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Distribution and Pattern of availability of Storage starch and cell death of Ray parenchyma cells of a Conifer Tree (*Larix kaempferi*)

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Abstract

Starch in the ray parenchyma cells of Larix kaempferi tree varies considerably on the position of trunk. The maximum starch granules observed in sapwood, mostly outer to middle part. Most starch grains were localized in middle lines of parenchyma cells rather than upper and lower lines. Parenchyma cells of the phloem part also content starch rather than outer bark due to content dead cell almost. The distribution of nuclei in secondary xylem cells of L. kaempferi resembled cell death. Starch disappearing occurred prior to cell death in secondary xylem cells. Some characteristics heartwood inducing substances synthesized just after starch depletion, which might be deposited in cell wall. Ray parenchyma cells remained alive for several years. The timing of cell death of middle ray cells different from upper and lower radial ray cells within a ray. Our results also indicate that the position of starch depletion within a ray might affect the timing of cell death. The distribution pattern of storage starch and cell death was inter-linked with each other. This relationship controls the formation of heartwood in conifer trees. This report would be helpful for further research to clarify the heartwood formation in conifers, which has importance in tree breeding program and improvement of quality wood.

Keywords: *Larix kaempferi*, storage materials, ray parenchyma cell, conifer, heartwood formation.

Introduction

Starch is a major reserve product of trees, and accumulates particularly in ray cells of the xylem and cambium. Ray parenchyma cells remain alive for several years at least, and they play an important role in the storage and transport of nutrients[1, 2]. During transporting of such nutrients the amount varied on position of tissue and annual ring. The reserve materials (e.g. starch) have been removed or converted into heartwood substances[3]. Some species form colored heartwood because xylem parenchyma cells synthesize heartwood substances such as polyphenols that contribute to increases in the decay resistance of tree trunk, prior to their death[4 -7].

Cell death plays important roles in the function of secondary xylem cells in woody plants. Tracheary elements lose their organelles immediately after their differentiation[8] and after cell death; tracheary elements play a critical role in the transport of water. The early death of ray parenchyma cells within upper and lower cell lines of a ray has been observed in hardwoods, such as *Magnolia obovata* and *Populus tremuloides*[9]. One research group observed the positional differences in the quantities of starch grains among ray parenchyma cells in the quantities of starch grains among ray parenchyma cells in some hardwoods, such as *Castanea crenata*[10]. Therefore, we postulated that positional information might be an important determinant of the control of cell death, differentiation and function of ray parenchyma cells in hardwoods, as is the case in conifers.

The significance of differences in elemental distribution between sapwood (SW) and heartwood (HW) is still unclear. Since the complex process of formation of heartwood from sapwood consists of a chain of events involving many activities so that our present study will find out such information of storage substances, their occurrences and degradation among the different parts of ray parenchyma cells, which would be a part of one activity[8,11]. In this article we demonstrate the chronological steps of starch appearance and their degradation patterns within the ray parenchyma cells. The distribution of starch among the sapwood-heartwood of *L. kaempferi* is discussed from a histochemical viewpoint. This report contributes to the understanding of the physiological changes associated with the senescence of ray parenchyma cells and storage starch distribution. A comparison study on the disappearing pattern of storage starch and nuclei within cells was made. However, the detailed mechanism of histochemical changes in transition zone from sapwood to heartwood is remaining limited. Therefore, in the present study, we investigated histochemical changes of starch from sapwood to heartwood in Japanese larch (*Larix kaempferi*) tree to make a clear conception about the mechanism of cell death in transition zone and heartwood formation.

Material and Methods

Plant materials: A Japanese larch (*Larix kaempferi*) trees of approximately 16 years of age, grown at tree breeding center,

FFPRI (Forestry and Forest Products Research Institute), Nagano prefecture, Japan used in this study. Discs from stem trunk (at breast height) were collected just after felling the tree in May 2011 (season of active cambium). Samples were fixed in 4% glutaraldehyde in 0.1M phosphate buffer (pH 7.3) at room temperature.

Preparation of samples for observation of starch: Fixed samples were washed in distilled water followed by trimming into small pieces and, sections (radial, transverse, and tangential) were cut at a thickness of approx. 40 µm with a stainless steel microtome blade on the freezing stage of a sliding microtome (MA-101; Komatsu Electronics, Tokyo, Japan) and washed with phosphate-buffered saline (PBS; 137mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.0 mM $NaHPO_4$, adjusted to pH 7.3)[11]. For light microscopic observations of starch grains, sections were stained with iodine-potassium iodide (I_2-KI) for 2-3 min[11-14]. After staining, sections were washed with distilled water.

Semi-quantitative analysis of starch and brown unknown materials*:* For the semi-quantification of starch, the desired portion of phloem, cambium, and xylem ray parenchyma cells were cut from the original images. Each category of cells in the image was segmented by manually drawing an outline, and the images were converted to binary images. The total analyzed-section area and total starch grains area were measured with the particle analysis command of Image J. The percentage of starch for specific area was calculated for each xylem ray parenchyma cells[14]. According to the above mentioned method the semi-quantification of unknown brown colored substances were done.

Preparation of samples for observation of nuclei: After cutting the sections using sliding microtome (as above), sections were stained with 1% aqueous solution of acetocarmine followed by washing with distilled water and series of ethanol (from 30% to 100% ethanol) for observation of nuclei[11-12]. Stained sections were observed under a light microscope (Axioscop; Carl Zeiss, Oberkochen, Germany). For observation of the autofluorescence of nuclei, radial sections of 40 µm thickness were examined with a fluorescence microscope (BX61; Olympus, Tokyo, Japan) under epifluorescence illumination (excitation/emission combination, BP 460-495/BA 510IF)[12].

Results and Discussion

The color of the heartwood of *L. kaempferi* was dark brown and the sapwood was light yellowish. The sapwood-heartwood (SW-HW) boundary occurred between mid of 7th and latewood of 8th annual ring (figures 1a, b) from the cambium. Gradually the color of the sapwood changed to dark yellowish to light brown in the heartwood. Transition zone occurred in between sapwood and heartwood region (figure 1b). Some morphological basic labeling and different angular anatomical views were shown schematically in figure 1c. In case of our study, only radial surface of the sample was examined.

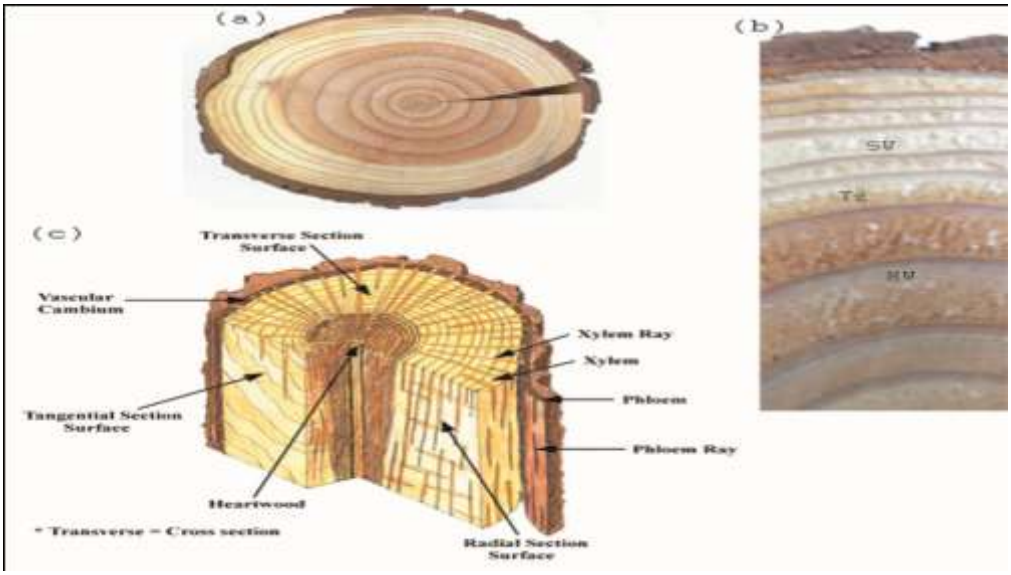

Figure-1

Photographs (a and b) and sketch diagram (c) showing morphological views of *Larix kaempferi* by naked eye. a) a collected stem disc, b) enlarged view of a part of stem disc; c) different parts of a stem disc showing with different angular views. SW, sapwood; TZ, transition; HW, heartwood

The distribution of reserve starch was observed in the trunk wood of *L. kaemferi*. The starch granules were observed as black mass and black grain like structure under light microscope. Visualization of starch covered from phloem to heartwood of the sample. There was no remarkable starch found in phloem and cambial zone. Limited starch grains viewed within phloem parenchyma cells, but not in uniform distribution (figure 2a). Our observation also revealed that there was complete lack of starch in current year xylem (figure 2a) and second year xylem from the cambium (data not shown). From third annual ring to inner sapwood the starch granules increased with depth. The abundant starch granules visualized in mid early wood of 7th annual ring (figure 2b). There were numerous heterogeneous brown colored materials appeared in ray parenchyma cells of 3rd growth ring to inside of trunk (figure 2b). The complete depletion of starch observed in 8th growth ring counted from the cambial zone (figure 2c).

To investigate the sources of brown colored unknown substance, we observed a similar section with and without I_2-KI staining. Results on such investigation showed the brown colored unknown substances appeared in both sections. So, the origin of such materials not comes from stain. Only brown materials visualized in control observation (figure 3a). On the other hand, both starch and brown materials available in ray parenchyma cells together (figure 3b). So, there was no change of brown colored materials observed after I_2-KI reaction. Beside those, the distribution pattern of storage starch within different parts of an annual ring was investigated, which depicted in figure 4. The outer early and mid of the early wood showed maximum deposition of starch in ray parenchyma cells of an annual ring (figure 4a), but decreasing the starch deposition at the innermost early wood portion (figure 4b). On the other hand, there was complete devoid of starch occurred in ray parenchyma cells of latewood potion in an annual ring (figure 4b).

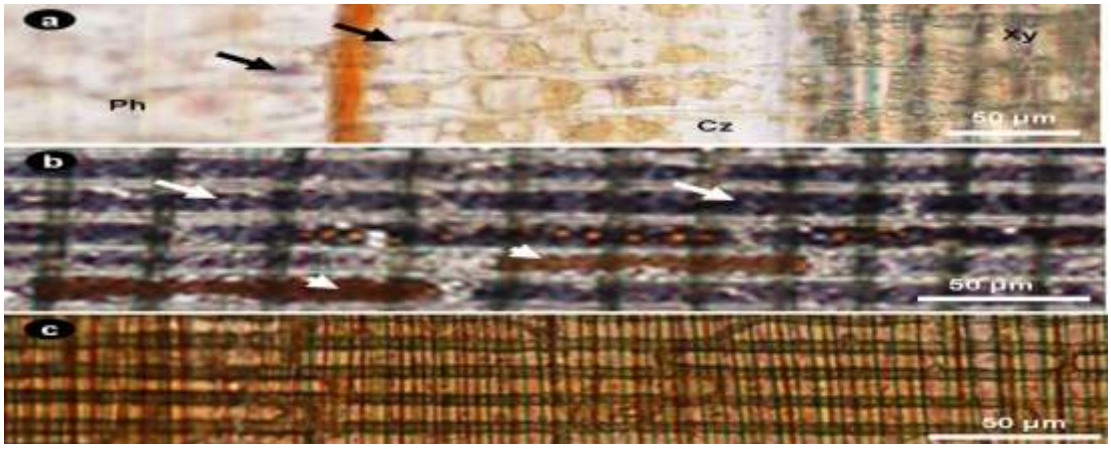

Figure - 2

Light micrographs of radial sections, stained with iodine potassium iodide showing starch grains around the phloem-cambium zone (a), sapwood (b) and heartwood (c) of *Larix kaempferi* stem. Ph, Phloem; Cz, Cambial zone; Xy, Xylem; arrows indicate starch grains; arrowheads indicate unknown brown colored materials

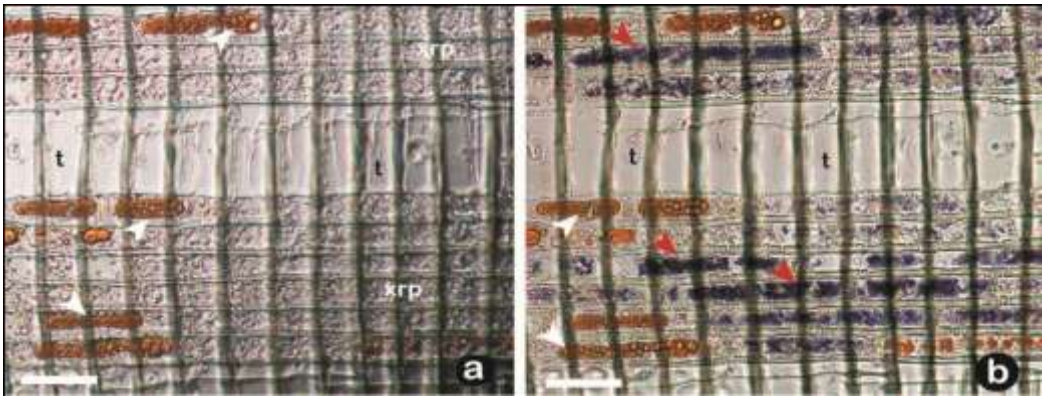

Figure - 3

Comparison of light micrographs between before (a) and after (b) staining with iodine potassium iodide in sapwood (around middle of 6th annual ring from cambium) of *Larix kaempferi* stem. White arrowheads indicate brown colored materials, red arrowheads indicate starch grains; xrp, xylem ray parenchyma cell; t, tracheid. Scale bars = 50 µm

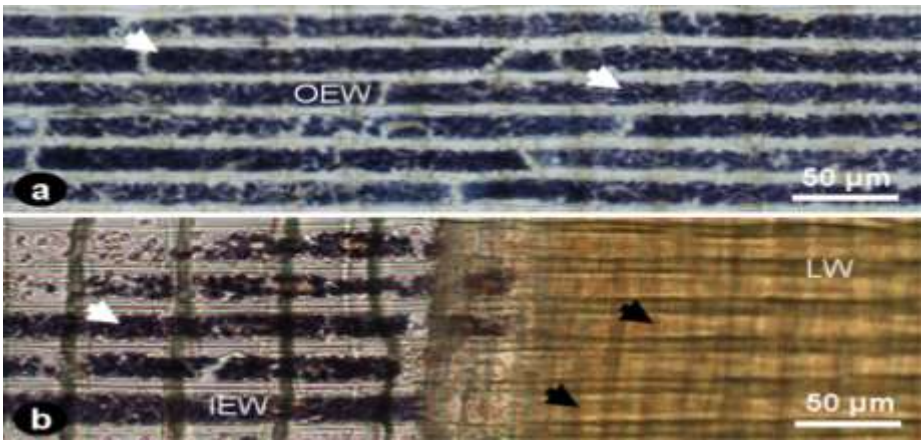

Figure - 4

Light micrographs of radial sections of ray parenchyma cells of *Larix kaempferi*, showing distribution pattern of starch grains within an annual ring (from outer early wood of 6th annual ring to latewood of 7th annual ring). Sections stained with iodine potassium iodide to localize starch grains. a) abundant of storage starch grains (white arrowheads) visualized from outer early wood (OEW) to mid early wood, b) innermost early wood (IEW) region of 6th annual ring showing starch grains (white arrowheads) and latewood (LW) region of 7th annual ring showed lack of starch (black arrowheads) in cells

Semi-quantification of starch and brown colored materials were examined in relation to different parts of sample. In starch quantification, the maximum occurrence observed in 7th annual ring, i.e. innermost part of sapwood (figure 5). Whereas the starch content was nil in phloem, cambial zone, 1st and 2nd annual ring (counted from the cambium). The quantity of starch showed increasing tendency from 3rd annual ring to 7th, dramatically it was also nil in 8th annual ring (figure 5). On the other hand, quantity of unknown brown colored substance showed relationship with starch appearance and depletion. Data showed the increasing tendency appeared from 6th to 7th annual ring, where maximum starch content abruptly depleted. This sudden depletion of starch may cause of increasing the amount of brown colored substances (figure 5). Rather than the all over parts from phloem to heartwood, we also investigated semi-quantification among different portions of 7th annual ring. Our findings reported the outer xylem ray parenchyma showed increasing tendency up to the mid early wood, but the degradation also appeared dramatically from mid early to inside of 7th annual ring (figure 6). The degradation pattern is very quickly occurred, where the quantity of brown colored materials extremely higher (figure 6). In the 7th annual ring the tendency of the amount of brown colored materials increased slowly before the middle early wood, then suddenly reached higher peak just after depletion of starch (figure 6). The highest availability of starch can be observed in the mid of 7th annual ring (figure 6) and decreases with increasing depth into the trunk and ceases at the sapwood-heartwood boundary. A marked decrease in starch content occurred during transformation of sapwood into heartwood. This zone is located between mid and innermost part of 7th annual ring. In the outer heartwood no trace amount of starch were present (figure 6).

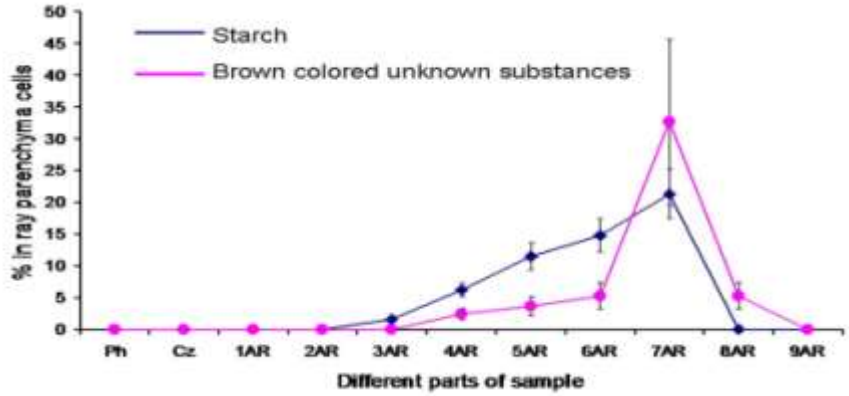

Figure-5

Line graphs showing percentage of starch and brown colored substances in ray parenchyma cells of different portion of *Larix kaempferi* stem. Ph, phloem; Cz, cambial zone; AR, annual ring. Annual ring numbers were counted from the cambial zone. Vertical bars indicate standard errors

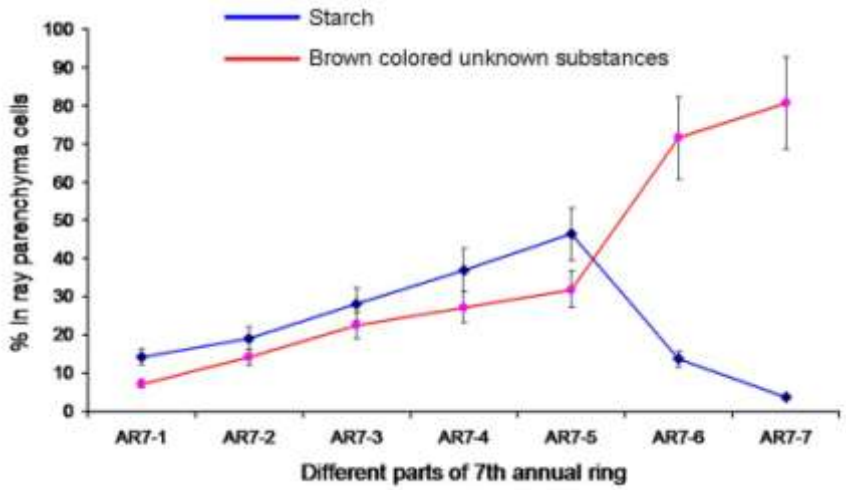

Figure – 6

Line graphs showing percentage of starch and brown colored materials in xylem ray parenchyma cells of different portion of 7th annual ring of *Larix kaempferi* stem. AR7-1 to AR7-7 indicates the sequential parts of xylem ray parenchyma cells within the 7th annual ring. Annual ring counted from the cambial zone. Vertical bars indicate standard errors

Nuclei observation was illustrated in figure 7. The shape and size of nuclei vary with location and types of cell. By using acetocarmine stain, the nuclei of live cells became red colored. The outer phloem cells were devoid of nuclei since those cells dead (figure 7a). The live cells in inner bark tissue content relatively small sized nuclei. Elliptical shaped nuclei visualized in phloem parenchyma cells observed (figure 7b). Cells of cambial zone content round shaped nuclei (figure 7c). Ray parenchyma cells in middle sapwood content various shaped nuclei (figures 7c-e). Nuclei were not visualized in the heartwood cells, i.e. dead cells (figure 7f). The variation of the shape of nuclei clearly visualized between early wood and latewood under fluorescence microscope (figure 8). Usually fusiform shaped nuclei visualized in early wood portion of the xylem tissue. The small and spherical shaped nuclei noticed in latewood portion of xylem tissue. This difference occurred due to the size of ray parenchyma cells or species specific genetical factors. Rather than the differences between latewood and early wood of xylem, the size and shape of nuclei also varied due to different physiological factors which are demonstrated in figure 9.

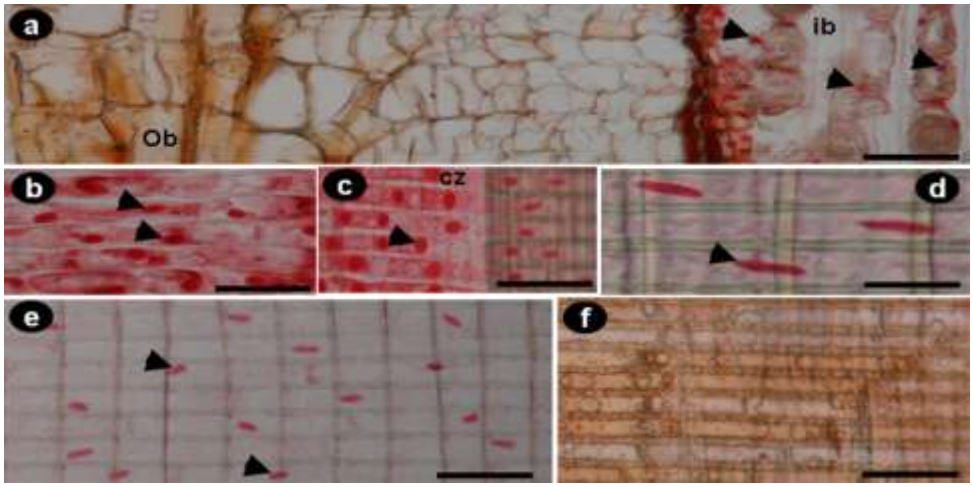

Figure-7

Light micrographs of radial observation of nuclei of different parts of stem of *Larix kaempferi*, stained with acetocarmine. a) phloem tissue, b) phloem ray parenchyma cells, c) ray parenchyma cells of camial zone and current year xylem, d) Slender nuclei in ray parenchyma cells of mid sapwood, e) ray parenchyma cells in early wood of 5th annual ring, f) heartwood zone, ray parenchyma cells devoid of nuclei. Arrowheads indicate nuclei within cells; ob, outer bark; ib, inner bark; cz, cambial zone. Scale bars 50 µm

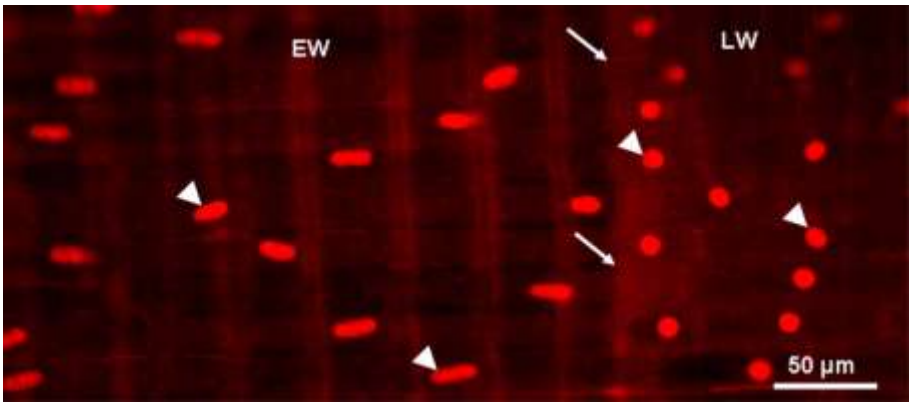

Figure – 8

Illustration on nuclei between early wood and latewood of an annual ring of *Larix kaempferi* stem under epifluorescence illumination (excitation/emission combination, BP 460-495/BA 510IF). EW, early wood; LW, latewood; arrows indicate boundary between 6th and 7th annual ring, counted from cambium; arrowheads indicate nuclei in ray parenchyma cells

Nuclei visualized up to the middle of 7th annual ring uniformly, and then disintegration of nuclei observed up to latewood of 8th growth ring in *L. kaempferi*. There were some morphological changes in nuclei observed in xylem ray parenchyma cells. All ray parenchyma cells from the phloem to cambial zone, had spherical nuclei, relatively smaller spherical shaped nuclei observed in current year xylem. Mostly fusiform and spherical nuclei visualized from 2nd annual ring to inner region of 7th annual ring, rather than variation of size between latewood and early wood. Deformed and relatively smaller sized nuclei visualized from innermost region of 7th annual ring to latewood of 8th annual ring (figure 9). There were varieties of shape and size remarked in sapwood, intermediate portion between sapwood and heartwood. Xylem parenchyma cells of same species showed morphologically distinguished nuclei shape (figures 9a, b). The remarkable slenderness of nuclei in ray parenchyma cells of middle sapwood observed, which was not found in sapwood near to cambial zone (figure 9b). The nuclei become smaller and distorted into abnormal shapes just before their disappearance (figure 9c), which is observed in transition area between sapwood and heartwood of *L. kaempferi* stem. Most of the nuclei were not visualized under light microscope due to presence of unknown substance in the transition area (figure 9d). The different shape and size of nuclei in parenchyma cells of a ray of an individual annual ring were noticed in this investigation also.

The schematic representation on the distribution and pattern of starch and nuclei was illustrated in figure 10. Nuclei were uniformed visualized from phloem to 6th annual ring. Nuclei of mid of 7th annual ring also visualized uniformly within the cells in outer part (figure 10) rather than one or two cells become dead (i.e. lack of nuclei) in inner part. Nuclei disappearing or losing started from the boundary area between sapwood and heartwood, which located from innermost part of 7th to latewood part of 8th growth ring. There was no nucleus present in heartwood region, i.e. after latewood of 8th annual ring (figure 10). The localization result revealed that starch depletion within the ray parenchyma cells occurred prior to the death of cells. No living cells were found in the heartwood zone. There are numerous heterogeneous unknown substances observed in SW-HW boundary followed by disappearing starch droplets (figure 10). It indicated the formation of such unknown substances may be the results of starch degradation. The live and dead cells were also easily distinguished in the schematic diagram (figure 10). The brown colored substances visualized from 4th growth ring to inside (i.e. outer xylem part). The occurrence of such substances increased with the inside of xylem tissue towards the transition zone (figure 10). In heartwood region, the absence of such brown substances is remarkable (figure 10). Such kind of substances may be intermediate products to induce heartwood, which may be produced from storage starch within the cells. The remarkable cell wall thickening also characterized between current year xylem (figure 10a) and innermost part of 7th annual ring (figure 10b). Thus, brown colored substances might play an important role in the formation of heartwood or heartwood inducing substances and the transport of such materials that are related to cell death.

The differences in terms of the timing of cell death, differentiation and appearance of storage starch in ray parenchyma cells of *L. kaempferi* were schematically shown also in figure 10. The positional patterns of disappearance and overall distribution of storage starch also illustrated there. No considerable amount of starch grains visualized in phloem, cambial zone and outermost xylem ray parenchyma cells. There were abundant storage starch observed from 4th to middle part of 7th growth ring, after that no starch was found inside the trunk. The distribution patterns and the quantitative variation of storage materials among the annual rings of *Larix kaempferi* demonstrated in figure 10. Results revealed that distribution patterns indicate a characteristic features, which would be more important to study the physiology as well as heartwood

formation. Quantitatively, storage starch increased with the depth of annual ring from the cambium zone, where outermost 2 annual rings showed almost devoid of storage starch. The increasing tendency observed with the depth of xylem tissue up to their final degradation. This degradation directly emphasized on the part for synthesis of unknown brown substances (figure 10).

The overall starch disappearing zone, increasing tendency of brown colored substances and their depletion in xylem ray parenchyma cell were outlined in figure 11. In short, the different zone between 7th and 8th growth ring and the localization of starch, brown colored materials, and nuclei in relation to sapwood, heartwood and their intermediate zone (i.e. transition zone) were illustrated at a glance. Furthermore, we also observed differences in the timing of ray parenchyma cell death (figure 11). All of the cells started to die from inner region of 7th annual ring to latewood of 8th annual ring (from the cambium). The appearance of unknown brown colored substances enormously increased by the mid of 7th annual ring just after disappearing of storage materials (figure 11). In heartwood portion (from latewood of 8th to inward of trunk), such brown unknown substances are deposited into the cell wall, followed by natural color of the cells become darker than outer sapwood.

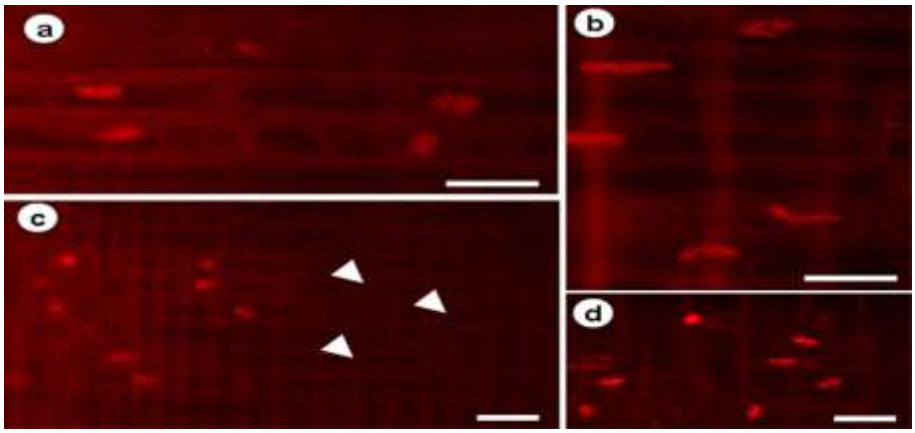

Figure - 9

Light microscopic features of nuclei in ray parenchyma cells of different parts of *Larix kaempferi* stem; a) cells of sapwood, mid of 7th annual ring, b) outer sapwood region (7th annual ring from the cambium), c) intermediate/transition zone between sapwood and heartwood (latewood region of the 8th annual ring from cambium), arrowheads indicate dead cells, d) mid 7th annual ring zone. Scale bars 50 µm

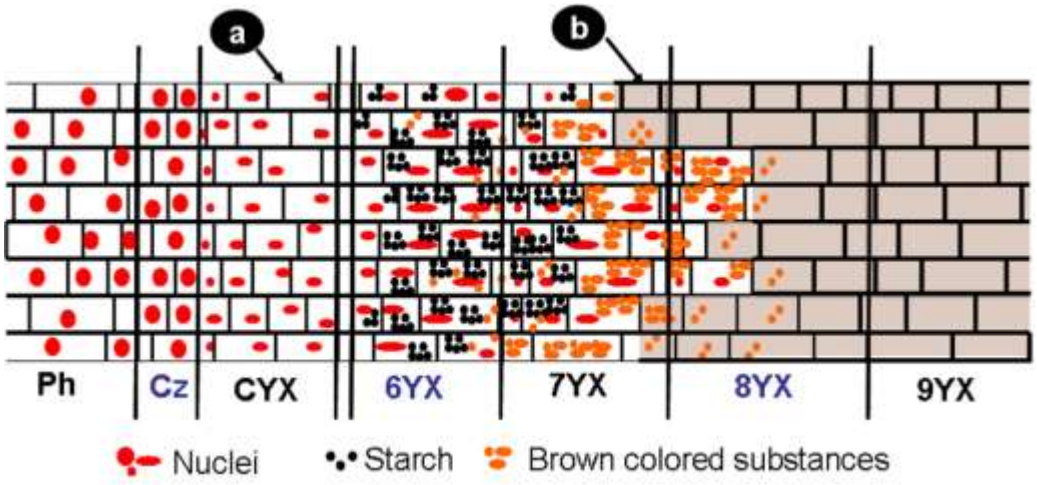

Figure - 10

Diagram on distribution patterns of storage starch and nuclei in ray parenchyma cells of *Larix kaempferi* stem. Ph, Phloem; Cz, Cambial zone; CYX, Current year xylem; YX, year xylem (e.g. 6YX indicates 6th year xylem); a, narrower cell wall in current year xylem; b, thickening of cell wall in innermost early wood of 7th annual ring. Annual rings counted from cambial zone

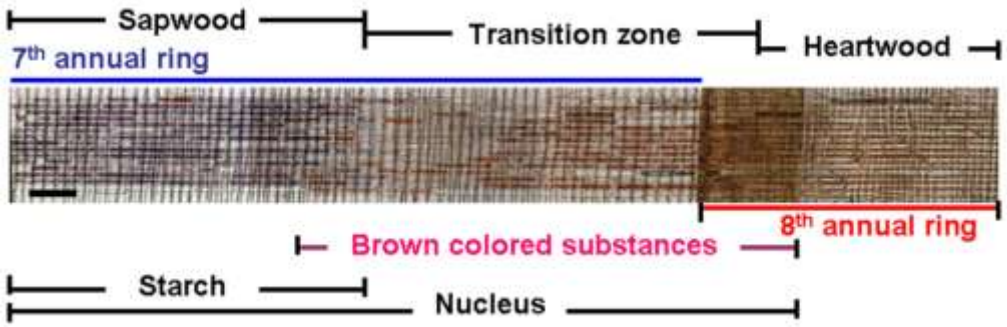

Figure – 11

Outline view on distribution of starch, nuclei and brown colored substances in respect of sapwood, transition zone and heartwood between 7th and 8th annual ring of *Larix kaempferi* stem. Horizontal and vertical bars indicate distribution and limit of such observation. Scale bar 100 µm

Discussion

It is well known that some of photosynthates move inward from the inner bark through the rays to the cambium. Rest of the photosynthates after consumed for different physiological functions, moving along the rays toward the Frances J Sharom center of the tree. Some of them may begin to accumulate and over time begin to break down to form a variety of compounds collectively as extractives. In a living tree most of the sapwood cells are dead (i.e., lack of protoplast); they live, on average, only about three weeks after they are formed. The food storage cells, however, live for a much longer time, and it is the death of these cells that marks the formation of heartwood. The patterns of cell death and distribution pattern of storage substances in the secondary xylem play an important role in the functions of heartwood formation. Most wood cells die after differentiation and cell wall lignifications. Only ray parenchyma and resin canal epithelial cells remain alive. However, starting from the cambium, and proceeding to inner sapwood, a gradual degradation of the cytoplasm in parenchyma cells occurs. During heartwood formation extractives are gathered in the cell walls and, eventually, even ray parenchyma cells die[15-16]. Wall thickening and lignifications of the ray parenchyma cells[9, 17] and also the radial resin canal tissue have been reported to be closely associated with the heartwood formation in the genus *Pinus*[18].

Hydrolysis of storage starch could partly account for the larger amount of unknown brown colored substances, synthesized through entire sapwood, because the amount of storage materials decreased towards the transition zone, while the heartwood extractives increased (figures 9, 10). Although previous reports could be easily assumed that heartwood extractives are responsible due to their color and exclusive existence in the formation of heartwood. Using UV-visible microscopic spectrometry, a research work reported that these materials had absorption in the UV range, indicating that they were heartwood phenolics[19]. Therefore, it was suggested that ray parenchyma cells could biosynthesize and accumulate different kinds of heartwood extractives. It has been reported that both axial and ray parenchyma cells are involved in heartwood formation with their different functions[20-21]. Our observations revealed that heartwood extractives were formed in situ at the heartwood periphery from translocated starch. The starch granules were degraded mostly at the sapwood-heartwood transition zone[22]. The composition of the substances formed is under genetic control and the amount formed is significantly influenced by the physiological conditions impinging on the parenchyma at the time of formation. In *Robinia pseudoacacia* (black locust), as in other heartwood forming species, the transition zone between sapwood and heartwood shows an enhanced metabolic activity at certain times of the vegetation period[23-25].

The formation and accumulation of heartwood inducing substances related with the transformation of sapwood into heartwood, and it is attractive to speculate that the building blocks of these secondary substances derive from storage starch. One of the past research report was the best evidence to supports that the polyphenols are formed in situ from the carbohydrates in the dying sapwood cells[26]. Our result suggested such unknown brown colored heterogeneous substances either partly or fully responsible for heartwood formation. Those substances enormously increased followed by disappearing of starch grains (figures 4, 9, 10). The differences in timing pattern of the disappearance of starch suggested different roles for directly synthesis of unknown brown colored heterogeneous substances within ray parenchyma cells, which play an important role to form heartwood inducing substances or directly to form heartwood.

In present study, we investigated the disappearance of nuclei as an indicator of cell death of ray parenchyma cells. The partial disappearance of nuclei from ray parenchyma cells started from the upper or lower radial cell lines of each ray[11-12, 27-28]. Similarly, in *Larix kaempferi*, cell death of ray parenchyma cells in the upper and lower cell lines occurred earlier than that of ray parenchyma cells that were located within the other cell lines (figure 9). Those observations revealed that different positions

within a ray are associated with differences in the timing of cell death of ray parenchyma cells in conifers. By contrast, ray parenchyma cells remained alive with protoplasm from the sapwood to intermediate wood. Research on *Acacia auriculiformis* indicated that nuclear slenderness ration increased in ray cells contiguous to vessels but gradually decreased in ray cells away from vessels[27]. In this study, nuclear slenderness clearly observed in ray parenchyma cells of *L. kaempferi* from the cambial zone to transition zone of sapwood-heartwood region. Ray parenchyma cells could survive for at least 7 years, i.e. the growth time from cambium zone towards intermediate wood in *L. kaempferi*. The disappearance of nuclei was a signal of the death of ray parenchyma which marked the formation of heartwood.

Our study revealed, starch grains were localized in ray parenchyma cells of outer sapwood of *L. kaempferi*. Most of the starch grains found in the middle ray parenchyma cells (figures 2, 3). The disappearing pattern of starch within ray cells is observed in a pattern, which related to timing of cell death. In a previous report[11], the position with ray might be an important factor in the control of timing of cell death, the pattern of differentiation and function of ray parenchyma cells in conifer, *A. sachalinensis*. In present study, we found evidence that position of starch availability, chronological changes of quantity, pattern of disappearing within a ray might be affect cell death and deposition of heartwood substance within the cell wall in *Larix kaempferi*. The order of disappearance of studied storage starch corresponded to that of cell death among the ray parenchyma cells around the sapwood-heartwood region of *L. kaempferi.* It also reported that the disappearance of starch related to the various parenchyma cell deaths of *Robinia pseudoacacia* L var. *inermis*[28]. Therefore, information on the distributing pattern studied storage materials appears to be an important factor in the disappearing of cell contents, regulation of cell death, ultimate formation of heartwood.

Investigations on the nature and radial distribution of reserve materials showed that in general the outer sapwood contains relatively higher amount of starch, while the heartwood is almost free of such storage materials. This is valid for soft- and hardwood species[24,29]. It has been suggested that precursors of heartwood constituents are translocated via the rays of the site of their synthesis[5]. In any case, storage starch in the living cells of sapwood must play a crucial role in the synthesis of heartwood constituents. We conclude that the substrates for the synthesis of heartwood compounds derived in part from the breakdown of starch. A translocation of heartwood extractives via the phloem and the rays to sites of accumulation at the sapwood-heartwood boundary seems to be unlikely.

Histochemical parameters in terms of starch and nuclei have been studied intensively in our reports. Considerable histochemical variations and their patterns of disappearance have been noticed during transition from the sapwood to the heartwood. This study emphasized on the further works on the analysis of sapwood, heartwood and their transition zone to understand the biochemical mechanism involved in the heartwood formation. It also serves some physiological findings between starch and nuclei which might be involved directly and /or indirectly in the formation of heartwood in conifers.

Conclusion

The starch contents from outer sapwood to inner sapwood and their gradual degradation from inner sapwood to outer heartwood suggesting that heartwood extractives are formed partly by hydrolysis of starch. The decrease in the amount of starch with the depth of the heartwood indicating reduced vitality and membrane deterioration of the xylem ray parenchyma cells in the heartwood portion. It also affects on the cell death, which is one important feature of heartwood. Abrupt increase of unknown brown colored substances just after depletion of starch indicates the raw materials supplied from degraded starch. Those substances deposited in cell wall, that's why the thickening of cell wall observed after formation of such brown colored substances. Moreover, the differences of starch localization and disintegration from sapwood to heartwood and availability of unknown materials that was observed in this study, would give information for further research regarding heartwood formation in conifers. This research would be helpful for further physiological works to improve wood quality.

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