Observation on Oil Canals of Umbelliferous Condiment Herbs of Japanese Origin

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Abstract

This study has observed the development, structure and distribution of oil canals in mitsuba, seri, ashitaba and hamabohu, that are condiment herbs belonging to the Umbelliferae family, using a light microscope. Oil canals were found in the petioles, leaf blades, stems, roots, hypocotyls and cotyledons. Oil canals were formed at early stages of hypocotyl and cotyledon development. These oil canal distribution in petioles can be classified into distribution due to ring vascular bundles, as in mitsuba and seri, and distribution due to diffuse vascular bundles, as in ashitaba and hamabohu. Oil canal development in the cortex due to petiole thickening was followed by the development of collenchyma and vascular bundles. However, no vascular bundles were formed in some cases. Many oil canals were found in the periphery of the petioles. Oil canals in leaf blades were found on the adaxial and abaxial sides on the veins. Those around the main veins were larger. Steam oil canals were found in the cortex and pith in mitsuba and seri, and in the cortex and fundamental tissues around the xylem, in ashitaba and hamabohu, while those in the roots were found in the pericycle in mitsuba and seri, and in the collenchyma-like tissues and phloem in ashitaba and hamabohu. The transverse sections of oil canals were round or elliptical.

The secretory cells in the cortex and pith were smaller than the neighboring parenchyma cells, while they were larger than the neighboring parenchyma cells in the phloem.

Key words : Condiment herb, Intercellular space, Japanese origin, oil canal, Umbelliferae, Vascular bundle

要　約

日本原産의 미나리科香辛野菜인 파드득나물, 미나리, 明日葉 및 갯방풍의 油道의 發生, 構造및 分布에 대하여 光學顯微鏡으로 觀察하였다.

油道는 葉柄, 葉身, 莖, 根, 胚軸및 子葉에 存在했다. 油道는 胚軸과 子葉의 發生初期에 形成되었다. 葉柄에 있어서의 油道는 파드득나물, 미나리에서와 같이 輪狀維管束에 相伴하여 나타나는 分布와, 明日葉, 갯방풍에서와 같이 散在維管束에 相伴하여 나타나는 分布로 區分할 수 있었다. 葉柄이 肥大해짐에 따라 皮層에 形成되는 油道는, 처음에 油道가 發生하고, 그 다음에 厚角組織과 維管束이 發達함을 알 수 있었다. 그러나, 維管束의 形成을 相伴하지 않는 경우도 있었다. 油道는 葉柄에서는 周邊部에 많이 存在했다. 葉身에서는 葉脈의 向軸側과 背軸側에 存在했고, 主脈에 있는 油道가 컸다. 莖에서의 油道는, 파드득나물과 미나리는 皮層과 髓에, 明日葉과 갯방풍은 皮層과 木部周邊의 基本組織에 分布했다. 根에서는, 파드득나물과 미나리는 내초에, 明日葉과 갯방풍은 厚角性組織과 篩部에 分布했다. 油道의 橫斷面은 圓形 혹은 楕圓形이었다. 分泌細胞는 皮層과 髓에서는 周邊의 柔細胞보다 작았으며, 篩部에서는 컸다.

Introduction

The Umbelliferae family includes a number of vegetables. The umbelliferous condiment herbs in Japan are mitsuba(*Cryptotaena japonica* Hassk.), seri(*Oenanthe javanica* DC.), ashitaba(*Angelica Keiskei* Koidz.) and hamabohu(*Glehnia littoralis* Fr.Schm.).

As these four species have characteristic flavors, they have been widely used in various Japanese cuisines. The leafy parts of these plants are consumed raw or cooked.

Mitsuba and seri grow best in damp and lightly shaded stityation. Ashitaba and hamabohu, on the other hand, are seen in coasted and grow well in a wide range of soil types. The flavors of umbellifers are originated by essential oil produced in their secondary process. The essential oil is accumulated in secretory tissues called oil canals in the body[1,2,3)]. It is known that this tissue is intercellular space that is formed schizogenously[6,9)].

Recently, the demand for condiment herbs is increasing and new growers increasing the area of production, with growing interests in flavors. In order to consider the culture and quality of these herbs, it is of great importance to reveal the tissues containing essential oil.

Warning[12)] reported a morphological observation on vegetative organs of parsnip. Esau[1)] investigated the relation between the collenchyma of petioles and vascular bundles of celery. Esau[2)] also noted a morphological observation on carrot roots, and Harvis[5)] studied the structure of hypocotyls and roots of carrots. Although these studies were not focused upon oil canals, they mentioned the tissue of oil canal. Fujita[4)] investigated the relation between collenchyma-like tissues and secretory tissues in the roots of umbelliferous medicinal plant. Paupardin et al.3) reported on the histological and physiological relation between essential and secretory organs in fennels.

For umbelliferous condiment herbs in Japan, however, no morphological report has been made to date. Hence, this experiment was carried out to investigate the development, structure, and distribution of oil canals of four different umbelliferous plants.

Materials and Methods

Seedlings and rooted cuttings were used in this investigation. Mitsuba, ashitaba and hamabohu started from seeds, whereas seri was rooted cuttings. On March 22, 1986, seeds were sown in 18cm black plastic pots with a potting medium consisting of equal parts of soil and bark compost. All plants were grown in the greenhouse where night temperature was 10°C and day temperature fluctuated with ambient.

Samples of plants were taken seeding at the cotyledon stage and then at a plant height of 25cm. The leaves used were the first true leaf and the fourth true leaf.

The materials were prepared by the freehand section and microtome section methods. The microtome sections were stained with the sudan Ⅳ and anilin blue. Observation was made using a light microscope. The oil canals in the petioles, leaf blades, stems, roots, hypocotyls and cotyledons were observed.

Results

1. Development of Oil Canal

To clarify the stage of oil canal development, transverse sections of hypocotyl and cotyledon of mitsuba at the cotyledon stage were observed (Figs.1 and 2). In the hypocotyl, oil canals were found growing near to the outer part of the pericycle. In the cotyledon, they were found developing on both adaxial and abaxial sides of the main vein. Thus it has been found out that the oil canals were formed at a very early stage after germination.

In the transverse section of the petiole of hamabohu, the oil canals were found in the cortical parenchyma close to the epidermis, where cell division was actively in progress, and in the fundamental tissues around the xylem of the vascular bundles (Figs. 3 and 4).

The oil canals around the xylem developed, in the first place, in contact with the xylem and, later, become detached as the number of the cells increased. Three canals were formed in this part; one in the mid portion of the xylem developed first, from which the other two occurred to the right and left almost at the same time(Fig.4). Canals developed earlier tended to be larger. Looking at the oil canal development in the periphery of xylem in ashitaba, one can see that two or three small secretory cells are formed(Fig.5), which split away as they increase in number, thus forming schizogenous intercellular space(Fig.6). This space grows larger to form an oil canal.

The development of oil canals due to the thickening of the petiole showed slightly different processes among different species. The mitsuba and seri developed their oil canals in the cortical parenchyma between collenchyma. In other words, in the transverse section of the petiole of mitsuba(Fig.7), two small secretory cells appeared below the two layers of parenchyma cells containing chlorophyll, immediately below the epidermis.

When oil canals were clearly recognized, the collenchyma differentiated close to the canals toward the epidermis. Then vascular bundles were formed in five to seven layers of cells inward from the canals. However, in some cases with mitsuba and seri, no vascular bundles were found. In such cases, the oil canals were extremely small.

Petioles of hamabohu and ashitaba had adaxial and abaxial side. The adaxial side presented a crescent shape. The oil canals differentiated in the fundamental tissues at both ends of the crescent. The canals moved inward as the petioles grew thicker(Fig.8).

In the case of ashitaba and mitsuba, oil canals also occurred in the phloem of the vascular bundles(Fig.9). These oil canals were formed after the phloem had developed to some degree. At first, two secretory cells that were larger than the neighboring cells emerged in the phloem, which later increased in number and schizogenously formed intercellular space that would become and oil canal. The position of the oil canal development inside the phloem was irregular.

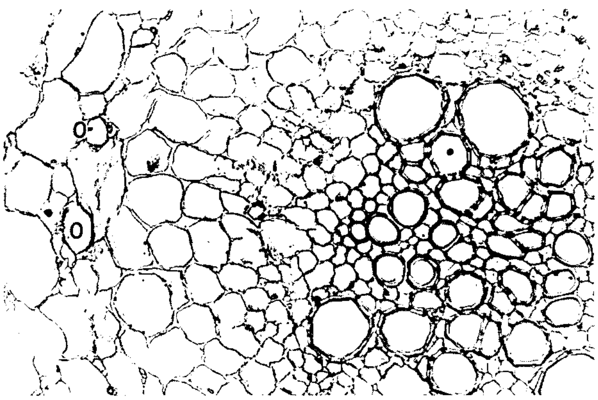

Fig. 1 Transverse section of mitsuba hypocotyl showing oil canals in pericycle, ×135.

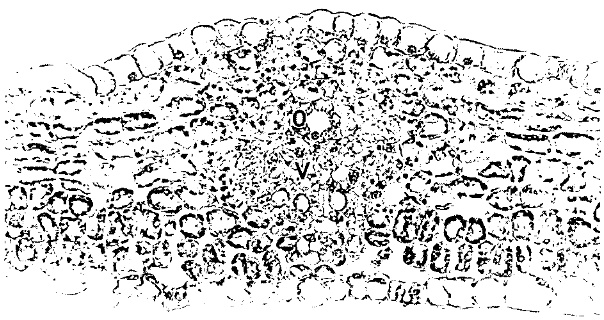

Fig. 2 Transverse section of mitsuba cotyledon showing oil canals in periphery of midrib, ×270

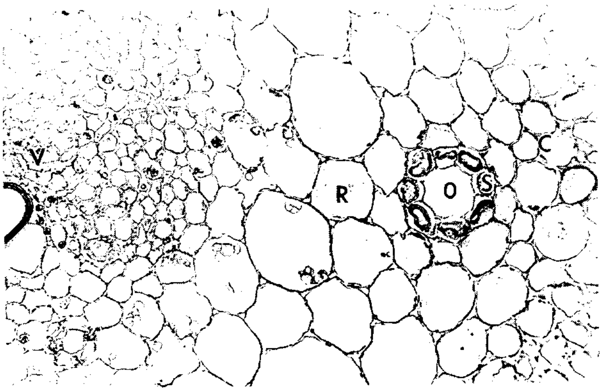

Fig. 3 Transverse section of hamabohu petiole showing oil canals between the vascular bundle and collenchyma, ×270.

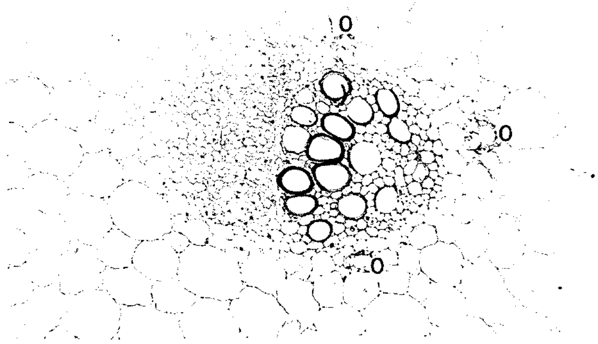

Fig. 4 Transverse section of hamabohu petiole. Three oil canals are distributed in the peripheral portion of the xylem, ×135.

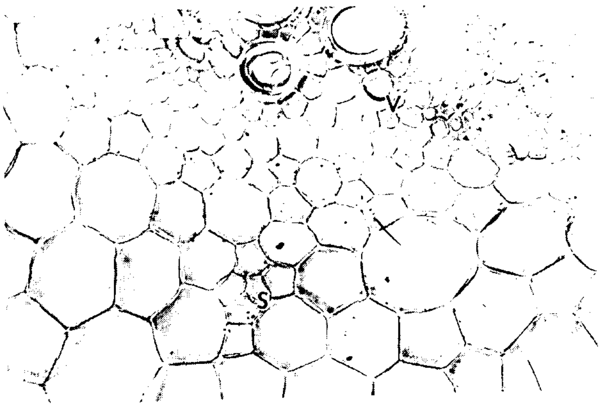

Fig. 5 Trånsverse section of ashitaba petiole showing initiation of secretory cells near to the peripheral portion of xylem, ×270.

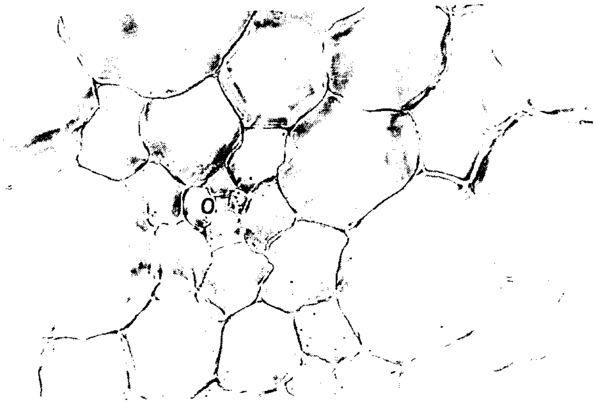

Fig. 6 Transverse section of ashitaba petiole showing the formation of oil canals, ×270.

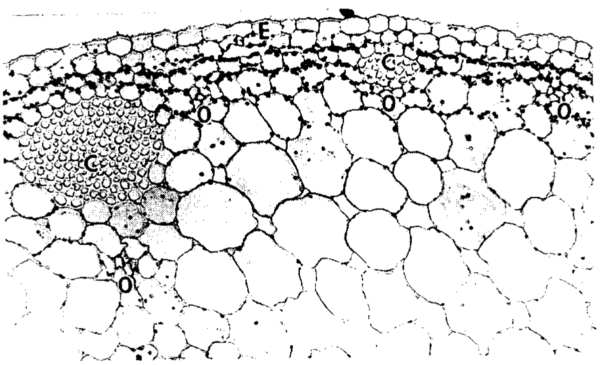

Fig. 7 Transverse section of mitsuba petiole showing progressive stages in development of oil canals in the cortical parenchyma, ×135.

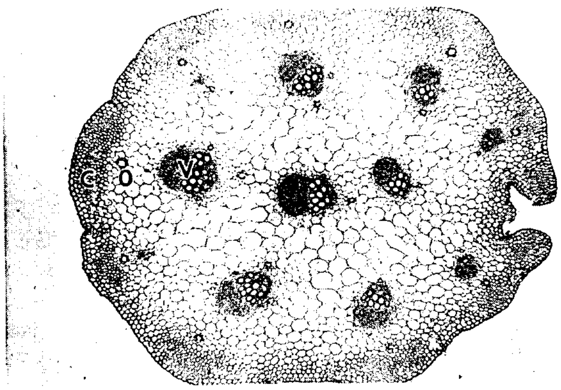

Fig. 8 Transverse section of hamabohu petiole showing random distribution of oil canals and vascular bundles, ×25.

2. Structure of Oil Canal

The oil canals developed between the vascular bundles and the collenchyma showed a rounded shape surrounded by a layer of eight secretory cells. The canals at early stages of growth were angular and the number of the cells was relatively samall, but they became round or elliptical as they grew. The diameters of canals were closely relateld to the number of the secretory cells. As the number of the cells increased, the diameter became larger(Table 1) and the shape became smoothly round. The oil canals could be easily identified as the secretory cells in this part were smaller than the neighboring parenchyma cells. The oil canals around the xylem of the vascular bundles and those around the leaf veins had the same structure.

The oil canals developed in the phloem had three or four angular secretory cells each. It was characteristic that these cells were larger than the neighboring cells(Fig.9). The canals were mostly quadrangular and 5.0 to 6.5μm in size, regardless of the species. They were considerably smaller than those found in other parts.

The oil canals in hypocotyls had a smaller number of secretroy cells that were round or elliptical. The cells were smaller than the neighboring cells(Fig.1).

The oil canals in the roots were all polygonal and had elliptical secretory cells that were smaller than the neighboring cells. They contained starch grains(Fig.10). The structure of the oil canals make little difference in species.

We observed a petiole of hamabohu clarify the structure of oil canals as viewed in a longitudinal section. The canal was long and tubular. No septa were observed(Fig.11).

3. Distribution of Oil Canal

The transverse section of a petiole of the mitsuba shows a row of elliptical epidermal cells, inside which are two layers of cells. Further inward, collenchyma are scattered surrounding the petiole. The number of the cells increased as the petiole thickened(Fig.12). The cortex consisted of six to seven cell layers and a row of vascular bundles was arranged in a ring on the inside. One oil canal was found between collenchyma and each vascular bundle, and another two or three in the periphery of the xylem of each vascular bundle. The oil canals developed in the cortex were larger than those found around the xylem. The oil canals also developed in the phloem, which increased as the phloem became larger. No oil canals were formed on the epidermis, collenchyma or vascular bundles. Nor were they found in the core of the pith. More oil canals were found in the periphery of the petiole than the core.

In the transverse section of the petiole of the hamabohu, unlike mitstba and seri, the vascular bundles did not form a ring. They existed in multitude, resembling the arrangement of vascular bundles of monocotyledon(Fig.8). The vascular bundles ranged from the adaxial side to the abaxial side, with the phloem sides facing outside. Those found farther from the adaxial side tended to be larger, unlike diffuse vascular bundles. One oil canal was found in the fundamental tissue between the collenchyma and each vascular bundles, and another three in the periphery of the xylem of the vascular bundle. Unlike mitsuba and seri, oil canals were found even in the core of the petiole.

In the transverse section of a leaf blade of the hamabohu, the oil canals could be easily identified as their secretory cells did not contain chlorophyll. Around the main vein, one oil canal was found on the adaxial side and three on the abaxial side. On the other hand, only one canal was found around each veinlet(Figs.13 and 14). The canals around the main vein were larger and had more secretory cells compared to those around veinlets. The canal found near the adaxial side were larger than those near the adaxial side. Also in the case of the leaf blade, the distribution of oil canals and

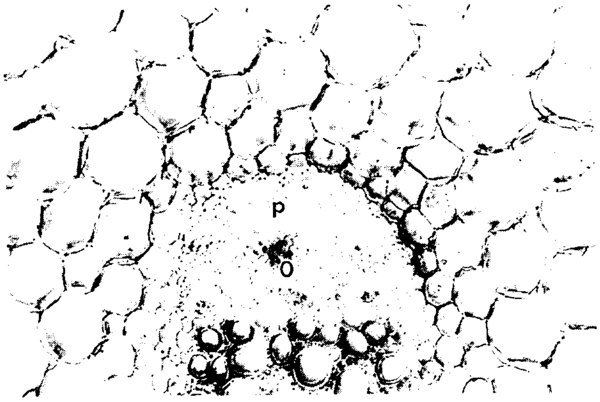

Fig. 9 Transverse section of mitsuba petiole showing oil canals within the phloem, ×135.

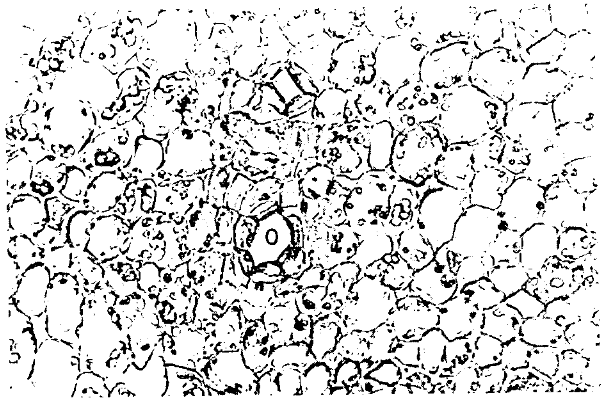

Fig. 10 Transverse section of ashitaba root showing oil canals, ×270.

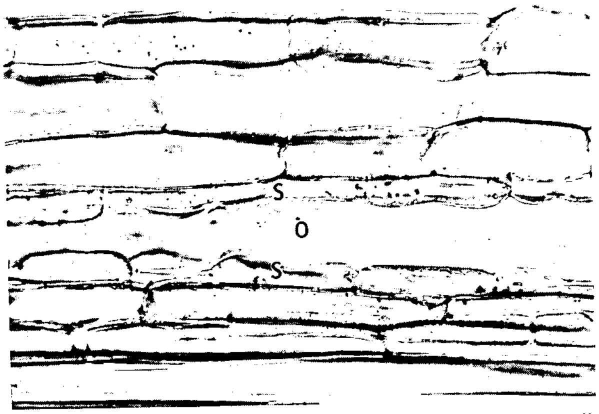

Fig. 11 Longitudinal section of a vascular bundle of ashitaba petiole showing oil canal, ×270.

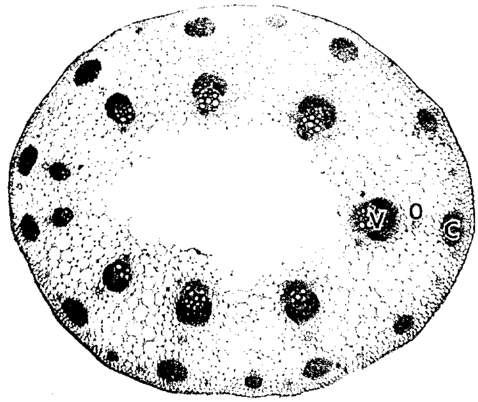

Fig. 12 Transverse section of mitsuba petiole with hollow core showing oil canals and ring vascular bundles, ×27.

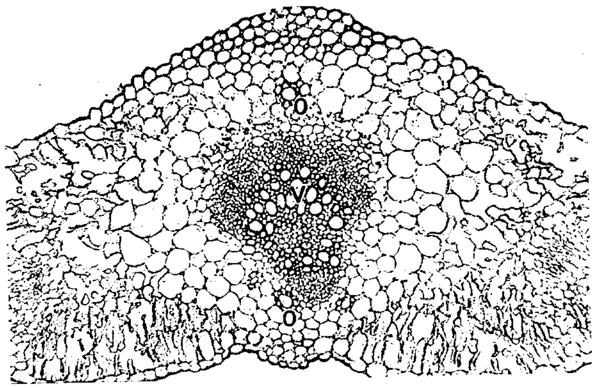

Fig. 13 Transverse section of main vein of hamabohu leaf showing oil canals lie above and below the vein, ×135.

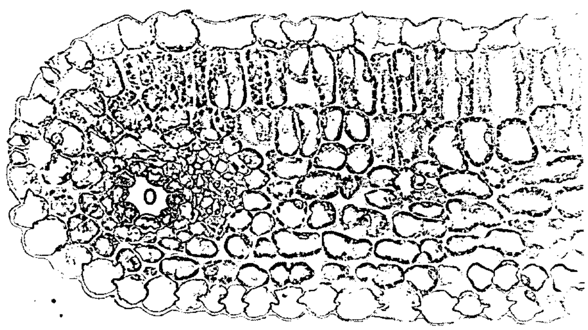

Fig. 14 Transverse section of hamabohu leaf showing oil canals at the extreme outer margin, × 270.

Table 1. Number and size of oil canal in the petiole* of Umbelliferous condiment herbs.

	Leaf length (cm)	Petiole Length (cm)	Width (mm)	Thickness (mm)	No. of vascular bundle	No of collenchyma	Cortex No. of oil canal	Diam. of oil canal (μm)	No. of secretory cell	Pitth No. of oil canal	Diam.of oil canal (μm)	No. of secretory cell
Seri	28.1*2	15.4	2.7	2.9	9.4	26.2	34.1	15.0	5.6	19.6	5.0	4.4
Mitsuba	30.8	23.8	3.1	3.4	7.8	12.2	19.5	22.5	6.0	8.1	10.0	4.5
Ashitaba	16.9	8.1	3.2	3.4	14.5	20.1	22.3	37.5	7.6	13.3	14.2	6.6
Hamabohu	23.4	14.7	3.1	3.4	10.1	10.3	10.6	32.5	9.5	17.2	15.2	7.0

*Materials measured the fourth leaf counted from the base.
*2 Values show the mean of 5 leaves.

that of vascular bundles were closely related. Oil canals were only found where vascular bundles existed.

The stem of the hamabhu had epidermis consisting of one layer, under which lied two or three layers of collenchyma cells surrounding the stem. Further inside, a few layers of cortical cells were found. Unlike the petiole, vascular bundles were arranged in a ring. Oil canals were found in the cortex, fundamental tissues in the periphery of the xylem of the vascular bundles, and phloem, as they were in the petiole. The oil canals in the stem were large and 20 to 30μm in diameter. No oil canals were found in the core of the pitch(Fig.15).

The stem of mitsuba had epidermis consisting of one layer, under which were collenchyma scattered near the periphery. The vascular bundles were ring vascular bundles with well-developed canbium rings. Oil canals were found in the cortex and pith. Oil canals were found in the core of the pith as well(Fig.16). These canals were extremely small and 10 to 15μm in diameter.

The root of the ashitaba had periderm consisting of six to seven layers, under which were two to three layers of collenchyma-like tissue. Oil canals were found in this tissue(Fig.17). Also in the phloem, polygonal oil canals surrounded by five to seven secretory cells were sparsely distributed. The secretory cells were smaller than the neighboring cells and contained no starch grains, making it easy to identify them. No oil canals were found in the pith.

The root of the mitsuba had dpidermis consisting of a layer of cells, under which three layers of oblong cells lied. Intercellular space was formed in the cortex. Oil canals were formed near the pericycle. They varied greatly in their size and shape. No oil canals were found in the pith(Fig.18). The root of the seri had two or three layers of cork cells under the epidermis. Parenchyma was developed between the cork cells and the pith, occupying more than two-thirds of the root diameter. A few oil canals were found only in the pericycle of the pith(Fig.19)

The oil canals in the hypocotyl located in the periphery of the pericycle, and none located in the pith or cortex(Fig.1). In the case of the petiole of ashitaba, oil canals were occasionally found in the collenchyma as the petiole grew thick(Fig.20).

4. Relation between Oil Canal, Vascular Bundle and Collenchyma

As the development of oil canals was considered closely related to vascular bundles and collenchyma, we have summarized the observation of the oil canals, vascular bundles and collenchyma in Table 1. Ashitaba had 14 vascular bundles, more than the others, followed by hamabohu, which had 10, seri 9, and mitsuba 7. Relatively great numbers of collenchymas were found in the seri and ashitaba, 26 and 20 respectively. The numbers of collenchymas found in mitsuba and hamabohu were much the same as the numbers of the vascular bundles.

The number of oil canals in the cortex was the grestest with seri, which had 44, followed by ashitaba, mitsuba and hamabohu. Seri had three times as many oil canals as hamabohu. This indicates that the development of oil canals in the cortex become active as the petiole grew thick. In the case of hamabohu, on the other hand, no oil canals were developed in the cortex and therefore the number of oil canals was as little as that of vascular bundles and collenchymas.

The oil canals found in seri were 15μm in diameter, the smallest of the four species and smaller than one-third of ashitaba and hamabohu. The numbers of secretory cells surrounding the oil canals were similar, ranging betweem five and nine. However, the results showed that larger oil canals had more secretory cells.

In the pith, the seri had more oil canals the other species. The pith of mitsuba collapses its core and becomes hollow as the plant grows, thus resulting in a small number of oil canals. The pith of seri also collapses its core and becomes hollow, but at a later stage compared to mitsuba. In diameter, hamabohu and ashitaba had larger oil canals, showing the same tendency as those around he cortex. In all the plants observed, oil canals in the cortex were smaller than half of those in the pith.

Discussion

To clarify the stage of the development of oil canals, we observed a hypocotyl and cotyledon of mitsuba at the cotyledon stage. In the hypocotyl, oil canals were found in contact with outer part of the pericycle. The oil canals were round or elliptical in shape. In the cotyledon,oil canals were found around the main vein, showing the same shape as in the adult leaf. Warning[12] found out that hypocotyl and cotyledon of parsnip had oil canals. Takahashi[11] carried out research on varnish tree and found out that resin canals were developed at an early stage after germination. In our study, no species difference was observed in the distribution and form of oil canals in the hypocptyls and cotyledons of four umbelliferous herbs observed. In the petioles of mitsuba and seri, no oil canals were found in the pith cores, while oil canals were found in the pith cores of ashitaba and hamabohu. This difference can be explained as the consequence of the difference of the vascular bundle arrangement. Morphologically, dicotyledon are supposed to have ring vascular bundles and monocotyledon diffuse vascular bundles. However, ashitaba and hamabohu are found to have a arrangement similar to diffuse vascular bundles, although they are not completely diffuse vascular bundles as they have distinct adaxal and abaxial sides. Both plants naturally grow in coastal areas. It can be assumed that severe ecological environments caused the bundle arrangement to change, resulting in the different distribution of oil canals.

In the petiole, oil canals were formed in the cortical parenchyma between vascular bundles and collenchyma and in the fundamental tissues around the xylem of vascular bundles. In the case of mitsuba and ashitaba, oil canals were also formed in the phloem of vascular bundles. No oil canals were observed in epidermis, xylem or collenchyma except for the ashitaba, which showed some oil canals in the collenchyma. Esau[1] carried out a study on the development of collenchyma and vascular bundles in celery petioles and found out that oil canales were formed in the periphery of the vascular bundles. Takahashi[11] carried out a study on resin canals in varinish trees and found out that there always were resin canals where there is a vascular bundles. These reports suggest that the development of vascular bundles causes the development of oil canals.

In the cortex of a petiole that is thickened, collenchyma and vascular bundles are formed after the development of oil canals and, in some cases, the vascular bundles were not developed(Fig.7). Therefore, it can be assumed that oil

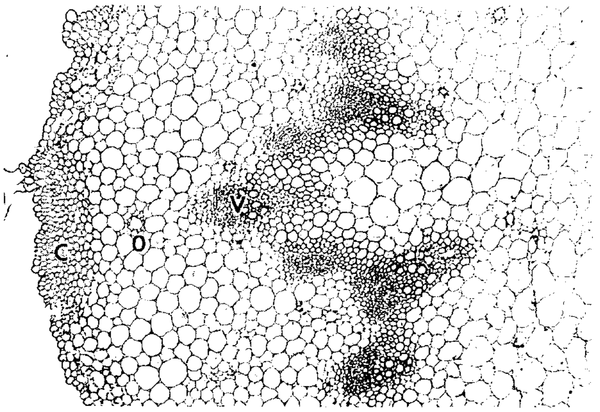

Fig. 15 Transverse section of hamabohu stem showing distribution of oil canals and vascular bundles, ×67.

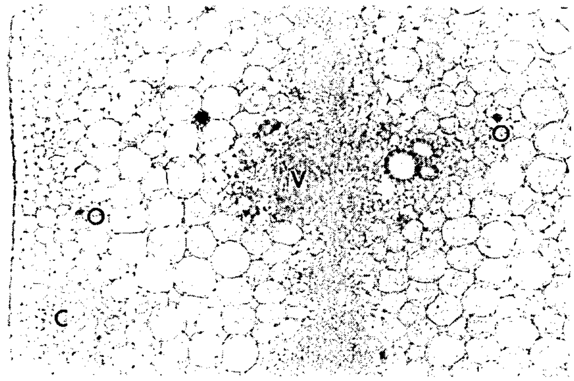

Fig. 16 Transverse section of mitsuba stem. Oil canals distributed throughout section but were more numerous in cortical parenchyma, ×25.

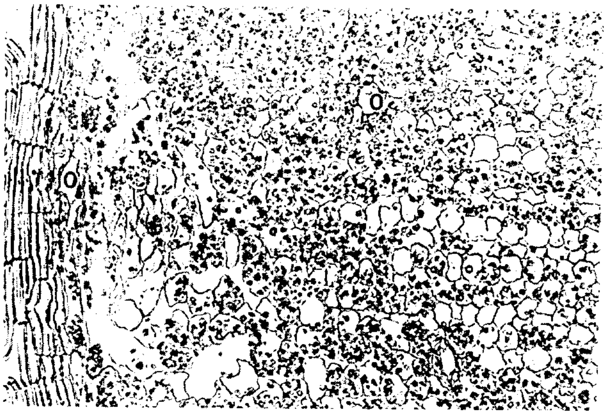

Fig. 17 Trânsverse sesction of ashitaba root showing oil canals in collenchyma-like tissue, ×135.

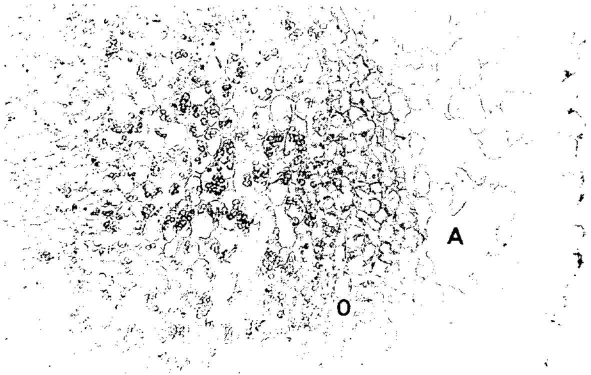

Fig. 18 Transverse section of mitsuba root showing canals and aerenchyma, X135.

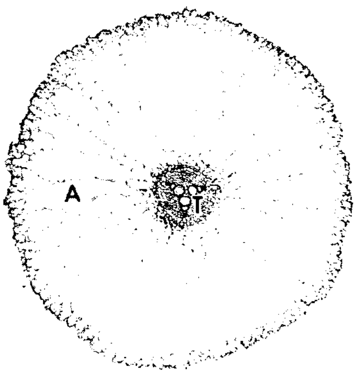

Fig. 19 Transverse section of Seri root with aerenchyma. Oil canals occurred in the pericycle, ×27.

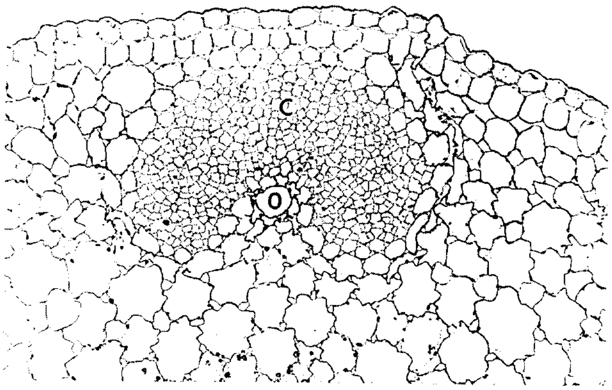

Fig. 20 Transverse section of ashitaba petiole showing oil canals within collenchyma, ×135.

canals do not cccur accompanying with the appearance of vascular bundles, but the collenchyma and vascular bundles form as the oil canal develops.

The oil canals of the mitsuba and seri were smaller than those of ashitaba and hamabohu. Mitsuba and seri had well-developed intercellulaar space in the roots, presenting a structure suitable for wet soils. Hamabohu and ashitaba, on the other hand, showed a structure suitable for well-drained soil in coastal areas, as indicated by the vascular bundle arrangement. Essential oil of plants is considered to help prevent the body temperature from rising. It can therefore be assumed that this is why oil canals are well-developed in ashitaba and hamabohu. On the other hand, mitsuba and seri, that prefer partially shaded wet soils, are not likely to meet high temperature, hence the difference in the development of oil canals.

It is said that the development of tissues containing essential oil can be classified into schizogenous development and lysogenous development. Warning[12] carried out histological observation on parsnip petioles and reported that oil canals were fomed schizogenously. Esau[1] also observed that oil canals were schizogenously formed in celery petioles. Our study also confirmed that oil canals in mitsuba and hamabohu were formed schizogenously, initiated with two or three secretory cells which developed to form larger intercellular space.

In the transverse section of the root of the ashitaba, there were two or three layers of oblong collenchyma cells under the periderm, within which oil canals were formed. Fujita[4] examined the same kind of tissue from the root of Nothosmyrnium japonicum and identified it as a collenchyma-like tissue. Konoshima *et al.*[7] observed the same kind of tissue from saiko (Bupheurum falcatum) and called it collenchyma. This tissue is called differently. We suggest that it is reasonable to call this a collenchyma-like tissue, considering the observation results of the root of ashitaba.

Mitsuba, seri, ashitaba and hamabohu have their origin in Japan. Their raw leaves alone are used as condiment herbs. For cooking, they are picked before the leaves have fully grown. Considering that the development of oil canals is closely related to that of vascular bundles and collenchyma, it can be said that the leaves are used before these tissues are fully grown, in order to enjoy the tender leaves with delicate flavor. These plants are often used after blanching, presumably for the same reason.

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