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ORIGINAL ARTICLE

Hiroshi Matsunaga · Rika Shiotari · Junji Matsumura
Kazuyuki Oda · Yasuhiro Utsumi · Koichi Yamamoto

Potassium distribution in black heartwood of sugi (*Cryptomeria japonica*) I: localization in axial parenchyma cells

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Abstract This is the first report to ascertain potassium location in black heartwood of sugi (*Cryptomeria japonica*). The objective of this study was to understand the distribution of potassium in sugi black heartwood in connection with anatomical morphology. Scanning electron microscopy with energy dispersive X-ray analysis (SEM-EDXA) was used to investigate the distribution of inorganic elements, especially potassium. In black heartwood, potassium was detected in all tissues (tracheids, ray parenchyma, and axial parenchyma), but its concentrations were different in each xylem tissue. Potassium was particularly accumulated in the droplets in axial parenchyma cells. In addition, calcium was also detected in all tissues. Phosphorus sometimes existed in the granules in ray parenchyma cells. From optical microscopy, the axial parenchyma cells were observed to be arranged in a tangential direction on transverse section and the droplets in these cells were distributed in areas of high ash and green moisture content. For red heartwood, potassium detected by SEM-EDXA was far lower in all tissues than in the black heartwood, while the trend for calcium was the converse. Potassium in axial parenchyma cells was more abundant in black heartwood than in typical pale red heartwood.

Key words *Cryptomeria japonica* · Black heartwood · Axial parenchyma cells · Potassium · Inorganic elements localization

H. Matsunaga (✉) · K. Yamamoto
Department of Wood Improvement, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba 305-8687, Japan
Tel. +81-29-873-3211 ext. 534; Fax +81-29-874-3720
e-mail: mhiroshi@ffpri.affrc.go.jp

R. Shiotari
Forest and Forest Products Science, School of Agriculture, Kyushu University, Fukuoka 812-8581, Japan

J. Matsumura · K. Oda · Y. Utsumi
Department of Forest and Forest Products Sciences, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan

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Introduction

Sugi (*Cryptomeria japonica* D. Don) is the most important commercial wood species in Japan. Its heartwood usually forms a reddish-pink color. However, it has long been known in Japan that changes in color often occur in some sugi heartwood, from reddish-pink to black, after the trees are felled. Black-colored heartwood of sugi greatly reduces the commercial value due to its ugly appearance, yet its strength properties are not significantly different from normal-colored wood. In addition, black-colored heartwoods have a high green moisture content,[1–8] which imposes costs that are higher than normal for transport and drying. Furthermore, the color cannot be determined until after a tree is felled, which means that adequate production control is impossible.

The blacking phenomenon in sugi heartwood has been studied by several investigators.[4–6,8–14] It is believed to result from oxidative polymerization of phenolic substances under weak alkaline conditions brought about by exposure to air after being harvested.[4] Abe and Oda[11] showed that potassium hydrogencarbonate is one of the substances that causes the discoloration of sugi heartwood from red to black. In addition, sugi heartwood coloration has been generally ascribed to some norlignans.[15,16] Takahashi[13,14] suggested that the blacking phenomenon might be due to changes in the main norlignans, that is agatharesinol and sequirin-C, under weak alkaline conditions in the presence of potassium hydrogencarbonate.

Some recent studies have suggested a number of points that may be relevant to the occurrence of black heartwood:

1. The ash content in black heartwood is higher than in normal heartwood (reddish-pink).[6,9,12,17]
2. The pH values of typical black heartwoods are weakly alkaline, while the pH values of normal red heartwoods are weakly acidic.[9–14]
3. The total content of Ca, Mg, Na, and especially K, is greater in black heartwoods than in red heartwoods.[6,9,12,13]

4. Black heartwoods contain more extractives than red heartwoods.[4,5,12–14]

However, it has not been revealed how large quantities of water, and alkaline earth and alkali metals (especially potassium) are transported into heartwood and accumulate in it. In addition, there is still no detailed information on potassium distribution in black heartwood.

In this study, the differences in the distribution of potassium in tissues of black-colored sugi heartwood in connection with anatomical morphology were clarified using scanning electron microscopy with energy dispersive X-ray analysis (SEM-EDXA) and optical microscopy.

Materials and methods

Plant materials

Four sugi (*Cryptomeria japonica*) trees that were 50 years old and approximately 25 cm in diameter at breast height (DBH) were felled in the Miyazaki Experimental Forests of Kyushu University. Their cultivars were all Obi-sugi group, two had typical reddish-pink-colored heartwoods and the other two had typical black-colored heartwoods. The individuals used as black-colored heartwoods became black after the trees were felled. Disks (20 cm thick) were obtained from below breast height (1.4 m) of each tree and fresh blocks were successively sampled from sapwood to heartwood, including intermediate wood. Some of the fresh blocks were used to measure the moisture content and the others were immediately fixed in a 3% glutaraldehyde solution for observation and elemental analysis.

Green moisture, potassium, and ash content

Fresh blocks were immediately weighed after cutting from disks and again after drying for 24 h at 103°C to determine the moisture content. After measuring, the samples were ashed in muffle furnaces at 200°C for 3 h, then at 300°C for 3 h, at 500°C for 3 h, and finally at 700°C for 3 h. The ash contents were measured and then the ash samples were dissolved in 6 N hydrochloric acid for potassium analysis by atomic absorption spectrophotometry (AAS; Shimadzu AA-6400F).

SEM-EDXA technique

Transverse sections (10 μm thick) taken from the fresh blocks fixed in 3% glutaraldehyde with a sliding microtome were examined by SEM-EDXA. The sections were attached to carbon specimen stubs using double-sided carbon-cello tape and were carbon-coated in a vacuum evaporator (Jeol JEC520). The surfaces of the carbon specimen stubs were polished following the example of Kobayashi et al.[18] By using the carbon stubs with a polished mirror surface as a support and slicing off an appropriately thick biosection, the samples were stabilized against the intense electron beam.[18–20]

These samples were examined with a Jeol JSM-5600LV scanning electron microscope equipped with a Jeol JED-2140 energy dispersive X-ray analyzer, which detects elements with an atomic number higher than sodium. The conditions used in this study were as follows: accelerating voltage 15 kV; illuminating current 1.5 nA; working distance 20 mm; takeoff angle 30°; tilt angle 0°; dead time 15%–20%; window width K-Kα: 3.25–3.38 keV, Ca-Kα: 3.63–3.76 keV, P-Kα: 1.20–1.30 keV. Backscattered electron images (BEI) that produced both specimen shapes and contrast by atomic number were mainly observed. The X-ray intensities were measured by 100 live second point analysis. Point analyses were carried out for ten places in each tissue of each growth ring.

Observation under optical microscope

The fresh block samples fixed with 3% glutaraldehyde were gathered from sapwood, intermediate wood, and heartwood. After observation using a zoom stereo microscope, transverse and tangential sections of 20 μm thickness were observed under optical microscope. These sections were not stained so as to retain fresh hues and were not embedded with resin so as to prevent droplets in axial parenchyma cells from falling off.

Results and discussion

Green moisture, ash, and potassium content at breast height of the stem

Figure 1 shows the radial variation of green moisture, ash, and potassium contents at breast height of the stem. Figure 1A shows data for a black heartwood specimen and Fig. 1B shows data for a red heartwood specimen. Black heartwood had higher green moisture content than red heartwood. Ash and potassium contents in black heartwood were clearly related to green moisture content (Fig. 1A). The other red and black heartwoods had similar radial variations of green moisture, ash, and potassium contents at breast height of the stem. Morikawa et al.[12] reported that there was a significant correlation (at 1% or 5% level) between green moisture content, ash content, and potassium content in heartwood.

Potassium distribution in various tissues

Figures 2A and 3A show backscattered electron images of transverse sections of black heartwoods. These images show droplets in the axial parenchyma cells. Most droplets in the axial parenchyma cells were distributed in the latewood, but occasionally appeared in the earlywood (see the arrow in Fig. 2A).

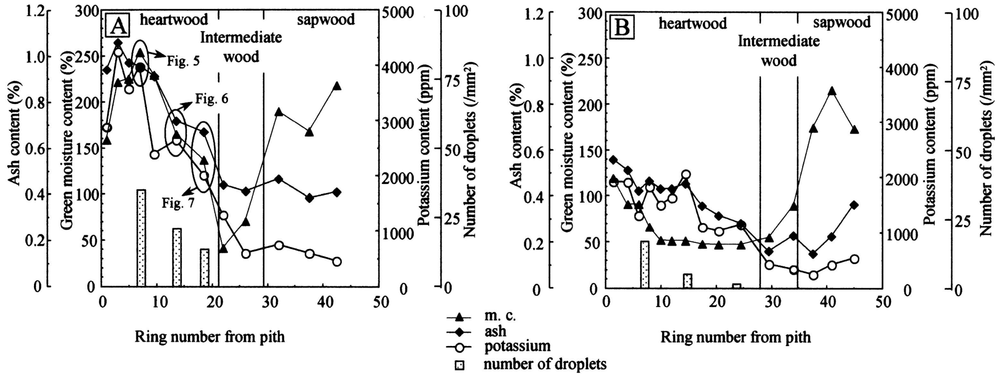


Fig. 1. Radial variation of green moisture, ash, and potassium contents at breast height of sugi and number of droplets in axial parenchyma cells for each unit area for **A** black heartwood and **B** red heartwood

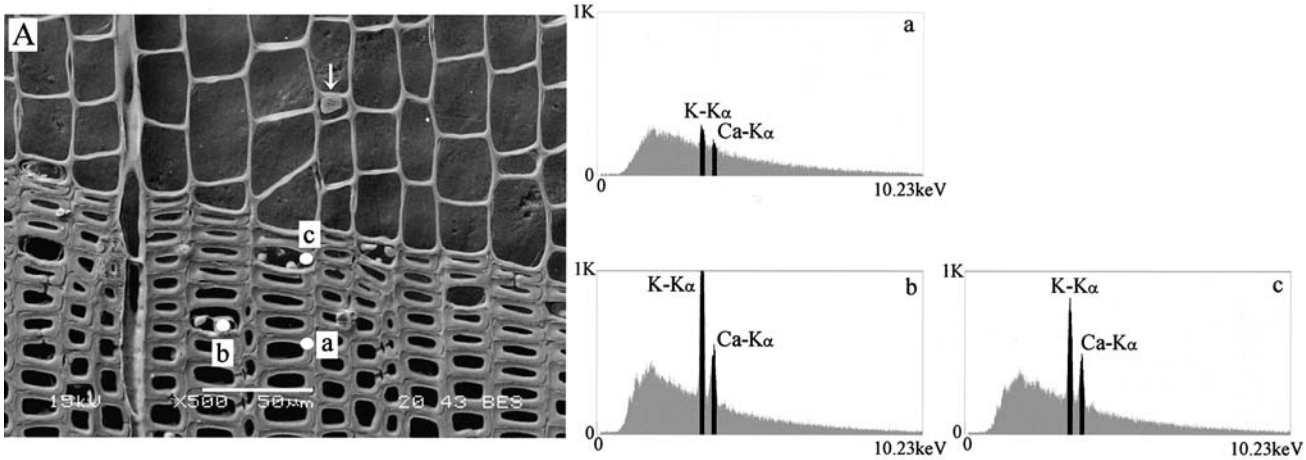


Fig. 2. Backscattered electron image (**A**) and energy dispersive X-ray analysis (EDXA) spectra obtained from each tissue of a transverse section of sugi black heartwood (**a**–**c**). **a** Secondary wall in a tracheid; **b** and **c** droplets in axial parenchyma cells. Point analysis locations of each spectrum are shown in **A**. *Arrow* indicates a droplet in the axial parenchyma cells of the earlywood

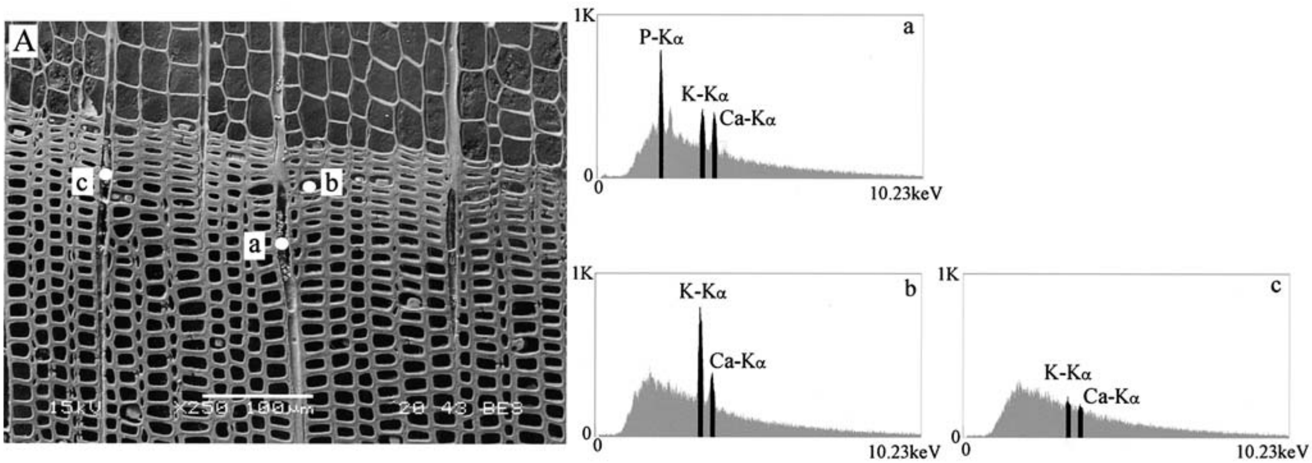


Fig. 3. Backscattered electron image (**A**) and EDXA spectra obtained from each tissue of a transverse section of sugi black heartwood (**a**–**c**). **a** Granule in a ray parenchyma cell; **b** droplet in an axial parenchyma cell; **c** granule in a ray parenchyma cell. Point analysis locations of each spectrum are shown in **A**

Point analyses were carried out to clarify the distribution of potassium in various tissues. Figures 2a–c and 3a–c show representative examples of the spectra by point analyses for cell walls in tracheids, droplets in the axial parenchyma cells, and granules in the ray parenchyma cells. From each spectrum, potassium was detected in every tissue. However, the potassium concentrations were different in each tissue. Figures 2b, c, and 3b show that potassium was much more heavily concentrated in the axial parenchyma cells than in the cell walls (Fig. 2a) in tracheids. On the other hand, the

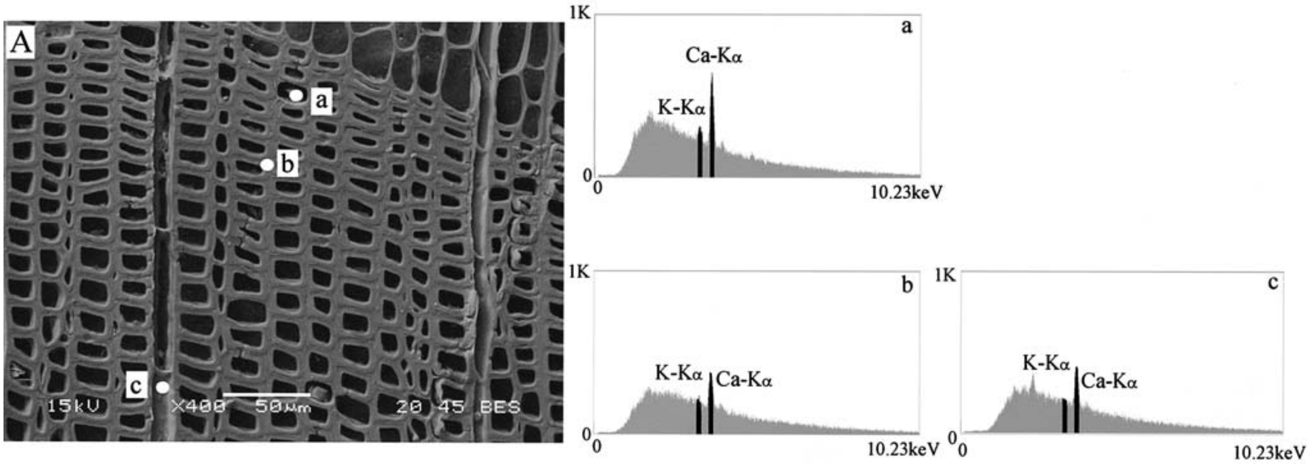


Fig. 4. Backscattered electron image (**A**) and EDXA spectra obtained from each tissue of a transverse section of sugi red heartwood (**a**–**c**). **a** Droplet in an axial parenchyma cell; **b** secondary wall in a tracheid; **c** granule in a ray parenchyma cell. Point analysis locations of each spectrum are shown in **A**

granules (Fig. 3a) in ray parenchyma cells showed potassium but the concentration was not higher than in the axial parenchyma cells. There existed many granules (Fig. 3c) in ray parenchyma cells in which potassium was not detected so that no clear tendency of potassium distribution was found in ray parenchyma cells. For every tissue in which potassium was detected, calcium was also detected. In the ray parenchyma cells (Fig. 3a), phosphorus was only occasionally detected.

Figure 4A shows a backscattered electron image of transverse sections of red heartwoods. Figure 4a–c shows the spectra by point analyses for the droplets in the axial parenchyma cells, cell walls in tracheids, and the granules in the ray parenchyma cells. Calcium was detected in all spectra (the same as in black heartwood), but potassium concentration was very low compared with that in black heartwood. These results obtained in both black and red heartwoods were recognized in each growth ring.

SEM-EDXA revealed that potassium was not high in the tissues of red-colored heartwood, while black heartwood contained extraordinary accumulations of potassium in the axial parenchyma cells. Similar results were obtained in the other red and black heartwoods. These results suggest that the axial parenchyma cells may play an important role in the black heartwood phenomenon.

Distribution of droplets in axial parenchyma cells in transverse section

It is noted above that axial parenchyma cells are very important when potassium distribution is considered. As Fig. 1A indicates, potassium radial variation and concentration differ in each growth ring in the transverse section. The distribution of the droplets in axial parenchyma cells in the transverse section where potassium was especially heavily localized in black heartwood, was investigated under an optical microscope.

Figures 5, 6, and 7 show the distribution of the droplets in axial parenchyma cells in black heartwood; part **a** in each figure shows transverse sections and part **b** shows tangential sections. The sample locations are shown in Fig. 1A, that is, the 7th growth ring for Fig. 5, the 12th growth ring for Fig. 6, and the 18th growth ring for Fig. 7.

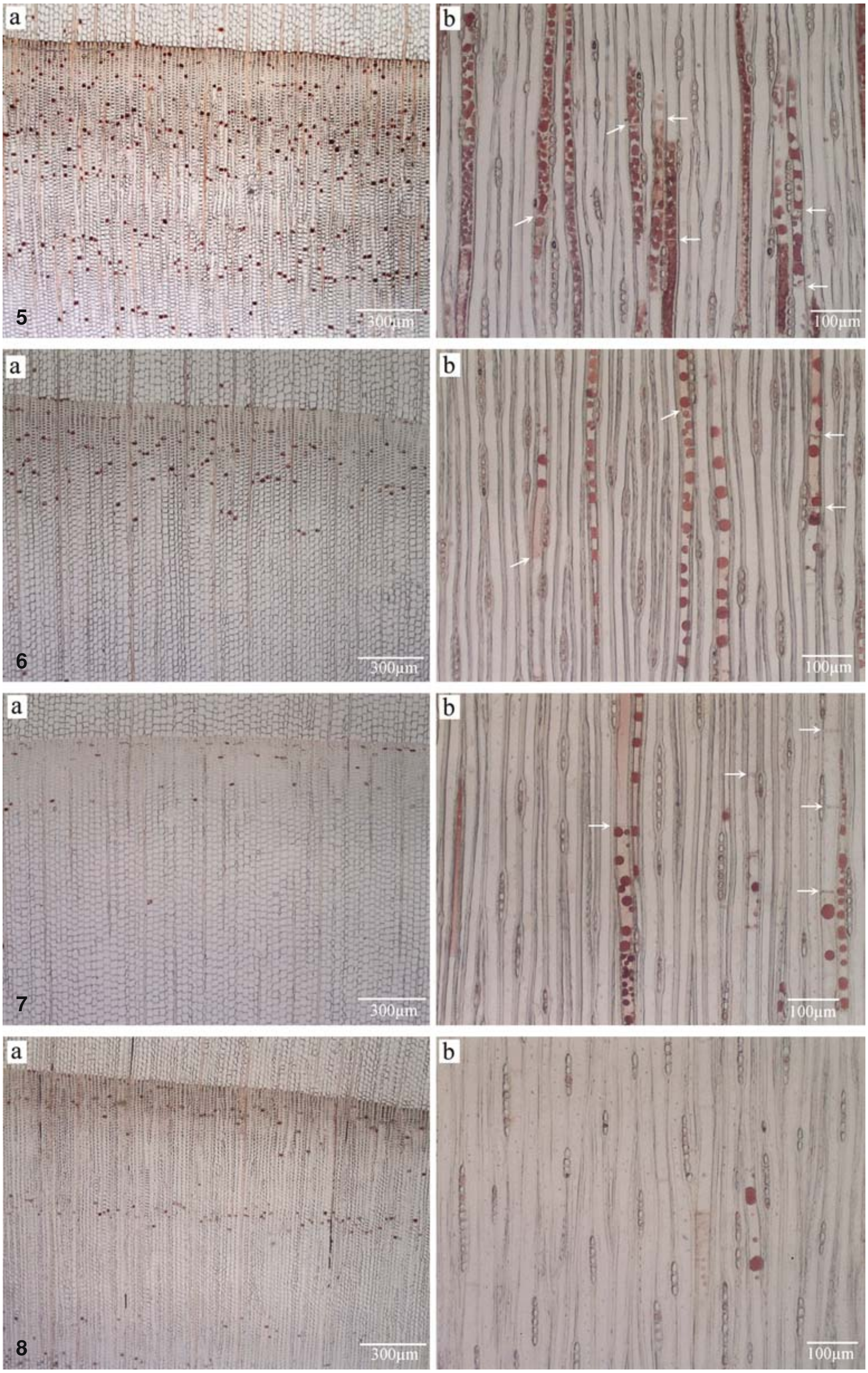
Figures 5–7 show different distributions of the droplets in axial parenchyma cells in each growth ring of the transverse section. In Fig. 5, there are many droplets, most of which are distributed in latewood and in the tangential direction. In tangential sections (Figs. 5b, 6b, and 7b), these droplets existed in axial parenchyma cells, which were constructed with strand end walls (see the arrows). Therefore, the numbers of droplets in the axial parenchyma cells in these sections were counted (Fig. 1). The most important finding is that these droplets increase, as the amount of potassium, ash, and green moisture become larger.

In red heartwood, the droplets in the axial parenchyma cells were less distributed and lighter in hue when compared with black heartwood (Figs. 8, 1).

Kuroda and Shimaji[21] reported that microspectrophotometric analyses indicated that different kinds of phenolic substances were produced between ray and axial parenchyma cells in sugi heartwood. In immunohistochemical studies, Nagasaki et al.[22] also indicated that the chemical structure of the contents of ray and axial parenchyma cells were different and that agatharesinol was localized in ray parenchyma cells, while agatharesinol was not detected in axial parenchyma cells in sugi heartwood. In the present study, the potassium distribution in sugi black heartwood was also different between ray and axial parenchyma cells. This finding suggests that these two types of cells have different functions in heartwood formation, and result in potassium being particularly accumulated in the axial parenchyma cells.

The subjects in this study were four individuals in Obi cultivar groups with samples taken breast-height portions.

Fig. 5a,b. Distribution of the droplets in axial parenchyma cells at the seventh growth ring in black heartwood. **a** Transverse section; **b** tangential section. *Arrows* show the end walls of the axial parenchyma cells **Fig. 6a,b.** Distribution of the droplets in axial parenchyma cells at the 12th growth ring in black heartwood. **a** Transverse section; **b** tangential section. *Arrows* show the end walls of axial parenchyma cells. **Fig.7a,b.** Distribution of the droplets in axial parenchyma cells at the 18th growth ring in black heartwood. **a** Transverse section; **b** tangential section. *Arrows* show the end walls of axial parenchyma cells. **Fig. 8a,b.** Distribution of the droplets in axial parenchyma cells at the seventh growth ring in red heartwood. **a** Transverse section; **b** tangential section



It is necessary to confirm whether the findings of this study can be found in other cultivars and clones in the further studies. This research represents part one of a two-part study. Part two investigates the vertical potassium distribution in trees related with various cultivars and clones.

Conclusions

In this study, the potassium distribution in sugi heartwood was investigated. The following points summarize the investigation findings:

1. In black heartwood, potassium exists in tracheids and ray and axial parenchyma.
2. In black heartwood, potassium is extraordinarily abundant in the axial parenchyma cells.
3. In black heartwood, the droplets of the axial parenchyma cells tend to arrange in the area of the high ash and green moisture content.
4. In red heartwood, the potassium content in tracheids and the droplets of the parenchyma cells is lower than in black heartwood as determined by SEM-EDXA point analysis.
5. For the Obi-sugi investigated in this study, in red heartwood, the droplets of the axial parenchyma cells are less distributed than in black heartwood.

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