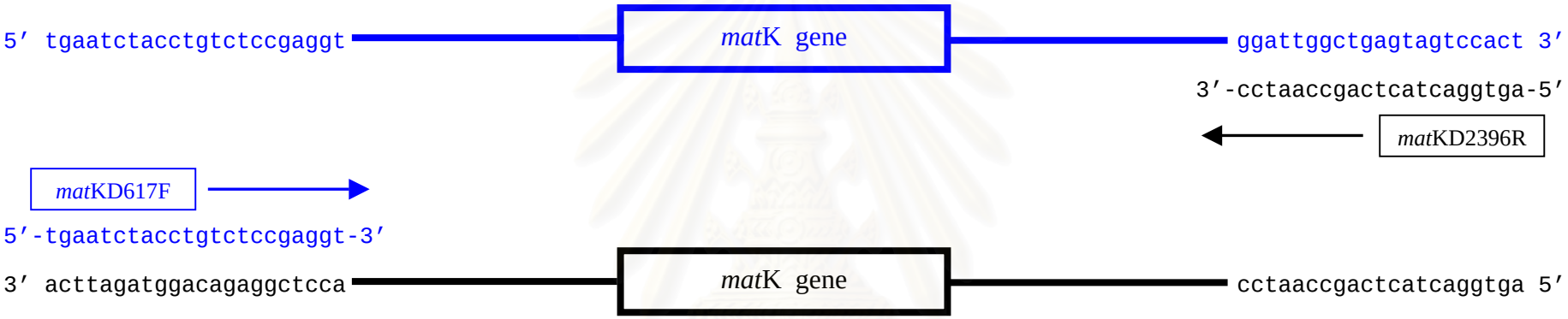



Figure 12. The hybridization of PCR primers, *mat*KD617F and *mat*KD2396R on *trn*K/*mat*K gene template. The single stranded DNA is the format representation of nucleotides as marked in the blue color.

### 1.3 The *mat*K amplification, sequencing and sequence alignment

The complete *mat*K gene (including partial *trn*K gene) was amplified by PCR technique using the primers, *mat*KD617F and *mat*KD2396R. The optimum of PCR condition and cycles were described in the Table 3. The annealing temperature at 58°C generated the high yield of PCR products. The 1.5 mM concentration of $MgCl_2$ was adequate for PCR amplification in this experiment. The PCR products of all durian cultivars from Chumporn and Chanthaburi provinces were about 1,780 bp, performed on 1.5% agarose gel electrophoresis as shown in the Figure 13 and 14, respectively. However, PCR products of some cultivars, 'Kradumthong' (DZ-KDJ 2 and DZ-KDJ 4) and 'Monthong' (DZ-MTJ 3 and DZ-MTJ 4) from Chanthaburi were slightly present. The PCR yields of 'Monthong' were increased when the annealing temperature was adjusted to 60°C as shown in the Figure 15. Although, PCR conditions were varied both $MgCl_2$ concentration and annealing temperature, two of five 'Kradumthong' samples as shown in Figure 14 were still present slightly.

PCR products were then sequenced by sequencing primers, walkingC/F15, walkingB/F8 and walkingD/R20 provided by BioService Unit (BSU), BIOTEC, Thailand. The fragment sequences of all durian cultivars obtained from each of sequencing primers were aligned by multiple sequence alignment. The results showed that the complete *mat*K gene region was 1,509 bp in length comparable to the previous report in GenBank, accession no. AY321188 of *Durio zibethinus*. Unfortunately, any specific cultivar was not indicated. The multiple sequence alignment of *mat*K sequences of certain durian cultivars from Chanthaburi and Chumporn provinces were illustrated in the Figure 16 and 17, respectively

According to the results of the sequence alignment, the cytosine substitutions were found at the position 275 in the *mat*K gene of 'Monthong' and 'Chani' cultivars from both provinces, and 'Kradumthong' from Chanthaburi province. Interestingly, 'Pauenmuang' cultivar presented either adenosine or cytosine substitutions at the same position. In addition, the *mat*K sequences of all tested durian cultivars were also found the cytosine and thymidine substitutions at the position 860 and 862, respectively. Finally, the complete *mat*K sequences of cultivated-durians; 'Monthong', 'Chani' and 'Kradumthong' from Chanthaburi province, and

‘Monthong’, ‘Chani’ and ‘Pauenmuang’ from Chumporn province were deposited in DDBJ/EMBL/GenBank database. The data of base substitution and submissiom were summarized in the Table 4. Hence, The *mat*K alignment of *Adansonia digitata* was outgroup samples as shown in appendix H.

Although the relatively rapid rate of evolution of *mat*K compared to other conservative genes, it is suitable for identification in taxonomic levels at the generic, species and even population, and the flanking *trn*K is easily designed primers to amplify and sequence total *mat*K gene (Soltis et al, 1998). Even if the *mat*K gene is applicable for studying taxonomy in generic and species levels, according to the characterization of *Fagopyrum* species (Ohsako and Ohnishi, 2001), the plant systematics of 112 species in Crassulaceae family (Mort et al., 2001), 57 species of Saxifragaceae family (Soltis et al., 2001), *Rheum* species (Yang et al., 2004), etc. However, the *mat*K gene sequences is also used to evaluate the genetic differentiation of radish cultivars but the relationship between cultivated and wild radishes were still obscure (Yamane et al., 2005). Yamane and co-workers have developed primers to study other gene in chloroplast genome by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). Like radish, *mat*K gene seemed not to give enough information to characterize durian cultivars because the evolution rate of *mat*K is slow and it was only one gene in total genomic DNA. Wissemann and Ritz (2005) used both ITS-1 of nDNA and IGS of *atp*B-*rbc*L in cpDNA to study the taxonomy of genus *Rosa*. Both the *mat*K and ITS sequence data were determined the phylogeny of the large genus *Valeriana* (Hidalgo et al., 2004). However, the *mat*K was found the base variation at positon 275 in the group of ‘Pauenmuang’ samples. In this study, RAPD analysis was preliminary examined by using RAPD marker to provide more information for identification of durian cultivars, this technique is simple, inexpensive and the technique does not needed DNA database (Atienzar and Jha, 2006).

Table 3. The optimised PCR condition of *mat*K gene amplification.

| **PCR parameters** | **Optimised condition of *mat*K gene amplification** |
|---|---|
| PCR buffer | 1X (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25 °C) and 0.1% Triton X-100) |
| $MgCl_2$ concentration | 1.5 mM |
| Primer concentration | 0.5 μM of each specific primers |
| dATP, dTTP, dCTP, dGTP mixing | 0.2 mM |
| *Taq* DNA polymerase Unit | 1.5 U |
| Amount of DNA | 100 ng per a reaction |
| Total volume | 50 μL |
| Thermal cycling condition | First cycle: 95°C for 2 min.<br>35 cycles: 95°C for 40 sec, 58°C for 40 sec, 72°C for 2 min.<br>Last cycle: 72°C for 10 min. |

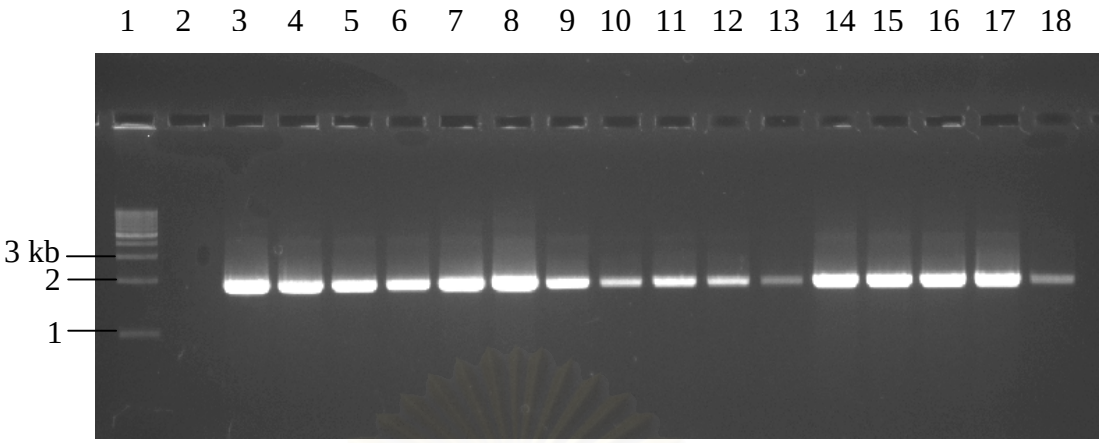



Figure 13. The PCR products of different durian cultivars from Chumporn province were about 1,780 bp in length using *mat*KD617F and *mat*KD2396R.
Lane 1: 1 Kb DNA Ladder.
Lane 2: Negative control.
Lane 3-8: Six samples of ‘Pauenmuang’ (DZ-PMC O - DZ-PMC 5).
Lane 9-13: Five samples of ‘Monthong’ (DZ-MTC 1 - DZ-MTC 5).
Lane 14-18: Five samples of ‘Chani’ (DZ-CNC 1 - DZ-CNC 5).

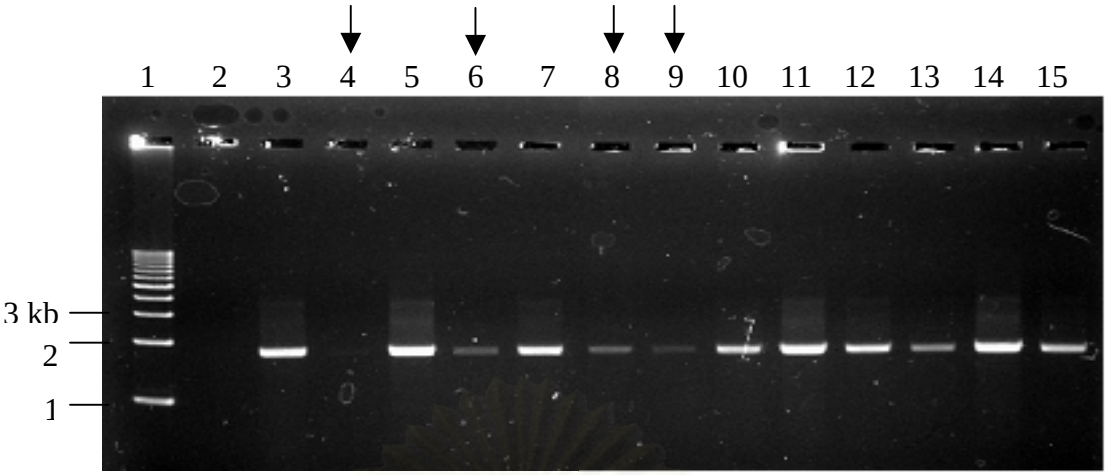



Figure 14. The PCR products of different durian cultivars from Chanthaburi province were about 1,780 bp in length using *mat*KD617F and *mat*KD2396R. The arrows above indicated the low yields of PCR products.

Lane 1: 1 Kb DNA Ladder.

Lane 2: Negative control.

Lane 3-7: Five samples of 'Kradumthong' (DZ-KDJ 1 - DZ-KDJ 5).

Lane 8-10: Three samples of 'Monthong' (DZ-MTJ 3 - DZ-MTJ 5).

Lane 11-15: Five samples of 'Chani' (DZ-CNJ 1 - DZ-CNJ 5).

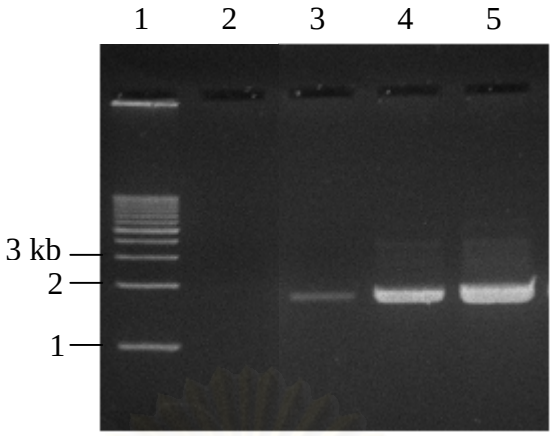



Figure 15. The PCR products of ‘Monthong’ cultivars from Chanthaburi province were about 1,780 bp in length using *mat*KD617F and *mat*KD2396R (the annealing temperature at 60°C).

Lane 1: 1 Kb DNA Ladder

Lane 2: Negative control

Lane 3-5: 3 samples of ‘Monthong’ (DZ-MTJ 3 - DZ-MTJ 5).

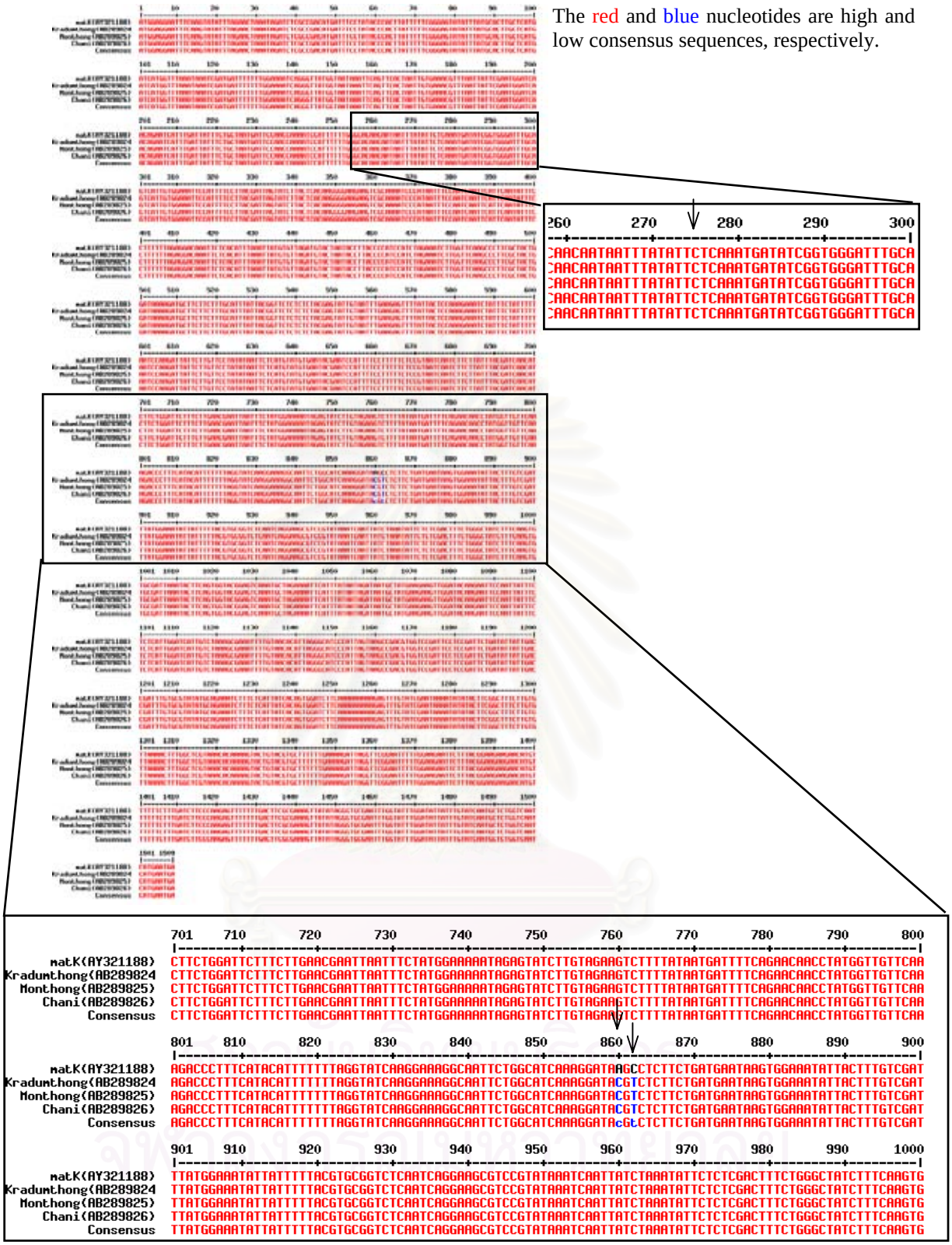



Figure 16. The sequence alignment of durian cultivars, ‘Kradumthong’ (AB289824), ‘Monthong’ (AB289825) and ‘Chani’ (AB289826) from Chanthaburi province was compared to *mat*K gene (AY321188) in GenBank database. The complete *mat*K gene was 1509 bp in length.

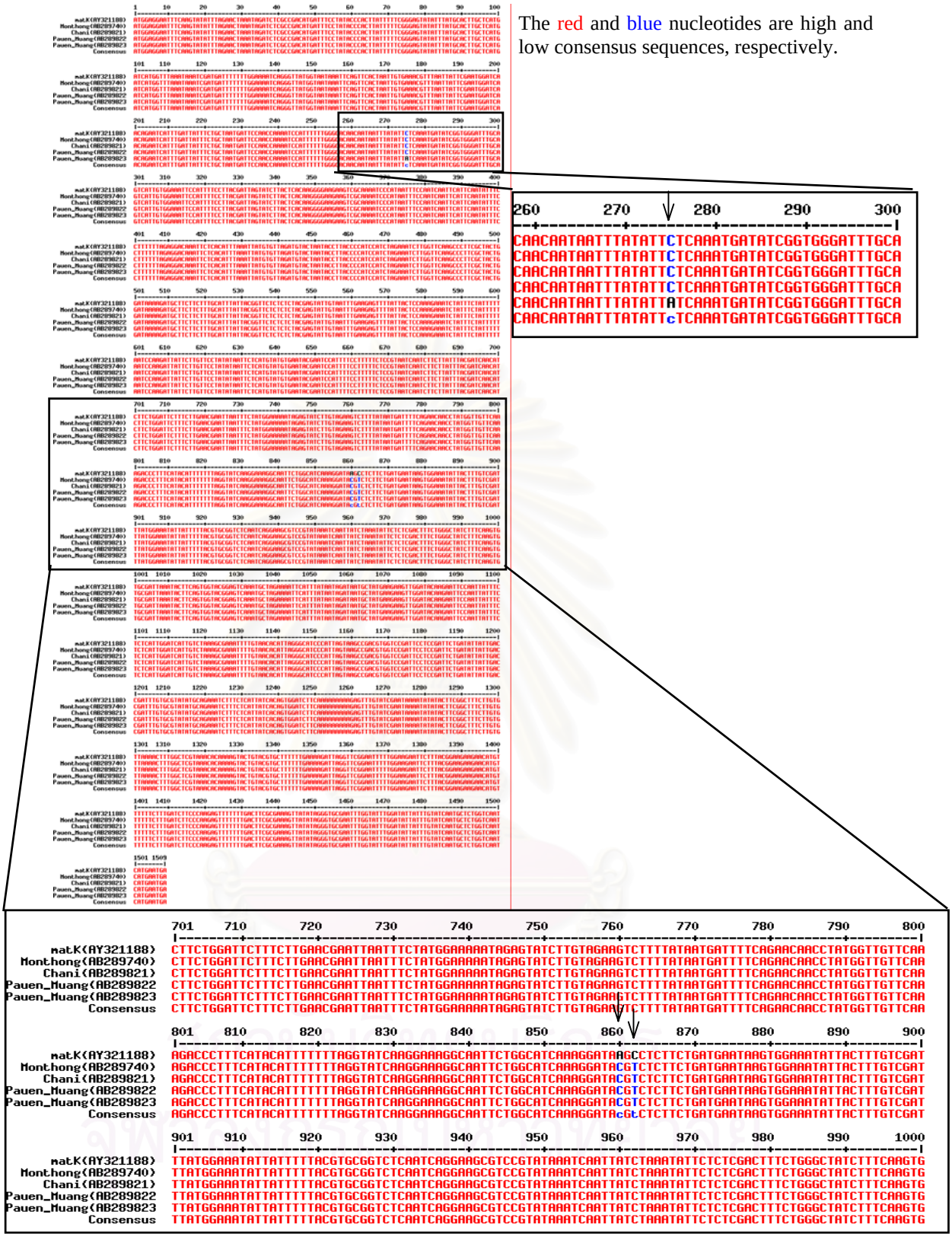



Figure 17. The sequence alignment of durian cultivars, ‘Monthong’ (AB289740), ‘Chani’ (AB289821), ‘Pauenmuang’1 (AB289822) and ‘Pauenmuang’2 (AB289823) from Chumporn province was compared to *mat*K gene (AY321188) in GenBank database. The complete *mat*K gene was 1,509 bp in length.

Table 4. The summarization of nucleotide substitution in *mat*K sequences of cultivated-durian from Chanthaburi and Chumporn provinces comparing with GenBank database, accession no. AY321188.

| **Species** | **Provinces** | **Cultivars** | **Nucleotide positions** | | | **Accession no.** |
|---|---|---|---|---|---|---|
| | | | 275 | 860 | 862 | |
| *Durio zibethinus* Murr. | GenBank database | non-identified | C | A | C | AY321188 |
| | Chanthaburi | Kradumthong | * | C | T | AB289824 |
| | | Monthong | * | C | T | AB289825 |
| | | Chani | * | C | T | AB289826 |
| | Chumporn | Monthong | * | C | T | AB289740 |
| | | Chani | * | C | T | AB289821 |
| | | Pauenmuang 1 | * | C | T | AB289822 |
| | | Pauenmuang 2 | A | C | T | AB289823 |

Asterisks (*) show the sequence identical to AY321188

### 1.4 RAPD analysis

Four random primer sets (20 primers per each set), SN01-20, SD01-20, SO01-20 and OPA 01-20 were scanned. The polymorphic band patterns of preliminary RAPD analysis were generated by only three oligoprimers, SN06 (5′-gag acg cac a-3′), SN20 (5′-ggt gct ccg t-3′) and SO15 (5′-tgg cgt cct t-3′) by PCR condition according to the Table 5. The fragments of PCR products were performed on 1.5% agarose gel electrophoresis as shown in the Figure 18, 19 and 20. Each sample was triplicated in the same PCR condition and reaction. The ranges of DNA fragments of primers, SN06, SN20 and SO15 were about 230-1,000, 300-1,250 and 250-2,300, respectively. The SN06 primer generated the clear unique profiles of cultivated-durians that were 1,000, 800 and 550 bp of 'Kradumthong', 800, 550 and 275 bp of 'Monthong', 1,000, 800, 750, 550 and 275 bp of 'Chani' and 1,000, 800, 750, 500, 325 and 275 bp of 'Pauenmuang'. They were indicated by one direction arrows in Figure 18. The SN06 primer also produced the polymorphic bands of different durian cultivars, which were indicated by one direction arrows as illustrated in Figure 19. However, the profiles of SO15 primers were obscure.

The only clear, strong and reproducible bands were scored as "present" or "absent" for each primers and transferred to a binary code with 1 or 0, respectively. Table 6 and 7 are summary of the total and polymorphic bands, and binary code of each primer for durian cultivars from both Chanthaburi and Chumporn provinces. Dendrogram of all durian specimens was generated using binary code by PAUP program. The dendrogram was the relationship of durian cultivars as shown in Figure 21. From that picture, the durian specimens can be divided into two main groups. The first was 'Pauenmuang' which was the native cultivars. The second was commercially cultivated durians which were 'Kradumthong', 'Monthong' and 'Chani' from Chanthaburi and/or Chumporn province. In addition, the results among cultivated-durian groups were subdivided into 3 groups. Group I was 'Kradumthong' (Chanthaburi) and 'Chani' (Chumporn). Group II was 'Monthong' from Chanthaburi and Chumporn provinces and the last was Group III, 'Chani' from Chanthaburi province. In assumption, Group I should be 'Chani' from both two provinces whereas Group III should belong to 'Kradumthong'.

Table 5. The optimised PCR condition for RAPD analysis

| **PCR parameters** | **Optimised condition of RAPD analysis (Atienzar and Jha, 2006)** |
|---|---|
| PCR buffer | 1X (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25 °C) and 0.1% Triton X-100) |
| $MgCl_2$ concentration | 3 mM |
| Primer concentration | 2 μM |
| dATP,dTTP,dCTP,dGTP mixing | 0.33 mM |
| *Taq* DNA polymerase Unit | 2 U |
| Amount of DNA | 100 ng per a reaction |
| Total volume | 20 μL |
| Thermal cycling condition | First cycle: 95°C for 4 min.<br>39 cycles: 95°C for 1 min, 50°C for 1 min, 74°C for 1 min.<br>Last cycle: 95°C for 1 min, 50°C for 1 min, 74°C for 10 min. |

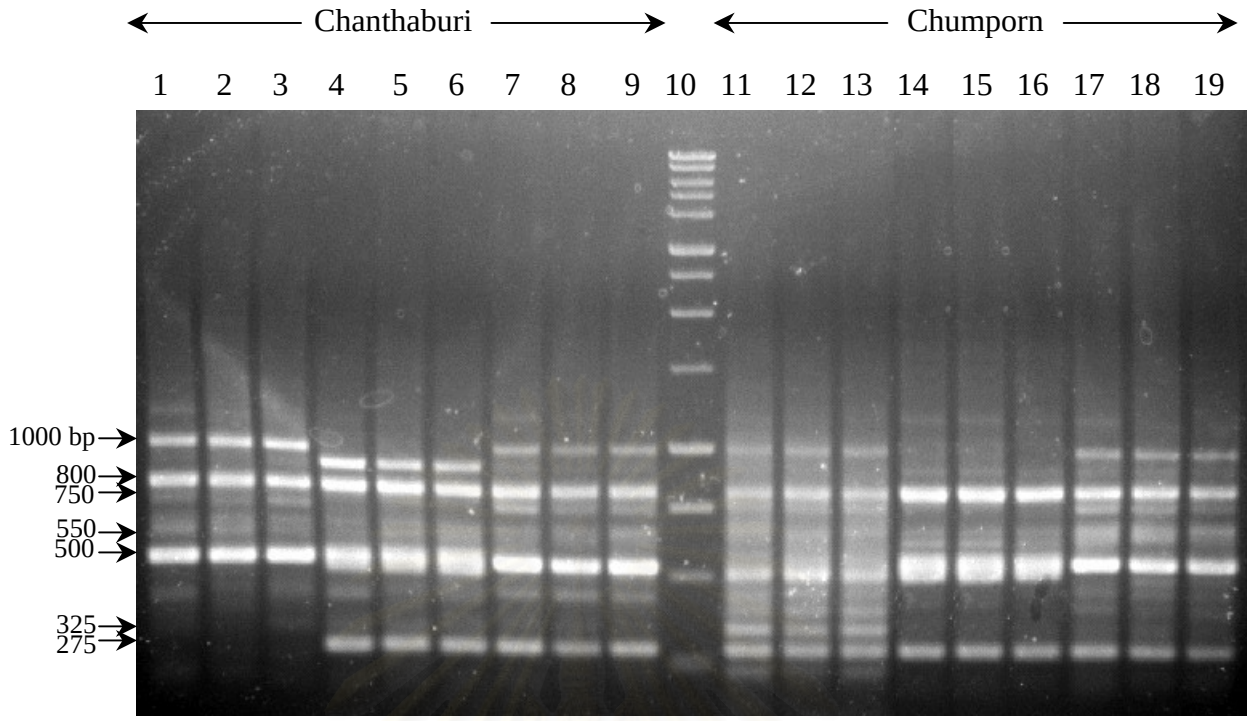



Figure 18. The RAPD profiles were triplicated in the same PCR condition and cycles by using SN06 primer. Electrophoresis was performed on 1.5% agarose gel.

Lane 1-3: 'Kradumthong'

Lane 4-6: 'Monthong'

Lane 7-9: 'Chani'

Lane 10: 1 Kb DNA Ladder (10000, 8000, 6000, 5000, 4000, 3000, 2500, 2000, 1500, 1000, 750, 500 and 250/253 bp from top to bottom)

Lane 11-13: 'Pauenmuang'

Lane 14-16: 'Monthong'

Lane 17-19: 'Chani'

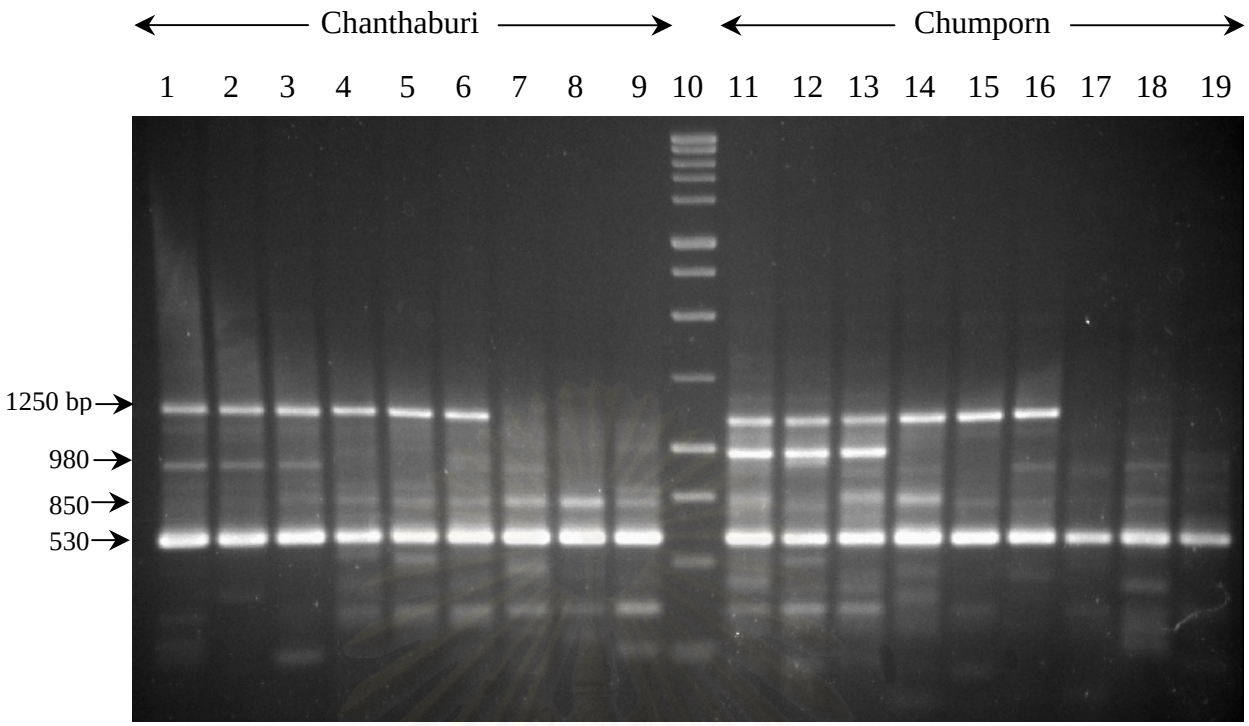



Figure 19. The RAPD profiles were triplicated in the same PCR condition and cycles by using SN20 primer. Electrophoresis was performed on 1.5% agarose gel.

Lane 1-3: 'Kradumthong'

Lane 4-6: 'Monthong'

Lane 7-9: 'Chani'

Lane 10: 1 Kb DNA Ladder (10000, 8000, 6000, 5000, 4000, 3000, 2500, 2000, 1500, 1000, 750, 500 and 250/253 bp from top to bottom)

Lane 11-13: 'Pauenmuang'

Lane 14-16: 'Monthong'

Lane 17-19: 'Chani'

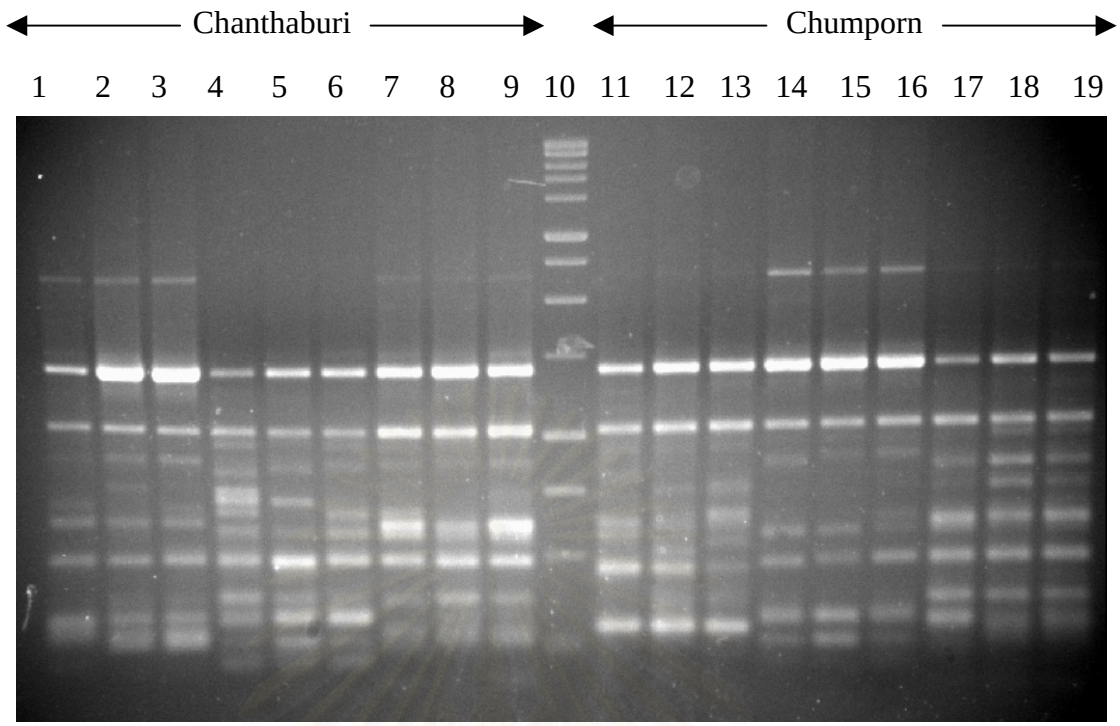



Figure 20. The RAPD profiles were triplicated in the same PCR condition and cycles by using SO15 primer. Electrophoresis was performed on 1.5% agarose gel.

Lane 1-3: 'Kradumthong'

Lane 4-6: 'Monthong'

Lane 7-9: 'Chani'

Lane 10: 1 Kb DNA Ladder (10000, 8000, 6000, 5000, 4000, 3000, 2500, 2000, 1500, 1000, 750, 500 and 250/253 bp from top to bottom)

Lane 11-13: 'Pauenmuang'

Lane 14-16: 'Monthong'

Lane 17-19: 'Chani'

Table 6. The amount of the total band profile and the polymorphic band of each primer from agarose gel electrophoresis.

| Primers | Total band per primer | Polymorphic band/primer | | | | | |
|---|---|---|---|---|---|---|---|
| | | Chanthaburi | | | Chumporn | | |
| | | Kradumthong | Monthong | Chani | Pauenmuang | Monthong | Chani |
| SN06 | 13 | 4 | 8 | 7 | 10 | 6 | 8 |
| SN20 | 7 | 4 | 4 | 3 | 5 | 4 | 1 |
| SO15 | 14 | 7 | 7 | 9 | 7 | 8 | 8 |
| Total | 34 | 15 | 19 | 19 | 22 | 18 | 17 |

Table 7. Binary code of each primer with 1 or 0 as the“present” or “absent” bands, respectively.

| Province | Primers | SN06 | SN20 | SO15 |
|---|---|---|---|---|
| Chanthaburi | Kradumthong | 1 0 1 0 1 0 1 0 0 0 0 0 | 1 0 1 1 0 1 0 | 0 1 1 0 1 0 1 0 1 0 0 1 0 1 |
| | Monthong | 0 1 1 0 1 1 1 0 1 0 1 1 0 | 1 0 0 1 1 1 0 | 0 1 1 0 0 0 0 1 1 0 1 1 0 1 |
| | Chani | 1 0 1 1 1 0 1 0 1 0 0 1 0 | 0 0 0 0 1 1 1 | 1 1 1 1 1 0 0 1 1 0 1 0 1 0 |
| Chumporn | Pauenmuang | 1 0 1 1 1 0 0 1 1 1 1 1 1 | 1 1 0 0 1 1 1 | 0 1 1 1 0 1 1 0 0 1 0 0 1 0 |
| | Monthong | 0 1 1 0 1 1 1 0 0 0 0 1 0 | 1 0 1 0 1 1 0 | 1 1 1 0 1 0 0 1 1 0 0 1 0 1 |
| | Chani | 1 1 1 1 1 0 1 0 1 0 0 1 0 | 0 0 0 0 0 1 0 | 0 1 1 0 1 1 1 0 1 0 1 1 0 0 |

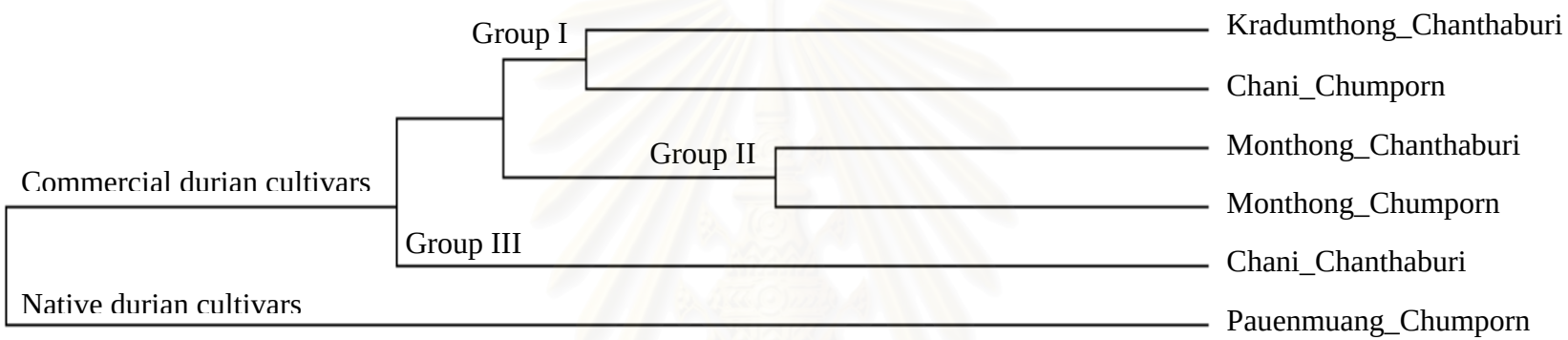



Figure 21. Dendrogram of durian cultivars, ‘Monthong’, ‘Chani’ and ‘Kradumthong’ from Chanthaburi province, and ‘Monthong’, ‘Chani’ and ‘Pauenmuang’ from Chumporn province.

The preliminary RAPD study demonstrated the molecular variation of durian cultivars, even between same cultivars but different location. In previous study, Somsri et al (2005) investigated the phylogeny of genus *Durio* and fifty six cultivars of *D. zibethinus*. They suggested that fifty six cultivars of *D. zibethinus* were very nearly relationship, they were almost identical. Thus, not only the amount of primers should be increased but also the outgroup plant samples within Bombacaceae family should be investigated. The bootstap tree should be also estimated for each clade of an observed tree by Felsenstein method (1985). Efron, et al (1996) showed that Felsenstein's method is not biased.

## 2. Analysis of Polysaccharide Gel (PG) from durian fruit-rinds

### 2.1 Isolation and yield of PG from dried fruit-rinds of durian

In this experiment, the total yield of dried powder of PG isolated from fruit-rinds of different durian cultivars including 'Kradumthong', 'Monthong' and 'Chani' from Chanthaburi province; 'Pauenmuang', 'Monthong' and 'Chani' from Chumporn province was 8.501±0.984%, 8.586±1.370%, 9.152±1.163%, 7.448±0.925%, 9.287±1.279% and 7.774±1.173% (w/w) of dried fruit-rinds, respectively. The number of raw data were shown in appendix D. Table 8 illustrated the percentage of yield and statistic analysis of PG yield. PG yield of 'Monthong' (commercially cultivated) from Chumporn gave significantly higher percent yield than that of 'Pauenmuang' and 'Chani' cultivars (naturally cultivated) in the same province ($P < 0.05$). On the other hand, the PG yield of three durian cultivars cultivated commercially from Chanthaburi province was not significant difference from each other and also not significant difference from that of 'Monthong' cultivated commercially from Chumporn ($P > 0.05$). PG yield of 'Chani' cultivated commercially from Chanthaburi provinces was higher than that of 'Chani' cultivated naturally from Chumporn province, whereas the PG yields of 'Monthong' cultivated commercially from the two provinces were not significantly different. All samples from Chanthaburi and 'Monthong' from Chumporn province were collected within durian plantation for commercial seemed to produce high PG yield of fruit-rinds, whereas naturally planted 'Pauenmuang' and 'Chani' from Chumporn seemed to provide low PG yield.

Table 8. The percentage of PG yield.

| **Provinces** | **Cultivars** | **% yield** | **Plantation** |
|---|---|---|---|
| Chanthaburi | Kradumthong | $8.501 \pm 0.984^{a, b}$ | Commercially |
| | Monthong | $8.586 \pm 1.370^{a, b}$ | Commercially |
| | Chani | $9.152 \pm 1.163^{a}$ | Commercially |
| Chumporn | Pauenmuang | $7.448 \pm 0.925^{b}$ | Naturally |
| | Monthong | $9.287 \pm 1.279^{a}$ | Commercially |
| | Chani | $7.774 \pm 1.173^{b}$ | Naturally |

a, b = the significant difference between groups ($P < 0.05$)

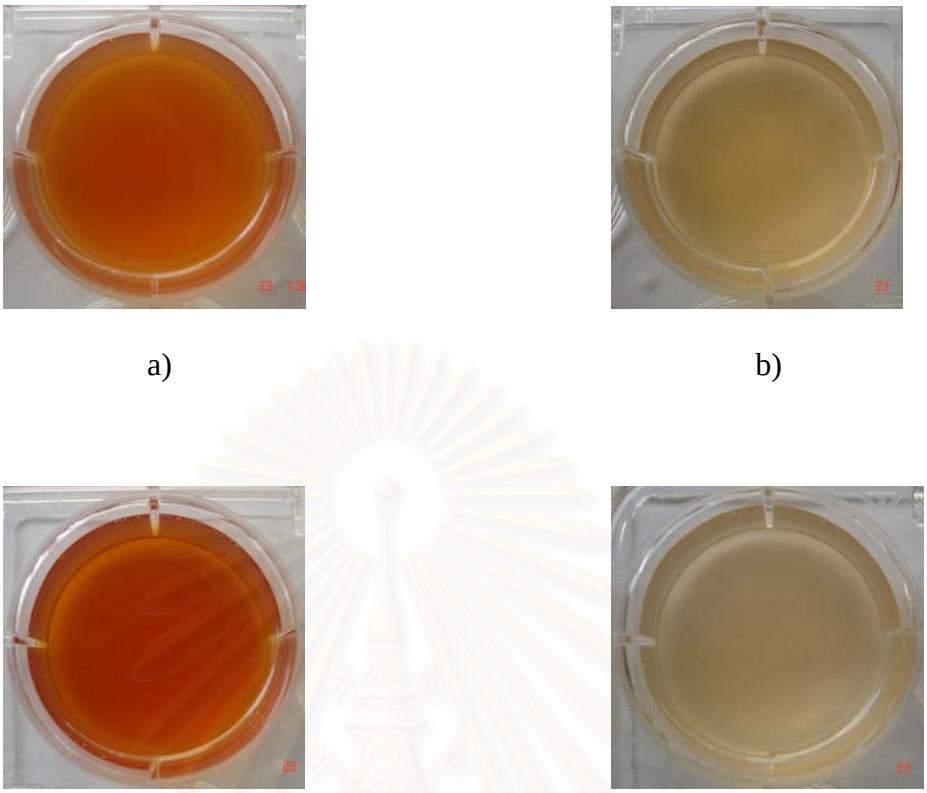
An aqueous solutions of PG from ‘Kradumthong’, ‘Pauenmuang’, ‘Chani’ and ‘Monthong’ from either Chanthaburi or Chumporn province were dark orange, light brown, orange and slightly yellow respectively as shown in the Figure 22.

### 2.2 pH and viscosity of PG

The 3% w/v solution of PG in distilled water was measured the pH value by pH meter. The pH values of durian cultivars, ‘Kradumthong’, ‘Monthong’ and ‘Chani’ from Chanthaburi province, and ‘Pauenmuang’, ‘Monthong’ and ‘Chani’ from Chumporn province as shown in Table 9 were 2.526±0.782, 2.471±0.794, 2.437±0.049, 2.463±0.119, 2.491±0.089 and 2.499±0.059, respectively. The acid pH values were observed in PG solutions because the polysaccharide gel composes high content of polygalacturonic acid which is a weak acid compound. The pKa value of pectin of different degree of esterification (DE) ranges from 3.5 to 4.10 (Hou et al., 1999).

The viscosity of 3% w/v PG solutions was scanned at shear rate from 0 to 6000 1/s by Rheometer (Rheowin-RV1 software, HAAKE Rheowin) using C60/1 Ti as the sensor. The shear rate at 10 1/s was used to measure the viscosity of PG in this study. Table 9 shows the viscosity of PG from different durian cultivars, ‘Kradumthong’, ‘Monthong’ and ‘Chani’ from Chanthaburi province, and ‘Pauenmuang’, ‘Monthong’ and ‘Chani’ from Chumporn province were 577.989±547.261, 832.271±409.011, 705.929±449.796, 491.011±272.117, 1135.091±996.238 and 407.371±219.943 cPs, respectively. The broad ranges of viscosity were obtained. The PG of ‘Monthong’ from both provinces gave the highest viscosity ($P < 0.05$).

The viscosity of a pectin solution may be determined for the purpose of estimating the molecular weight of pectin or for evaluating the thickening effect of pectin. The viscosity of PG depended on size of the polysaccharide structure, and the process of isolation also effected the size of PG product. The pH also influences the



a) b)

c) d)

Figure 22. The aqueous solutions of 3% (w/v) PG. a) ‘Kradumthong’, b) ‘Pauenmuang’, c) ‘Chani’ and d) ‘Monthong’.

Table 9. The pH and viscosity of PG at concentration of 3% w/v PG.

| **Provinces** | **Cultivars** | **pH** | **Viscosity (cP)** |
|---|---|---|---|
| Chanthaburi | Kradumthong | 2.526 ± 0.782 | 577.989 ± 547.261 [b] |
| | Monthong | 2.471 ± 0.794 | 832.271 ± 409.011 [a] |
| | Chani | 2.437 ± 0.049 | 705.929 ± 449.796 [b] |
| Chumporn | Pauenmuang | 2.463 ± 0.119 | 491.011 ± 272.117 [b] |
| | Monthong | 2.491 ± 0.089 | 1135.091 ± 996.238 [a] |
| | Chani | 2.499 ± 0.059 | 407.371 ± 219.943 [b] |

a, b = significant difference between groups ($P < 0.05$).

viscosity of pectin solutions. In a calcium-free solution the viscosity drops when pH is increased. The same result was also observed with PG from fruit-rinds of 'Monthong' (Lertchaiporn, 2003; Paphattarapong, 2005). However, CP Kelco ApS company suggested that the viscosity should be determined in a calcium-free solution at a fixed pH. The information received on Feb 27, 2007 from the website, www.cpkelco.com. In this study, pH and viscosity of PG solution were measured only at 3% PG at room temperature at pH ranges of each extracted sample were 2.4-2.6.

### 2.3 FT-IR spectra

Infrared spectra of PG was determined by using a Fourier Transform Infrared Spectrometry (FT-IR). PG powder was directly examined using KBr disc. IR spectra of PG from 'Kradumthong', 'Monthong' and 'Chani' cultivars from Chanthaburi province, and 'Pauenmuang', 'Monthong' and 'Chani' cultivars from Chumporn province were compared with the spectrum of commercial pectin from citrus fruits (P9135 from Sigma company) with the degree of esterification (DE) of 60.97% and the galacturonic content of ≥ 74%. The FT-IR spectra of pectin standard and PG sample were illustrated in Figure 23. It was found that the profiles of FT-IR spectra of PG from durian cultivars were identical to that of the pectin standard from citrus fruits, suggested that PG from each durian cultivars was pectic polysaccharide. The 1260-830 $cm^{-1}$ region, which is referred to as the "finger print" region of polysaccharide, is unique to a PG sample. For polysaccharide this region is dominated by ring vibrations overlapped with streching vibrations of (C-OH) side groups and the (C-O-C) glycosidic bond vibration. The main absorbance regions are at 1,150, 1,103 and 1015 $cm^{-1}$. The specific bands at 1,747 and 1,640 indicated the ester carbonyl (COOR) and carboxylate ion (COO-) groups, respectively, (Sun et al, 2005; Fang et al., 2006). The results of FT-IR spectras were identical to FT-IR spectra of pectic polysaccharide PG characterization as prior previous studied (Gerddit, 2002). The results showed that PG extracted from different durian cultivars and different cultivated areas gave a pectic polysaccharide with identical profiles of FT-IR spectra (Figure 23).

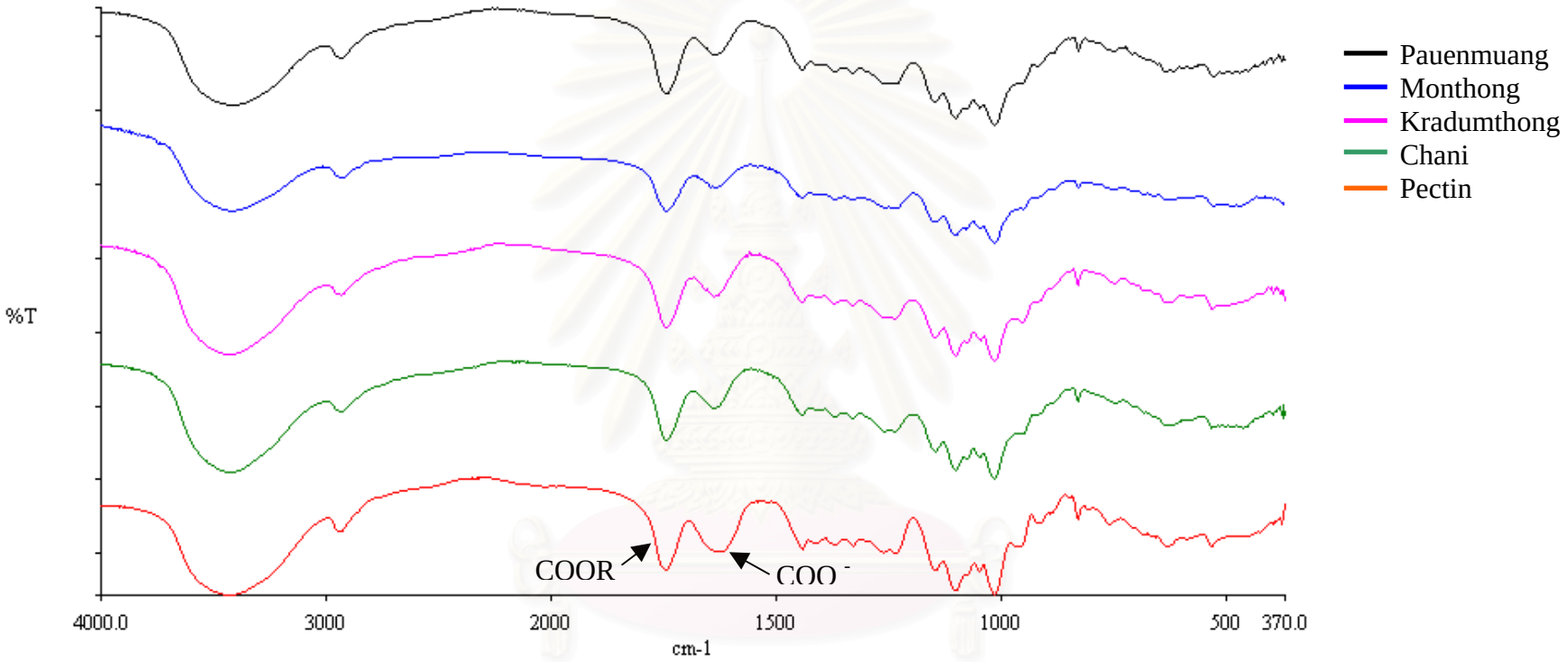



Figure 23. Fourier transform infrared spectra of polysaccharide gel (PG) from durian-fruit rinds of cultivars, 'Monthong', 'Pauenmuang', 'Chani', and 'Kradumthong' were compared to commercial pectin (P9135 from Sigma Co.) with the degree of esterification (DE) of 60.97% and the galacturonic content of ≥ 74%.

### 2.4 Galacturonic acid assay in PG

Galacturonic acid component in PG was analyzed by spectrophotometry assay using m-hydroxydiphenyl reagent (Filisetti-Cozzi et al.1991). D-(+)-Galacturonic acid standard at 50, 100, 150, 200 and 250 nmol concentrations, pectin (Galacturonic acid ≥74.0 %) at 0.01% concentration as a positive control and PG sample at 0.0125% solution in distilled water was determined. The concentration of galacturonic acid standard was plotted against absorbance at 525 nm. Absorbance of various concentration of standard was shown in Table 10 and the standard curve was plotted (Figure 24). Galacturonic acid in PG samples and pectin was determined by using the correlation of its absorbance and galacturonic acid concentration in solution from standard curve. The percentages of galacturonic acid in pectin and PG were calculated by the formular below. The X (nmol) was multipled with 0.53040 or 0.42432 for the calculation of galacturonic acid component in pectin standard or PG, respectively.

$$\% \text{ Galacturonic acid in Pectin} = (0.53040)\ X$$

$$\% \text{ Galacturonic acid in PG} = (0.42432)\ X$$

The galacturonic acid composition in PG of durian cultivars, ‘Kradumthong’, ‘Monthong’ and ‘Chani’ from Chanthaburi province, and ‘Pauenmuang’, ‘Monthong and ‘Chani’ from Chumporn province was 84.740±10.323%, 65.813±5.841%, 59.370±9.558%, 64.330±6.674%, 66.097±10.197% and 54.351±9.459%, respectively, data are shown in the Table 11. The number of raw data were shown in appendix E. The component of galacturonic acid in commercial pectin (Galacturonic acid ≥ 74%) was 94.588±10.718%. The galacturonic acid content in fruit-rinds of durian was different between cultivars. The results in Table 11 showed that PG from ‘Kradumthong’ composed of the highest galacturonic acid content. The lower galacturonic acid content was ‘Monthong’, ‘Pauenmuang’ and ‘Chani’, respectively. However, the galacturonic acid contents in PG of ‘Pauenmuang’ naturally cultivated and ‘Monthong’ commercially cultivated from different areas were not significant difference. Galacturonic acid content in PG

Table 10. The absorbance of D-(+)-galacturonic acid at 525 nm.

| Galacturonic acid (nmol) | Absorbance, 525 nm |
|---|---|
| 0 | 0 |
| 50 | 0.09 |
| 100 | 0.19 |
| 150 | 0.28 |
| 200 | 0.39 |
| 250 | 0.52 |

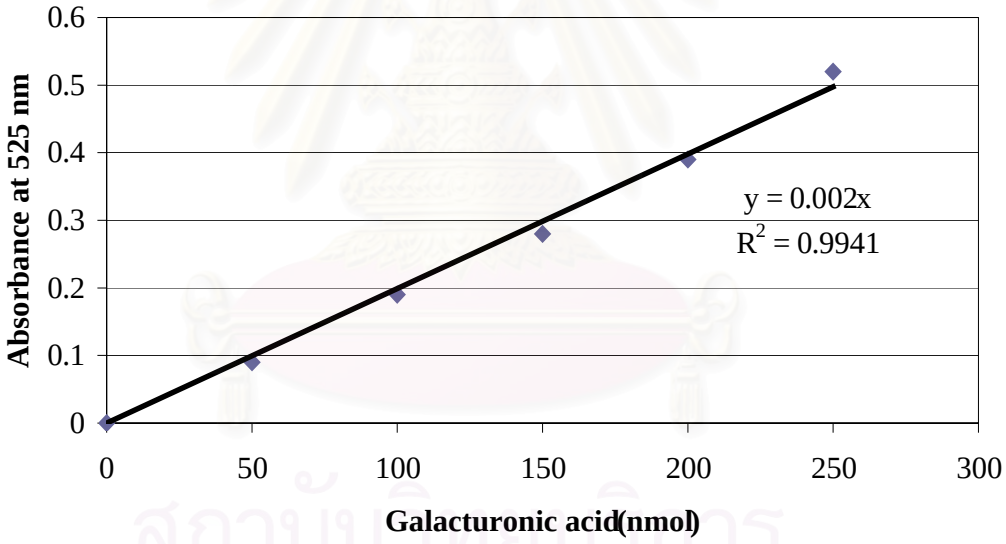



Figure 24. The standard curve of D-(+)-galacturonic acid versus absorbance.

Table 11. The galacturonic acid content in PG of durian cultivars.

| **Provinces** | **Cultivars** | **% galacturonic acid** | **Plantation** |
|---|---|---|---|
| Chanthaburi | Kradumthong | 84.740 ± 10.323 [a] | Commercially |
| | Monthong | 65.813 ± 5.841 [b,c] | Commercially |
| | Chani | 59.370 ± 9.558 [c, d] | Commercially |
| Chumporn | Pauenmuang | 64.330 ± 6.674 [b, c] | Naturally |
| | Monthong | 66.097 ± 10.197 [b] | Commercially |
| | Chani | 54.351 ± 9.459 [d] | Naturally |

a, b, c and d = the significant difference between groups ($P < 0.05$).

of ‘Monthong’ cultivar commercially cultivated from the two provinces was not significant difference. Galacturonic acid content in PG of ‘Chani’ commercially cultivated from Chanthaburi and ‘Chani’ naturally cultivated from Chumporn was also not significantly different. In this study suggested that the galacturonic acid components in PG of the same cultivars but different planted areas, ‘Monthong’ or ‘Chani’ were not significant difference, but galacturonic acid contents in PG of different cultivars such as ‘Kradumthong’, ‘Monthong’ and ‘Chani’ were significantly different. A comparison of galacturonic acid in PG between cultivars ‘Monthong’ and ‘Chani’, and planted areas can be summarized as follow:

| **Comparison** | **Cultivars** | **Areas** | **% Galacturonic acid in PG** |
|---|---|---|---|
| Monthong (Chanthaburi) Chani (Chumporn) | Different | Different | Significant difference |
| Monthong (Chumporn) Chani (Chanthaburi) | Different | Different | Significant difference |
| Monthong (Chumporn) Chani (Chumporn) | Different | Same | Significant difference |
| Monthong (Chanthaburi) Chani (Chanthaburi) | Different | Same | No significant difference |

The galacturonic acid in PG of three groups were significantly difference except the case of ‘Monthong’ (Chanthaburi) and ‘Chani’ (Chanthaburi). Because the gene expression of any metabolites depends on two factors. Firstly is genetic materials which referred to as the variation of different cultivars. Secondly is the environmental effects which meaned the planted areas including fertilizer used in commercial durian production. However, m-hydroxydiphenyl reagent was analyzed total uronic acid (Filisetti-Cozzi et al.1991). The percentage of galacturonic acid in PG of ‘Kradumthong’, ‘Chani’ and ‘Pauenmuang’ in this study might be included other uronic acids such as glucoronic acid, mannuronic acid, etc. Although, PG from ‘Monthong’ was not found other uronic acids (Gerddit, 2002; Hokputsa, 2004). The high resolution techniques should be used to ensure the galacturonic acid content in PG and other sugar components. The techniques were a gas chromatographic (GC) method (Jones and Albersheim, 1972), a high-performance liquid chromatographic

(HPLC) method or a liquid chromatography-mass spectrometry (LC-MS) (Sánchez-Machado et al, 2003).

Although, the galacturonic acid content in PG of ‘Kradumthong’ was the hightest value, but the pH value of its PG solution was not significantly different between cultivars.

### 2.5 Correlation of DNA fingerprint and PG analysis from fruit-rinds

According to the dendrogram of RAPD analysis suggested that durian cultivars, ‘Monthong’, ‘Chani’, ‘Kradumthong’ and ‘Pauenmuang’ had the variation of genetic materials. The results of the present study indicated that the composition of galacturonic acid in PG varied in accordance with the different durian cultivars ($P < 0.05$). The galacturonic acid content in PG might be the influence of the genetic materials. Nevertheless, the broad standard deviation (SD) of galactuoronic acid values maybe also indicated the variation of the environment factors of durian fruits such as extraction condition, fruit ripening, etc. On the other side, the yield of PG depended on the maintainance of commercially plantation of durian, espectially the PG yield of ‘Chani’ from naturally and commercially cultivated areas.

Normally, the correlation between chemicals and genetic materials was not successful like Italian rice (Brandolini et al., 2006), but achieved in Italian garlic (Brandolini et al., 2005). The DNA-based marker is appropriate for fast and simple techniques to identify two food additive polysaccharide, locust bean gum and guar gum, besides the chemically different ratio of galactose and mannose (Urdiain et al., 2004; Urdiain et al., 2005). Although the galacturonic acid content in PG did not relate to the durian classification of dendrogram analysis. However, RAPD analysis has just preliminary studied. The more number of the oligoprimers as well as the amount of durian samples should be examined. As the results, the polymorphic band profiles of RAPD may be used as the molecular marker together with galacturonic acid content in PG for characterization and identification of durian cultivars. The limitation of RAPD is reproducible, the optimized PCR condition should be confirmed by repeating the PCR reaction (Atienzar and Jha, 2006). Finally, other

sugar components of PG should be entirely determined or estimated to ratio of sugar, like the galactose and mannose ratios of locust bean gum and guar gum.

# CHAPTER IV

# CONCLUSION

## 1. DNA fingerprint analysis

**The *mat*K gene and sequencing:** The completed *mat*K gene of durian cultivars, 'Kradumthong', 'Monthong', 'Chani' and 'Pauenmuang' were 1,509 bp in length. The nucleotide substitutions were occured at the position of 275, 860 and 862 compared with *mat*K sequences of *Durio zibethinus* in GenBank, accession no. AY321188. The *mat*K of 'Pauenmuang' cultivar presented either adenosine or cytosine substitutions at the position 275, whereas 'Monthong' and 'Chani' cultivars from both provinces, and 'Kradumthong' from Chanthaburi province presented the cytosine substitutions at the same position as same as the previous in GenBank. The *mat*K sequences of all durian cultivars were also found the cytosine and thymidine substitutions at the position 860 and 862, respectively. The *mat*K gene was not represented a suitable molecular markers for durian identification in this study. However, the new *mat*K sequences in this study have been deposited on DDBJ/EMBL/GenBank database in accession no. AB289824, AB289825, AB289826, AB289822, AB289823, AB289740, and AB289821 for durian cultivars, 'Kradumthong', 'Monthong' and 'Chani' from Chanthaburi, and 'Pauenmuang' (cytosine substitution at the position 275), 'Pauenmuang' (adenine substitution at the position 275) 'Monthong' and 'Chani' from Chumporn province, respectively.

**RAPD analysis:** The RAPD technique generated the polymorphic band patterns of durian cultivars, 'Kradumthong', 'Monthong', 'Chani' and 'Pauenmuang' with the three primers, SN06, SN20 and SO15. The SN06 primer generated the clear unique profiles of cultivated-durians which were 1,000, 800 and 550 bp of 'Kradumthong', 800, 550 and 275 bp of 'Monthong', 1,000, 800, 750, 550 and 275 bp of 'Chani' and 1,000, 800, 750, 500, 325 and 275 bp of 'Pauenmuang'. The DNA fragments were analyzed to binary code, 1 and 0 for present and absent bands, respectively, and then constructed to the dendrogram using PAUP program. As the result of the dendrogram, the durian specimens were divided into two main groups,

naturally cultivated or ‘Pauenmuang’ cultivar and commercially cultivated durian cultivars. The commercially cultivated durian cultivars were subdivided into 3 minor groups. Group I was ‘Kradumthong’ (Chanthaburi) and ‘Chani’ (Chumporn). Group II was ‘Monthong’ from Chanthaburi and Chumporn provinces and the last was Group III, ‘Chani’ from Chanthaburi province. However, the RAPD can be used as the molecular marker for identification of cultivated-durians by using polymorphic band patterns, but the more number of primers should be investigated.

**2. Analysis of PG in fruit-rinds**

**The yield of PG:** In this experiment, the total yield of PG isolated from fruit-rinds of durian cultivars, ‘Kradumthong’, ‘Monthong’ and ‘Chani’ from Chanthaburi province; ‘Pauenmuang’, ‘Monthong’ and ‘Chani’ from Chumporn province was 8.501±0.984%, 8.586±1.370%, 9.152±1.163%, 7.448±0.925%, 9.287±1.279% and 7.774±1.173% by weight of dried fruit-rinds, respectively. PG from the fruit rinds of ‘Monthong’ gave the highest yield. The percentage of PG yield perhaps depended on durian plantation according to the total PG yield of ‘Chani’ cultivated commercially from Chanthaburi gave higher PG yield ($P < 0.05$) than that of ‘Chani’ cultivated naturally from Chumporn. Commercially planted gave high total yield of PG in durian fruit-rinds

**pH and viscosity of PG:** The pH values of durian cultivars at 3% w/v PG, ‘Kradumthong’, ‘Monthong’ and ‘Chani’ from Chanthaburi province, and ‘Pauenmuang’, ‘Monthong’ and ‘Chani’ from Chumporn province were 2.526±0.782, 2.471±0.794, 2.437±0.049, 2.463±0.119, 2.491±0.089 and 2.499±0.059, respectively. Although, the galacturonic acid content in PG of ‘Kradumthong’ was the hightest value but pH in its PG solution was not significantly the lowest among other cultivars. The viscosity of PG from different durian cultivars, ‘Kradumthong’, ‘Monthong’ and ‘Chani’ from Chanthaburi province, and ‘Pauenmuang’, ‘Monthong’ and ‘Chani’ from Chumporn province were 577.989±547.261, 832.271±409.011, 705.929±449.796, 491.011±272.117, 1135.091±996.238 and 407.371±219.943 cPs, respectively. PG solution of ‘Monthong’ gave the highest viscosity ($P < 0.05$).

**Galacturonic acid content in PG:** The galacturonic acid composition in PG of durian cultivars, ‘Kradumthong’, ‘Monthong’ and ‘Chani’ from Chanthaburi province, and ‘Pauenmuang’, ‘Monthong’ and ‘Chani’ from Chumporn province was 84.740±10.323%, 65.813±5.841%, 59.370±9.558%, 64.330±6.674%, 66.097±10.197% and 54.351±9.459%, respectively. The highest galacturonic acid content in PG was from ‘Kradumthong’ ($P < 0.05$), followed by galacturonic acid content in PG from ‘Monthong’, ‘Pauenmuang’ and ‘Chani’, respectively. Galacturonic acid in PG of ‘Monthong’ and ‘Chani’ from different provinces was not significant difference ($P > 0.05$). The content of galacturonic acid in PG of ‘Pauenmuang’ was not significantly different from Monthong. However, m-hydroxydiphenyl reagent is analyzed total uronic acid (Tullia et al.1991) so the high resolution techniques should be studied to ensure the galacturonic acid content in PG and other sugar components. Although, PG from ‘Monthong’ was not found other uronic acids (Gerddit, 2002; Hokputsa, 2004).

### 3. Correlation of DNA fingerprint and PG from fruit-rinds

The polymorphic band profiles of RAPD may be used as the specific marker together with the galacturonic acid content in PG. Moreover, the more number of the oligoprimers as well as the amount of durian samples should be examined. Finally, other sugar constituent should be also determined.

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# APPENDICES

# APPENDIX A

Table A1. Lists of durian plantation owner.

| No. | Durian plantation owner | เจ้าของสวนทุเรียน |
|---|---|---|
| 1 | Mrs. Dutsadee Manthasatian<br>Amphoe Klung, Chanthaburi province | สวนคุณดุษฎี มันตเสถียร<br>อำเภอ ขลุง จังหวัด จันทบุรี |
| 2 | Amphoe Lhangsaun, Chumporn province | อ. หลังสวน จ. ชุมพร |
| 3 | Mr. Boonpaem Chaoungsom<br>Tambon Taamsinhg, Amphoe Muang,<br>Chumporn province | สวนนายดาบตำรวจ บุญเพิ่ม ช่วงสม<br>244 หมู่6 ต.ถ้ำสิงห์ อ. เมือง ชุมพร |

# APPENDIX B

## Reagents

1. 4 M sulfamic acid-potassium sulfamate (pH 1.6), total volume of 25 ml

The weight of sulfamic acid is 9.709 g. It is added with a half of total volume of water (12.5 ml). Then, pH is adjusted to 1.6 by saturated KOH. The final concentration will be reached to 4 M sulfamic acid-potassium sulfamate at pH 1.6, total volume of 25 ml.

2. $H_2SO_4$ (96.4% assay) containing 75 mM sodium tetraborate, total volume of 1000 ml

The weight of sodium tetraborate is 15.103 g. It is stirred overnight in $H_2SO_4$ (96.4% assay). The solution should be prepared in 1000 ml of volumetric flask. The final concentration will be reached to75 mM sodium tetraborate in 96.4% of $H_2SO_4$.

3. 0.15% (w/v) m-hydroxydiphenyl in 0.5% (w/v) NaOH, total volume of 10 ml

The weight of m-hydroxydiphenyl is 15 mg. It is dissolved in 0.5% (w/v) NaOH. The solution should be prepared in 10 ml of volumetric flask. The final concentration will be reached to 0.15% (w/v) m-hydroxydiphenyl in 0.5% (w/v) NaOH. The solution should be freshly prepared.

# APPENDIX C

## 1. Galacturonic calculation

### 1.1 Pectin standard

The 400 μl of pectin solution at 0.01% w/v concentration is

| | | | |
|---|---|---|---|
| Solution volume | 100 ml | composes of pectin matter | 0.01 g |
| Solution volume | 1 ml | composes of pectin matter | 0.1 mg |
| Solution volume | 1000 μl | composes of pectin matter | 0.1 mg |
| **So, Solution volume** | **400 μl** | **composes of pectin matter** | **0.04 mg** |

400 μl of pectin standard is determined the galacturonic acid content in solution (X) by standard curve of D-galacturonic acid at 525 nm.

| | | | |
|---|---|---|---|
| pectin matter | 0.04 mg | composes of galacturonic acid | X nmol |
| pectin matter | 100 mg | composes of galacturonic acid | (2500)X nmol |

Note: The molecular weight of D-galacturonic acid is 212.16 g/mol.

| | | |
|---|---|---|
| The weight of galacturonic acid in pectin is | $(212.16)\ (2500 \times 10^{-9})(X)$ | g |
| The weight of galacturonic acid in pectin is | $(212.16)\ (2500 \times 10^{-9})(X)$ | g |
| So, The weight of galacturonic acid in pectin is | (0.5304)X | mg |

**Finally: % galacturonic acid (in 100 mg pectin) = (0.5304) X**

### 1.2 PG sample

The 400 μl of PG solution at 0.0125% w/v concentration is

| | | | |
|---|---|---|---|
| Solution volume | 100 ml | composes of PG matter | 0.0125 g |
| Solution volume | 1 ml | composes of PG matter | 0.125 mg |

| | | | |
|---|---|---|---|
| Solution volume | 1000 μl | composes of PG matter | 0.125 mg |
| **So, Solution volume** | **400 μl** | **composes of PG matter** | **0.05 mg** |

400 μl of PG solution is determined the galacturonic acid content in solution (X) by standard curve of D-galacturonic acid at 525 nm.

| | | | |
|---|---|---|---|
| PG matter | 0.05 mg | composes of galacturonic acid | X nmol |
| PG matter | 100 mg | composes of galacturonic acid | (2000)X nmol |

Note: The molecular weight of D-galacturonic acid is 212.16 g/mol.

| | | |
|---|---|---|
| The weight of galacturonic acid in PG is | $(212.16)x(2000 \times 10^{-9})(X)$ | g |
| The weight of galacturonic acid in PG is | $(212.16)x(2000 \times 10^{-9})(X)$ | g |
| So, The weight of galacturonic acid in PG is | (0.42432)X | mg |

Finally, **% galacturonic acid (in 100 mg PG) = (0.42432) X**

# APPENDIX D

Table D1. The data of the total yield of PG

| Province | Cultivars | N | % yield of PG |
|---|---|---|---|
| Chanthaburi | Kradumthong | 13 | 7.96 9.84 7.54 8.10 9.55 9.90 7.63 6.69 8.31 9.06 9.44 8.18 8.32 |
| | Monthong | 8 | 7.19 7.33 8.10 8.79 11.61 8.48 8.93 8.27 |
| | Chani | 17 | 8.90 9.92 9.82 7.77 10.86 9.23 9.55 10.43 11.32 9.65 8.77 8.90 8.41 8.05 8.24 9.11 6.67 |
| Chumporn | Pauenmuang | 10 | 6.12 5.99 7.43 8.28 8.22 7.28 7.61 8.84 7.86 6.87 |
| | Monthong | 19 | 9.73 9.30 10.87 9.76 9.46 10.90 10.79 10.56 7.28 7.44 8.79 8.59 8.80 10.68 10.1 10.38 7.55 7.57 7.92 |
| | Chani | 18 | 6.50 8.97 6.55 7.38 9.26 7.97 9.20 8.42 10.35 6.70 6.76 6.55 7.68 8.36 6.77 8.39 6.39 7.72 |

N = Number of data

**The statistics of the total yield of PG**

**Oneway**

**Descriptives**

Total_yield

| | N | Mean | Std. Deviation | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | | |
| Kradumthong_ Chanthaburi | 13 | 8.5014 | .98447 | 7.9065 | 9.0963 | 6.69 | 9.90 |
| Monthong_ Chanthaburi | 8 | 8.5864 | 1.37098 | 7.4402 | 9.7325 | 7.19 | 11.61 |
| Chani_ Chanthaburi | 17 | 9.1515 | 1.16330 | 8.5534 | 9.7496 | 6.67 | 11.32 |
| Pauenmuang_ Chumporn | 10 | 7.4483 | .92464 | 6.7869 | 8.1097 | 5.99 | 8.84 |
| Monthong_ Chumporn | 19 | 9.2869 | 1.27953 | 8.6702 | 9.9037 | 7.28 | 10.90 |
| Chani_ Chumporn | 18 | 7.7744 | 1.17273 | 7.1912 | 8.3576 | 6.39 | 10.35 |
| Total | 85 | 8.5372 | 1.32038 | 8.2524 | 8.8220 | 5.99 | 11.61 |

**ANOVA**

Total_yield

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 39.463 | 5 | 7.893 | 5.828 | .000 |
| Within Groups | 106.984 | 79 | 1.354 | | |
| Total | 146.447 | 84 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

Dependent Variable: Total_yield
Tukey HSD

| (I) Cultivars | (J) Cultivars | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Kradumthong _Chanthaburi | Monthong_Chanthaburi | -.08499 | .52292 | 1.000 | -1.6124 | 1.4424 |
| | Chani_Chanthaburi | -.65014 | .42876 | .655 | -1.9025 | .6022 |
| | Pauenmuang_Chumporn | 1.05308 | .48948 | .272 | -.3766 | 2.4828 |
| | Monthong_Chumporn | -.78556 | .41886 | .425 | -2.0090 | .4379 |
| | Chani_Chumporn | .72700 | .42356 | .525 | -.5102 | 1.9642 |
| Monthong _ Chanthaburi | Kradumthong_Chanthaburi | .08499 | .52292 | 1.000 | -1.4424 | 1.6124 |
| | Chani_Chanthaburi | -.56515 | .49894 | .866 | -2.0225 | .8922 |
| | Pauenmuang_Chumporn | 1.13807 | .55200 | .318 | -.4742 | 2.7504 |
| | Monthong_Chumporn | -.70057 | .49046 | .710 | -2.1331 | .7320 |
| | Chani_Chumporn | .81199 | .49448 | .574 | -.6323 | 2.2563 |
| Chani _ Chanthaburi | Kradumthong_Chanthaburi | .65014 | .42876 | .655 | -.6022 | 1.9025 |
| | Monthong_Chanthaburi | .56515 | .49894 | .866 | -.8922 | 2.0225 |
| | Pauenmuang_Chumporn | 1.70323(*) | .46377 | .006 | .3486 | 3.0578 |
| | Monthong_Chumporn | -.13542 | .38850 | .999 | -1.2702 | .9993 |
| | Chani_Chumporn | 1.37714(*) | .39357 | .010 | .2276 | 2.5267 |
| Pauenmuang _Chumporn | Kradumthong_Chanthaburi | -1.05308 | .48948 | .272 | -2.4828 | .3766 |
| | Monthong_Chanthaburi | -1.13807 | .55200 | .318 | -2.7504 | .4742 |
| | Chani_Chanthaburi | -1.70323(*) | .46377 | .006 | -3.0578 | -.3486 |
| | Monthong_Chumporn | -1.83865(*) | .45464 | .002 | -3.1666 | -.5107 |
| | Chani_Chumporn | -.32609 | .45897 | .980 | -1.6667 | 1.0145 |
| Monthong _ Chumporn | Kradumthong_Chanthaburi | .78556 | .41886 | .425 | -.4379 | 2.0090 |
| | Monthong_Chanthaburi | .70057 | .49046 | .710 | -.7320 | 2.1331 |
| | Chani_Chanthaburi | .13542 | .38850 | .999 | -.9993 | 1.2702 |
| | Pauenmuang_Chumporn | 1.83865(*) | .45464 | .002 | .5107 | 3.1666 |
| | Chani_Chumporn | 1.51256(*) | .38277 | .002 | .3946 | 2.6306 |
| Chani _ Chumporn | Kradumthong_Chanthaburi | -.72700 | .42356 | .525 | -1.9642 | .5102 |
| | Monthong_Chanthaburi | -.81199 | .49448 | .574 | -2.2563 | .6323 |
| | Chani_Chanthaburi | -1.37714(*) | .39357 | .010 | -2.5267 | -.2276 |
| | Pauenmuang_Chumporn | .32609 | .45897 | .980 | -1.0145 | 1.6667 |
| | Monthong_Chumporn | -1.51256(*) | .38277 | .002 | -2.6306 | -.3946 |

* The mean difference is significant at the .05 level.

**Homogeneous Subsets**

Total_yield

Tukey HSD

| Cultivars | N | Subset for alpha = .05 | |
|---|---|---|---|
| | | 1 | 2 |
| Pauenmuang_Chumporn | 10 | 7.4483 | |
| Chani_Chumporn | 18 | 7.7744 | |
| Kradumthong_Chanthaburi | 13 | 8.5014 | 8.5014 |
| Monthong_Chanthaburi | 8 | 8.5864 | 8.5864 |
| Chani_Chanthaburi | 17 | | 9.1515 |
| Monthong_Chumporn | 19 | | 9.2869 |
| Sig. | | .145 | .531 |

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 12.795.

b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

## APPENDIX E

Table E1. The data of the total galacturonic acid content in PG

| Province | Cultivars | N | % galacturonic acid of PG | | | | |
|---|---|---|---|---|---|---|---|
| Chanthaburi | Kradumthong | 36 | 98.264 | 98.264 | 102.73 | 96.030 | 96.030 |
| | | | 96.030 | 82.631 | 91.564 | 89.331 | 69.231 |
| | | | 69.231 | 73.698 | 66.998 | 71.464 | 66.998 |
| | | | 89.331 | 91.564 | 91.564 | 98.264 | 93.797 |
| | | | 93.797 | 78.164 | 73.698 | 80.397 | 71.464 |
| | | | 75.931 | 71.464 | 89.331 | 89.331 | 89.331 |
| | | | 84.864 | 87.097 | 87.097 | 80.397 | 82.631 |
| | | | 82.631 | | | | |
| | Monthong | 21 | 58.597 | 68.699 | 70.720 | 60.617 | 78.802 |
| | | | 72.741 | 66.679 | 66.679 | 72.741 | 62.638 |
| | | | 62.638 | 70.720 | 58.597 | 62.638 | 62.638 |
| | | | 56.576 | 58.597 | 64.658 | 66.679 | 66.679 |
| | | | 72.741 | | | | |
| | Chani | 49 | 73.292 | 73.292 | 65.577 | 67.505 | 67.505 |
| | | | 71.363 | 54.004 | 63.648 | 65.577 | 69.434 |
| | | | 63.648 | 67.505 | 69.434 | 63.648 | 69.434 |
| | | | 55.933 | 63.648 | 69.434 | 73.440 | 76.704 |
| | | | 75.072 | 52.224 | 44.064 | 48.960 | 53.856 |
| | | | 58.752 | 63.648 | 53.856 | 58.752 | 57.120 |
| | | | 66.912 | 65.280 | 48.960 | 47.328 | 44.064 |
| | | | 47.328 | 50.592 | 55.488 | 55.488 | 52.224 |
| | | | 55.488 | 47.328 | 45.696 | 44.064 | 58.752 |
| | | | 42.432 | 52.224 | 55.488 | 63.648 | |
| Chumporn | Pauenmuang | 60 | 59.036 | 60.881 | 63.648 | 71.808 | 75.771 |
| | | | 58.752 | 60.298 | 60.384 | 66.912 | 63.648 |
| | | | 66.912 | 70.105 | 71.808 | 72.741 | 77.485 |
| | | | 60.384 | 60.617 | 60.617 | 63.648 | 71.464 |
| | | | 78.164 | 57.586 | 57.586 | 63.648 | 63.648 |
| | | | 68.544 | 69.231 | 71.808 | 78.164 | 60.881 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 62.726 | 63.648 | 65.280 | 69.710 | 72.741 |
| | | 47.328 | 48.494 | 53.598 | 53.598 | 60.617 |
| | | 62.531 | 51.365 | 57.120 | 62.016 | 63.648 |
| | | 66.679 | 69.710 | 63.648 | 65.770 | 65.770 |
| | | 66.415 | 66.679 | 69.710 | 70.105 | 60.298 |
| | | 60.298 | 60.617 | 60.617 | 63.648 | 69.231 |
| Monthong | 57 | 69.231 | 71.464 | 78.164 | 49.132 | 44.665 |
| | | 53.598 | 55.832 | 49.132 | 66.998 | 82.631 |
| | | 87.097 | 73.698 | 58.065 | 58.065 | 60.298 |
| | | 53.598 | 60.112 | 61.880 | 56.576 | 61.880 |
| | | 63.648 | 61.880 | 61.880 | 53.040 | 63.648 |
| | | 70.720 | 74.256 | 70.720 | 74.256 | 86.632 |
| | | 63.648 | 74.256 | 61.880 | 72.488 | 72.488 |
| | | 74.256 | 81.007 | 71.363 | 65.577 | 61.719 |
| | | 65.577 | 63.648 | 59.791 | 75.220 | 81.007 |
| | | 82.935 | 79.078 | 69.434 | 48.218 | 61.719 |
| | | 61.719 | 52.076 | 52.076 | 67.505 | 65.577 |
| | | 71.363 | 79.078 | | | |
| Chani | 54 | 63.648 | 63.648 | 55.488 | 39.168 | 34.272 |
| | | 39.168 | 39.168 | 32.640 | 44.064 | 56.576 |
| | | 58.597 | 56.576 | 74.761 | 68.699 | 68.699 |
| | | 52.535 | 50.514 | 50.514 | 58.597 | 60.617 |
| | | 66.679 | 46.473 | 50.514 | 60.617 | 56.576 |
| | | 58.597 | 52.535 | 42.432 | 42.432 | 40.411 |
| | | 46.473 | 52.535 | 52.535 | 54.555 | 72.741 |
| | | 72.741 | 54.555 | 58.597 | 54.555 | 44.453 |
| | | 50.514 | 54.555 | 62.638 | 64.658 | 56.576 |
| | | 48.494 | 54.555 | 54.555 | 52.535 | 52.535 |
| | | 58.597 | 60.617 | 56.576 | 58.597 | |

N = Number of data

**The statistics of the total galacturinic acid content of PG**

**Oneway**

**Descriptives**

Galacturonic_acid

| | N | Mean | Std. Deviation | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | | |
| Kradumthong_ Chanthaburi | 36 | 84.73993 | 10.323440 | 81.24698 | 88.23288 | 66.998 | 102.730 |
| Monthong_ Chanthaburi | 21 | 65.81290 | 5.841214 | 63.15401 | 68.47179 | 56.576 | 78.802 |
| Chani_ Chanthaburi | 49 | 59.36968 | 9.558395 | 56.62419 | 62.11517 | 42.432 | 76.704 |
| Pauenmuang_ Chumporn | 60 | 64.32990 | 6.674380 | 62.60572 | 66.05407 | 47.328 | 78.164 |
| Monthong_ Chumporn | 57 | 66.09646 | 10.196949 | 63.39084 | 68.80207 | 44.665 | 87.097 |
| Chani_ Chumporn | 54 | 54.35107 | 9.459066 | 51.76924 | 56.93290 | 32.640 | 74.761 |
| Total | 277 | 64.63564 | 12.592911 | 63.14613 | 66.12515 | 32.640 | 102.730 |

**ANOVA**

Galacturonic_acid

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 21777.417 | 5 | 4355.483 | 53.673 | .000 |
| Within Groups | 21991.052 | 271 | 81.148 | | |
| Total | 43768.469 | 276 | | | |

**Post Hoc Tests**

**Multiple Comparisons**

Dependent Variable: Galacturonic_acid
Tukey HSD

| (I) Cultivars | (J) Cultivars | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Kradumthong _Chanthaburi | Monthong_Chanthaburi | 18.927032(*) | 2.473517 | .000 | 11.82748 | 26.02659 |
| | Chani_Chanthaburi | 25.370253(*) | 1.977418 | .000 | 19.69461 | 31.04589 |
| | Pauenmuang_ Chumporn | 20.410032(*) | 1.899097 | .000 | 14.95919 | 25.86087 |
| | Monthong_ Chumporn | 18.643473(*) | 1.917746 | .000 | 13.13911 | 24.14784 |
| | Chani_ Chumporn | 30.388861(*) | 1.938258 | .000 | 24.82562 | 35.95210 |
| Monthong _Chanthaburi | Kradumthong_Chanthaburi | -18.927032(*) | 2.473517 | .000 | -26.02659 | -11.82748 |
| | Chani_Chanthaburi | 6.443221 | 2.349523 | .070 | -.30044 | 13.18688 |
| | Pauenmuang_ Chumporn | 1.483000 | 2.283997 | .987 | -5.07259 | 8.03859 |
| | Monthong_ Chumporn | -.283559 | 2.299527 | 1.000 | -6.88372 | 6.31661 |
| | Chani_ Chumporn | 11.461829(*) | 2.316661 | .000 | 4.81249 | 18.11117 |
| Chani _Chanthaburi | Kradumthong_Chanthaburi | -25.370253(*) | 1.977418 | .000 | -31.04589 | -19.69461 |
| | Monthong_Chanthaburi | -6.443221 | 2.349523 | .070 | -13.18688 | .30044 |
| | Pauenmuang_ Chumporn | -4.960220 | 1.734515 | .051 | -9.93867 | .01823 |
| | Monthong_ Chumporn | -6.726779(*) | 1.754914 | .002 | -11.76378 | -1.68978 |
| | Chani_ Chumporn | 5.018608 | 1.777305 | .057 | -.08266 | 10.11988 |
| Pauenmuang _ Chumporn | Kradumthong_Chanthaburi | -20.410032(*) | 1.899097 | .000 | -25.86087 | -14.95919 |
| | Monthong_Chanthaburi | -1.483000 | 2.283997 | .987 | -8.03859 | 5.07259 |
| | Chani_Chanthaburi | 4.960220 | 1.734515 | .051 | -.01823 | 9.93867 |
| | Monthong_ Chumporn | -1.766559 | 1.666166 | .897 | -6.54883 | 3.01571 |
| | Chani_ Chumporn | 9.978829(*) | 1.689734 | .000 | 5.12891 | 14.82875 |
| Monthong _ Chumporn | Kradumthong_Chanthaburi | -18.643473(*) | 1.917746 | .000 | -24.14784 | -13.13911 |
| | Monthong_Chanthaburi | .283559 | 2.299527 | 1.000 | -6.31661 | 6.88372 |
| | Chani_Chanthaburi | 6.726779(*) | 1.754914 | .002 | 1.68978 | 11.76378 |
| | Pauenmuang_ Chumporn | 1.766559 | 1.666166 | .897 | -3.01571 | 6.54883 |
| | Chani_ Chumporn | 11.745388(*) | 1.710667 | .000 | 6.83538 | 16.65539 |
| Chani _ Chumporn | Kradumthong_Chanthaburi | -30.388861(*) | 1.938258 | .000 | -35.95210 | -24.82562 |
| | Monthong_Chanthaburi | -11.461829(*) | 2.316661 | .000 | -18.11117 | -4.81249 |
| | Chani_Chanthaburi | -5.018608 | 1.777305 | .057 | -10.11988 | .08266 |
| | Pauenmuang_ Chumporn | -9.978829(*) | 1.689734 | .000 | -14.82875 | -5.12891 |
| | Monthong_ Chumporn | -11.745388(*) | 1.710667 | .000 | -16.65539 | -6.83538 |

* The mean difference is significant at the .05 level.

**Homogeneous Subsets**

**Galacturonic_acid**

Tukey HSD

| Cultivars | N | Subset for alpha = .05 | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| Chani_ Chumporn | 54 | 54.35107 | | | |
| Chani_Chanthaburi | 49 | 59.36968 | 59.36968 | | |
| Pauenmuang_ Chumporn | 60 | | 64.32990 | 64.32990 | |
| Monthong_Chanthaburi | 21 | | | 65.81290 | |
| Monthong_ Chumporn | 57 | | | 66.09646 | |
| Kradumthong_Chanthaburi | 36 | | | | 84.73993 |
| Sig. | | .127 | .135 | .951 | 1.000 |

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 40.395.

b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

# APPENDIX F

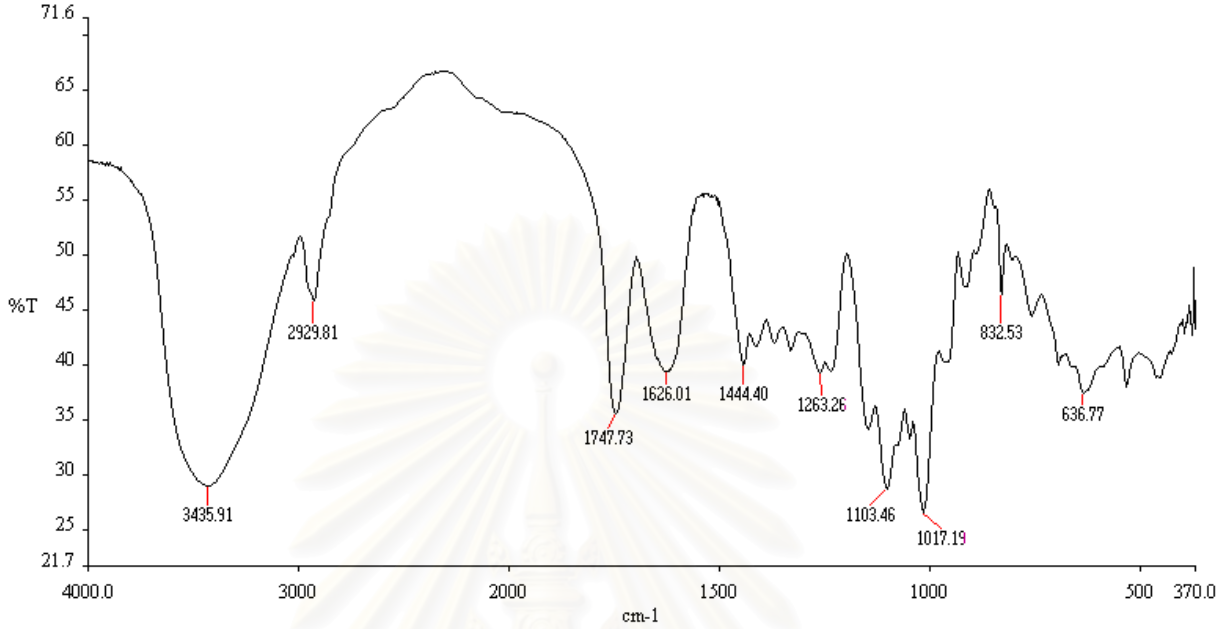


Figure F1. FT-IR spectra of pectin from citrus fruits.

Table F1. The band intensity of pectin from citrus fruits.

| Wavelength ($cm^{-1}$) | %T |
|---|---|
| 3435.91 | 28.98 |
| 2929.81 | 45.86 |
| 1747.73 | 35.59 |
| 1626.01 | 39.41 |
| 1444.4 | 39.99 |
| 1263.26 | 39.32 |
| 1103.46 | 28.78 |
| 1017.19 | 26.61 |
| 832.53 | 46.4 |
| 636.77 | 37.41 |

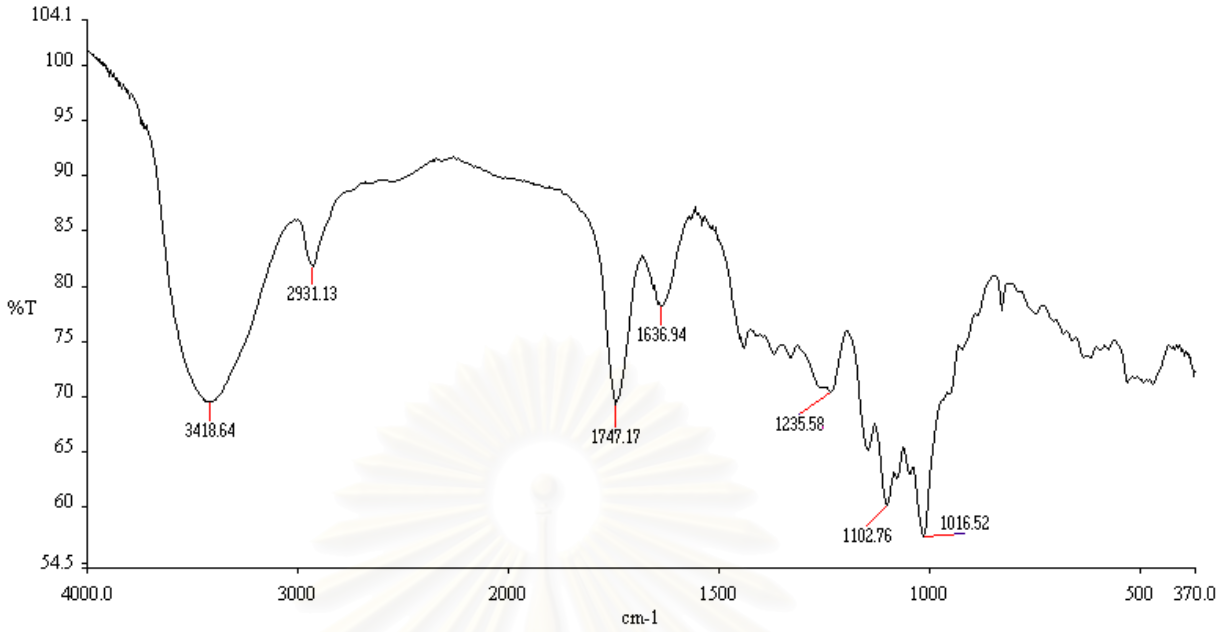


Figure F2. FT-IR spectra of ‘Monthong’ cultivar.

Table F2. The band intensity of ‘Monthong’ cultivar.

| **Wavelength ($cm^{-1}$)** | **%T** |
|---|---|
| 3418.64 | 69.5 |
| 2931.13 | 81.73 |
| 1747.17 | 69.28 |
| 1636.94 | 78.16 |
| 1235.58 | 70.44 |
| 1102.76 | 60.17 |
| 1016.52 | 57.29 |

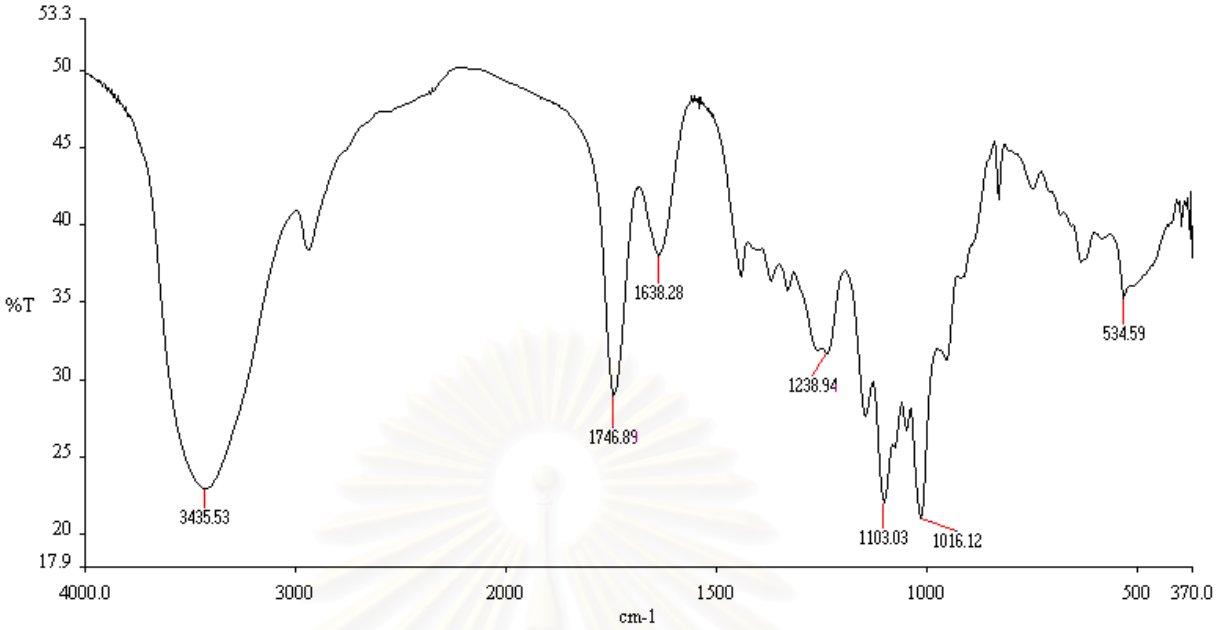


Figure F3. FT-IR spectra of ‘Chani’ cultivar.

Table F3. The band intensity of ‘Chani’ cultivar.

| **Wavelength ($cm^{-1}$)** | **%T** |
|---|---|
| 3435.53 | 22.91 |
| 1746.89 | 28.96 |
| 1638.28 | 37.96 |
| 1238.94 | 31.67 |
| 1103.03 | 22.05 |
| 1016.12 | 21.02 |
| 534.59 | 35.22 |

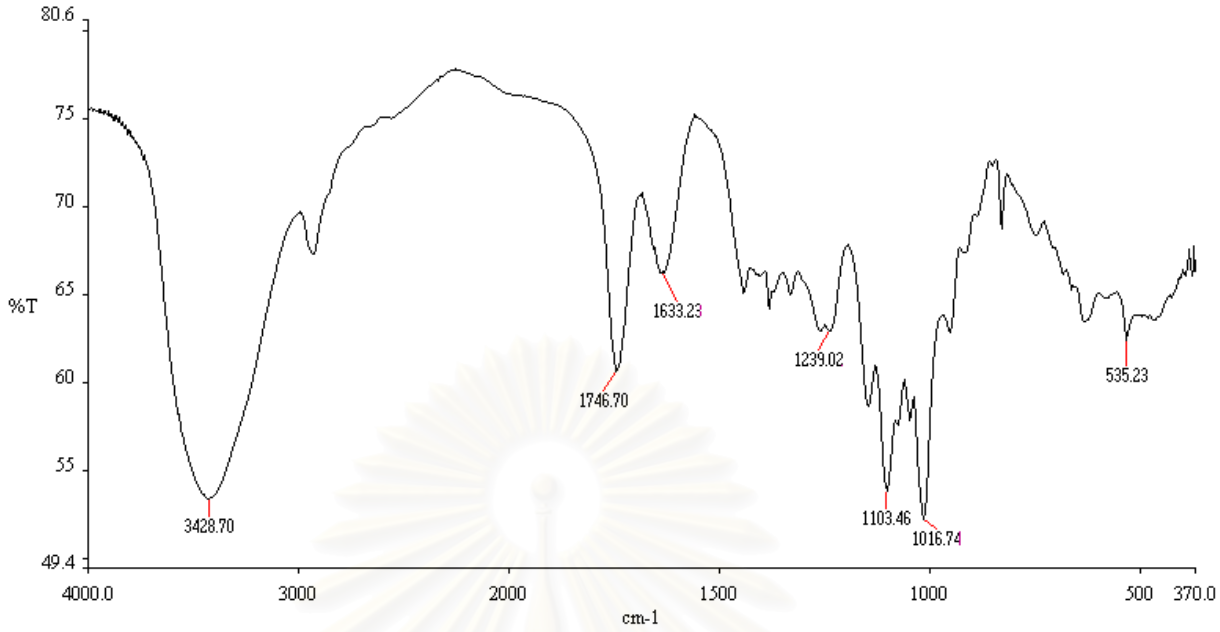


Figure F4. FT-IR spectra of ‘Pauenmuang’ cultivar.

Table F4. The band intensity of ‘Pauenmuang’ cultivar.

| **Wavelength ($cm^{-1}$)** | **%T** |
|---|---|
| 3428.7 | 53.39 |
| 1746.7 | 60.64 |
| 1633.23 | 66.19 |
| 1239.02 | 62.9 |
| 1103.46 | 53.85 |
| 1016.74 | 52.24 |
| 535.23 | 62.43 |

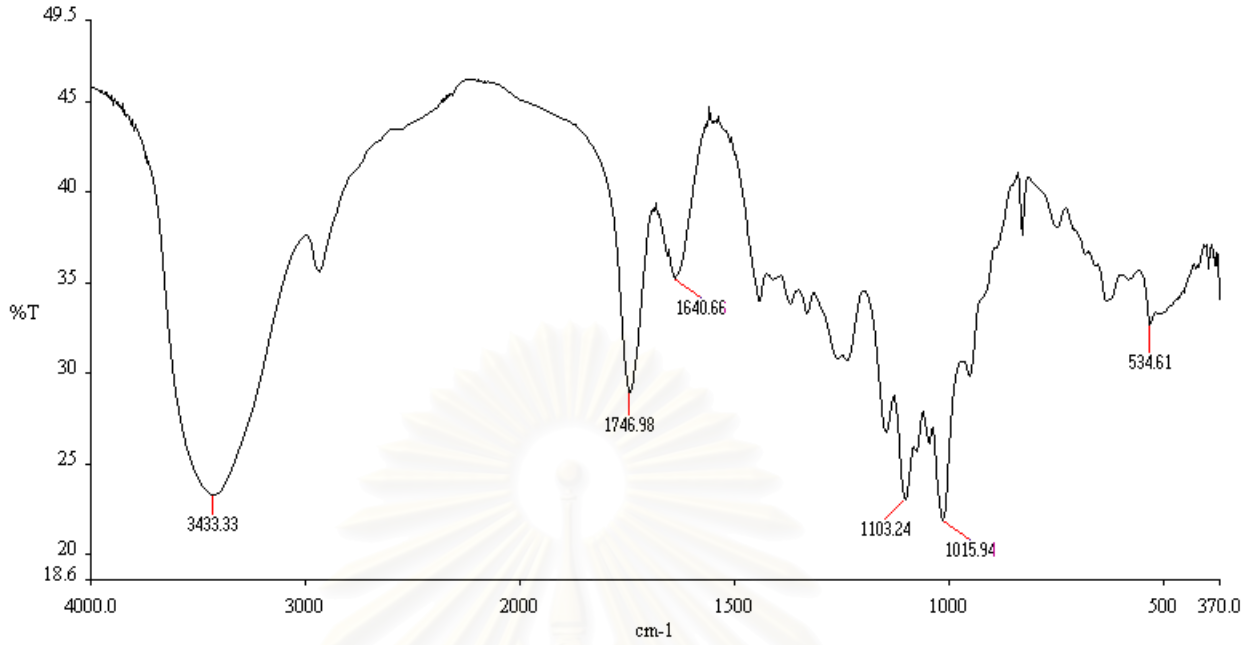


Figure F5. FT-IR spectra of ‘Kradumthong’ cultivar.

Table F5. The band intensity of ‘Kradumthong’ cultivar.

| **Wavelength ($cm^{-1}$)** | **%T** |
|---|---|
| 3433.33 | 23.26 |
| 1746.98 | 28.93 |
| 1640.66 | 35.24 |
| 1103.24 | 22.97 |
| 1015.94 | 21.85 |
| 534.61 | 32.66 |

# APPENDIX G

1. **The data of DNA Sequencing which was submitted to DDBJ database**

### 1.1 Kradumthong from Chanthaburi

EntryID : 20070109001548.48858

[Contact Person]

E-mail: ssukrong@hotmail.com

Name: Suchada Sukrong

Institution: Chulalongkorn university

Department: Pharmacognosy

Country: Thailand

City: Bangkok

Street: Phayathai

Zip code: 10330

Phone: 6622188364

Fax: 6622188357

submitter(s): Suchada Sukrong

[Hold-date]

Immediate release: No

Hold-date: 2007/12/31

Kind of data: General data

[REFERENCE No.1]

Status: In Preparation

Year: 2007

Title: DNA fingerprint analysis of Durian cultivars selected in Thai comparing their polysaccharide contents

author(s): S Sukrong, S Pongsamart

[Sequence]

length: 1509

Sequence:

```
  1 atggaggaat ttcaagtata tttagaacta aatagatctc gccgacatga tttcctatac
 61 ccacttattt ttcgggagta tatttatgca cttgctcatg atcatggttt aaataaatcg
121 atgatttttt tggaaaatca gggttatggt aataaattca gttcactaat tgtgaaacgt
181 ttaattattc gaatggatca acagaatcat ttgattattt ctgctaatga ttccaaccaa
241 aatccatttt ttgggcacaa caataattta tattctcaaa tgatatcggt gggatttgca
301 gtcattgtgg aaattccatt ttccttacga ttagtatctt actcacaagg ggaagaagtc
361 gcaaaatccc ataatttcca atcaattcat tcaatatttc ctttttaga ggacaaattc
421 tcacatttaa attatgtgtt agatgtacta ataccttacc ccatccatct agaaatcttg
481 gttcaagccc ttcgctactg gataaaagat gcttcttctt tgcatttatt acggttctct
541 ctctacgagt attgtaattt gaagagtttt attactccaa agaaatctat ttctattttt
601 aatccaagat tattcttgtt cctatataat tctcatgtat gtgaatacga atccattttc
661 ctttttctcc gtaatcaatc ttcttattta cgatcaacat cttctggatt ctttcttgaa
721 cgaattaatt tctatggaaa aatagagtat cttgtagaag tcttttataa tgattttcag
781 aacaacctat ggttgttcaa agacccttc atacattttt ttaggtatca aggaaaggca
841 attctggcat caaaggatac gtctcttctg atgaataagt ggaaatatta ctttgtcgat
901 ttatggaaat attatttta cgtgcggtct caatcaggaa gcgtccgtat aaatcaatta
961 tctaaatatt ctctcgactt tctgggctat ctttcaagtg tgcgattaaa tacttcagtg
1021gtacggagtc aaatgctaga aaattcattt ataatagata atgctatgaa gaagttggat
1081acaagaattc caattatttc tctcattgga tcattgtcta aagcgaaatt ttgtaacaca
1141ttagggcatc ccattagtaa gccgacgtgg tccgattcct ccgattctga tattattgac
1201cgatttgtgc gtatatgcag aaatctttct cattatcaca gtggatcttc aaaaaaaaag
1261agtttgtatc gaataaaata tatacttcgg ctttcttgtg ttaaaacttt ggctcgtaaa
1321cacaaaagta ctgtacgtgc ttttttgaaa agattaggtt cggaattttt ggaagaattc
1381tttacggaag aagaacatgt tttttctttg atcttcccaa gagtttttt gacttcgcga
1441aagttatata gggtgcgaat ttggtatttg gatattattt gtatcaatgc tctggtcaat
1501catgaatga
```

[Organism]

| | |
|---|---|
| organism | *Durio zibethinus* |
| mol_type | genomic DNA |
| collected_by | Suchada Sukrong |
| collection_date | 2005 |
| country | Thailand:Chantaburi |
| cultivar | Kradumthong |
| identified_b | Suchada Sukrong |
| organelle | plastid:chloroplast |
| specimen_voucher | Kradumthong |
| Genetic code | 11 |

[CDS Feature No.1]

Location 1..1509

| | |
|---|---|
| product | maturaseK |
| gene | *mat*K |
| transl_table | 11 |
| translation | |

```
MEEFQVYLELNRSRRHDFLYPLIFREYIYALAHDHGLNKSMIFLENQGYGNKFSSLIVKRLIIRMDQQN
HLIISANDSNQNPFFGHNNNLYSQMISVGFAVIVEIPFSLRLVSYSQGEEVAKSHNFQSIHSIFPFLED
KFSHLNYVLDVLIPYPIHLEILVQALRYWIKDASSLHLLRFSLYEYCNLKSFITPKKSISIFNPRLFLF
LYNSHVCEYESIFLFLRNQSSYLRSTSSGFFLERINFYGKIEYLVEVFYNDFQNNLWLFKDPFIHFFRY
QGKAILASKDTSLLMNKWKYYFVDLWKYYFYVRSQSGSVRINQLSKYSLDFLGYLSSVRLNTSVVRSQM
LENSFIIDNAMKKLDTRIPIISLIGSLSKAKFCNTLGHPISKPTWSDSSDSDIIDRFVRICRNLSHYHS
GSSKKKSLYRIKYILRLSCVKTLARKHKSTVRAFLKRLGSEFLEEFFTEEEHVFSLIFPRVFLTSRKLY
RVRIWYLDIICINALVNHE
```

note an open reading frame (ORF) located within the intron of the transfer RNA gene for lysine

### 1.2 Monthong from Chanthaburi

EntryID : 20070108235842.22750


[Contact Person]

| | |
|---|---|
| E-mail: | ssukrong@hotmail.com |
| Name: | Suchada Sukrong |
| Institution: | Chulalongkorn university |
| Department: | Pharmacognosy |
| Country: | Thailand |
| City: | Bangkok |
| Street: | Phayathai |
| Zip code: | 10330 |
| Phone: | 6622188364 |
| Fax: | 6622188357 |
| submitter(s): | Suchada Sukrong |


[Hold-date]

| | |
|---|---|
| Immediate release: | No |
| Hold-date: | 2007/12/31 |

Kind of data: General data

[REFERENCE No.1]

Status: In Preparation

Year: 2007

Title: DNA fingerprint analysis of Durian cultivars selected in Thai comparing their polysaccharide contents

author(s): S Sukrong, S Pongsamart

[Sequence]

length: 1509

Sequence:

```
  1 atggaggaat ttcaagtata tttagaacta aatagatctc gccgacatga tttcctatac
 61 ccacttattt ttcgggagta tatttatgca cttgctcatg atcatggttt aaataaatcg
121 atgatttttt tggaaaatca gggttatggt aataaattca gttcactaat tgtgaaacgt
181 ttaattattc gaatggatca acagaatcat ttgattattt ctgctaatga ttccaaccaa
241 aatccatttt ttgggcacaa caataattta tattctcaaa tgatatcggt gggatttgca
301 gtcattgtgg aaattccatt ttccttacga ttagtatctt actcacaagg ggaagaagtc
361 gcaaaatccc ataatttcca atcaattcat tcaatatttc ctttttaga ggacaaattc
421 tcacatttaa attatgtgtt agatgtacta ataccttacc ccatccatct agaaatcttg
481 gttcaagccc ttcgctactg gataaaagat gcttcttctt tgcatttatt acggttctct
541 ctctacgagt attgtaattt gaagagtttt attactccaa agaaatctat ttctattttt
601 aatccaagat tattcttgtt cctatataat tctcatgtat gtgaatacga atccattttc
661 ctttttctcc gtaatcaatc ttcttattta cgatcaacat cttctggatt ctttcttgaa
721 cgaattaatt tctatggaaa aatagagtat cttgtagaag tcttttataa tgattttcag
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841 attctggcat caaaggatac gtctcttctg atgaataagt ggaaatatta ctttgtcgat
901 ttatggaaat attatttta cgtgcggtct caatcaggaa gcgtccgtat aaatcaatta
961 tctaaatatt ctctcgactt tctgggctat ctttcaagtg tgcgattaaa tacttcagtg
1021gtacggagtc aaatgctaga aaattcattt ataatagata atgctatgaa gaagttggat
1081acaagaattc caattatttc tctcattgga tcattgtcta aagcgaaatt ttgtaacaca
1141ttagggcatc ccattagtaa gccgacgtgg tccgattcct ccgattctga tattattgac
1201cgatttgtgc gtatatgcag aaatctttct cattatcaca gtggatcttc aaaaaaaaag
1261agtttgtatc gaataaaata tatacttcgg ctttcttgtg ttaaaacttt ggctcgtaaa
1321cacaaaagta ctgtacgtgc ttttttgaaa agattaggtt cggaatttt ggaagaattc
1381tttacggaag aagaacatgt tttttctttg atcttcccaa gagtttttt gacttcgcga
1441aagttatata gggtgcgaat ttggtatttg gatattattt gtatcaatgc tctggtcaat
1501catgaatga
```

[Organism]

organism *Durio zibethinus*

mol_type genomic DNA

collected_by Suchada Sukrong

collection_date 2005

country Thailand:Chantaburi

| | |
|---|---|
| cultivar | Monthong |
| identified_by | Suchada Sukrong |
| organelle | plastid:chloroplast |
| specimen_voucher | Monthong1 |
| Genetic code | 11 |

[CDS Feature No.1]

| | |
|---|---|
| Location | 1..1509 |
| product | maturaseK |
| gene | *mat*K |
| transl_table | 11 |
| translation | |

```
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HLIISANDSNQNPFFGHNNNLYSQMISVGFAVIVEIPFSLRLVSYSQGEEVAKSHNFQSIHSIFPFLED
KFSHLNYVLDVLIPYPIHLEILVQALRYWIKDASSLHLLRFSLYEYCNLKSFITPKKSISIFNPRLFLF
LYNSHVCEYESIFLFLRNQSSYLRSTSSGFFLERINFYGKIEYLVEVFYNDFQNNLWLFKDPFIHFFRY
QGKAILASKDTSLLMNKWKYYFVDLWKYYFYVRSQSGSVRINQLSKYSLDFLGYLSSVRLNTSVVRSQM
LENSFIIDNAMKKLDTRIPIISLIGSLSKAKFCNTLGHPISKPTWSDSSDSDIIDRFVRICRNLSHYHS
GSSKKKSLYRIKYILRLSCVKTLARKHKSTVRAFLKRLGSEFLEEFFTEEEHVFSLIFPRVFLTSRKLY
RVRIWYLDIICINALVNHE
```

note an open reading frame (ORF) located within the intron of the transfer RNA gene for lysine

### 1.3 Chani from Chanthaburi

EntryID : 20070109000746.66658

[Contact Person]

| | |
|---|---|
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| Institution: | Chulalongkorn university |
| Department: | Pharmacognosy |
| Country: | Thailand |
| City: | Bangkok |
| Street: | Phayathai |

Zip code: 10330
Phone: 6622188364
Fax: 6622188357
submitter(s): Suchada Sukrong

[Hold-date]
Immediate release: No
Hold-date: 2007/12/31
Kind of data: General data

[REFERENCE No.1]
Status: In Preparation
Year: 2007
Title: DNA fingerprint analysis of Durian cultivars selected in Thai comparing their polysaccharide contents
author(s): S Sukrong, S Pongsamart

[Sequence]
length: 1509
Sequence:

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   1 atggaggaat ttcaagtata tttagaacta aatagatctc gccgacatga tttcctatac
  61 ccacttattt ttcgggagta tatttatgca cttgctcatg atcatggttt aaataaatcg
 121 atgatttttt tggaaaatca gggttatggt aataaattca gttcactaat tgtgaaacgt
 181 ttaattattc gaatggatca acagaatcat ttgattattt ctgctaatga ttccaaccaa
 241 aatccatttt ttgggcacaa caataattta tattctcaaa tgatatcggt gggatttgca
 301 gtcattgtgg aaattccatt ttccttacga ttagtatctt actcacaagg ggaagaagtc
 361 gcaaaatccc ataatttcca atcaattcat tcaatatttc ctttttaga ggacaaattc
 421 tcacatttaa attatgtgtt agatgtacta ataccttacc ccatccatct agaaatcttg
 481 gttcaagccc ttcgctactg gataaaagat gcttcttctt tgcatttatt acggttctct
 541 ctctacgagt attgtaattt gaagagtttt attactccaa agaaatctat ttctattttt
 601 aatccaagat tattcttgtt cctatataat tctcatgtat gtgaatacga atccattttc
 661 ctttttctcc gtaatcaatc ttcttattta cgatcaacat cttctggatt ctttcttgaa
 721 cgaattaatt tctatggaaa aatagagtat cttgtagaag tcttttataa tgattttcag
 781 aacaacctat ggttgttcaa agacccttc atacattttt ttaggtatca aggaaaggca
 841 attctggcat caaaggatac gtctcttctg atgaataagt ggaaatatta ctttgtcgat
 901 ttatggaaat attattttta cgtgcggtct caatcaggaa gcgtccgtat aaatcaatta
 961 tctaaatatt ctctcgactt tctgggctat ctttcaagtg tgcgattaaa tacttcagtg
1021gtacggagtc aaatgctaga aaattcattt ataatagata atgctatgaa gaagttggat
1081acaagaattc caattatttc tctcattgga tcattgtcta aagcgaaatt ttgtaacaca
1141ttagggcatc ccattagtaa gccgacgtgg tccgattcct ccgattctga tattattgac
1201cgatttgtgc gtatatgcag aaatctttct cattatcaca gtggatcttc aaaaaaaaag
1261agtttgtatc gaataaaata tatacttcgg ctttcttgtg ttaaaacttt ggctcgtaaa
1321cacaaaagta ctgtacgtgc ttttttgaaa agattaggtt cggaattttt ggaagaattc
```

```
1381tttacggaag aagaacatgt tttttctttg atcttcccaa gagtttttt gacttcgcga
1441aagttatata gggtgcgaat ttggtatttg gatattattt gtatcaatgc tctggtcaat
1501catgaatga
```

[Organism]

| | |
|---|---|
| organism | *Durio zibethinus* |
| mol_type | genomic DNA |
| collected_by | Suchada Sukrong |
| collection_date | 2005 |
| country | Thailand:Chantaburi |
| cultivar | Chani |
| identified_by | Suchada Sukrong |
| organelle | plastid:chloroplast |
| specimen_voucher | Chani1 |
| Genetic code | 11 |

[CDS Feature No.1]

| | |
|---|---|
| Location | 1..1509 |
| product | maturaseK |
| gene | matK |
| transl_table | 11 |
| translation | |

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MEEFQVYLELNRSRRHDFLYPLIFREYIYALAHDHGLNKSMIFLENQGYGNKFSSLIVKRLIIRMDQQN
HLIISANDSNQNPFFGHNNNLYSQMISVGFAVIVEIPFSLRLVSYSQGEEVAKSHNFQSIHSIFPFLED
KFSHLNYVLDVLIPYPIHLEILVQALRYWIKDASSLHLLRFSLYEYCNLKSFITPKKSISIFNPRLFLF
LYNSHVCEYESIFLFLRNQSSYLRSTSSGFFLERINFYGKIEYLVEVFYNDFQNNLWLFKDPFIHFFRY
QGKAILASKDTSLLMNKWKYYFVDLWKYYFYVRSQSGSVRINQLSKYSLDFLGYLSSVRLNTSVVRSQM
LENSFIIDNAMKKLDTRIPIISLIGSLSKAKFCNTLGHPISKPTWSDSSDSDIIDRFVRICRNLSHYHS
GSSKKKSLYRIKYILRLSCVKTLARKHKSTVRAFLKRLGSEFLEEFFTEEEHVFSLIFPRVFLTSRKLY
RVRIWYLDIICINALVNHE
```

note an open reading frame (ORF) located within the intron of the transfer RNA gene for lysine

### 1.4 Pauenmuang1 from Chumporn

EntryID : 20070108233400.40228

[Contact Person]

| | |
|---|---|
| E-mail: | ssukrong@hotmail.com |
| Name: | Suchada Sukrong |
| Institution: | Chulalongkorn university |
| Department: | Pharmacognosy |
| Country: | Thailand |
| City: | Bangkok |
| Street: | Phayathai |
| Zip code: | 10330 |
| Phone: | 6622188364 |
| Fax: | 6622188357 |
| submitter(s): | Suchada Sukrong |

[Hold-date]

| | |
|---|---|
| Immediate release: | No |
| Hold-date: | 2007/12/31 |
| Kind of data: | General data |

[REFERENCE No.1]

| | |
|---|---|
| Status: | In Preparation |
| Year: | 2007 |
| Title: | DNA fingerprint analysis of Durian cultivars selected in Thai comparing their polysaccharide contents |
| author(s): | S Sukrong, S Pongsamart |

[Sequence]

| | |
|---|---|
| length: | 1509 |

Sequence:

```
   1 atggaggaat ttcaagtata tttagaacta aatagatctc gccgacatga tttcctatac
  61 ccacttattt ttcgggagta tatttatgca cttgctcatg atcatggttt aaataaatcg
 121 atgatttttt tggaaaatca gggttatggt aataaattca gttcactaat tgtgaaacgt
 181 ttaattattc gaatggatca acagaatcat ttgattattt ctgctaatga ttccaaccaa
 241 aatccatttt ttgggcacaa caataattta tattctcaaa tgatatcggt gggatttgca
 301 gtcattgtgg aaattccatt ttccttacga ttagtatctt actcacaagg ggaagaagtc
 361 gcaaaatccc ataatttcca atcaattcat tcaatatttc ctttttaga ggacaaattc
 421 tcacatttaa attatgtgtt agatgtacta ataccttacc ccatccatct agaaatcttg
 481 gttcaagccc ttcgctactg gataaaagat gcttcttctt tgcatttatt acggttctct
 541 ctctacgagt attgtaattt gaagagtttt attactccaa agaaatctat ttctattttt
 601 aatccaagat tattcttgtt cctatataat tctcatgtat gtgaatacga atccattttc
 661 ctttttctcc gtaatcaatc ttcttattta cgatcaacat cttctggatt ctttcttgaa
 721 cgaattaatt tctatggaaa aatagagtat cttgtagaag tcttttataa tgattttcag
 781 aacaacctat ggttgttcaa agacccttc atacattttt ttaggtatca aggaaaggca
 841 attctggcat caaaggatac gtctcttctg atgaataagt ggaaatatta ctttgtcgat
 901 ttatggaaat attatttta cgtgcggtct caatcaggaa gcgtccgtat aaatcaatta
 961 tctaaatatt ctctcgactt tctgggctat ctttcaagtg tgcgattaaa tacttcagtg
1021gtacggagtc aaatgctaga aaattcattt ataatagata atgctatgaa gaagttggat
1081acaagaattc caattatttc tctcattgga tcattgtcta aagcgaaatt ttgtaacaca
1141ttagggcatc ccattagtaa gccgacgtgg tccgattcct ccgattctga tattattgac
1201cgatttgtgc gtatatgcag aaatctttct cattatcaca gtggatcttc aaaaaaaaag
1261agtttgtatc gaataaaata tatacttcgg ctttcttgtg ttaaaacttt ggctcgtaaa
1321cacaaaagta ctgtacgtgc ttttttgaaa agattaggtt cggaattttt ggaagaattc
1381tttacggaag aagaacatgt tttttctttg atcttcccaa gagtttttt gacttcgcga
1441aagttatata gggtgcgaat ttggtatttg gatattattt gtatcaatgc tctggtcaat
1501catgaatga
```

[Organism]

| | |
|---|---|
| organism | *Durio zibethinus* |
| mol_type | genomic DNA |
| collected_by | Suchada Sukrong |
| collection_date | 2005 |
| country | Thailand:Chumporn |
| cultivar | Pauenmuang |
| identified_by | Suchada Sukrong |
| organelle | plastid:chloroplast |
| specimen_voucher | Pauenmuang |
| Genetic code | 11 |

[CDS Feature No.1]

| | |
|---|---|
| Location | 1..1509 |
| product | maturaseK |
| gene | *mat*K |
| transl_table | 11 |

translation

```
MEEFQVYLELNRSRRHDFLYPLIFREYIYALAHDHGLNKSMIFLENQGYGNKFSSLIVKRLIIRMDQQN
HLIISANDSNQNPFFGHNNNLYSQMISVGFAVIVEIPFSLRLVSYSQGEEVAKSHNFQSIHSIFPFLED
KFSHLNYVLDVLIPYPIHLEILVQALRYWIKDASSLHLLRFSLYEYCNLKSFITPKKSISIFNPRLFLF
LYNSHVCEYESIFLFLRNQSSYLRSTSSGFFLERINFYGKIEYLVEVFYNDFQNNLWLFKDPFIHFFRY
QGKAILASKDTSLLMNKWKYYFVDLWKYYFYVRSQSGSVRINQLSKYSLDFLGYLSSVRLNTSVVRSQM
LENSFIIDNAMKKLDTRIPIISLIGSLSKAKFCNTLGHPISKPTWSDSSDSDIIDRFVRICRNLSHYHS
GSSKKKSLYRIKYILRLSCVKTLARKHKSTVRAFLKRLGSEFLEEFFTEEEHVFSLIFPRVFLTSRKLY
RVRIWYLDIICINALVNHE
```

note an open reading frame (ORF) located within the intron of the transfer RNA gene for lysine

### 1.5 Pauenmuang2_Chumporn

EntryID : 20070108234909.49564

[Contact Person]

| | |
|---|---|
| E-mail: | ssukrong@hotmail.com |
| Name: | Suchada Sukrong |
| Institution: | Chulalongkorn university |
| Department: | Pharmacognosy |
| Country: | Thailand |
| City: | Bangkok |
| Street: | Phayathai |
| Zip code: | 10330 |
| Phone: | 6622188364 |
| Fax: | 6622188357 |
| submitter(s): | Suchada Sukrong |

[Hold-date]

| | |
|---|---|
| Immediate release: | No |
| Hold-date: | 2007/12/31 |
| Kind of data: | General data |

[REFERENCE No.1]

| | |
|---|---|
| Status: | In Preparation |
| Year: | 2007 |
| Title: | DNA fingerprint analysis of Durian cultivars selected in Thai comparing their polysaccharide contents |
| author(s): | S Sukrong, S Pongsamart |

[Sequence]

length: 1509

Sequence:

```
  1 atggaggaat ttcaagtata tttagaacta aatagatctc gccgacatga tttcctatac
 61 ccacttattt ttcgggagta tatttatgca cttgctcatg atcatggttt aaataaatcg
121 atgatttttt tggaaaatca gggttatggt aataaattca gttcactaat tgtgaaacgt
181 ttaattattc gaatggatca acagaatcat ttgattattt ctgctaatga ttccaaccaa
241 aatccatttt ttgggcacaa caataattta tattatcaaa tgatatcggt gggatttgca
301 gtcattgtgg aaattccatt ttccttacga ttagtatctt actcacaagg ggaagaagtc
361 gcaaaatccc ataatttcca atcaattcat tcaatatttc ctttttaga ggacaaattc
421 tcacatttaa attatgtgtt agatgtacta ataccttacc ccatccatct agaaatcttg
481 gttcaagccc ttcgctactg gataaaagat gcttcttctt tgcatttatt acggttctct
541 ctctacgagt attgtaattt gaagagtttt attactccaa agaaatctat ttctattttt
601 aatccaagat tattcttgtt cctatataat tctcatgtat gtgaatacga atccattttc
661 ctttttctcc gtaatcaatc ttcttattta cgatcaacat cttctggatt ctttcttgaa
721 cgaattaatt tctatggaaa aatagagtat cttgtagaag tcttttataa tgattttcag
781 aacaacctat ggttgttcaa agacccttc atacattttt ttaggtatca aggaaaggca
841 attctggcat caaaggatac gtctcttctg atgaataagt ggaaatatta ctttgtcgat
901 ttatggaaat attatttta cgtgcggtct caatcaggaa gcgtccgtat aaatcaatta
961 tctaaatatt ctctcgactt tctgggctat ctttcaagtg tgcgattaaa tacttcagtg
1021gtacggagtc aaatgctaga aaattcattt ataatagata atgctatgaa gaagttggat
1081acaagaattc caattatttc tctcattgga tcattgtcta aagcgaaatt ttgtaacaca
1141ttagggcatc ccattagtaa gccgacgtgg tccgattcct ccgattctga tattattgac
1201cgatttgtgc gtatatgcag aaatctttct cattatcaca gtggatcttc aaaaaaaaag
1261agtttgtatc gaataaaata tatacttcgg ctttcttgtg ttaaaacttt ggctcgtaaa
1321cacaaaagta ctgtacgtgc ttttttgaaa agattaggtt cggaattttt ggaagaattc
1381tttacggaag aagaacatgt tttttctttg atcttcccaa gagtttttt gacttcgcga
1441aagttatata gggtgcgaat ttggtatttg gatattattt gtatcaatgc tctggtcaat
1501catgaatga
```

[Organism]

| | |
|---|---|
| organism | *Durio zibethinus* |
| mol_type | genomic DNA |
| collected_by | Suchada Sukrong |
| collection_date | 2005 |
| country | Thailand:Chumporn |
| cultivar | Pauenmuang |
| identified_by | Suchada Sukrong |

| | |
|---|---|
| organelle | plastid:chloroplast |
| specimen_voucher | Pauenmuang |
| Genetic code | 11 |

[CDS Feature No.1]

| | |
|---|---|
| Location | 1..1509 |
| product | maturaseK |
| gene | *mat*K |
| transl_table | 11 |
| translation | |

```
MEEFQVYLELNRSRRHDFLYPLIFREYIYALAHDHGLNKSMIFLENQGYGNKFSSLIVKRLIIRMDQQN
HLIISANDSNQNPFFGHNNNLYYQMISVGFAVIVEIPFSLRLVSYSQGEEVAKSHNFQSIHSIFPFLED
KFSHLNYVLDVLIPYPIHLEILVQALRYWIKDASSLHLLRFSLYEYCNLKSFITPKKSISIFNPRLFLF
LYNSHVCEYESIFLFLRNQSSYLRSTSSGFFLERINFYGKIEYLVEVFYNDFQNNLWLFKDPFIHFFRY
QGKAILASKDTSLLMNKWKYYFVDLWKYYFYVRSQSGSVRINQLSKYSLDFLGYLSSVRLNTSVVRSQM
LENSFIIDNAMKKLDTRIPIISLIGSLSKAKFCNTLGHPISKPTWSDSSDSDIIDRFVRICRNLSHYHS
GSSKKKSLYRIKYILRLSCVKTLARKHKSTVRAFLKRLGSEFLEEFFTEEEHVFSLIFPRVFLTSRKLY
RVRIWYLDIICINALVNHE
```

note an open reading frame (ORF) located within the intron of the transfer RNA gene for lysine

### 1.6 Monthong_Chumporn

EntryID : 20070108223220.40395

[Contact Person]

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| Department: | Pharmacognosy |
| Country: | Thailand |
| City: | Bangkok |
| Street: | Phayathai |
| Zip code: | 10330 |
| Phone: | 6622188364 |
| Fax: | 6622188357 |

submitter(s): Suchada Sukrong

[Hold-date]

Immediate release: No

Hold-date: 2007/07/08

Kind of data: General data

[REFERENCE No.1]

Status: In Preparation

Year: 2007

Title: DNA fingerprint analysis of Durian cultivars selected in Thai comparing their polysaccharide contents

author(s): s Sukrong, S Pongsamart

[Sequence]

length: 1509

Sequence:

```
   1 atggaggaat ttcaagtata tttagaacta aatagatctc gccgacatga tttcctatac
  61 ccacttattt ttcgggagta tatttatgca cttgctcatg atcatggttt aaataaatcg
 121 atgatttttt tggaaaatca gggttatggt aataaattca gttcactaat tgtgaaacgt
 181 ttaattattc gaatggatca acagaatcat ttgattattt ctgctaatga ttccaaccaa
 241 aatccatttt ttgggcacaa caataattta tattctcaaa tgatatcggt gggatttgca
 301 gtcattgtgg aaattccatt ttccttacga ttagtatctt actcacaagg ggaagaagtc
 361 gcaaaatccc ataatttcca atcaattcat tcaatatttc ctttttaga ggacaaattc
 421 tcacatttaa attatgtgtt agatgtacta ataccttacc ccatccatct agaaatcttg
 481 gttcaagccc ttcgctactg gataaaagat gcttcttctt tgcatttatt acggttctct
 541 ctctacgagt attgtaattt gaagagtttt attactccaa agaaatctat ttctattttt
 601 aatccaagat tattcttgtt cctatataat tctcatgtat gtgaatacga atccattttc
 661 ctttttctcc gtaatcaatc ttcttattta cgatcaacat cttctggatt ctttcttgaa
 721 cgaattaatt tctatggaaa aatagagtat cttgtagaag tcttttataa tgattttcag
 781 aacaacctat ggttgttcaa agacccttc atacattttt ttaggtatca aggaaaggca
 841 attctggcat caaaggatac gtctcttctg atgaataagt ggaaatatta ctttgtcgat
 901 ttatggaaat attatttta cgtgcggtct caatcaggaa gcgtccgtat aaatcaatta
 961 tctaaatatt ctctcgactt tctgggctat ctttcaagtg tgcgattaaa tacttcagtg
1021gtacggagtc aaatgctaga aaattcattt ataatagata atgctatgaa gaagttggat
1081acaagaattc caattatttc tctcattgga tcattgtcta aagcgaaatt ttgtaacaca
1141ttagggcatc ccattagtaa gccgacgtgg tccgattcct ccgattctga tattattgac
1201cgatttgtgc gtatatgcag aaatctttct cattatcaca gtggatcttc aaaaaaaaag
1261agtttgtatc gaataaaata tatacttcgg ctttcttgtg ttaaaacttt ggctcgtaaa
1321cacaaaagta ctgtacgtgc ttttttgaaa agattaggtt cggaattttt ggaagaattc
1381tttacggaag aagaacatgt tttttctttg atcttcccaa gagtttttt gacttcgcga
1441aagttatata gggtgcgaat ttggtatttg gatattattt gtatcaatgc tctggtcaat
1501catgaatga
```

[Organism]

| | |
|---|---|
| organism | *Durio zibethinus* |
| mol_type | genomic DNA |
| collected_by | Suchada Sukrong |
| collection_date | 2005 |
| country | Thailand:Chumporn |
| cultivar | Monthong |
| identified_by | Suchada Sukrong |
| organelle | plastid:chloroplast |
| specimen_voucher | Monthong |
| Genetic code | 11 |

[CDS Feature No.1]

| | |
|---|---|
| Location | 1..1509 |
| product | maturaseK |
| gene | *mat*K |
| transl_table | 11 |
| translation | |

```
MEEFQVYLELNRSRRHDFLYPLIFREYIYALAHDHGLNKSMIFLENQGYGNKFSSLIVKRLIIRMDQQN
HLIISANDSNQNPFFGHNNNLYSQMISVGFAVIVEIPFSLRLVSYSQGEEVAKSHNFQSIHSIFPFLED
KFSHLNYVLDVLIPYPIHLEILVQALRYWIKDASSLHLLRFSLYEYCNLKSFITPKKSISIFNPRLFLF
LYNSHVCEYESIFLFLRNQSSYLRSTSSGFFLERINFYGKIEYLVEVFYNDFQNNLWLFKDPFIHFFRY
QGKAILASKDTSLLMNKWKYYFVDLWKYYFYVRSQSGSVRINQLSKYSLDFLGYLSSVRLNTSVVRSQM
LENSFIIDNAMKKLDTRIPIISLIGSLSKAKFCNTLGHPISKPTWSDSSDSDIIDRFVRICRNLSHYHS
GSSKKKSLYRIKYILRLSCVKTLARKHKSTVRAFLKRLGSEFLEEFFTEEEHVFSLIFPRVFLTSRKLY
RVRIWYLDIICINALVNHE
```

note an open reading frame (ORF) located within the intron of the transfer RNA gene for lysine

### 1.7 Chani_Chumporn

EntryID : 20070108231721.41046

[Contact Person]

| | |
|---|---|
| E-mail: | ssukrong@hotmail.com |
| Name: | Suchada Sukrong |
| Institution: | Chulalongkorn university |

Department: Pharmacognosy
Country: Thailand
City: Bangkok
Street: Phayathai
Zip code: 10330
Phone: 6622188364
Fax: 6622188357
submitter(s): Suchada Sukrong

[Hold-date]
Immediate release: No
Hold-date: 2007/12/31
Kind of data: General data

[REFERENCE No.1]
Status: In Preparation
Year: 2007
Title: DNA fingerprint analysis of Durian cultivars selected in Thai comparing their polysaccharide contents
author(s): S Sukrong, S Pongsamart

[Sequence]
length: 1509
Sequence:

```
  1 atggaggaat ttcaagtata tttagaacta aatagatctc gccgacatga tttcctatac
 61 ccacttattt ttcgggagta tatttatgca cttgctcatg atcatggttt aaataaatcg
121 atgatttttt tggaaaatca gggttatggt aataaattca gttcactaat tgtgaaacgt
181 ttaattattc gaatggatca acagaatcat ttgattattt ctgctaatga ttccaaccaa
241 aatccatttt ttgggcacaa caataattta tattctcaaa tgatatcggt gggatttgca
301 gtcattgtgg aaattccatt ttccttacga ttagtatctt actcacaagg ggaagaagtc
361 gcaaaatccc ataatttcca atcaattcat tcaatatttc ctttttaga ggacaaattc
421 tcacatttaa attatgtgtt agatgtacta ataccttacc ccatccatct agaaatcttg
481 gttcaagccc ttcgctactg gataaaagat gcttcttctt tgcatttatt acggttctct
541 ctctacgagt attgtaattt gaagagtttt attactccaa agaaatctat ttctattttt
601 aatccaagat tattcttgtt cctatataat tctcatgtat gtgaatacga atccattttc
661 ctttttctcc gtaatcaatc ttcttattta cgatcaacat cttctggatt ctttcttgaa
721 cgaattaatt tctatggaaa aatagagtat cttgtagaag tcttttataa tgattttcag
781 aacaacctat ggttgttcaa agacccttc atacattttt ttaggtatca aggaaaggca
841 attctggcat caaaggatac gtctcttctg atgaataagt ggaaatatta ctttgtcgat
901 ttatggaaat attatttta cgtgcggtct caatcaggaa gcgtccgtat aaatcaatta
961 tctaaatatt ctctcgactt tctgggctat ctttcaagtg tgcgattaaa tacttcagtg
```

```
1021gtacggagtc aaatgctaga aaattcattt ataatagata atgctatgaa gaagttggat
1081acaagaattc caattatttc tctcattgga tcattgtcta aagcgaaatt ttgtaacaca
1141ttagggcatc ccattagtaa gccgacgtgg tccgattcct ccgattctga tattattgac
1201cgatttgtgc gtatatgcag aaatctttct cattatcaca gtggatcttc aaaaaaaaag
1261agtttgtatc gaataaaata tatacttcgg ctttcttgtg ttaaaacttt ggctcgtaaa
1321cacaaaagta ctgtacgtgc ttttttgaaa agattaggtt cggaatttt ggaagaattc
1381tttacggaag aagaacatgt tttttctttg atcttcccaa gagtttttt gacttcgcga
1441aagttatata gggtgcgaat ttggtatttg gatattattt gtatcaatgc tctggtcaat
```
1501catgaatga

[Organism]

| | |
|---|---|
| organism | *Durio zibethinus* |
| mol_type | genomic DNA |
| collected_by | Suchada Sukrong |
| collection_date | 2005 |
| country | Thailand:Chumporn |
| cultivar | Chani |
| identified_by | Suchada Sukrong |
| organelle | plastid:chloroplast |
| specimen_voucher | Chani |
| Genetic code | 11 |

[CDS Feature No.1]

| | |
|---|---|
| Location | 1..1509 |
| product | maturaseK |
| gene | *mat*K |
| transl_table | 11 |
| translation | |

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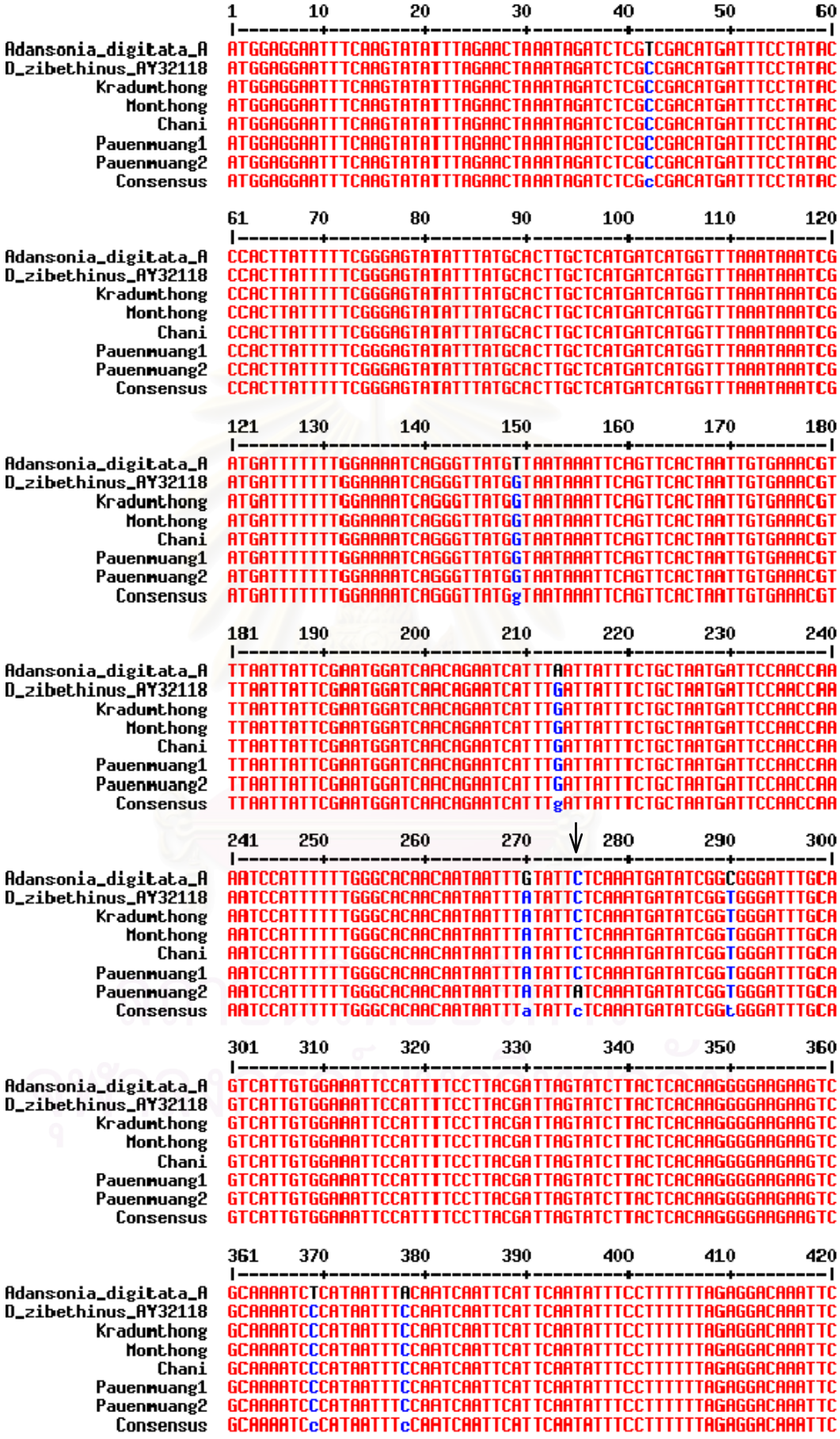
note an open reading frame (ORF) located within the intron of the transfer RNA gene for lysine

# APPENDIX H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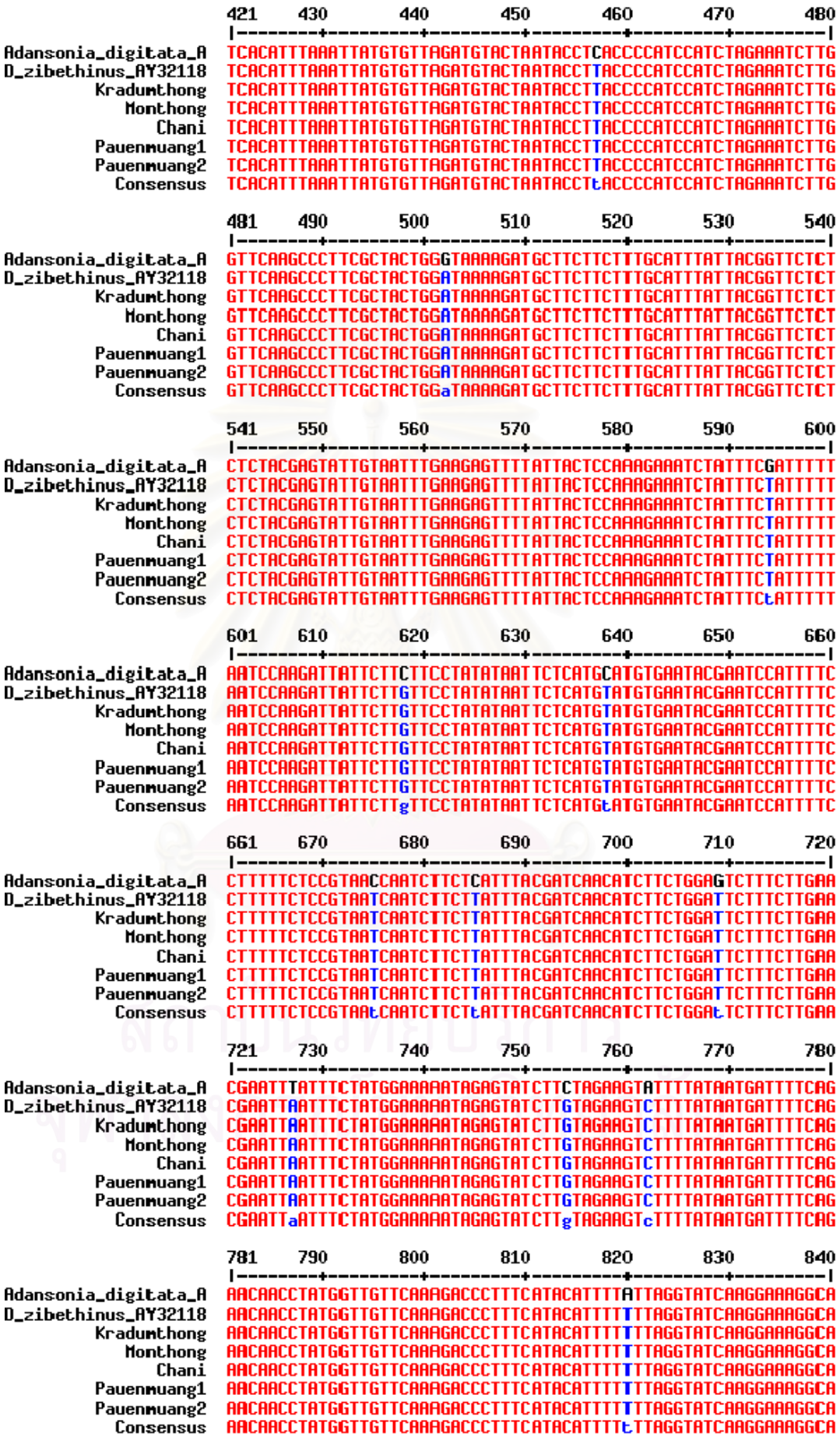
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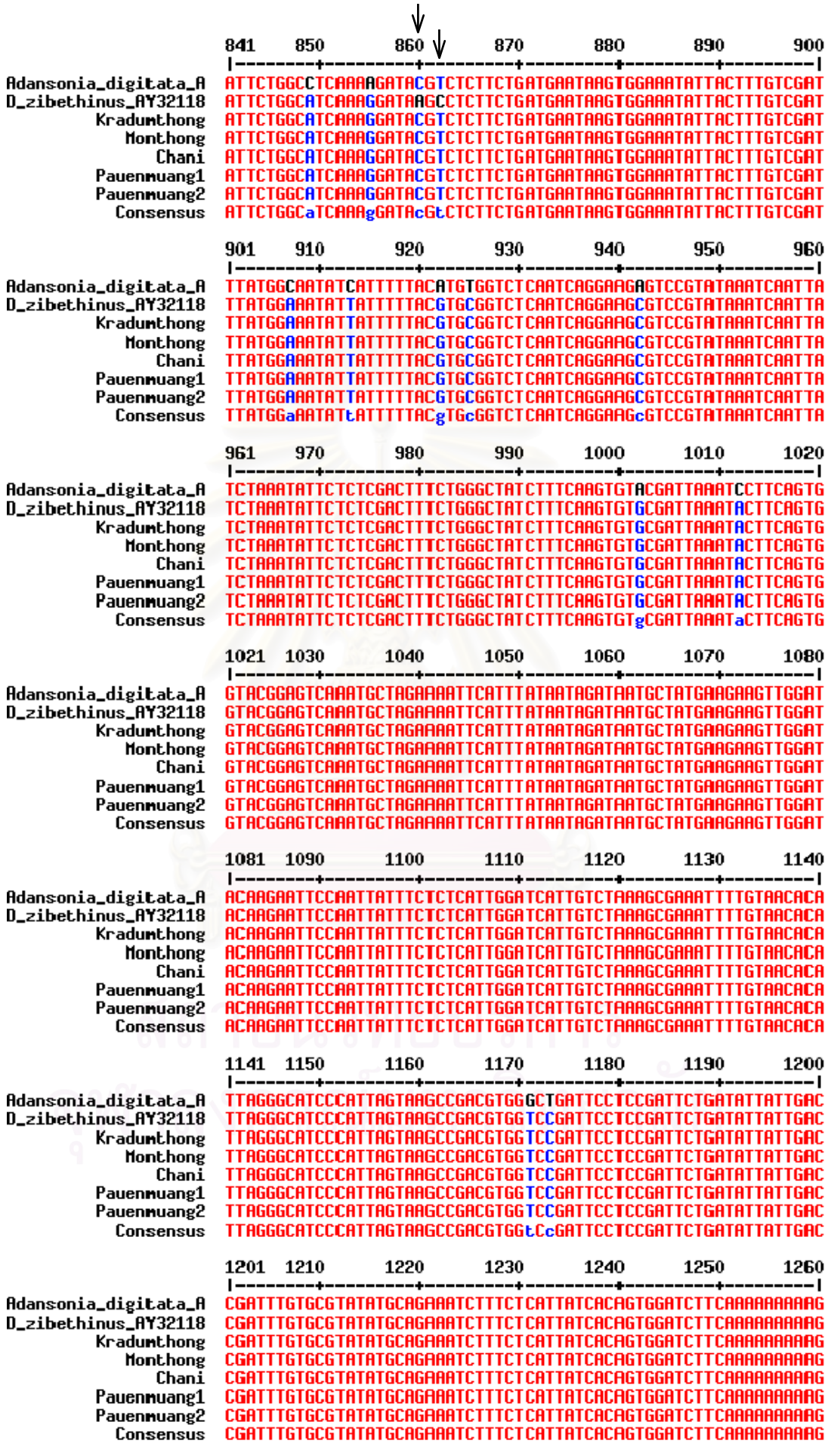
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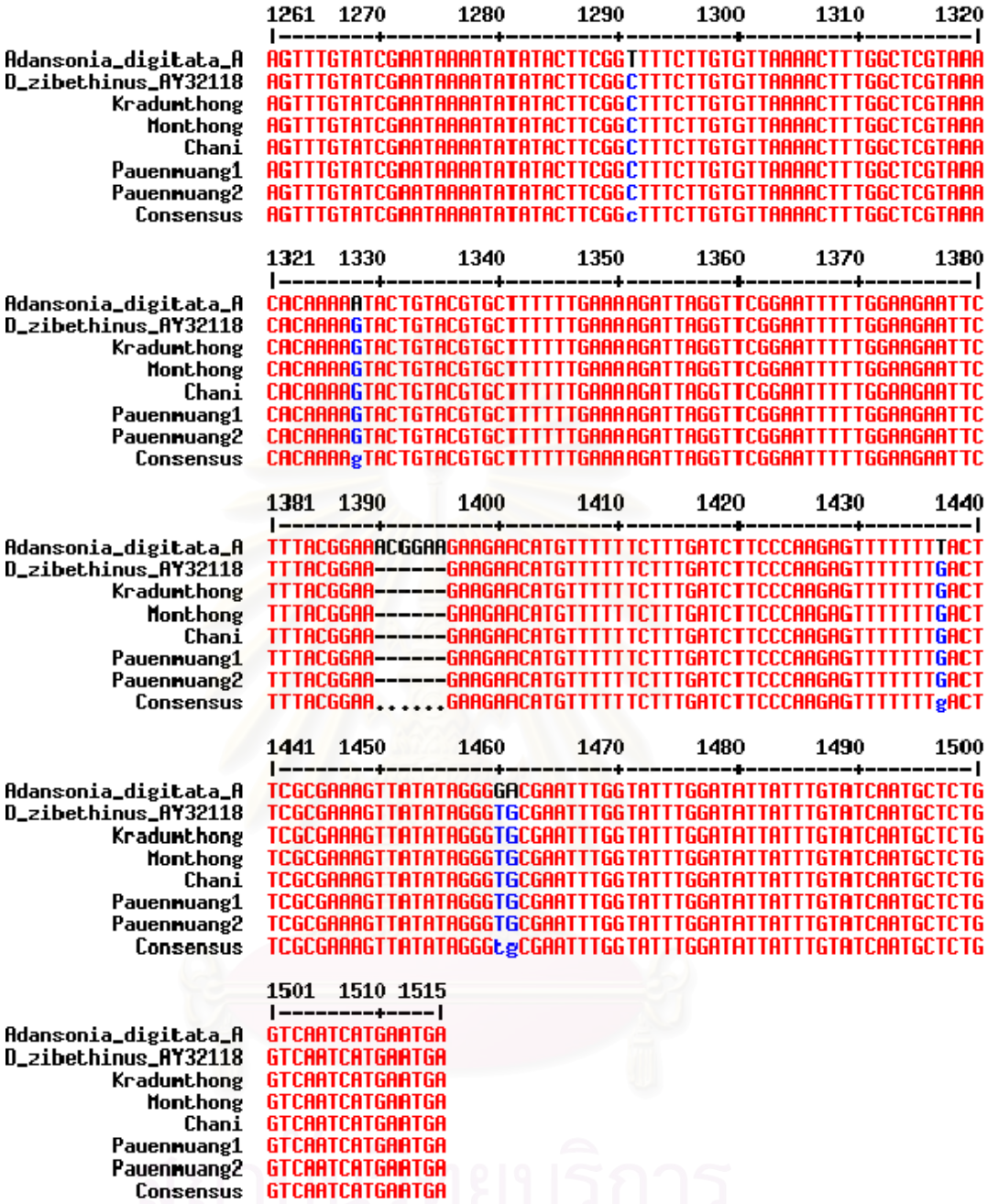


Figure H1. The sequence alignment of durian cultivars, ‘Kradumthong’ ‘Monthong’, ‘Chani’, ‘Pauenmuang’1 and ‘Pauenmuang’2 was compared to *mat*K gene of *Adansonia digitata* (AY321168) in GenBank database as out group samples.

# VITA

Miss Onanong Nuchuchua was born on March 28, 1982 in Bangkok, Thailand. She graduated Bechelor's Degree in Biochemistry in 2003 from Department of Biochemistry, Faculty of Science, Chulalongkorn University.