短 報

Differentiation of Genera *Pinus, Picea,* and *Abies* by the Transfusion Tracheid Phytoliths of Pinaceae Leaves

Renzo KONDO[*1], Kiyoshi TSUTSUKI[*1], Masayuki TANI[*1] and Ryuichi MARUYAMA[*2]

Keywords : Opal phytoliths, Biotic Silica, Transfusion tracheid, Brodered Pit, Northern Conifer

1. Introduction

Information on the opal phytoliths originating from conifers is very scarce compared with those from Poaceae and Cyperaceae (Piperno, 1987; Kondo and Sase, 1989). Kondo and Sumida (1978) reported the silicification patterns and morphological characteristics of the leaves of 24 species in several families (Pinaceae, Taxodiaceae, Podacarpaceae *etc.*), which grow naturally or planted in Japan. Klein and Geis (1978) reviewed the degree of silicification, the contents of biogenic silica in leaves of 15 species of the Pinaceae, and referred to the cells producing these phytoliths. According, to them, the quantity of opal phytoliths in leaves of Pinaceae rarely exceeds 1 %, and is usually in very low level from 0.1 to 0.4 %. They clarified that the cells with high degree of silicification are transfusion tracheid cells, endodermal cells, and epidermal cells.

Kondo *et al.* (1997) studied in detail the contents and morphological characteristics of opal phytoliths contained in native and introduced conifer leaves of 5 genera and 54 species of Pinaceae grown in the Experimental Forest of the Faculty of Agriculture, Tokyo University at Furano city in Hokkaido. The most outstanding observation is the presence of opal phytoliths drived from the transfusion tracheid cells, which were detected in all conifer species and was different from the similar opal phytoliths originating from Poaceae and other plants. However, it is difficult to distinguish this phytolith only from its morphology and size among different species of Pinaceae. Therefore, we directed our attention to the sizes of bordered pits on the surface of the transfusion tracheid phytoliths and studied its difference among *Pinus, Picea,* and *Abies.*

The purpose of this study is to clarify whether there is a difference or not in the size of bordered pits on the transfusion tracheid phytoliths found in the Pinaceae leaves. Especially, if there is a significant difference between *Abies* and *Picea,* it will become possible to assess the distribution and succession of northern conifers (*Abies sachalinensis* and *Picea jezoensis var.jezoensis*). Furthermore, it will contribute to clarify the change in forest structure and the co-existing mechanism of northern conifers by the use of opal phytolith analysis.

2. Materials and Methods

Pinaceae leaves used in this study (older than one year) were collected in the Experimental Forest of the Faculty of Agriculture, Tokyo University located in the mountain region of Furano city in Hokkaido. Most of the soils developed in these area are classified into the Udepts.

Used samples belonged to 10 species of *Pinus,* 11 species of *Picea,* and 11 species of *Abies,* 32 species in total (Table 1). These leaf samples were soaked in dilute HCL for twenty-four hours. During the soaking, the sample was treated with ultrasonics (350 W, 20 Kc/s, 3-5 min) several times. Then, the leaves were washed thoroughly with water and dried at 60 ℃. Separation of opal phytoliths from the leaves was carried out by the wet digestion

*1 Laboratory of Land Resourece Science, Obihiro University of Agriculture and Veterinary Medicine , Inada-chyo, Obihiro 080-8555
*2 Docon Co. Ltd., 4-1, 1 Jo, Atsubetu-chyo, Atsubetu-ku , Sapporo 004-0051

Table 1. Average sizes* of bordered pits on the transfusion tracheid phytoliths separated from Pinaceae leaves.

Samples	Size (μm)	Samples	Size (μm)	Samples	Size (μm)
Genus Pinus		*Genus Picea*		*Genus Abies*	
P. cembra	5.63	*P. abies*	4.12	*A. alba*	2.80
P. contorta	6.43	*P. bicolor*	4.16	*A. balsamea*	2.99
P. contorta var. latifolia	6.27	*P. glauca*	4.78	*A. homolepis*	3.94
P. koraiensis	6.56	*P. glehnii*	5.00	*A. grandis*	3.00
P. monticola	5.93	*P. jezoensis var. jezoensis*	3.84	*A. lasiocarpa*	2.88
P. nigra	5.47	*P. koraiensis*	3.54	*A. mariesi*	2.67
P. Peuce	5.92	*P. koyamae var. koyamae*	4.73	*A. nephrolepis*	2.96
P. pumila	5.97	*P. mariana*	4.69	*A. nordmanniana*	3.05
P. pungesu	6.66	*P. obovata*	4.05	*A. sachalinensis*	2.95
P. resinosa	5.99	*P. ruben*	4.37	*A. sibirica*	3.25
		P. sithensins	3.55	*A. veitchii*	3.34
Mean	6.08		4.26		3.08
Standard Deviation	0.39		0.50		0.34

* About thirty phytoliths were counted per one sample, and the average was shown
The difference among the genera was significant at 0.1% level (Fisher's LSD test)

method (Kondo, 2000) using the mixed solution of nitric acid, sulfuric acid, and perchloric acid (10 : 1 : 4). Particles finer than 5μm were removed by ultrasonic treatment and sedimentation method. Particles larger than 5μm were washed with centrifuge, dried at 110℃ for 24 hours, and offered for microscopic observation. Measurement of particle sizes was carried out using the tablet unit for micro-measurement manufactured by Olympus Co. Ltd.

3. Results and Discussion

Opal phytoliths contained in the Pinaceae leaves are mainly from epidermal cells, transfusion tracheid cells, endodermal cells, and stoma cells. They rarely originate from sub-epidermal cells, mesophyll cells, and Astrosclereids (Klein and Geis, 1978; Kondo *et al.* 1997).

Opal phytoliths from transfusion tracheid cells and endodermal cells are large (20 ~ 80μm) and similar in the morphology as a whole (Kondo *et al.* 1997). Cubic shapes of opal phytoliths from transfusion tracheid cells and endodermal cells of Pinaceae leaves are very similar to that of bulliform cell phytoliths of *Poaceae* (Kondo and Sumida, 1978). Because the opal phytoliths of endodermal cells do not have bordered pits which are universal in transfusion tracheid cells, they are easily mistaken by researcher with little experience. On the other hand, the transfusion tracheid phytoliths have about 4 ~ 12 navel-like bordered pit impressions on one aspect, and the size of these pits seem to differ among different species of Pinaceae leaves. Therefore, we measured the size of bordered pits using a tablet unit for micro-measurement in order to clarify the difference in the level of genus. Thirty phytoliths were counted per one sample, and the average was shown.

Table 1 shows the average sizes of bordered pits on the transfusion tracheid phytoliths originating from *Pinus*, *Picea*, and *Abies*.

Sizes of bordered pits of *Pinus* leaves were largest among Pinaceae leaves (5.47 ~ 6.66μm), followed by those of *Picea* (3.55 ~ 5.00μm) and *Aabies* (2.67 ~ 3.94μm) in this order. Difference among the average values for these genera, *Pinus* (mean ± 1s.d., 6.08 ± 0.39μm), *Picea* (4.26 ± 0.50μm), and *Abies* (3.08 ± 0.34μm), was examined statistically (Fisher's least significant difference test). The difference among the genera was significant at 0.1 % level. This result suggests that Pinaceae can be discriminated at the level of genera.

Difference among species is not clear due to the lack of sample numbers. However, considerable difference was observed between *Picea glehnii* and *P. sitchensis, P. koraiensis, P. jezoensis nar. Jezoensis.*

The statistic significance of this difference should be examined in detail by increasing sample numbers.

In any case, *Picea jezoensis* var. *jezoensis* and *Abies sachalinensis* are likely to be discriminated according to the sizes of bordered pits on the transfusion tracheid

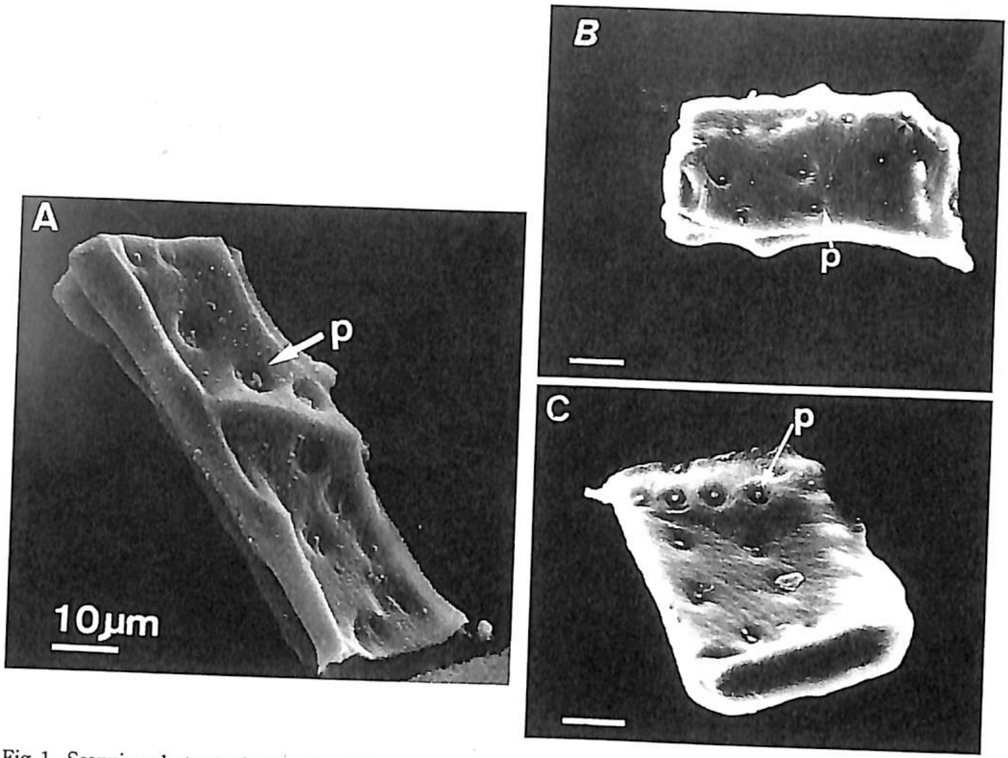

Fig. 1. Scanning electron micrographs of the transfusion traxheid separated from *Pinus, Picea,* and *Abies* leaves.
A : *Pinus pumila,* B : *Picea jezoensis var. jezoensis,* C : *Abies sachalinensis,* p : bordered pit

phytoliths. This is very useful for clarifying the distribution and succession of northern conifers in post glacial erae in Hokkaido. Further study is necessary to compare statistically the sizes of bordered pits on the transfusion tracheid phytoliths using many individual *Picea jezoensis* var *jezoensis* and *Abies sachalinensis.*

Acknowledgements

We are grateful to Ms. Satoko Shiomi, the student of our laboratory (presently working for Hiyama branch of Hokkaido prefecture office), and to Mr. Shinsaku Shibano of the Experimental Forest of the Faculty of Agriculture, Tokyo University, who allowed and helped us in collecting leaf samples of various conifers.

References

Klein, R.L, and Geise, J.W. 1978. Biogenic silica in the Pinaceae. Soil Sci., 126 (3), 145- 155.

Kondo, R. 2000. II-1-3-6 Opal phytoliths. In The Fossil Research Group eds. Methds of fossil study. -From collection to the latest analytical methods-, 59-63, Tokyo, Kyouritsu Press, (J).

Kondo and Sase, 1986. Opal phytolityhs, their nature and application. The Quaternary Research, 25 31-63 (J).

Kondo R.. Siomi, S., Tsutsuki, K. and Sibano, S. 1997. Characteristics of opal phytolith derived from *Pinaceae* leaves. Abstracts of the annual meeting, Japanese Soil Society of Science and Plant Nutrition. 43, 132 (J).

Kondo, R. and T. Sumita 1978. The study of opal phytoliths in tree leaves. I. Opal phytoliths in gymnosperm and monocotyledonous angiosperm tree leaves. Journal of the Science of Soil and Manure (Nihon Dojyo Hiryogaku-zasshi), 49,138-144 (J+E).

Piperno, D. L 1988. Phytolith Analysis: An Archaeological and Geological Perspective. New York, Academic Press, 280p.

Summary

We directed our attention to the sizes of bordered pits on the surface of the transfusion tracheid phytoliths in and studied its difference among *Pinus, Picea,* and *Abies.* The sizes tended to increase in the order of *Pinus* (6.02 ± 0.42μm) > *Picea* (4.31 ± 0.49μm) > *Abies* (3.07 ± 0.33μm). The difference in average value was significant at 0.1% level. This suggests that we can assess the distribution and succession of northern conifers (*Abies sachalinensis* and *Picea jezoensis* var. *jezoensis*) by investigating the density of opal phytolith originating from transfusion tracheid cells and sizes of bordered pits on them. Though the sizes of bordered pits on the transfusion tracheid phytoliths did not differ largely among the conifers belonging to the same genus, there seemed to be a little difference among *Picea glehnii, P. hitchensis, P. kaoraiensis,* and *P. jezoensis.* Further study is nessessary to compare statistically

The size of bordered pits on the transfusion tracheid phytoliths using many individual *Picea jezoensis* var *jezoensis* and *Abies sachalinensis.*