# アケビ果実の成長に伴う果肉組織の形態的変化


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Original

# Anatomical and Histological Changes of the Pulp Tissue in Developing Akebi (*Akebia pentaphylla*) Fruit

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**Abstract** The growth of akebi (*Akebia pentaphylla*) fruits was investigated to analyze the edible portion of the fruit in relation to its cell structure throughout the fruit growing period. Samples ranging from flowers to mature fruits were collected weekly from mature trees. The growing period from anthesis to fruit maturity lasted 24 weeks. The flesh of akebi fruit arose as projections from the placenta derived from the endocarp just before and at flowering. During the fruit growing period, the expansion of the pulp tissue was observed as placentation. The tip of the protrusion of the pulp tissue continued to erupt with a volcano-like appearance, and functioned as meristematic tissue. The contact interface at the tip portion of the pulp tissues fused with each other. Finally, the pulp tissues filled the entire carpel and packed many seeds. At fruit maturity, a crevice between the basal part of pulp tissue and rind appeared, and then the rind dehisced along the ventral suture.

**Key words:** Lardizabalaceae, placentation, pulp tissue, rind dehiscence

アケビ果実の成長に伴う果肉組織の形態的変化（葉　麗紅 *・宋　　陽 *・中尾義則 **・新居直祐 *）

**要約**　ゴヨウアケビ果実の成長過程における細胞組織構造の変化を検討するために，開花期から果実成熟期の期間，毎週果実を採取して調査した．果実は開花期から成熟期までに24週を要した．アケビ果実の果肉は開花期前後に内果皮から発達した胎座の表層部から突起状に発生した．その後，果実の成長期間を通じて果肉組織は胎座型（placentation）として拡大を続けた．果肉組織の突起状の先端部では常に細胞が飛散していて火山の噴火に類似した形状が観察され，細胞の分裂も見られた．果肉組織の先端部位では隣接する他の組織と接触し，その結果，組織同士が融合した．最終的には果肉組織は多くの種子を包みながら心皮内に充満した．果実は完熟期近くになると果肉組織の基部と果皮の間に裂け目が生じ，最終的には果肉が果皮から完全に離れ，果皮は腹合線に沿って裂開した．

## Introduction

Morphologically, akebi fruits have fleshy follicles (Payne and Seago, 1968; Wang et al., 2005), and the growth and development of the edible pulp tissue in these species have distinctive and unique features. In the reviews by Wang et al. (2005) and Li et al. (2010), the horticultural aspects of akebi fruits were as follows. The surface color of rind at maturity of akebi fruit varies, being lilac, brown, and yellow and so on. The flesh is fine and smooth with a milky white color at maturity. There are many bright black or rufous seeds embedded in the flesh. The fruit weight presents a wide range from 80 to 120 g in the wild fruit (Wang et al., 2005) or from 25 to 300 g with the maximum of 546 g (Li et al., 2010), with a thick peel (4–8 mm thick), numerous seeds (100–300 seeds/fruit) and a relatively small proportion of edible flesh. The percentage of rind, seed and flesh on the basis of fruit fresh weight is approximately 55–70%, 10–25% and 15–25%, respectively. The fruit pulp accounts for 17 to 40% of fruit weight. In comparison with other common fruits, akebi species appear to be extremely rich in vitamin C (108 to 930 mg/100 g fresh weight); e.g. comparable to kiwifruit (50 to 300 mg/100 g fresh weight). When the fruit attains to ripeness on the vines, the rind splits naturally and its shape looks like a flying butterfly. There are abundant *Akebia spp.* resources with diverse fruit types and different maturing periods.

In recent years, the fruit of *Akebia spp.* in the Lardizabalaceae has been widely researched because of its edibility and medical value (Li et al., 2010; Wang et al., 2005). Akebi has been used in particular as a traditional Chinese medicine. Its roots, stems, leaves, and fruits can be used as medicines for relieving internal heat and diuresis, improving blood circulation, detoxification and anti-cancer properties.

Wang et al. (2005) and Li et al. (2010) also described akebi as a valuable wild fruit and a potential new fruit crop in

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China. In Japan, domestication and breeding of akebi has also taken place. Farmers have made superior selections of akebi from the wild and cultivated them. Most reports have emphasized cultivation for production of herbal medicines rather than fruit. Commercially, akebi species appear to have several selling points (Li et al., 2010), such as: (1) the eye-appealing quality of the fruit shape and color; (2) the nutritional and cosmetic value of the fruit; and (3) the medicinal properties of the fruit.

The morphological characteristics of floral sexual dimorphism and anatomy of floral development in Lardizabalanceae have also been investigated by some researchers (Yoshida and Michikawa, 1973; Zhang and Ren, 2011). Although akebi plants have been researched in many respects, as mentioned above, and the morphological features of akebi fruits are quite unique, there are few reports investigating the morphology and anatomy of pulp tissue in akebi fruits. Even in the relatively new field of fruit science, it is necessary to observe the anatomical changes of fruit throughout the growing season. A study of the anatomy of akebi fruits would contribute to the information in morphological development of edible portions of fruits.

## Materials and Methods

*Plant materials*

The flowers and developing fruits of *Akebia pentaphylla* were used as research material. Samples of flowers at anthesis and fruits were collected weekly until harvest, which extended from mid-April to mid-October. For fruit set, pistillate flowers were hand-pollinated by cross-pollination between the two species. Data were collected during two seasons in 2010–2011.

Numerous small fruits (n = 15–20) were collected early in the season to provide sufficient material for various measurements. The number of fruit per sample was later reduced (n = 7–10) to ensure an adequate supply of fruit until the end of the harvest. Fruit diameters and FWs were determined on all fruit per sampling date.

*Tissue preparation and light microscopy observations*

Fruits were then photographed using a digital camera (Caplio R5, Ricoh, Tokyo, Japan). The samples were then cut into cross-sections for photography with a microscope (SZX12, Olympus, Tokyo, Japan). These photographs were then studied to determine the interior structures. For anatomical studies, the flowers and fruits were excised and fixed in 3% (v/v) glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2. All specimens were initially stored at 4°C and then dehydrated through ethanol solutions graded from 30% (v/v) to 100% (v/v) for 5–24h, depending on tissue size, and then embedded in Technovit 7100 resin (Heraeus Kulzer GmbH & Co., Wehrheim, Germany). Sections of tissue, 1.5 $\mu$m thick, were cut using a glass knife and then stained with 1% (w/v) methylene blue for one minute, transferred to a slide, and photographed under a microscope (BX60, Olympus, Tokyo, Japan).

## Results and Discussion

Full bloom occurred in mid-April and the fruit was harvested in early October for *A. pentaphylla* (Fig. 1A, D). The

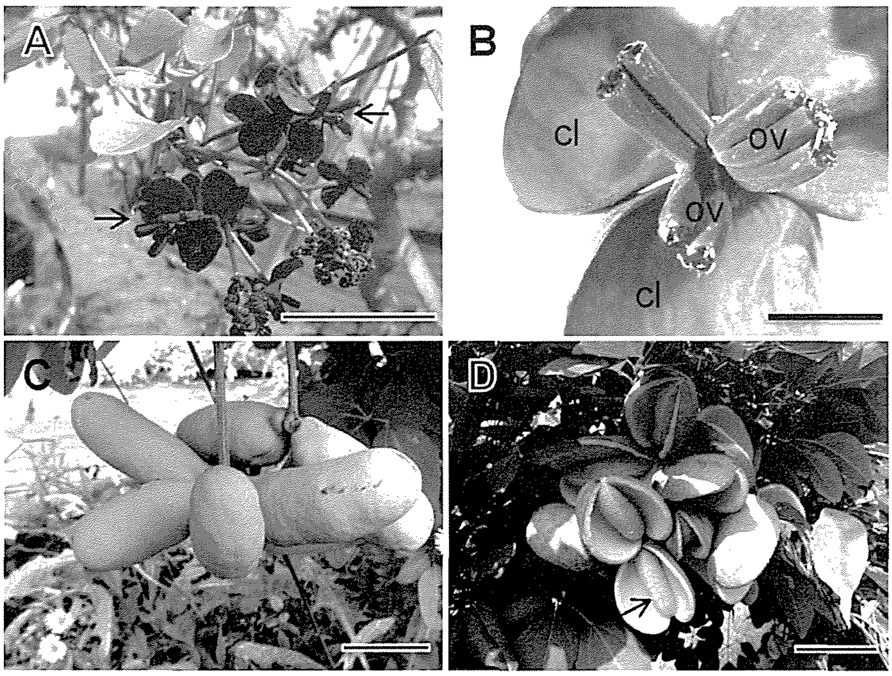


Fig. 1. Morphological changes from flowering to fruit maturity in *Akebia pentaphylla*.
A, inflorescence of pistillate flower (arrows); B, anthesis on 18 April, three calyx lobes (cl) and seven ovaries (ov); C, fruit on 12 June; D, mature fruit and pulp tissue (arrow) on 2 October. Scale bars = 5 mm (D), 20 mm (A), 40 mm (C), and 100 mm (B).

growing period from anthesis to fruit maturity lasted 24 weeks in *A. pentaphylla*. Akebi flowers have been classified as monoecious with functionally unisexual flowers (Li et al., 2010; Wang et al., 2005; Yoshida and Michikawa, 1973; Zhang and Ren, 2011). A racemose inflorescence was pendulous on the current shoots. At the basal part of the raceme, 1–3 pistillate flowers generated. At the upper part of the raceme, staminate flowers generated. Staminate flowers have six or seven incurved stamens, short filaments, and oblong anthers. Pistillate flowers had three or four large calyx lobes without petals (Fig. 1B). Inside the calyx lobes, there were three to nine individual carpels (Li et al., 2010; Yoshida and Michikawa, 1973) or three to twelve (Wang et al., 2005) ones with stick-like shape ovaries. Each stigma secreted a large, viscous drop of fluid that received pollen. Fig. 1C shows a representative fruit bearing at the enlargement stage of fruit development.

Transverse sections of the ovary showed a carpel with many ovules and three main vascular bundles: a single dorsal bundle and two ventral bundles (Fig. 2A, B; Fig. 3A, a). The ovary wall developed into a thick rind during the growing period (Fig. 2 C, D, F). The edible portion of the akebi fruit, which was referred to pulp tissues in the present study, initiated on the locular side of the placenta derived from the endocarp (laminar placentation) just before and at anthesis (Fig. 3B, b). Three weeks after anthesis, when the fruits were approximately 9 mm in length, the pulp-tissue primordia started to swell, then commenced rapid growth and became cylindrical in shape (Fig. 3B, b). There were no vascular bundles in the pulp tissue. With the swelling of the pulp tissue, the placenta tissue developed towards the hollow carpel (Fig. 2C, c). Furthermore, placenta tissue packed the seeds completely (Fig. 2C, D). At fruit maturity, a crevice between the pulp tissue and rind appeared before dehiscence of the fruit rind, and visible vascular tissue could be seen adhering to the inner portion of the rind (Fig. 2D, E).

An increase in cell number at the initiating region of the pulp tissue continued to fill the locule (Fig. 3b, C). All the cells of the swelling region, especially the cells at the front end (the pulp-initiating region), actively divided (Fig. 3c). The tip of the pulp tissue expanded in a manner similarly to a volcano (Fig. 3b). When contact occurred among the pulp tissues at the tip portion, fusion was observed (Fig. 3C, c).

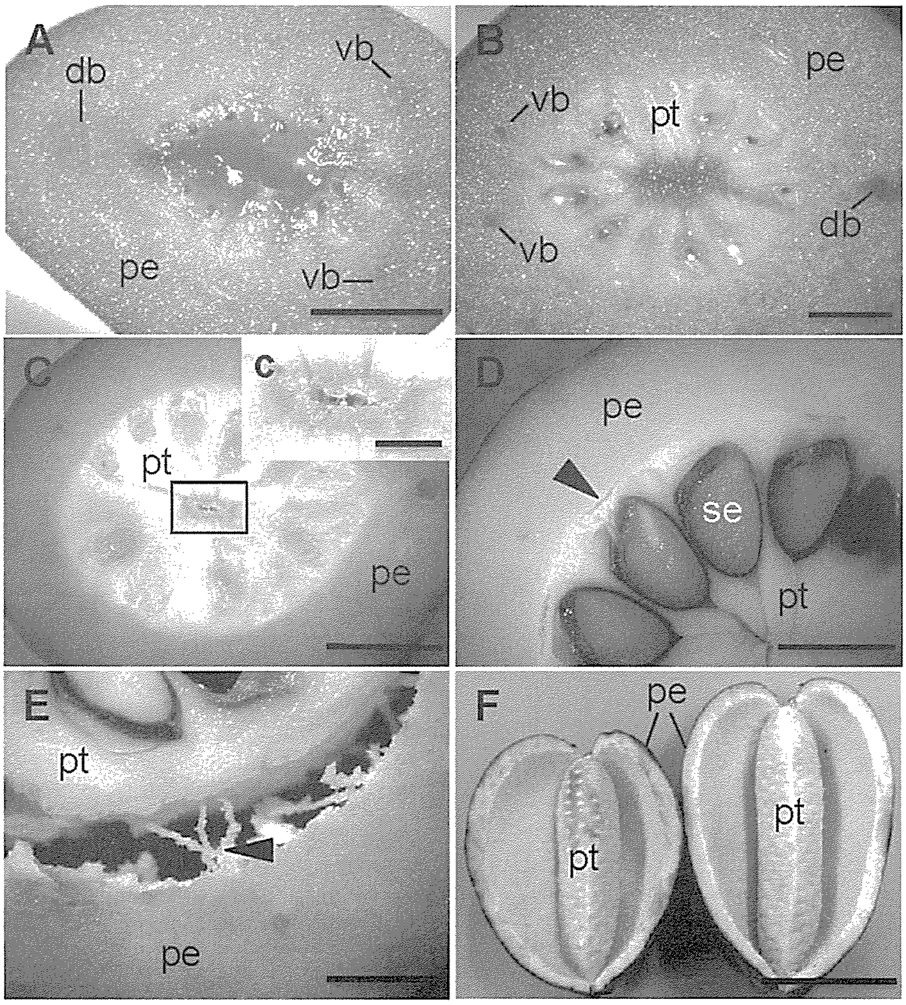


Fig. 2. Transections of developing pulp sacs and internal morphology of *Akebia pentaphylla* fruits.
A, fruit on 1 May; B, fruit on 8 May; C, fruit on 5 June and c is the magnification of the boxed area in C; D, fruit on 19 September; E, fruit on 9 October; F, fruit on 2 October, rind splitting naturally. pe = pericarp (rind); pt = pulp tissue; se = seed; db = dorsal bundle; vb = ventral bundle; arrowhead = vascular bundle. Scale bars = 1 mm (A, B, c), 5 mm (C, D, E), 50 mm (F).

During June, after emergence of the pulp tissues, the major event in the development of the fruit was that the placenta tissue (pulp tissues) nearly filled the entire carpel and all the ovules were enclosed by expansion of pulp tissues (Fig. 2C). Because of seed enlargement, the basal portion of the pulp tissue became narrow (Fig. 2D).

At the time these events occurred and the fruit increased considerably in diameter. The pattern of fruit enlargement was distinguishable into three stages (Fig. 4). Stage 1 continued for 8 weeks (from anthesis to early June) and was characteristic of tissue extension of the pulp sac according to cell increase. Stage 2 was seed growth (enlargement and hardening) and pulp tissue expansion up to 16 weeks after anthesis. Stage 3 was the maturation stage until maturity and needed more than 8 weeks, in which the pulp softened and became succulent and edible. The transverse diameter and vertical diameter of fruits increased quickly until early June (Fig. 4). During this period, the pulp tissue developed by swelling to fill the whole carpel, and the seed also developed quickly. The parenchyma cells in the pulp tissues enlarged as the fruit matured. It is probable that the increase in diameter of the fruit was at least in part due to the parenchyma cell enlargement.

The anatomical features of akebi fruits were some similar to the development of juice sac in citrus, juice column in red bayberry, and pulp in banana fruits (Nii and Coombe, 1988; Nii et al., 2008; Ram et al., 1962). In banana fruit, the pulp tissue developed from the pulp-initiating cells lying immediately below the epidermis of the endocarp (Ram et al., 1962). By 12 weeks after emergence of pulp tissue in banana, the loculi were filled with the ingrowths of the pulp parenchyma. Finally the pulp tissue at matured stage in banana fruit was quite similar to akebi flesh. Citrus juice sac and red bayberry juice column had also similar features of pulp tissue in akebi, although the parent body and developing structures were completely different. The citrus juice sac appears as a gland from the endocarp, and the juice column of red bayberry develops as flesh from the exocarp, but the pulp tissue in akebi fruit initiate from the placenta derived from endocarp. Juice sac cells in citrus have prominent features and cylindrical stages. The mature sac is formed by swelling of the distal sac body and delineation of

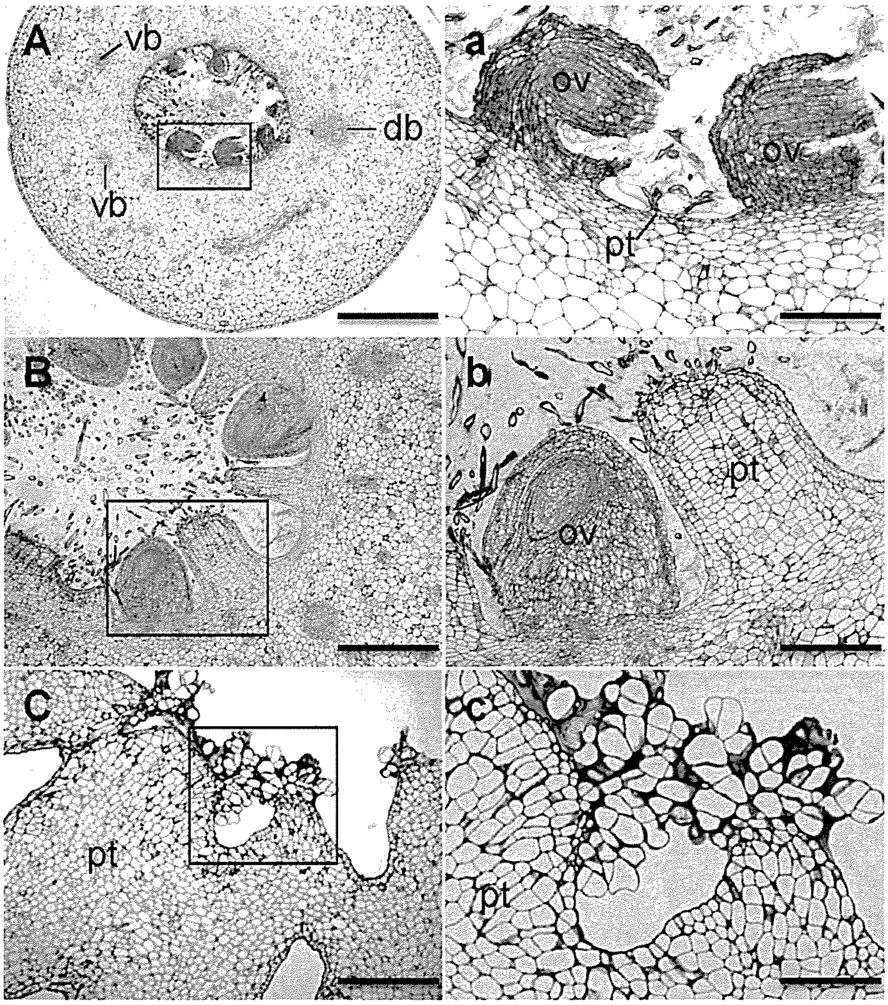


Fig. 3. Cross-sections of pulp-sacs developed from placenta in *Akebia pentaphylla* fruits.
A, transverse section of ovary at anthesis on 18 April; a, the placenta with the appearance of filament hairs just before the initiation of pulp tissue primordia among ovules; B, b, pulp tissue on 1 May, several pulp tissue enlarged inward the locule cavity; C, c, the tip portion of the pulp tissue on 12 June; a, b, c are the magnifications of boxed area of A, B, C, respectively. db = dorsal bundle; vb = ventral bundle; ov = ovule; pt = pulp tissue. Scale bars = 100 $\mu$m (a); 200 $\mu$m (b, c); 500 $\mu$m (A, B, C).

the proximal stalk. In red bayberry fruit, juice columns develop from lump to the cylindrical stage. In akebi fruit, the pulp tissues were initiated as bulges on the epidermal side of the placenta (endocarp), and elongated cylindrically, although the loculi were filled with the pulp tissues until fruit maturity.

The pulp tissue of akebi fruit is the organ that accumulates and stores sugars and other solutes. Numerous sections were examined, but no vascular connections were found between the rind and pulp tissue in akebi fruit. The edible portion of akebi fruit poses an interesting problem in solute translocation because of the long distance from the vascular tissue to pulp tissues as well as citrus juice sacs (Koch, 1984; 1985; Koch et al., 1986; Nii and Coombe, 1988). Juice sac primordia arise at a distance from the vascular bundles in the albedo and vascular elements do not enter the juice sac stalk. On the other hand, in red bayberry fruit, juice column from the outer layers of the pericarp develops outwards and the vascular connection developed between the mesocarp and the juice column (Nii et al., 2008).

We described the structure of akebi fruit for the first time, with particular attention to the pulp tissue cells derived from the placenta (endocarp). The structure of akebi fruit is quite unique, although the arrangement of the edible portion and its development were similar to those of juice sacs in citrus, juice column in red bayberry, and pulp in banana.

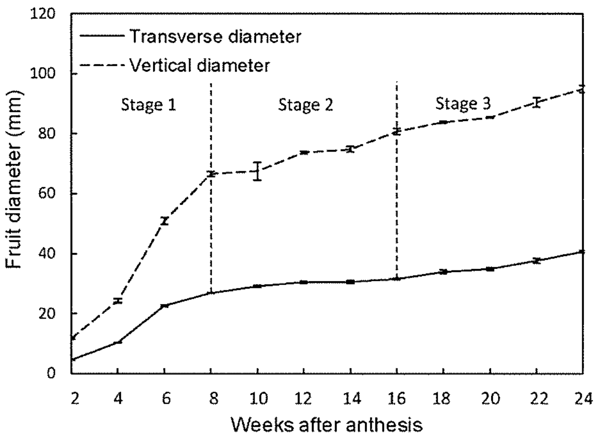


Fig. 4. The growth curve of *Akebia pentaphylla* fruit.
The vertical bars show ± SE (n = 7–10)

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