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Histochemical features of ray parenchyma cells of broad-leaved trees in the storage of starch.

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Histochemical investigation of the ray parenchyma cells was carried out for the elucidation of the physiological features of ray tissues in broad-leaved trees. Seasonal fluctuation of starch was mainly observed in fifteen species which were selected based on the morphological features of ray tissues. Results are summarized as (1) In the heterogeneous ray tissues upright or square cells usually stored more starch than procumbent cells. (2) In homogeneous ray tissues which are constituted only by procumbent cells, the cells situated in the upper and lower marginal positions showed different morphological features from the cells situated in the central part of ray tissues. That is; the cells of upper and lower marginal positions generally were shorter in cell length. These cells preferentially stored starch. (3) Contact-cells which are connected with the vessel through ray—vessel pitting had usually a lesser amount of starch. They had, however, a higher amount of enzymes—acid phosphatase, succinate dehydrogenase. Active metabolism of carbohydrates transformation is considered to be occurred in these cells. (4) From the histochemical investigation of the cells constituting ray tissues it was revealed that the morphological types of ray parenchyma cells had strong relationship with the storage of starch.
1. Introduction

Ray tissues are mainly concerned with storage and mobilization of reserve substances. Towards cambial side, they supply carbohydrates for the cambial activity and towards inner side for the heartwood formation. Comparing ray tissues of coniferous trees and broad-leaved trees, the latter generally has complicated structure.

Ray tissues of broad-leaved trees generally are composed of ray parenchyma cells of different morphological types that is, the combination of upright cells, square cells and procumbent cells. Ray tissues are divided into two groups—homogeneous ray tissues constituted only by procumbent cells and heterogeneous ray tissues which certainly have upright or square cells. It is important to study the physiological roles of individual ray parenchyma cells of different morphological types in the storage and mobilization of reserve substances.

Many reports have been published concerning the reserve substances of ray tissues in broad-leaved trees. In most of these reports, however, the behaviour of reserve substances was investigated mainly in tissue level. Sauter was one of the few authors who investigated reserve substances of ray tissues in cell level. He divided ray tissues into two types of cell row—the cell rows connected with the vessels and the cell rows isolated from the vessels, and reported their behaviour in the starch deposition.

The species that Sauter used in his experiment was poplar. Poplar belongs to the group having homogeneous ray tissues. It is, therefore, insufficient to clarify the relationship between the morphological types of ray tissues and the reserve substances.

In this report, the relationship between the morphological types of ray parenchyma cells and the reserve substances during the storage and mobilization have been studied in the broad-leaved trees which were selected from different morphological types of cells constituting ray tissues. That is, the features of individual ray parenchyma cells in the storage and mobilization of reserve substances have been investigated through the seasonal fluctuation of starch deposition.

The authors thank the personnel of the Experimental Forests of Kyoto University for their co-operation and providing the necessary wood samples.

2. Materials And Methods

2.1. Materials

Sample trees and their morphological features of ray tissues are listed in Table 1. Classification of morphological types of ray tissues based on Broun was adopted. All
Table 1 Sample species and their typical morphological types of ray tissues.

<table>
<thead>
<tr>
<th>Species</th>
<th>Type of ray tissues</th>
</tr>
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<tbody>
<tr>
<td>Katsura (Ceroidiphyllum japonica Sieb. et Zucc.)</td>
<td>C(I)-UP-u</td>
</tr>
<tr>
<td>Koshiabura (Acanthopanax sieboldiyoides Fr. et Sav.)</td>
<td>C(CI)-SP-m</td>
</tr>
<tr>
<td>Onigurumi (Juglans mandshurica subsp. Sieboldiana Kitam.)</td>
<td>C(I)-SP-m</td>
</tr>
<tr>
<td>Hoonoki (Magnolia obovata Thunb.)</td>
<td>C(CD)-SP-u</td>
</tr>
<tr>
<td>Itayakaede (Acer Mono Maxim).</td>
<td>C(CI)-MP-m</td>
</tr>
<tr>
<td>Tochinoki (Aesculus turbinata Blume)</td>
<td>C(CD)-MP-u</td>
</tr>
<tr>
<td>Keyaki (Zelkova serrata (Thunb.) Makino)</td>
<td>C(C)-SP-m</td>
</tr>
<tr>
<td>Akanebashiwa (Mallotus japonica Muill. Arg.)</td>
<td>C(C)-SP-u</td>
</tr>
<tr>
<td>Kusunoki (Cinnamomum Camphora (Linn.) Sieb.)</td>
<td>C(C)-SP-u</td>
</tr>
<tr>
<td>Nemunoki (Albizia Julibrissin Durazz.)</td>
<td>C(C)-MP-m</td>
</tr>
<tr>
<td>Kuri (Castanea crenata Sieb. et Zucc.)</td>
<td>C(C)-MP-u</td>
</tr>
<tr>
<td>Konara (Quercus serrata Thunb.)</td>
<td>I-P-mf</td>
</tr>
<tr>
<td>Buna (Pagus crenata Blume)</td>
<td>I-P-mf</td>
</tr>
<tr>
<td>Hannoki (Alnus japonica Steud.)</td>
<td>I-P-a</td>
</tr>
<tr>
<td>Yamagurumia (Trochodendron aralioides Sieb. et Zucc.)*</td>
<td>C(I)-UP-m</td>
</tr>
</tbody>
</table>

Notes; Type of ray tissue is expressed as the combination of contact or isolation of ray tissues, cell types constituting ray tissues, and uniseriate (and/or biseriate) or multiseriate. C: contact-cell, I: isolation-cell, U: upright cell, S: square cell, P: procumbent cell, M: M-cell (see text), u: uniseriate or biseriate ray, m: multiseriate ray, mf: broad ray, a: aggregate ray.

* Contact or isolation of ray tissues was decided in relation to tracheids.

were the mature trees.
Wood blocks taken from the breast height from the ground level by an increment borer were fixed in 3% glutaraldehyde. The sample collections were carried out bimonthly during one year (May, 1979–Apr., 1980).
In koshiabura, wood blocks were collected in every month for the histoenzymatic observation.

2.2 Methods
(1) Histochemistry of cell contents
Radial and tangential sections, 20–30 μm thick, were cut from the wood blocks fixed in glutaraldehyde solution. The sections stained with I₂-KI (starch), Sudan IV (lipids), and Haematoxylin (nucleus) were observed under a light microscope.
Small wood blocks, 1×1×5 mm, taken from the glutaraldehyde-fixed blocks were post fixed in 2% KMnO₄, dehydrated with EtOH series and embedded in Epon 812. Ultrathin sections were observed under a transmission electron microscope (JEM-7, 80kV).
For the measurement of starch content in light microscopy, the weight of the objects in the enlarged photographic paper was measured and the percentage of starch area to the ray parenchyma cell area was calculated.

(2) Histoenzymatic observation

Acid phosphatase (ACPase) which was reported to relate with starch dissolution and succinate dehydrogenase (SDH) which has the role of intra-cellular respiration were investigated.

For ACPase, wood blocks fixed in 3% glutaraldehyde (in acetic acid buffer, pH 5.0) were used based on the methods of Jensen. For SDH, fresh blocks stored in phosphate buffer were used based on the method of Defendi and Pearson.

3. Results and Discussion

All of the sample trees were confirmed to belong to the "starch tree". Living ray parenchyma cells, therefore, generally contained starch grains. Starch content, showed seasonal fluctuation.

In the radial direction, seasonal fluctuation was more conspicuous towards the cambial side. The large fluctuation of starch in the outer sapwood is reasonable because one of the starch utilization is for the cambial activity.

Comparing deciduous and ever green trees, the former showed larger seasonal changes of starch. The difference of seasonal fluctuation between them is thought to be the difference in the ability in photosynthesis especially in autumn and winter seasons. The observation and measurement were carried out mainly in the outer sapwood of deciduous trees.

(1) Starch deposition in heterogeneous ray tissues

Katsura, koshiabura, akamegashiwa, kusunoki, hoonoki, onigurumi, keyaki, buna, and yamaguruma belong to the heterogeneous ray tissue. The seasonal fluctuation of starch content in square cells and procumbent cells of koshiabura are shown in Fig. 1. In the process of the increase of starch content from spring to summer, square cells preferentially stored starch (Photo. 1). The storage of starch in procumbent cells was delayed. Starch content increased to the maximum in autumn, decreased in mid winter and increased again towards spring. In this seasonal fluctuation square cells usually store more starch than procumbent cells. The decrease of starch in mid winter are considered to be the conversion of starch to sugars under low temperatures.

The other species showed almost the same seasonal fluctuation as koshiabura.

![Seasonal fluctuation of starch content of square and procumbent cells in koshiabura of heterogeneous ray tissues](image-url)
That is, upright or square cells showed different features in storage of starch from procumbent cells.

(2) Starch deposition in homogeneous ray tissues

Nemunoki, kuri, tochinoki, itayakaede, hannoki, and konara belong to the homogeneous ray tissue. That is, only procumbent cells constitute the ray tissues.

In detailed observation, however, some special shaped cells generally were observed in the upper and lower marginal positions of ray tissues. These cells showed same vertical height as ordinary procumbent cells situated in the central part of ray tissues but showed shorter cell length. The results of the measurement of cell length are shown in Fig. 2.

In this report shorter cells in marginal positions of ray tissues were distinguished from the ordinary procumbent cells and called "M-cells" for convenience.

The starch content was compared between procumbent cells and M-cells. In many species of this group the differences were observed between them. An example of kuri is shown in Fig. 3. The seasonal fluctuation of starch content was the same as in the heterogeneous ray tissues. It was noticeable that M-cells showed preferential storage in the increase of starch content from spring to summer. This means that M-cells are the same features as upright or square cells of heterogeneous ray tissues in the storage of starch. Photo. 2 shows the preferential storage in M-cells in nemunoki (September).

Many species in this type showed same pattern in storage of starch as kuri. In tochinoki and hannoki, however, M-cells showed almost the same features in the storage of starch as procumbent cells. These species are considered to be the proper homogeneous ray tissues in terms of physiology.

It is noticeable that many species belonging to homogeneous ray tissues showed different features in starch deposition depending on the morphological types of ray parenchyma cells. Although not so much conspicuous as in the upright or square cells in heterogeneous ray tissues, some functional differentiations are thought to be occurred in homogeneous
Ray tissues. In tangential section M-cells obviously show the different shape from cells situated in the central position of ray tissues. The special shape of the cells in marginal position is observed not only in broad-leaved trees but also in coniferous tree. In intermediate wood of sugi, for example, ray parenchyma cells began to die in the upper and/or lower position of ray tissue. These cells are different in their shape from the cells situated in the central part of ray tissues.

From the observation mentioned above, it was indicated that the morphological types of ray parenchyma cells have strong relationship with the storage of starch.

(3) **Contact-cell**

Ray tissue more or less has the connection with vessels by special contact-cells. In storage of starch contact-cells generally had extremely small amount of starch. They contained, however, much lipid. Even in autumn when the storage of starch showed the maximum content, very small amount of starch was observed in contact-cell. One example of transmission electron micrographs is shown in Photo. 3 (November).

The peculiarity of contact-cells in the storage of starch was observed regardless of the shapes of ray parenchyma cells. In homogeneous ray tissue of tochinoki, for example, only contact-cells have no starch grains (Photo. 4).

Contact-cells which are connected through large pits with vessels (ray-vessel pitting) and generally have extremely small amount of starch are considered to have the function of carbohydrates mobilization between ray tissue and vessel as Sauter stated. Histoenzymatic observation of ray tissues was, therefore, carried out in koshiabura.

The distribution of ACPase and SDH revealed that outer part of sapwood had a larger amount of enzymes. In ray tissues contact-cells accumulated more enzymes than isolation-cells (Photos. 5, 6). Sauter reported that the high accumulation of ACPase was related with the dissolution of starch and mobilization of sugars. As SDH showed higher accumulation in contact-cells in addition to ACPase, active metabolism of carbohydrates is considered to occur in contact-cells. Isolation-cells, on the other hand, had smaller amount of enzymes. Slow metabolism of carbohydrates is supposed to occur in isolation-cells.

(4) **Relationship between the shapes of ray parenchyma cells and the features in storage of starch**

A part from contact-cells, the morphological types of ray parenchyma cells were supposed to have strong relation with the storage of starch both in homogeneous and heterogeneous ray tissues. Some considerations on this point have been carried out.

In the observation of seasonal changes in starch content in ray tissues of poplar, Sauter compared the contact-cell rows which are connected with the vessels through contact-cells and the isolation-cell rows which are isolated from the vessels. He stated that the former preferentially stored starch and asserted the importance of cell rows in storage and mobilization of starch. If his statement is true, differences will be observed between contact-cell rows and isolation-cell rows in the storage of starch also in homogeneous ray tissue. In homogeneous ray tissues of this experiment (for example—tochinoki, itaya-kaede, hannoki, buna), there was no differences between contact-cell rows and isolation-cell
rows (Photo. 4).

In konara, moreover, cells constituting broad ray are all isolation-cells and generally procumbent cells. In some instances, however, square cells were observed in broad ray and they preferentially stored starch grains (Photo. 7). This feature emphasizes that the shape of ray cells is strongly related with the storage of starch regardless of contact-cell rows or isolation-cell rows.

In poplar the cells constituting contact-cell rows are considered to be like M-cells. The morphological difference of M-cells from procumbent cells other than marginal position is thought to show the difference in the storage of starch. From the consideration of the relationship between cell shape and the storage of starch mentioned above, it is pointed out that upright, square or M-cells have a stronger tendency in the storage of starch than procumbent cells.

Strong relationship between morphological types of ray parenchyma cells and the storage of starch was clarified although ray tissues show complicated structure in broad-leaved trees. Individual cell rows of ray tissue generally are constituted of single-shaped cells although there are some exceptions. Cell rows of ray tissue, therefore, exhibit their function controlled by the function of individual cells which are originated from the morphology of the cells.

References

7) JENSEN, W. A.: The histochemical localization of acid phosphatase in root tip cells. Amer. J. Bot. 43. 50-54, 1956
Photo. 1. A light micrograph of a radial section showing the early deposition of starch in square cells (arrows) in koshiabura (August, outermost annual ring).

Photo. 2. A light micrograph of a radial section showing the preferential stock of starch in M-cells (arrows) in nemunoki (September, outermost annual ring). Starch grains also are stored in living wood fibers (LWF).

Photo. 3. An electron micrograph of a tangential section of koshiabura showing the deposition of starch in contact-cell (November, outermost annual ring). Notes; A: amiloplast, S: starch grain, L: lipid droplet.

Photo. 4. A light micrograph of a radial section showing the features in starch-deposition in tochinoki. Procumbent cells other than contact-cells (arrows) contain the almost equal amount of starch grains (August, outer sapwood).

Photo. 5. Histochemical localization of acid phosphatase (ACPase) in koshiabura (August, outermost annual ring). Contact-cells contain large amount of ACPase (arrow) (radial section).

Photo. 6. Histochemical localization of succinate dehydrogenase in koshiabura (January, outermost annual ring). Contact-cells contain large amount of SDH (arrows) (radial section).

Photo. 7. A light micrograph of a radial section showing the peculiar starch deposition in “the square cells” in broad ray (arrows) (konara, August, outermost annual ring).