Differentiation of Stylar Transmitting Tissue during Ovule Development in Female Flowers of Japanese Chestnut (*Castanea crenata* Sieb. et Zucc.)

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To understand the anatomical features and fertilization processes of female flowers in Japanese chestnut, we examined the differentiation of transmitting tissue. From the observation of serial transverse sections from the base of the ovary to the style, it was revealed that nine septa formed nine styles. The transmitting tissue in the style appeared in the boundary area where the two adjacent septa joined. The epidermal cells of each style were derived from the cortex parenchyma surrounding the transmitting tissue, and each style elongated cylindrically during flower development. The transmitting tissue, a solid type, was observed at the top of the stigma. Ovules with a short, thick funiculus were pendulous on the surface of the placenta under the style, and were anatropous. The interval between the terminal of the transmitting tissue and the top of the ovary contained no track for pollen tubes to migrate along. This interval might be important for pollen tube growth.

**Key Words:** Japanese chestnut (*Castanea crenata* Sieb. et Zucc.), locule, ovule, stylar transmitting tissue.

**Introduction**

In Japanese chestnut (*Castanea crenata* Sieb. et Zucc.), one to three female flowers usually develop at the basal portion of male catkins. Each bur generally has three ovaries, two external and one internal. Each ovary is generally divided by septa into nine loculi, each of which has an elongated style, although the number of loculi per ovary varies depending on the cultivar (Nakamura, 1986). There are usually two ovules per locule; however, only one ovule in each ovary is fertilized and develops into a seed (Nakamura, 1986, 1991, 1994, 2001). The unfertilized ovules degenerate in early July.

In contrast to most angiosperms, in which fertilization occurs several days after pollination, a long period, from 4 days to more than 1 year, is needed for fertilization in some plant orders, including Fagales. Why fertilization is delayed, and where and how pollen tubes stay in the pistil during the period are still unclear (Sogo and Tobe, 2006). Although in some species of fruit trees, fertilization after pollination requires more than 1 week, the chestnut, which belongs to the Fagales order, requires almost 1 month for fertilization (Nakamura, 1994). There have been many reports on pollination and fertilization in chestnut trees (Nakamura, 1986, 1991, 1994, 2001); however, there are few reports on the developmental process of stylar transmitting tissue at an anatomical level (Nakamura, 1992), although the structure of the transmitting tissue, in other plant species, has been observed in detail by light and transmission electron microscopy (Ciampolini et al., 1996; Considine and Knox, 1979; Erbar, 2003; Heslop-Harrison and Shivanna, 1977; Serrano et al., 2008). In angiosperm reproduction, pollen tubes elongate from the stigma to the ovary through the stylar transmitting tissue to deliver the male gametes for fertilization; therefore, the stylar transmitting tissue is important for pollen tube elongation (Erbar, 2003).

Erbar (2003) reviewed various histological modifications of pollen transmitting tissue, including bridging the gap between the pollen tube transmitting tissue and the ovule. The pollen tube transmitting tissue provides not only a convenient pathway connecting the stigma and ovules for pollen tube growth, but is also a site for pollen tube competition and attrition. Many functions of pollen tube transmitting tissue have been explained in reviews; however, the differentiation of pollen tube transmitting tissue remains unknown despite the importance of this structure.

The present study was conducted to clarify the anatomical characteristics of stylar transmitting tissue and ovule disposition in Japanese chestnut.
Materials and Methods

Plant materials

Three forty-year-old trees of Japanese chestnut cv. Tanzawa, an early ripening variety with a harvest period in mid-September, were used in this experiment. The trees were cultivated in an orchard of Meijo University Experimental Farm. Flowers or burs were randomly harvested at 7-day intervals from late May to mid-July. For each sampling, four to five burs were collected from current shoots in the middle part of the trees.

Tissue preparation and light microscopic observation

Female flowers and burs were observed under a light microscope (Olympus SZX12, Tokyo, Japan) fitted with a digital camera (Olympus U-TVO, 5XC-3, Tokyo, Japan) to understand the morphology of the flowers.

Female flowers and burs were then dissected to separate the style and locule, including the ovule, before being fixed in 3% glutaraldehyde (0.1 M cacodylate buffer, pH 7.4) at 4°C. The fixed specimens were then dehydrated through a graded ethanol solution from 30% to 100% (v/v) for 5 to 24 h depending on the tissue size, and then embedded in Technovit 7100 resin (Heracrus Kulzer GmbH, Wehrheim, Germany). Transverse or longitudinal sections (1.5 μm) of the tissues were prepared using an ultramicrotome with a glass knife and stained with methylene blue for histological examination.

Results and Discussion

Morphological development of female flowers

The stigmas appeared from a bur in late May and the styles elongated during ovary enlargement (Figs. 1A, B, 2, and 3A). Anthesis of the female flower was not easily recognized externally. We concluded that anthesis occurred on approximately June 12, judging from the maximum length of the style. Generally, female flowers or burs of Japanese chestnut are composed of three ovaries, each with nine loculi (Fig. 3B, C). Numerous vascular bundles were observed in the ovary wall and central axis (Fig. 3C). Although each locule contained two ovules, meaning that each ovary contained approximately 18 ovules, only one ovule can be fertilized (Fig. 3D). Consequently, only one to three seeds in each bur developed to maturity. Each locule was filled with spongy fibrous tissue (Fig. 3C).

Differentiation of style transmitting tissue

A series of cross-sections of a single ovary from the base to the style was made from female flowers on June 6 in order to investigate the developmental process in the transmitting tissues during style elongation (Fig. 4). The magnified structures of Figure 4F and G are shown in Figure 5 to clarify the formation process of the transmitting tissue. The differentiation process of the stylar transmitting tissue from the basal to the upper portion of the ovary was observed. The basal portion was partitioned into loculi and possessed a central axis with a vascular bundle (Fig. 4A); in the upper portion, two ovules appeared within a locule (Fig. 4B). Two small septa appeared between the large alternative septa in transverse sections, leading to three large and six small septa in an ovary (Fig. 4C, D, E). The stylar transmitting tissue was formed in the boundary layer between two adjacent septa and the stylar epidermis appeared (Fig. 4E, F, G); nine styles (one underdeveloped) appeared in the upper portion of the ovary (Fig. 4H). In addition, all of the septa were fused in the center of the ovary and a triangular-shaped vascular bundle was visible in the central axis (Fig. 4A). The vascular bundle in the axis was connected to the funiculus (ovule stalk) (Fig. 4B).

Figure 5 shows enlargements of the photographs in

Fig. 1. Development of style in Japanese chestnut. A, B, morphological features of the style. A, on May 29; B, on June 12. C, D, cross-sections at the middle part of the style. C, on May 29; D, on June 12; E, F, longitudinal section of the style on May 29. E, stigma; F, in the middle part of the style. st = style; tt = transmitting tissue; vb = vascular bundle. Scale bars = 20 μm (E, F); 50 μm (C, D); 2 mm (A, B).

Fig. 2. Changes of style length in Japanese chestnut from late May to early July. Vertical bars indicate ± SD (n = 5).
Figure 4F and G, which provide more details of the stylar transmitting tissue and vascular bundle in the style: the appearance of stylar transmitting tissue between two septa (Fig. 5A), the cell structure of transmitting tissue (Fig. 5a), the differentiation of the stylar epidermis (Fig. 5B, b), and the nearly well-developed styles with the transmitting tissue and vascular bundles (Fig. 5C, c).

Based on our observations, the chestnut style is of the solid type (Fig. 1C, D), and the stigma was not papillae, as reported earlier by Nakamura (1992). The cylindrical transmitting tissue was located in the center of the style and was continuous with the stigma (Fig. 1E). Vascular bundles were present in the parenchymatous cortex of the style. During style elongation, the vascular bundles developed (Fig. 1C, D). The cells of the transmitting tissue appeared to be round in transverse sections and elongated in longitudinal sections (Fig. 1C, D, F). The transmitting tissue showed two types of cells: one was cytoplasm-rich and the other had large vacuoles (Fig. 1F).

In the style, we distinguished three regions in transverse sections: (1) epidermis, (2) stylar cortex containing vascular bundles, and (3) a central region corresponding to the transmitting tissue, as described by Serrano et al. (2008). The epidermis of the style was composed of a single thickened layer of cells. Adjacent to the epidermis were several layers of vacuolated cortical cells. The stylar transmitting tissue was surrounded by vascular bundles, particularly xylem.

Ovule disposition
In the majority of angiosperms, pollen tubes enter the ovule for fertilization through the micropyle formed by the integument(s). This mode of fertilization based on the pollen tube path is known as porogamy (Maheshwari, 1950). Nakamura (2001) concluded that no pollen tubes penetrate the chalazal region, while in some ovules, some pollen tubes enter the micropyle or between the inner and outer integuments; therefore, he concluded that in chestnut, the entry of pollen tubes into the embryo sac is porogamous.

Ovules were observed in the loculi, each locule containing two anatropous ovules with micropyles oriented toward the upper part of the ovary (Fig. 6A, B). The ovule was attached pendulously to a short, thick funicle (Fig. 6C, D). The nucellus faced upward and was surrounded by the integuments. The seed development
system was multidimensional. This study mainly looked at selected aspects of the sequential process of the stylar transmitting tissue. The pollen tubes elongated into a specially differentiated track between the stigma and ovary. Erbar (2003) described in a review that the transmitting tissue provides not only guidance and an adequate environment for the growth of pollen tubes, namely, in the form of pollen tube nutrition, but possibly also the medium for the passage of electrical and other signals between the stigma and ovary. However, the interval between the lower terminal part of the transmitting tissue and the micropyle of the ovary contained no track for pollen tube elongation, and the distance between lower and upper parts was $716 \pm 49.0 \mu m$ (mean $\pm$ SE, $n = 5$).

Erbar (2003) reviewed various histological modifications of pollen transmitting tissue, including bridging the gap between the transmitting tissue and the ovule. The stylar transmitting tissue is also potentially where pollen tube elongation ends (Erbar, 2003). To understand flower development, it is very important to know the ovule arrangement and the distance between the end of the transmitting tissue and the micropyle. The present report is the first study of the anatomical process of stylar transmitting tissue formation in female flowers of Japanese chestnut.

In conclusion, the stylar transmitting tissue first appeared in the boundary layer between two adjacent septa and developed in close association with the development of ovule; however, many questions remain concerning the relationship between the stylar transmitting tissue and the pollen tube elongation required for fertilization. Various events occur within the style during the fertilization period. The role of the gaps in the transmitting tissue leading to the ovary should be investigated to determine their role in pollen tube elongation to the ovule.

**Literature Cited**


