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Seed Coat Formation in *Apostasia nipponica*

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ヤクシマランの種皮形成

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The seed coat formation was observed in *Apostasia nipponica* Masamune. The placental ridge and ovule formation began about 40 and 14 days before flowering, respectively. The funiculi are bent, and inner and outer integuments and megaspore mother cell are distinct at the time of flowering. The inner and outer integuments consist of two or three cell layers. The cells of the inner integument cover directly the embryo sack, and then are eventually disorganized. The cells of the outermost layer of the outer integument become transparent and the cells of the second layer of the outer integument become enlarged and sclerotic with dark deposits.

In most species of Orchidaceae, only the outermost layer of the outer integument persists as the seed coat, and the other layers are disorganized (Swamy 1949). The cells of the seed coat are usually dead and have transparent cell walls. In a few species the seed coat becomes thickened as seen in *Apostasia nuda, Selenipedium chica* and *Vanilla planifolia* (Garay 1960). In *Vanilla planifolia* both outer and inner integuments take part in seed coat formation and the outermost layer of the outer integument becomes highly sclerotic with dark deposits (Swamy 1947). In the other two genera the process of seed coat formation has not been described yet.

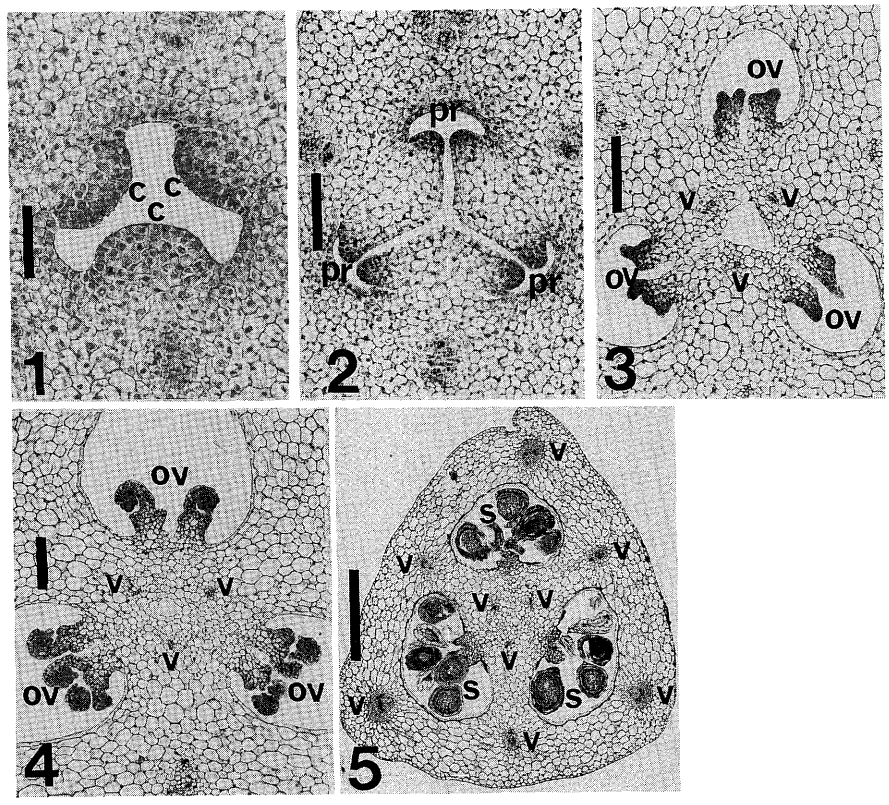
In this study the seed coat formation is reported in *Apostasia nipponica*.

Materials and Methods

The ovaries of *Apostasia nipponica* were collected during September 3 and March 19, 1989, during June 29 and September 3, 1990 and during May 11 and June 28, 1991. The materials for microtome sectioning were fixed in FAA containing 50% ethanol, dehydrated in *n*-butyl alcohol series, and embedded in paraffin. Serial sections were cut at ca. 6 μm and stained with Delafield's haematoxylin.

Results

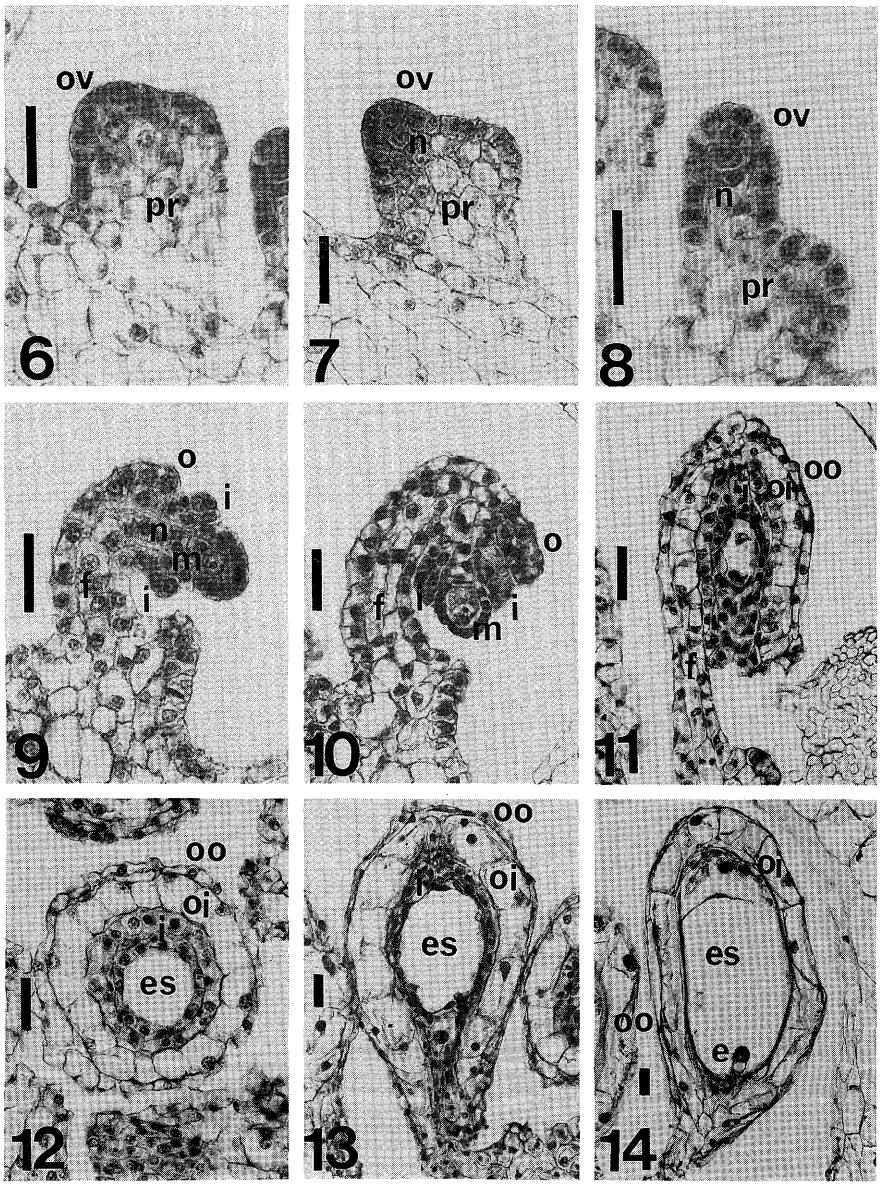
Ovary development–The ovary has a single cavity with three risings of ovary wall (Fig. 1). Placental ridges are formed from the margins of the ovary risings (Fig. 2). Two rows of ovules are usually produced on one placental ridge (Fig. 3). Placental



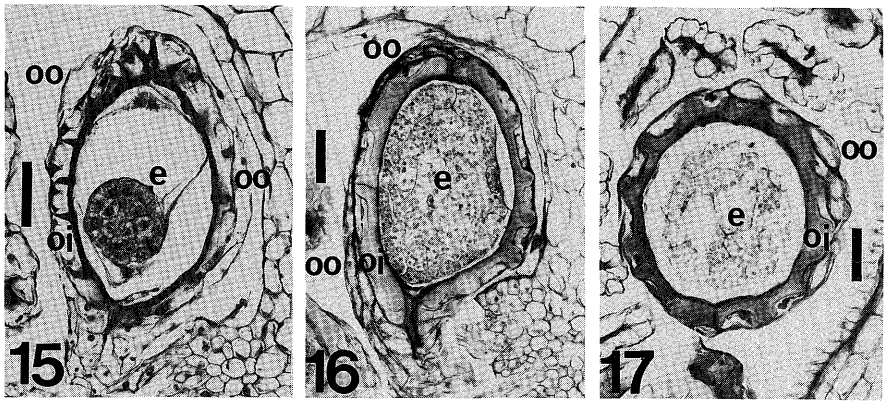
Figs. 1–5. Development of ovary. Fig. 1. An ovary showing risings of ovary wall (cross section). Fig. 2. Formation of placental ridges (cross section). Fig. 3. Development of the ridges (cross section). Fig. 4. Ovary showing three locules (cross section). Fig. 5. Ovary about 5 months after flowering (cross section). c = rising of ovary wall; ov = ovule; pr = placental ridge; s = seed; v = vascular bundle. Bars indicate 0.1 mm in Figs. 1–4 and 0.5 mm in Fig. 5.

ridges and ovules are visible about 40 and 14 days before flowering, respectively. The three ovary wall risings grow further and eventually fuse together to form three locules (Figs. 3–5). Thus, each of locule contains two rows of axial placenta and four rows of ovules.

Ovule development – One of the subepidermal cells of the placental ridge divides frequently (Fig. 6) and forms an ovule intial (Fig. 7). Then, the initial forms a linear row of nucellar cells besides an epidermal layer (Fig. 8). The funiculus bends, and outer and inner integuments are formed from the epidermal cells (Fig. 9). The terminal cell of the nucellus becomes a megaspore mother cell (Fig. 9). Flowers open at this stage of ovule development. The funiculus bends 360° and the tip of the ovule faces the foot of the funiculus. The outer and inner integuments consist of mostly two, sometimes three cell layers (Fig. 10). No cell layers are differentiated between the megaspore mother cell and the nucellar epidermis. The inner integumentary cells grow up over the nucellar epidermis and completely cover the embryo sack (Figs. 11, 12). These inner integumentary cells are disorganized when embryos start to develop (Figs. 13, 14). The outermost cell layer of the outer integument becomes thin and transparent (Figs. 13, 14). On the other hand, the cells of second layer of the outer integument become enlarged (Figs. 11–13). As the embryo devel-



Figs. 6–14. Development of ovule I. Fig. 6. Placental ridge showing an enlarged subepidermal cell (longisection). Fig. 7. Differentiation of nucellar cells (longisection). Fig. 8. Nucellar cells arranged in a line (longisection). Fig. 9. Formation of outer and inner integuments and megaspore mother cell (longisection). Fig. 10. Young ovule with elongating outer and inner integuments (longisection). Fig. 11. Mature ovule with thin outermost cell layer and thickened second cell layer of the outer integument (longisection). Fig. 12. Ovule at the same stage as Fig. 11 (cross section). Fig. 13. Mature ovule with further thickened second cell layer (longisection). Fig. 14. Ovule at the beginning of embryo development showing the membranous outermost cell layer and the thickened second cell layer (longisection). e = embryo; es = embryo sack; f = funiculus; i = inner integument; m = megaspore mother cell; n = nucellus; o = outer integument; oi = the second cell layer of outer integument; oo = the outermost cell layer of outer integument; ov = ovule; pr = placental ridge. Bars indicate 0.02 mm.



Figs. 15–17. Development of ovule II. Fig. 15. Ovule showing early stage of sclerotic cell wall of the second cell layer (longisection). Fig. 16. Ovule showing the sclerotic second cell layer (longisection). Fig. 17. Mature seed (cross section). e = embryo; oi = the second cell layer of outer integument; oo = the outermost cell layer of outer integument. Bars indicate 0.05 mm.

ops, about 60 days after flowering, the inner and side cell walls of the second cell layer become sclerotic with dark deposits (Fig. 15), but the outer cell wall does not become sclerotic (Figs. 16, 17). Seeds mature about 8 months after flowering.

Discussion

In *Apostasis nipponica* placenta and ovule formation begins about 40 days and 14 days before flowering, respectively, and inner and outer integuments and a megaspore mother cell are already differentiated at the time of flowering. In most species of Orchidaceae, except Cypripedioideae, ovule formation initiates after pollination by the stimulatory effect of an auxin from the pollen (Wirth and Withner 1959). In Cypripedioideae ovule formation begins before pollination; in *Cypripedium pubescens* an ovule possesses a megaspore mother cell and inner integuments, and funiculus is somewhat bent at the time of pollination (Duncan and Curtis 1942), and in *C. parviflorum* an ovule possesses a megaspore mother cell (Carlson 1940). These facts may indicate that *Apostasia* and *Cypripedium*, both of which are considered to be primitive in Orchidaceae, share the same characteristic of not demanding any stimulation by pollination on ovule formation.

In *Vanilla planifolia* both inner and outer integuments take part in seed coat formation and the outermost layer of the outer integument becomes sclerotic with black deposits (Swamy 1947). In *Apostasia nipponica* two cell layers of outer integument take part in seed coat formation, the outermost layer becomes thin and transparent, and the second layer becomes sclerotic with dark deposits. *Vanilla* and *Apostasia* are markedly different in the origin of the sclerotic seed coat, although their appearances are similar. The way of seed coat formation as in *Apostasia nipponica* has never been reported. This evidence supports an idea to separate Apostasioideae from Orchidaceae.

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要旨

ヤクシマランの胚珠の発育過程を，特に種皮の形態に注目して観察した．開花の約 40 日前に胎座の形成が始まり，約 14 日前に胚珠形成が始まる．開花時には内珠皮，外珠皮および胚嚢母細胞はすでに形成されている．内珠皮および外珠皮は 2–3 細胞層からなっている．内珠皮は胚嚢細胞形成から受精のころまでは胚嚢を包んでいるが，胚の成長とともに消失する．一方，外珠皮では外側から第 2 層の細胞が大きくなり，やがてそれぞれの細胞壁が肥厚し暗褐色になる．また，最外層の細胞は透明の膜状となる．

ラン科の大多数の種では，種皮は外珠皮の最外層が透明の膜状になったものであるが，いくつかの種では *Vanilla planifolia* のように種皮が肥厚して暗褐色になるものがある．しかし，その場合は外珠皮の最外層が厚膜化する．本研究で観察された *Apostasia nipponica* のように第 2 層目が厚膜化する例はこれまでには報告されていない．これはヤクシマラン亜科を独立したヤクシマラン科とする意見を支持する．