UNIVERSITI PUTRA MALAYSIA

DISTRIBUTION AND CHARACTERISTICS OF MINERAL INCLUSIONS IN Hopea odorata Roxb. AND Dryobalanops aromatic Geartn. f.

TOONG WEI CHING

FH 2012 21
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Master degree of Science

DISTRIBUTION AND CHARACTERISTICS OF MINERAL INCLUSIONS IN *Hopea odorata* Roxb. AND *Dryobalanops aromatic* Geartn. f.

By

TOONG WEI CHING

NOVEMBER 2012

Chairman: Professor Dr. Mohd. Hamami bin Sahri, PhD

Faculty: Faculty of Forestry

This study was to investigate the occurrence, distribution, morphology, and dimension of mineral inclusions (calcium crystal and siliceous inclusion) in *H. odorata* and *D. aromatic*. Specifically, this study also attempted to compare the different amount of calcium crystals with regard to its growing and age of the tree. These species were selected due to deposition of mineral inclusion which leads to caused wearing in the woodworking cutting tools which contributed to processing economic.

Siliceous inclusion deposited in *D. aromatic* has smooth and rough surface with globular, aggregate and irregular shape, and its size was ranging from 1.31 to 23.1 µm. Calcium crystal deposited was in druse form and that found
to has sizes in ranging from 7.9 to 23.1 µm. The siliceous inclusion commonly deposited in wood ray and phloem ray, and even pith, but occasionally in axial parenchyma of wood and phloem. Pith area in the branch was lacking of mineral inclusions. The amount and size of siliceous inclusion was increased toward inner part of secondary xylem. The size was decreased, while the amount was increased with the tree height. At the pith this inclusion showed a decreased in the amount and size with an increased in tree height. Calcium crystal coexisted with siliceous inclusion in phloem ray and the cortex of the barks, and commonly found in separate cells. Mineral inclusions in the trunk bark were decreased in size with an increased in tree height, while in the root bark was decreased with an increasing in depth within the soil. Siliceous inclusion was deposited in epidermis while the crystal was deposited in palisade and spongy mesophyll of the leaf blade, and in cortex of the midrib and petiole, but occasionally in the parenchyma cell adjacent to vascular bundles of the petiole. Crystal found in leaf blade had smaller size than in petiole.

*H. odorata* deposited the calcium crystal in form of druse and prismatic with four to eight planes, and with size ranging from 9.8 to 45.4 µm. Prismatic crystal was deposited in wood ray and the pith. The amount and the size of the crystals were increased toward inner part of secondary xylem, and decreased with an increasing in tree height. Druse crystal was commonly found in the phloem ray and the cortex, occasionally also found in wood ray.
and axial phloem parenchyma. The size was decreased in size with an increasing tree height.

Crystals in young *H. odorata*’s tree showed druse and prismatic form with four to ten planes in shape, and the size ranging from 7.4 to 53.3 µm. Both crystal forms were coexisting in wood ray, but druse crystal deposited in inflated ray cell or inflated chambered ray cell. Prismatic crystal was increased in size with an increasing tree height, while druse crystal size distribution was showed a reverse pattern. The crystal found in the pith was prismatic and showing decreasing size with an increased tree height. In the branch wood, the crystal was absent. Prismatic crystal was deposited in ray cells of root wood, but druse crystal was absent. The size of crystal in root wood was decreased as it goes deeper into soil. Druse crystals was deposited in spongy and palisade mesophyll of leaf blade, in the cortex of midrib and parenchyma and cortex of petiole in which size in petiole was larger than in leaf blade. Tree planted in Selangor which was older than tree planted in Perlis was showed fewer amount of crystals than in Perlis even in the young tree.

The finding from this study, occurrence of mineral inclusions has a significant in the taxonomical characteristic and wood identification. The dimension and distribution pattern can serve as basis for investigation into wearing of woodworking cutting tools. It also filled the gap of knowledge of mineral inclusions in various tree parts.
Abstrack tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains.

TABURAN DAN CIRI-CIRI INKLUSI GALIAN DALAM Hopea odorata Roxb. DAN Dryobalanops aromatic Gaertn. f.

Oleh
TOONG WEI CHING

NOVEMBER 2012

Pengerusi: Profesor Dr. Mohd. Hamami bin Sahri, PhD
Fakulti: Fakulti Perhutanan

Penyelidikan ini dijalankan untuk mengkaji kedapatan, taburan, morfologi, dan dimensi inklusi galian seperti kristal berkalsium dan inklusi bersilika dalam H. odorata dan D. aromatic. Secara khususnya, penyelidikan ini membandingkan jumlah kalsium yang berlainan dan jumlah kristal yang mendap berhubung kait dengan faktor pengaruh iaitu lokasi dan umur pokok berkenaan.

Inklusi bersilika yang termendap dalam D. aromatic mempunyai permukaan yang licin atau kasar dengan kewujudan dalam bentuk bulat, berkumpulan atau tidak teratur. Saiznya berjulat antara 1.31 hingga 23.1 μm. Kristal

*H. odorata* memendapkan kristal berkalsium dalam bentuk druse dan prisma yang mempunyai 4 hingga 8 permukaan serta dengan saiz antara 9.8 hingga

kekurangan berbanding dengan pokok yang ditanamkan di Perlis walaupun saiznya adalah kecil.

Dari penyelidikan ini, ia dapat mengimpulkan kewujudan inklusi galian yang mempunyai kepentingan dalam taxonomi tumbuhan. Sementara itu, dimensi dan taburannya boleh digunakan sebagai asas untuk mengkaji kehausan dan sebab ketumpulan kepada alat-alat pemotong kayu. Malahan, ini juga mampu mengisi kekurangan pengetahuan mengenai inklusi galian dalam bahagian pokok.
ACKNOWLEDGEMENTS

I desire to express my sincere appreciation to Prof. Dr. Mohd. Hamami Sahri, who is the chairman of my supervisory committee, for his mentoring and precious suggestions throughout this study. My grateful thanks also to Assoc. Prof. Dr. Mohd. Zin Jusoh, who is members of my advisory committee, for his suggestions and comments.

My warmest regards due to Prof. Dr. Tadashi Nobuchi for his concept and invaluable suggestion which made this study possible. I am also like to thank Dr. Rasmina Halis for her suggestion in improving the chemical analysis of this study. Special thanks are due to Dr. Mohamad Roslan Mohamad Kasim for his guidance in statistical analysis.

I desire to convey my deepest appreciation to my dearest family members for their warm support and encouragement. My appreciation from bottom of my heart to Son Mei Teng who consistently and unfailingly in giving encouragement and always for be there for me during my difficulty. I also wish to thanks to my friend Abdirizak for giving me hope and strength.
I certify that a Thesis Examination Committee has met on (insert 01 November 2012) to conduct the final examination of Toong Wei Ching on his thesis entitled “Distribution and characteristics of mineral inclusions in Hopea odorata and Dryobalanops aromatica” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the master of science.

Members of the Thesis Examination Committee were as follows:

Dr. H’ng Paik San
Associate Professor
Department of Forest Production, Faculty of Forestry
Universiti Putra Malaysia
(Chairman)

Dr. Edi Suhaimi Bakar
Associate Professor
Department of Forest Management, Faculty of Forestry
Universiti Putra Malaysia
(Internal Examiner)

Dr. Rasmina binti Halis
Senior Lecturer
Department of Forest Production, Faculty of Forestry
Universiti Putra Malaysia
(Internal Examiner)

Dr. Othman bin Sulaiman
Professor
Division of Bioresource, Paper & Coating Technology
Universiti Sains Malaysia
Malaysia
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

x
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the Master degree of Science. The members of the supervisory Committee were as follows:

Mohd Hamami bin Sahri, PhD  
Professor  
Faculty of Forestry  
Universiti Putra Malaysia  
(Chairman)

Mohd Zin bin Jusoh, PhD  
Associate Professor  
Faculty of Forestry  
Universiti Putra Malaysia  
(Member)

__________________________________  
BUJANG BIN KIM HUAT, PhD  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia  

Date:
DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____________________
TOONG WEI CHING

Date: 1 NOVEMBER 2012
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 3.1.</td>
<td>Description of Sampled Trees Collection on the Fields</td>
<td>30</td>
</tr>
<tr>
<td>Table 4.1.</td>
<td>Occurrence and morphology of mineral inclusions in the leaf of <em>D. aromatica.</em></td>
<td>51</td>
</tr>
<tr>
<td>Table 4.2.</td>
<td>Occurrence and morphology of mineral inclusions in the branch wood, trunk and root of <em>D. aromatica.</em></td>
<td>52</td>
</tr>
<tr>
<td>Table 4.3.</td>
<td>Occurrence and morphology of mineral inclusions in the bark of trunk and root of <em>D. aromatica.</em></td>
<td>53</td>
</tr>
<tr>
<td>Table 4.4.</td>
<td>Occurrence and morphology of mineral inclusions in the trunk of <em>H. odorata</em> (Selangor).</td>
<td>73</td>
</tr>
<tr>
<td>Table 4.5.</td>
<td>Occurrence and Morphology of calcium crystals in the wood of trunk and branch of <em>H. odorata</em> (Perlis’s Juvenile).</td>
<td>74</td>
</tr>
<tr>
<td>Table 4.6.</td>
<td>Occurrence and morphology of calcium crystal in the bark of <em>H. odorata</em> (Selangor).</td>
<td>82</td>
</tr>
<tr>
<td>Table 4.7.</td>
<td>Occurrence and morphology of calcium crystals in the leaf of <em>H. odorata</em> (Perlis’s Juvenile).</td>
<td>85</td>
</tr>
<tr>
<td>Table 4.8.</td>
<td>Occurrence and morphology of calcium crystal in the root of <em>H. odorata</em> (Perlis’s Juvenile).</td>
<td>88</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. 2.1</td>
<td>Anatomical characteristic on transverse plane and wood of <em>H. odorata</em>.</td>
<td>9</td>
</tr>
<tr>
<td>Fig. 2.2</td>
<td>Photographs of <em>H. odorata</em>.</td>
<td>10</td>
</tr>
<tr>
<td>Fig. 2.3</td>
<td>Anatomical characteristic on transverse plane and wood of <em>D. aromatica</em>.</td>
<td>12</td>
</tr>
<tr>
<td>Fig. 2.4</td>
<td>Photographs of <em>D. aromatica</em>.</td>
<td>13</td>
</tr>
<tr>
<td>Fig. 2.5</td>
<td>Diagrammatic presentation of siliceous inclusion.</td>
<td>15</td>
</tr>
<tr>
<td>Fig. 2.6</td>
<td>Scanning electron photomicrograph of morphology of siliceous inclusion.</td>
<td>15</td>
</tr>
<tr>
<td>Fig. 2.7</td>
<td>Conventional light photomicrograph of morphology of calcium crystal.</td>
<td>23</td>
</tr>
<tr>
<td>Fig. 2.8</td>
<td>Scanning electron photomicrographs of fresh and isolated calcium oxalate crystal from plants.</td>
<td>24</td>
</tr>
<tr>
<td>Fig. 3.1</td>
<td>Map of Perlis and Selangor State on Peninsular Malaysia.</td>
<td>29</td>
</tr>
<tr>
<td>Fig. 3.2</td>
<td>Schematic Illustration of Specimens Collection from Various Tree Parts and within in a Disc.</td>
<td>31</td>
</tr>
<tr>
<td>Fig. 3.3</td>
<td>Procedure of staining and mounting for conventional section.</td>
<td>34</td>
</tr>
<tr>
<td>Fig. 3.4</td>
<td>Procedure of staining and mounting for resin-embedded section.</td>
<td>35</td>
</tr>
<tr>
<td>Fig. 3.5</td>
<td>Procedure of sample preparation for scanning electron microscope and energy disperse X-ray.</td>
<td>36</td>
</tr>
<tr>
<td>Fig. 3.6</td>
<td>Procedure of histochemistry for siliceous inclusion and calcium crystal.</td>
<td>37</td>
</tr>
<tr>
<td>Fig. 3.7</td>
<td>Procedure of histochemistry for lipid.</td>
<td>38</td>
</tr>
</tbody>
</table>
Fig. 4.1. Conventional light photomicrographs present the various size of siliceous inclusion in comparing with size of starch grain of *D. aromatica* from radial section.

Fig. 4.2. Conventional light photomicrographs of siliceous inclusion in series of morphologies of *D. aromatica* from radial and transverse section.

Fig. 4.3. Conventional and partially polarized light photomicrographs of siliceous inclusions and calcium crystals which were occasionally occurred in more than singly per cell in wood ray parenchyma, phloem ray parenchyma and leaf of *D. aromatica* from radial and transverse section.

Fig. 4.4. Partially polarized light photomicrographs present the various size of calcium crystals in form of druse of *D. aromatica* from transverse section.

Fig. 4.5. Scanning electron photomicrograph and energy dispersive X-ray microanalysis of siliceous inclusion in wood of *D. aromatica*.

Fig. 4.6. Partially polarized light photomicrographs of druse crystal in which treated with hydrochloric acid of *D. aromatica* from transverse section.

Fig. 4.7. Conventional light photomicrographs of siliceous inclusion and calcium crystal in wood and pith of *D. aromatica* from radial and transverse section.

Fig. 4.8. Conventional and partially polarized light photomicrographs of siliceous inclusion and calcium crystal in bark of *D. aromatica* from transverse section.

Fig. 4.9. Partially polarized light photomicrographs of siliceous inclusion and calcium crystal in leaf of *D. aromatica* from transverse section.

Fig. 4.10. Comparing dimension of siliceous inclusions in radial direction of trunk at different level of *D. aromatica*.

Fig. 4.11. Variation in dimension of siliceous inclusion and calcium crystal in the various tissues of *D. aromatica*.
Fig. 4.12. Variation in dimension of siliceous inclusion and calcium crystal in the bark of trunk and root of *D. aromatica*.

Fig. 4.13. Variation in distribution of siliceous inclusion in different level of trunk of *D. aromatica*.

Fig. 4.14. Conventional light photomicrographs of prismatic crystals with series of morphologies of *H. odorata* from radial section.

Fig. 4.15. Partially polarized light photomicrographs of druse crystal which varying in term of degree of spikiness from transverse section of *H. odorata*.

Fig. 4.16. Scanning electron photomicrographs of druse crystal in crystal idioblast at palisade mesophyll layer of leaf blade from transverse section of *H. odorata*.

Fig. 4.17. Scanning electron photomicrographs of quadrilateral prismatic crystal in wood ray parenchyma from radial section of *H. odorata*.

Fig. 4.18. Conventional and partially polarized light photomicrographs of calcium crystals deposited more than singly per cell in wood and bark of *H. odorata* (Selangor) and *H. odorata* (Perlis’s Juvenile) from radial and transverse section.

Fig. 4.19. Energy disperse X-ray microanalysis of prismatic and druse crystals in *H. odorata*.

Fig. 4.20. Partially polarized light photomicrographs of druse crystal treated with hydrochloric acid and the control from transverse section of *H. odorata*.

Fig. 4.21. Conventional light photomicrographs of calcium crystals in secondary xylem and pith of *H. odorata* (Selangor) from radial and transverse section.

Fig. 4.22. Conventional light photomicrographs of prismatic crystal in secondary xylem and pith of *H. odorata* (Perlis's Juvenile) from radial and transverse section.

Fig. 4.23. Conventional light photomicrographs of druse crystal in ray parenchyma of *H. odorata* (Perlis's Juvenile) from radial section.
Fig. 4.24. Full and partially polarized light photomicrographs of calcium crystal in branch of *H. odorata* (Perlis’s Juvenile) from transverse section.

Fig. 4.25. Partially polarized light photomicrographs of druse crystals in bark of *H. odorata* (Selangor) from transverse section.

Fig. 4.26. Partially polarized light photomicrographs of druse crystals in blade, midrib and petiole of leaf of *H. odorata* (Perlis’s Juvenile) from transverse section.

Fig. 4.27. Full and partially polarized light photomicrographs of calcium crystal in root of *H. odorata* (Perlis’s Juvenile) from radial and transverse section.

Fig. 4.28. Comparing the dimension of calcium crystal in radial direction of trunk of a level and different level of *H. odorata* (Selangor).

Fig. 4.29. Variation in dimension of calcium crystals in the bark of trunk of *H. odorata* (Selangor).

Fig. 4.30. Dimension of prismatic crystal in secondary xylem and pith from root to trunk of *H. odorata* (Perlis’s Juvenile).

Fig. 4.31. Dimension of druse crystal in secondary xylem of different trunk level of *H. odorata* (Perlis’s Juvenile).

Fig. 4.32. Dimensional variation of calcium crystals in various parts of tree of *H. odorata* (Perlis’s Juvenile).

Fig. 4.33. Comparing the distribution of calcium crystal within a trunk level and different trunk level of *H. odorata* (Selangor).

Fig. 4.34. Partially polarized and conventional light photomicrographs of hesperidin crystal in phloem ray parenchyma.

Fig. 4.35. Comparison of distributional variation of calcium crystal in trunk of tree grew in Perlis and Selangor.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter</td>
</tr>
<tr>
<td>EDX</td>
<td>energy dispersive X-ray</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>IAWA</td>
<td>International Association of Wood Anatomists</td>
</tr>
<tr>
<td>IUCN</td>
<td>International Union for Conservation of Nature</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>m</td>
<td>meter</td>
</tr>
<tr>
<td>m²</td>
<td>square meter</td>
</tr>
<tr>
<td>m³</td>
<td>cubic meter</td>
</tr>
<tr>
<td>μm</td>
<td>micrometer</td>
</tr>
<tr>
<td>mm</td>
<td>millimeter</td>
</tr>
<tr>
<td>%</td>
<td>percentage</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscope</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>t-test</td>
<td>Student's t test</td>
</tr>
<tr>
<td>yr</td>
<td>year</td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ABSTRACT</th>
<th>ii</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRAK</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ix</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>x</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xviii</td>
</tr>
</tbody>
</table>

## CHAPTERS

### 1 INTRODUCTION
1.1 Background 1
1.2 Justification 3
1.3 Objective 4
1.4 Scope of This Study 4

### 2 LITERATURE REVIEW
2.1 Description of *Hopea odorata* 6
2.2 Description of *Dryobalanops aromatica* 11
2.3 Siliceous Inclusion
   2.3.1 Morphology of Siliceous Inclusion 14
   2.3.2 Chemical Nature of Siliceous Inclusion 16
   2.3.3 Organograhic Distribution of Siliceous Inclusion 17
   2.3.4 Dimensional Variation of Siliceous Inclusion in Wood 18
   2.3.5 Functions of Siliceous Inclusion to the Plant 18
2.4 Calcium Crystal
   2.4.1 Morphology of Calcium Crystal 20
   2.4.2 Chemical Nature of Calcium Crystal 25
   2.4.3 Distributional Variation of Calcium Crystal in the Trunk 26
   2.4.4 Organograhic Distribution of Calcium Crystal 26
   2.4.5 Functions of Calcium Crystal to the Plant 27

### 3 MATERIALS AND METHODS
3.1 Plant Materials 28
3.2 Field’s Samples Collection 30
3.3 Sample Block Preparation for Microtomy 32
3.4 Epoxy Embedding for Heterogeneous Tissues 32
3.5 Conventional and Polarized Light Microscopy 33
3.6 Scanning Electron Microscopy and X-ray Microanalysis
3.7 Histochemistry Identification of Cell Inclusions
  3.7.1 Siliceous Inclusion and Calcium Crystal
  3.7.2 Lipid
3.8 Quantitative Analysis
  3.8.1 Distributional Analysis
  3.8.2 Dimensional Measurement
3.9 Statistical Analysis
3.10 Plant Anatomical Terminology
3.11 Experimental Design

4 RESULTS AND DISCUSSIONS
4.1 Occurrence, Dimension, and Distribution of Siliceous Inclusion and Calcium Crystal in D. aromatica
  4.1.1 Physical Properties of Siliceous Inclusion and Calcium Crystal
  4.1.2 Chemical Composition of Siliceous Inclusion and Calcium Crystal
  4.1.3 Occurrence of Siliceous Inclusion and Calcium Crystal in the Various Types of Tissues
  4.1.4 Dimensional Variations of Siliceous Inclusion and Calcium Crystal in the Different Tissues of Tree
  4.1.5 Distributional Pattern of Siliceous Inclusion in Correlated with Positions in the Trunk
4.2 Occurrence, Distribution, and Dimension of Calcium Crystals in H. odorata
  4.2.1 Physical Properties of Calcium Crystal
  4.2.2 Chemical Composition of Calcium Crystal
  4.2.3 Organographic Distribution of Calcium Crystal in Various Tissues of Tree
  4.2.4 Dimensional Variations of Calcium Crystal
  4.2.5 Distributional Pattern of Calcium Crystal in the Trunk
  4.2.6 Deposition of Other Mineral Inclusion in Some Tissues
4.3 Variation in Amount of Calcium Crystal in H. odorata's Grow at Perlis and Selangor
4.4 Factors Affecting the Morphology and Organographic Distribution of Mineral Inclusions
4.5 Factors Influencing the Variation in Dimension and Distribution of Mineral Inclusions in the Tree
4.6 Theoretical Assumption Into Degree of Wearing of Woodworking Cutting Tools and its Relation with Variation in Amount and Size of Mineral Inclusions in the Trunk
5 CONCLUSION AND RECOMMENDATION
5.1 Conclusion 110
5.2 Recommendation for Future Research 111

REFERENCES 113