Formation and Distribution of Calcium Crystals in the Trunk of *Hopea odorata*

Toong Wei Ching, Tadashi Nobuchi, Mohd. Hamami Sahri, and Liew Chun Yu

Abstract

Some of trees accumulate calcium crystals, which cause damage to cutting tools in wood machinery. For the fundamental information on the formation and distribution of calcium crystal in a trunk, *Hopea odorata* was very important to be studied. Three discs from lower, middle and upper positions of the trunk were collected. In each disk, radial strip from bark to pith was fixed with 3% glutaraldehyde. In each disc, small blocks from outer, middle, inner part and pith were cut and used for microscopic observation. Sections were stained with safranin and fast green. Morphology and distribution of calcium crystals in a trunk (radial and longitudinal) and quantitative analysis were carried out. Acid treatments with hydrochloric acid or acetic acid were also applied to investigate the chemical characteristic of the crystals. For comparison of morphology of calcium crystals between wood and bark, bark block was embedded in epoxy resin, and sections were stained with safranin. Crystals in wood were exclusively included in square/ upright ray cells with very rare cases of crystals in procumbent ray cells of ray parenchyma. Their morphology was prismatic crystals in wood and pith. In the bark, crystals showed druses form. Through chemical treatments, calcium crystals were identified as calcium oxalate. In the trunk, crystals were increased from outer to inner in the radial direction, but decreased in the pith. In the longitudinal direction, crystals showed an increase from lower to upper position in the outer and middle part, but decreased at inner part. Pith did not show much difference from lower to upper position of the trunk.

Key words: formation, distribution, calcium crystal, calcium oxalate, *Hopea odorata.*

Introduction

Calcium is one of the essential elements for plant growth (Pallardy 2008). Essential elements for plant are carbon, hydrogen, oxygen, nitrogen, phosphorus, calcium, potassium, magnesium, and iron. Calcium plays a very important role in the plant elasticity of the cell wall as the calcium pectate. Surplus calcium is often accumulated as the calcium oxalate crystals (one of the mineral inclusions) in leaves and woody tissue in the plant. (Kramer and Kozlowski 1979).

Calcium crystal's type and location are often used in plant taxonomic classification (Solereder 1908; Hsieh and Huang 1974; Genua and Hillson 1985). Furthermore, calcium crystals are considered to be the end result of the detoxification mechanism of active plant cells (Steward 1960). Calcium crystals are also considered to be one of the waste product of active metabolism of cambial layer, etc. (Chattaway 1953)

Calcium crystals can be classified into five categories based on their morphology: Crystal sand, raphide, druse, styloid, and prismatic. Prismatic which is the solitary rhombohedral or octahedral crystals; Druse is a more or less spherical in shape, and many components protrude from the surface giving the star-shaped appearance; Styloid is a large crystal at least four times as long as width with pointed ends; Crystal sand is a granular mass composed of very small crystals; Raphide is a bundle of long needle-like crystals (IAWA Committee 1989).

Nakata (2003) reported the formation and function of calcium crystals in the plant. Ogata (1983) reported the occurrence of calcium crystals in the plant cells in family of Dipterocarpaceae, Monotoidae, and Pakaraimodae, in which crystals were present in axial parenchyma and fibres in some of the species. Negi *et al*. (2003) recorded the pattern and occurrence of calcium crystal and cells that occur in wood of the family Meliaceae by using authentic wood samples from xylarium. Wu and Kuo-Huang (1997) recorded the type, morphology, and distribution of calcium crystals in the mature leaves of nine species of family Moraceae.

From the perspective of wood machinery, the calcium crystals cause the damage to the cutting tools such as various types of saw used in the sawmill, which reduces their durability. In this research, *Hopea odorata* which is categorized as one of the commercial timbers was selected as an experimental tree species. The distribution of calcium crystals in a trunk was investigated focusing on the types of crystals and what types of cells include the crystals.

Materials and Methods

Plant Material

Hopea odorata (Family Dipterocarpaceae) was selected as the sample species based on Ogata (1983) who described that this species includes calcium crystals, and has no silica. Sample trees were collected from the plantation site at Forest Research Institute Malaysia (FRIM) in Perlis. Trees were planted in 1999 and sample trees were felled in 2009.

Sample Block Collection

Three discs from lower, middle and upper part of the trunk were collected. The heights from the ground level were 1.3, 5.0 and 10.0 m. The diameters of the disks were 16.2, 13.8 and 7.7 cm. A radial strip was cut in each disk and fixed with 3% glutaraldehyde (a fixative) immediately after collection to keep the structure in intact condition.

In each strip, small blocks for the sectioning were cut. The size was 1 x 1 x 2 cm (radial x tangential x longitudinal). The bark blocks were also prepared.

Sectioning and Staining

Radial and transverse sections were obtained. Sections with 20~30 μm in thickness were cut using a LEICA SM2000R sliding microtome. They were stained with safranin and fast green.

Acid Treatment

To investigate chemical characteristics of calcium crystals, acid treatment was carried out. Two treatments were selected (Shimaji *et al*. 1976). One was acetic acid. Another treatment was 1 Normal of hydrochloric acid (HCl). Sections were immersed in the chemical for 7 days. After treatment, they were washed with distilled water and stained in the same method as described above.

Epoxy Embedding of Bark Samples

The bark samples were embedded in Epoxy resin (Nobuchi and Sahri 2008) to get better sections. Sections were stained with 0.5% safranin in water.

Quantitative Analysis and Photography

Quantitative analysis of calcium crystals was carried out to determine the amount of calcium crystal distributions in the wood by counting the number of crystals and cells by following formula:

$$\frac{\text{Number of Square/Upright Ray Cells Included Crystals}}{\text{Number of Square/Upright Ray Cells}} \times 100\%$$

For the microscopic observation and photography LEITZ DMRB (polarized/light microscope equipped with Image Analysis System) was used.

Result and Discussion

Morphology of Calcium Crystals

In the secondary xylem, calcium crystals (here in after referred to as crystals) were included in upright/ square ray cells of ray parenchyma. They showed prismatic crystals (Figure 1a). The pith also included prismatic crystals (Figure 1b). In the bark crystals showed not prismatic but druse, a star like shape (Figure 1c).

Chemical Treatment of Calcium Crystals

From the microscopical observation, crystals were considered to be calcium crystals. Acid treatments (Shimaji *et al.* 1976) were applied to deepen the nature of crystals. Calcium carbonate will dissolve in both hydrochloric acid and acetic acid. Calcium oxalate will dissolve in hydrochloric acid but not dissolve in acetic acid. Silica inclusions will not dissolve in both hydrochloric acid and acetic acid. As the results of chemical treatment, crystals in *H. odorata* in both wood and bark were judged to be calcium oxalate.

Chemical treatment also showed that *H. odorata* does not have silica inclusions. This result is supported by the report of Ogata (1983) in which genus *Hopea* includes calcium crystals but not includes silica body. The results of chemical treatment also coincided with the definition by IAWA Committee (1989) which describes prismatic crystals are composed of calcium oxalate.

Occurrence of Calcium Crystals in Relation to Cell Type.

In the secondary xylem, crystals were observed in ray parenchyma cells but not observed in other cells such as axial parenchyma cells, fibers and vessel elements. Table 1 summarizes the occurrence of crystals and types of inclusion in *H. odorata.*

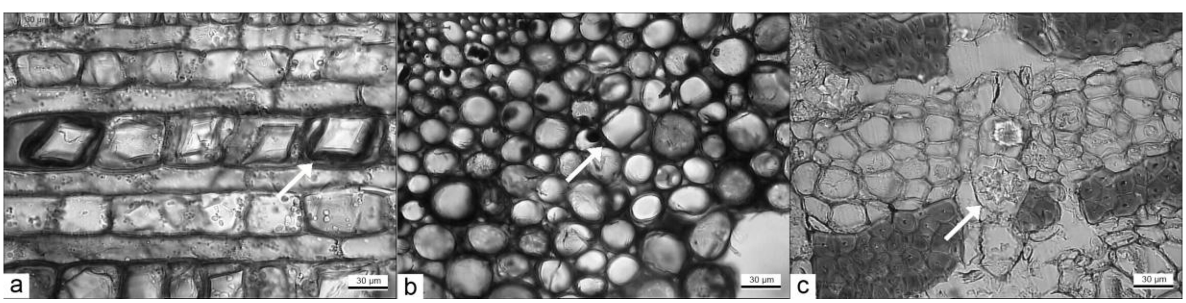

Figure 1. Light micrographs showing calcium crystals in secondary xylem (a), pith (b) and bark (c). Secondary xylem and pith have prismatic crystals and bark has druses. (a) is radial section (b~c) are transverse section. Arrow indicates a crystal.

Table 1. Occurrence of calcium crystals in *Hopea odorata.*

Tissue	Cell	Prismatic Crystal	Druse	Silica body
Wood				
Ray parenchyma	Square ray cell	+	-	-
	Upright ray cell	+	-	-
	Procumbent ray cell	±	-	-
Axial parenchyma		-	-	-
Fiber		-	-	-
Vessel		-	-	-
Bark		-	+	-
Pith		+	-	-

Distribution and Quantitative Analysis of Calcium Crystals in A Trunk

Quantitative analysis of crystals was carried out to investigate their distribution in a trunk. In each disk radial variation of crystals was first evaluated. Based on the data, longitudinal variation was also investigated.

For the quantitative analysis, the number of crystals and the number of cells were counted focusing on square/ upright ray cells. It is because almost all crystals were included in square/ upright ray cells and one single crystal was included in a single cell (with some exceptions described below).

Lower Position

Percentage of crystals in outer, middle and inner part of secondary xylem and pith in the disk were calculated. Figure 3 (a, b, c, d) shows the light micrographs of each part. Figure 2 indicates the percentage of crystals from outer to inner.

From the quantitative analysis, it was concluded that crystals increased from outer to inner in the secondary xylem but the pith had the smaller amount of crystals.

Middle Position

Figure 5 (a, b, c, d) show the light micrographs of outer, middle and inner part of secondary xylem and pith. Figure 4 indicates the percentage of crystals from outer to inner. The pattern of radial changes of crystals showed the basically similar pattern as lower disk.

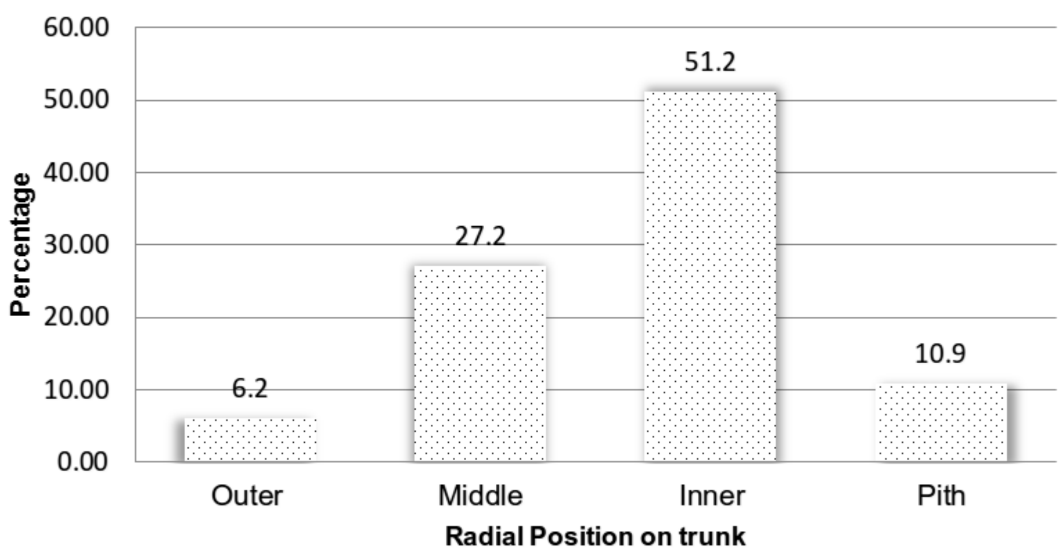

Figure 2. Percentage of crystals in outer, middle and inner part of secondary xylem and pith in the lower position of trunk. The light micrographs of each part are shown in Figure 3 (a, b, c, d).

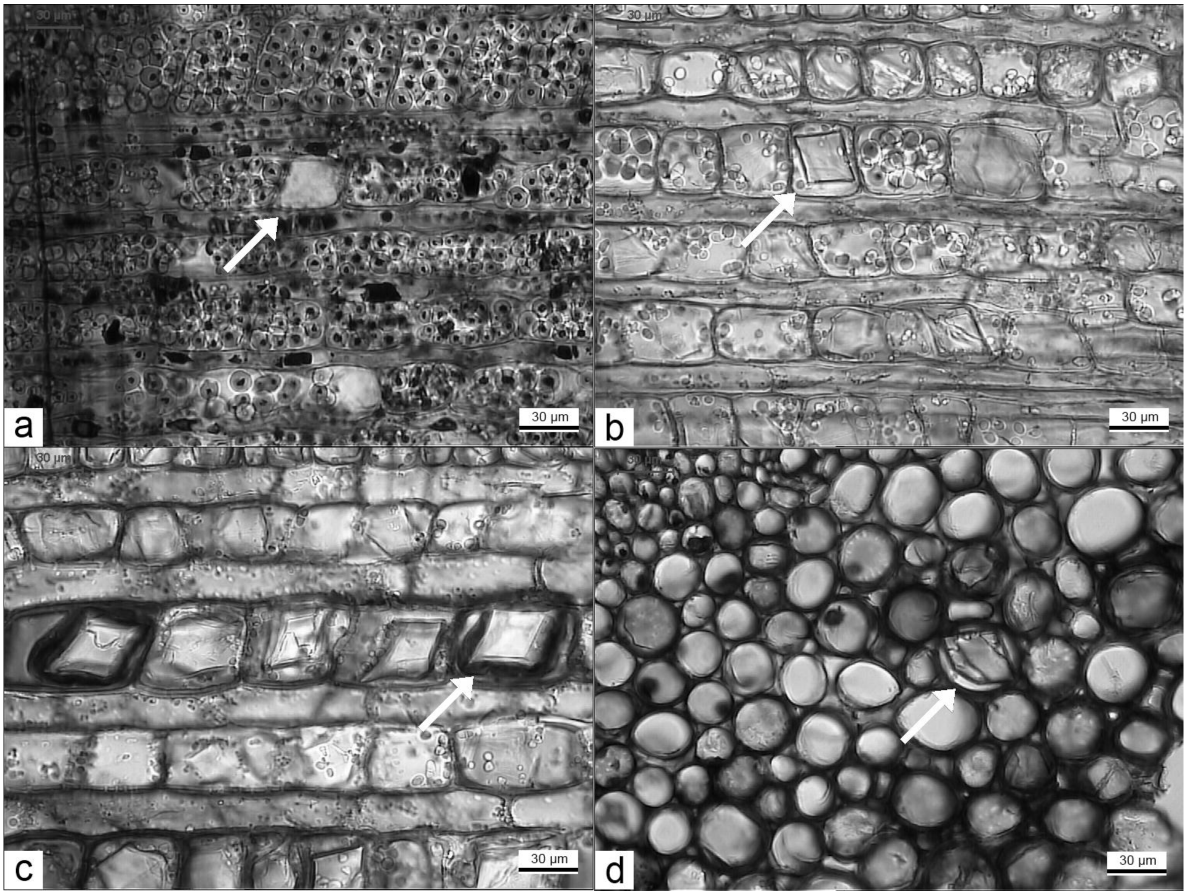

Figure 3. Light micrographs showing calcium crystals in radial direction at the lower position. (a) outer, (b) middle, (c) inner and (d) pith. Sections of secondary xylem are radial section (a~c) and section of pith is transverse section (d). Arrow indicates a crystal.

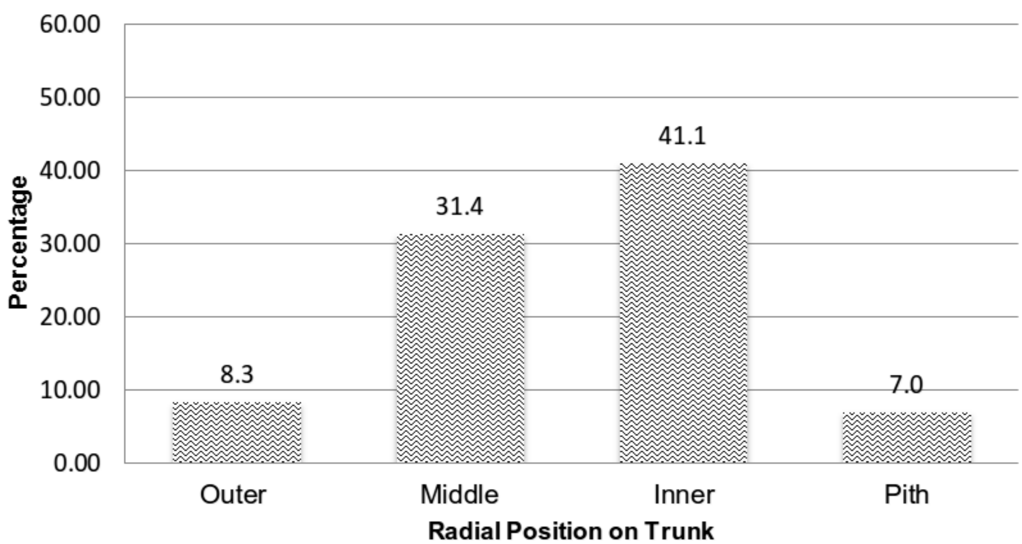

Figure 4. Percentage of crystals in outer, middle and inner part of secondary xylem and pith in the middle position of trunk. The light micrographs of each part are shown in Figure 5 (a, b, c, d).

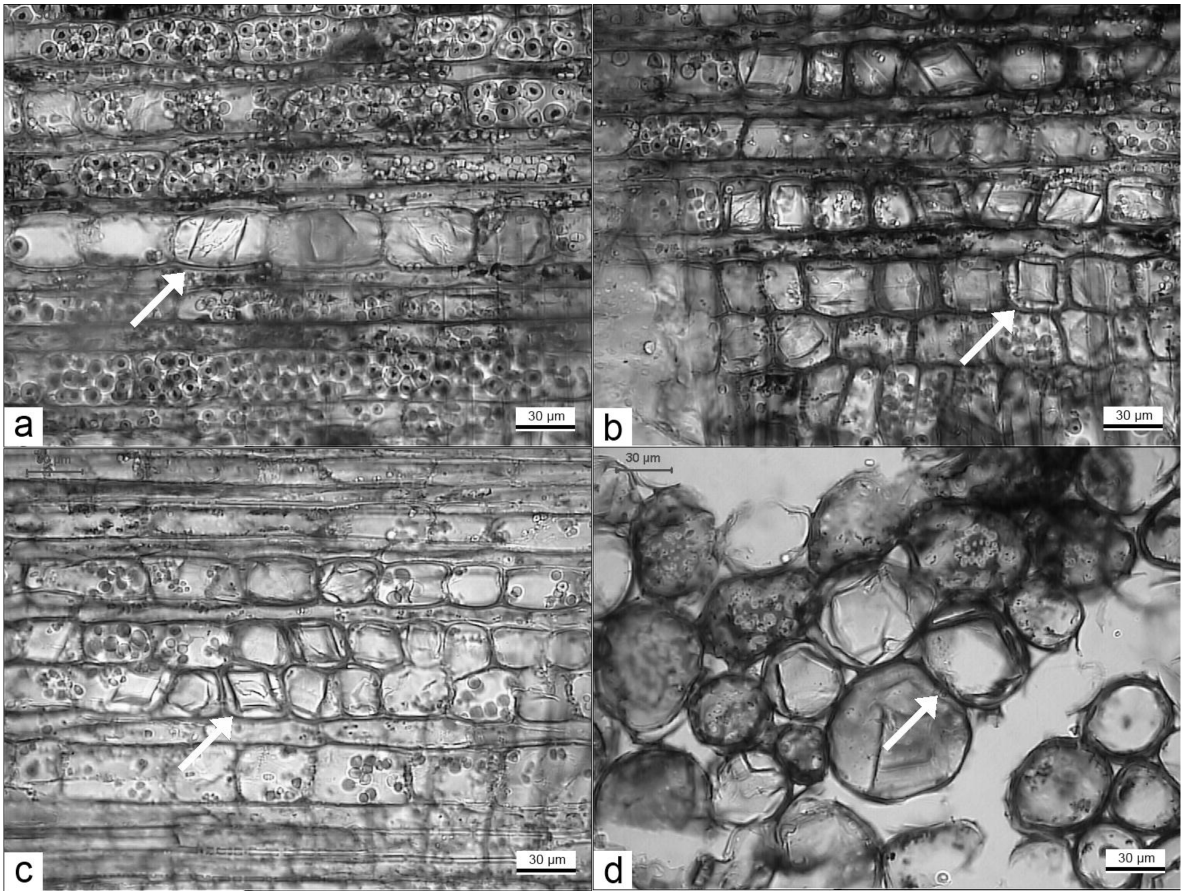

Figure 5. Light micrographs showing calcium crystals in radial direction at the middle position. (a) outer, (b) middle, (c) inner and (d) pith. Sections of secondary xylem are radial section (a~c) and section of pith is transverse section (d). Arrow indicates a crystal.

Upper Position

In the upper part of the trunk, the diameter of the disk was smaller than middle and lower position. In the radial direction secondary xylem was divided into outer and inner.

Figure 7 (a, b, c) show the light micrographs of outer and inner part of secondary xylem and pith. Percentages of crystals are indicated in Figure 6.

In the upper position, radial distribution pattern of crystals showed the similar tendency as lower and middle part. That is, in secondary xylem they increased from outer to inner and decreased in the pith. The rate of an increase from outer to inner, however, was not much.

From the quantitative analysis of crystals in the radial direction, it was clear that amount of crystals increased from outer to inner in the secondary xylem and decreased in the pith. The difference between inner and outer was the larger when the position was the lower. Distance (or years) from inner to outer is considered to be the important factor which correlates with the accumulation of the calcium element. Comparison of the crystal amounts in the longitudinal direction of the trunk is given in the next section together with the discussion.

Distribution of Calcium Crystals in Longitudinal Direction of the trunk

Radial variations of the amount of crystals at the lower, middle and upper position of the trunk (Figs. 2, 4 and 6) were summarized into one graph (Figure 8). Crystals increased from lower to upper in the outer and middle parts but decreased in inner part. Pith did not show much difference in the amount of crystals from lower to upper.

In Figure 2, 4 and 6, the amounts of an increase from outer to inner (or the difference between inner and outer) for lower, middle and upper positions of the trunk were 45.0, 32.8 and 2.3%, respectively. Changes of the amount of crystals from lower to upper were considered to be much less than those in the radial direction.

To consider factors affecting distribution of crystals in the trunk, water movement in a trunk is important. Calcium is considered to be absorbed from soil to roots together with water.

Another important point is that calcium is one of the essential elements for plant growth (Pallardy 2008) and surplus calcium is accumulated as the crystals (Kramer and Kozlowski 1979).

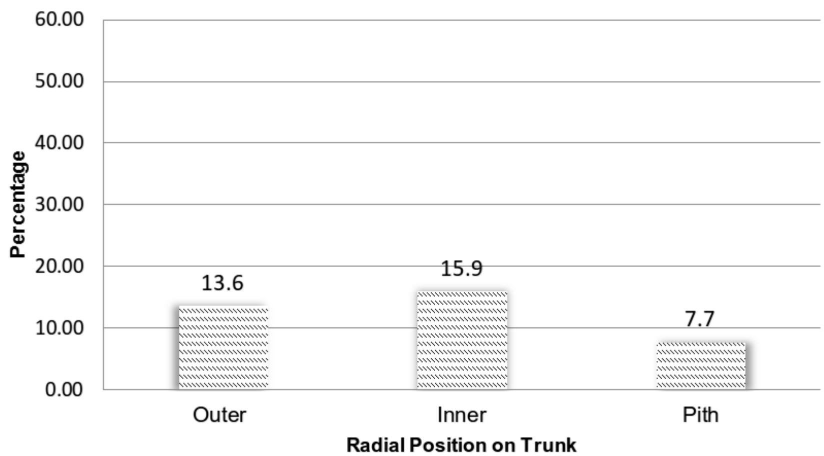

Figure 6. Percentage of crystals in outer and inner part of secondary xylem and pith in the upper position of trunk. The light micrographs of each part are shown in Figure 7 (a, b, c).

Figure 7. Light micrographs showing calcium crystals in radial direction at the upper position. (a) outer, (b) inner and (c) pith. Sections of secondary xylem are radial section (a~b) and section of pith is transverse section (c). Arrow indicates a crystal.

The main water movement (or mass flow) is the longitudinal direction from roots to leaves in which water is translocated outer part of the secondary xylem (Kramer and Kozlowski). Through this movement water is supplied for photosynthesis. Mass flow is considered to have the relatively short period in relation to seasons in a year. A part of the surplus calcium is released from the tree to outside when the leaves fall.

Another water movement in the trunk is radial water movement (Zimmermann 1983). This water movement is considered to have close relation with heartwood formation or aging. The period related to the radial movement, therefore, is a long term or several or more years. Different from the longitudinal water movement, calcium moved into inner part remains in the trunk. The amount of calcium increases following the accumulation during the long period. This point explains the reason why many amount of crystals were accumulated in the inner part of the trunk especially at the lower disk which has the larger diameter or is formed during many years.

Pith has no active role for the water translocation because it is formed in the process of the elongation growth of a tree. This is the possible reason for the low amount of crystals in the pith from lower to upper positions in the trunk.

Exceptional Patterns of the Occurrence of Calcium Crystals

In the most parts of secondary xylem, a single crystal was included in a square/ upright ray cell. Very limited exceptions, however, were observed. Two crystals were included in a square cell (Figure 9a and b). Their size was smaller than general crystals.

Another exception was that crystals were included in a procumbent ray cell (Figure 9c and d). The size was smaller (about one third in diameter) than a crystal in a square/ upright ray cell.

The actual process of calcium accumulation and crystal formation is still not clear. Following is one of the possible reasons. Calcium element is considered to be accumulated in a vacuole as the waste of metabolism.

Following the increase of calcium in the vacuole, the vacuole increases its size. However, the increase of size has the limitation because the rigid cell wall does not allow further expansion of the vacuole. This leads to the increase of the concentration of calcium and finally calcium is crystallized in the vacuole.

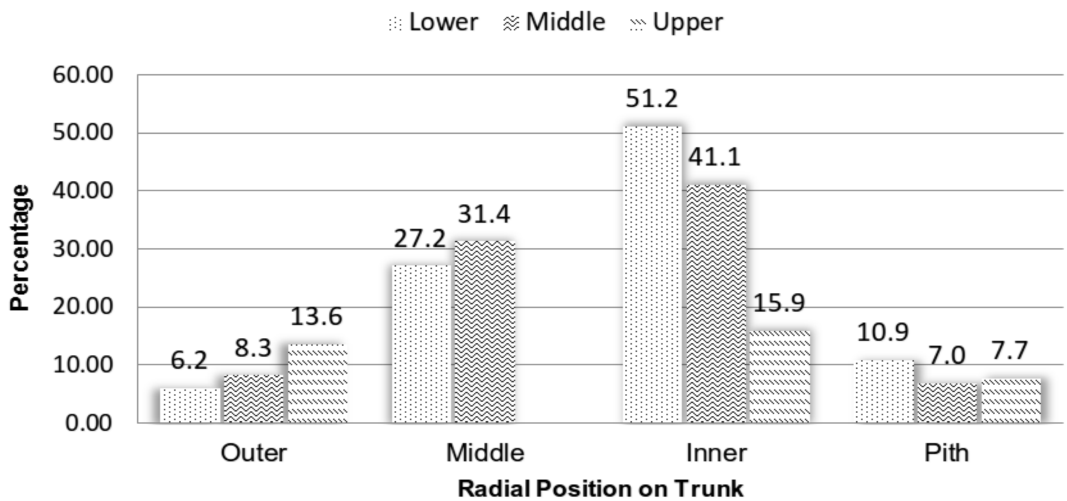

Figure 8. Comparison of percentages of calcium crystals among lower, middle and upper positions of trunk.

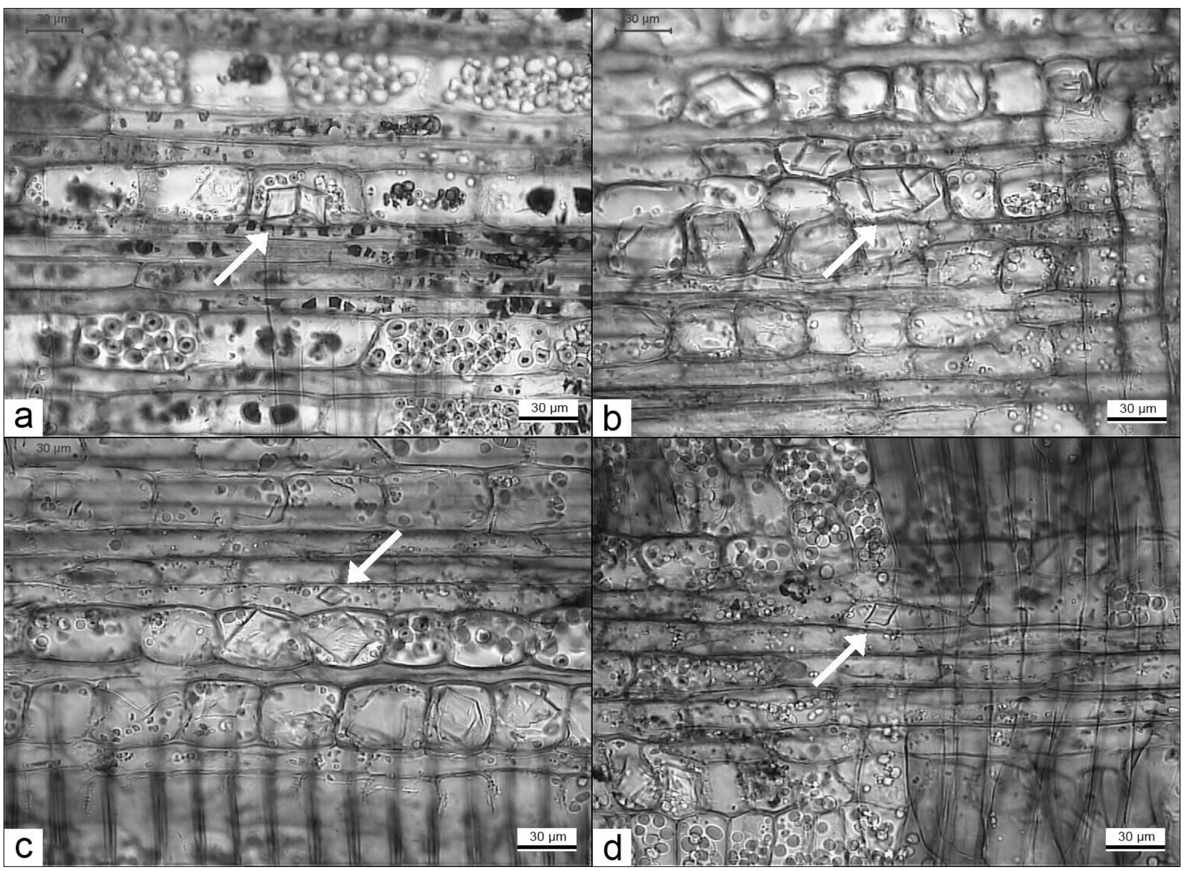

Figure 9. Light micrographs showing two calcium crystals in a square ray cell at the lower position (a~b) and calcium crystals in a procumbent ray cell at the middle position of the trunk (c~d). Arrow indicates a crystal.

Conclusions

Hopea odorata had calcium crystals in the ray parenchyma of the secondary xylem. Crystals were also included in pith and bark. In the ray parenchyma, they were included in square/ upright ray cells with very exceptional cases of crystal inclusion in procumbent ray cells. Different types of crystals were formed in wood and bark. The former had prismatic crystals, and the latter had druses. Chemical analysis revealed that crystals were calcium oxalate. Quantitative analysis of crystals in the trunk showed that their increase in the radial direction is more prominent than in the longitudinal direction. Study of crystal formation and distribution in a whole tree including root, trunk, bark and leaves is necessary in the future with particular reference to the water movement.

References

Chattaway, M.M. 1953. The Occurrence of Heartwood Crystals in Certain Timbers. Aust. J. Bot. 1: 27-88 (quoted by Rao and Dave 1984).

Genua, J.M.; C.J. Hillson. 1985. The Occurrence, Type and Location of Calcium Oxalate Crystals in the Leaves of Fourteen Species of Araceae. Ann. Bot. 56: 351-358 (quoted by Wu and Kuo-Huang 1997).

Hsieh, C.F.; T.C. Huang. 1974. The Acanthaceous Plants of Taiwan. Taiwania 19: 19-57 (quoted by Wu and Kuo-Huang 1997).

IAWA Committee. 1989. IAWA List of Microscopic Features for Hardwood Identification. IAWA Bulletin n. s., 10 (3): 219-332.

Kramer, P.J.; Kozlowski, T.T. 1979. Physiology of Woody Plants. Academic Press, New York, San Francisco, London: 340.

Kuo-Huang, L.L.; S.J. Chen. 1999. Subcellular Localization of Calcium in the Crystal-Forming Sclereids of *Nymphaea tetragona Georgi.* Taiwania 44(4): 520-528.

Nakata, P.A. 2003. Advance in Our Understanding of Calcium Oxalate Crystal Formation and Function in Plants. Plant Science 164: 901-909.

Negi, K.; S. Gupta; L. Chauhan; M. Pal. 2003. Patterns of Crystal Distribution in the Woods of Meliaceae from India. IAWA Journal 24(2): 155-162.

Nobuchi, T.; M.H. Sahri. 2008. The Formation of Wood in Tropical Forest Trees: A Challenge from the Perspective of Functional Wood Anatomy. Penerbit Universiti Putra Malaysia: 19-20.

Ogata, K. 1983. Summary of Anatomical Characteristic of Wood of Family Dipterocapceae. Mokuzai Kogyo 38(8): 385-386 (in Japanese).

Pallardy, S.G. 2008. Physiology of Woody Plants, 3rd ed. Academic Press, Amsterdam, Boston, Heildelberg, London, New York, Oxford, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo: 257-258.

Rao, K.S.; Y.S. Dave. 1984. Occurrence of Crystals in Vascular Cambium. Protoplasma 119: 219-221.

Shimaji, K.; Sudo, S.; Harada, H. 1976. Structure of Wood. Morikita-Shuppan: 166-169 (in Japanese).

Solereder, H. 1908. Systematic Anatomy of the Dicotyledons. Clarendon, Oxford (quoted by Wu and Kuo-Huang 1997).

Steward, C.M. 1960. Detoxication during Secondary Growth in Plants. Nature 186: 374-375 (quoted by Rao and Dave 1984).

Wu, C.C.; L.L. Kuo–Huang. 1997. Calcium Crystals in the Leaves of Some Species of Moraceae. Bot. Bull. Acad. Sin. 38: 97-104.

Zimmermann, M.H. 1983. Xylem Structure and the Ascent of Sap. p. 83-85. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo.

Wei Ching, Toong
Faculty of Forestry, Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia
Tel. : 6016-4015329
E-mail : osmosizching@gmail.com

Tadashi Nobuchi
E-mail : tmnobuchi.tm@nike.eonet.ne.jp

Mohd. Hamami Sahri
Faculty of Forestry, Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia
Tel. : 603-89467171
Fax. : 603-89432514
E-mail : hamami5253@gmail.com

Chun Yu, Liew
Faculty of Forestry, Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia
Tel. : 6016-8752360
E-mail : chunyu87@gmail.com