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FEMALE REPRODUCTIVE STRUCTURES AND SEED DEVELOPMENT IN *DIOSCOREA NIPPONICA* MAKINO (DIOSCOREACEAE)

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This work presents data on the genesis of ovule and seed structures in *Dioscorea nipponica* Makino and examines morphogenetic correlations in their development.

Key words: *Dioscorea*, reproductive biology, embryology, seed.

INTRODUCTION

The genus *Dioscorea* includes more than 600 species, most of which are not well studied embryologically, though different aspects of their reproductive biology are of great theoretical and practical importance (Shreter at al., 1965; Bouman, 1993).

This work is a detailed study of ovule and seed development in *D. nipponica* Makino, about which information is lacking in the literature.

MATERIALS AND METHODS

The material was fixed by FAA, dehydrated and embedded in paraffin, cut into 10–15 μm sections, and stained with fuchsin/sulfuric acid, alcian blue and hematoxylin. The material was studied by traditional histochemical methods (see: Shamrov, 2000).

RESULTS

The gynoecium is coenocarpous, trilocular. The ovary is inferior. Two ovules develop in each locule, one under the other on placental outgrowths. The mature ovule is anatropous, crassinucellate, bitegmic, with a well-differentiated nucellar cap, postament, podium, hypostase (in the understanding of Batygina and Shamrov; see: Shamrov, 1998), massive chalaza and placental obturator. Initiation of the podium, postament and hypostase occurs at the stage of archesporial cell differentiation and separation of the parietal cell: the podium and postament in the basal part of the nucellus, the hypostase at the border of the base of the nucellus (podium) and funiculus at the level of the bases of the inner and outer integuments, which arise at the same time (Figs. 1–3). The inner integument is of dermal origin; the outer integument is of dermal-subdermal origin, laid down at the base of the common initials [pachychalaza – juvenile variation, terminology according to Shamrov (1998)]. Growth of the integuments occurs mainly by division of terminal initial cells. A 1- or 2-layered parietal tissue and nucellar cap are formed during megasporogenesis (Fig. 4). Further development of nucellar structures is correlated with the beginning of embryo sac formation. During this process, the parietal tissue and adjacent cells of the nucellus degrade, and cell division activity in the postament and podium increase (Figs. 5–7a,b). In the mature ovule (Figs. 7a,b, 8) the nucellar cap becomes 2–3-layered;

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the postament is represented by axial rows of cells with dense cytoplasm, a cup-shaped podium formed by periclinal and anticlinal divisions of subepidermal cells of the nucellus (lateral part of podium), and cells in the basal part of the nucellus (central part of podium). The hypostase is 3–4-layered, consisting of tabulate cells with a dense cytoplasm. The inner integument is 3–4-layered in the basal part, 4–5-layered in the apical part, and 2-layered in the middle part. The outer integument consists of 4–5 layers of cells in its middle and basal parts, and 2–3 layers in the apical part.

The archesporium is 1-celled, sometimes 2-celled (Fig. 10), and transforms into the megasporocyte after separation of the parietal cell. The embryo sac is monosporic and develops according to the Polygonum type (Figs. 11–15). The mature embryo sac (before fertilization) (Fig. 16) has 7 cells and 8 nuclei. It is cylindrical in form, with a narrow chalazal end, well-differentiated egg apparatus (polarized egg cell, synergids with filiform apparatus and beak-shaped outgrowths), a central cell with polar nuclei in contact in the chalazal part (rarely in the micropylar part), 3 antipodal cells triangular in form with dense cytoplasm, and wall outgrowths lengthwise along the whole surface of contact with the embryo sac wall.

It should be mentioned that the ovules can be in different locations in the ovary and within one locule. Usually they are apotropous, rarely epitropous. As a rule the micropyle is turned toward the top of the ovary, but very seldom it is turned toward the base of the ovary (Fig. 17).

Double fertilization is of the premitotic type. Frequent cases of additional pollen tube entrance were noted (Fig. 18). The embryo develops according to the Asterad type. Its development is divided into two periods: (I) on the maternal plant, before dissemination, and (II) after separation from it (embryo postdevelopment). During period I the proembryo forms, and embryo organs initiate and develop. At the moment of dissemination the embryo consists of the suspensor, cotyledon, and the shoot and root apices. During period II the embryo organs develop further. The endosperm is nuclear. After fertilization the seed grows asymmetrically and flattens in the bilateral plane. The result of such growth is the arrangement of the vascular bundle of the raphe on the flat surface of the seed, not on the seed rib (Fig. 23).

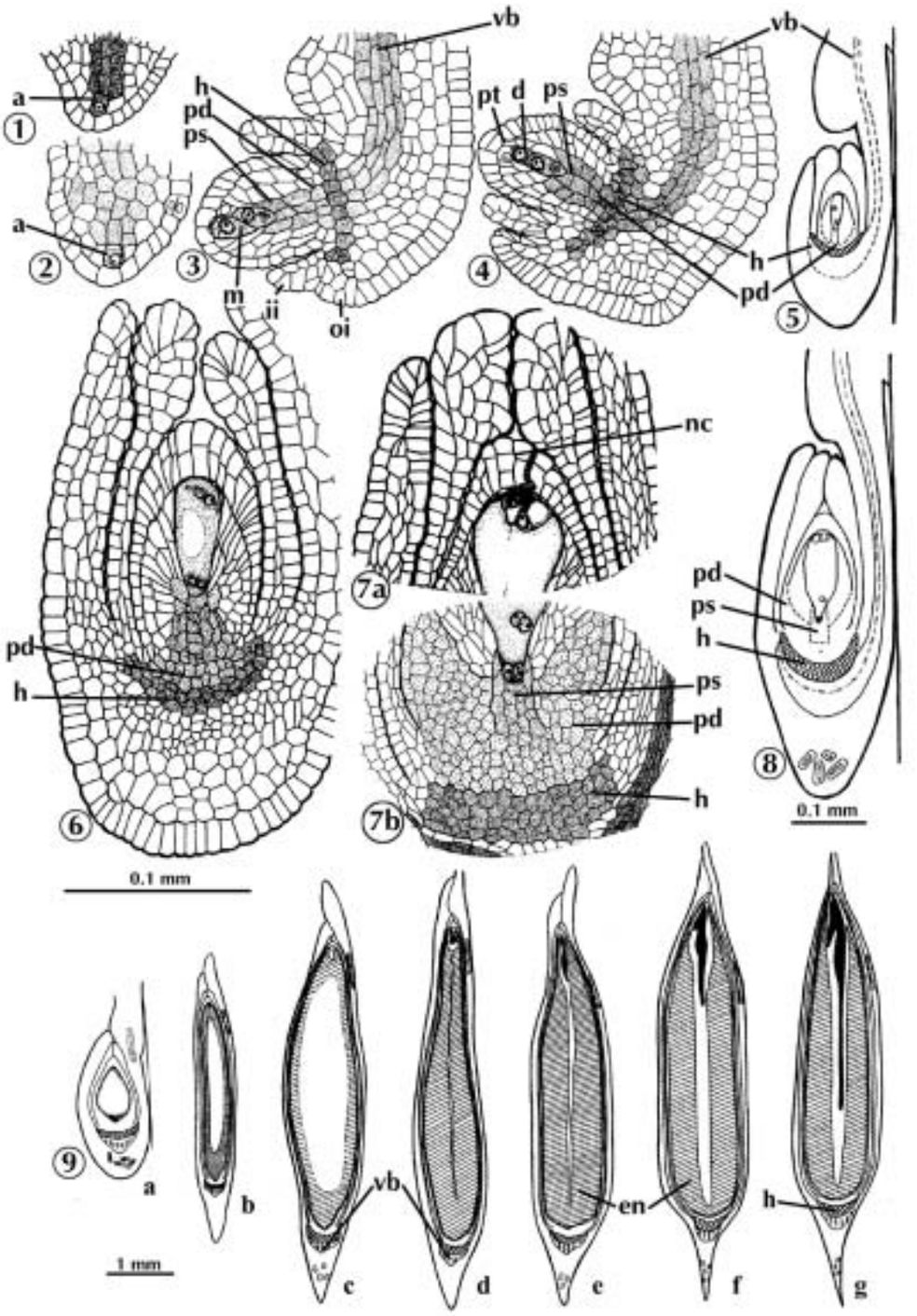
Seed development is shown in Figure 9. During early proembryo formation (4–10 DAP) (Fig. 9a) the coenocytic endosperm develops. It is characterized by heteromorphism of its nuclei in size and ploidy (especially in the chalazal part); it is accompanied by degradation of the postament and antipodal cells and by maximum development of the podium. At this stage the seed coat elements begin to form, with the enlargement of the extracellular spaces in the outer integument tissue (Fig. 19) and the appearance of thickened cell walls in the micropylar part of the inner integument and palisade-like cells in its outer epidermis.

During late proembryo formation (10–20 DAP) (Fig. 9b) cytokinesis occurs in the endosperm (from micropyle to chalaza), and the heteromorphism of its nuclei is preserved. The podium cells in contact with the chalazal part of the endosperm degrade, the hypostase cells specialize (appearance of thickened cell walls and tannins in them), and tannins begin to accumulate in the endotegmen cells.

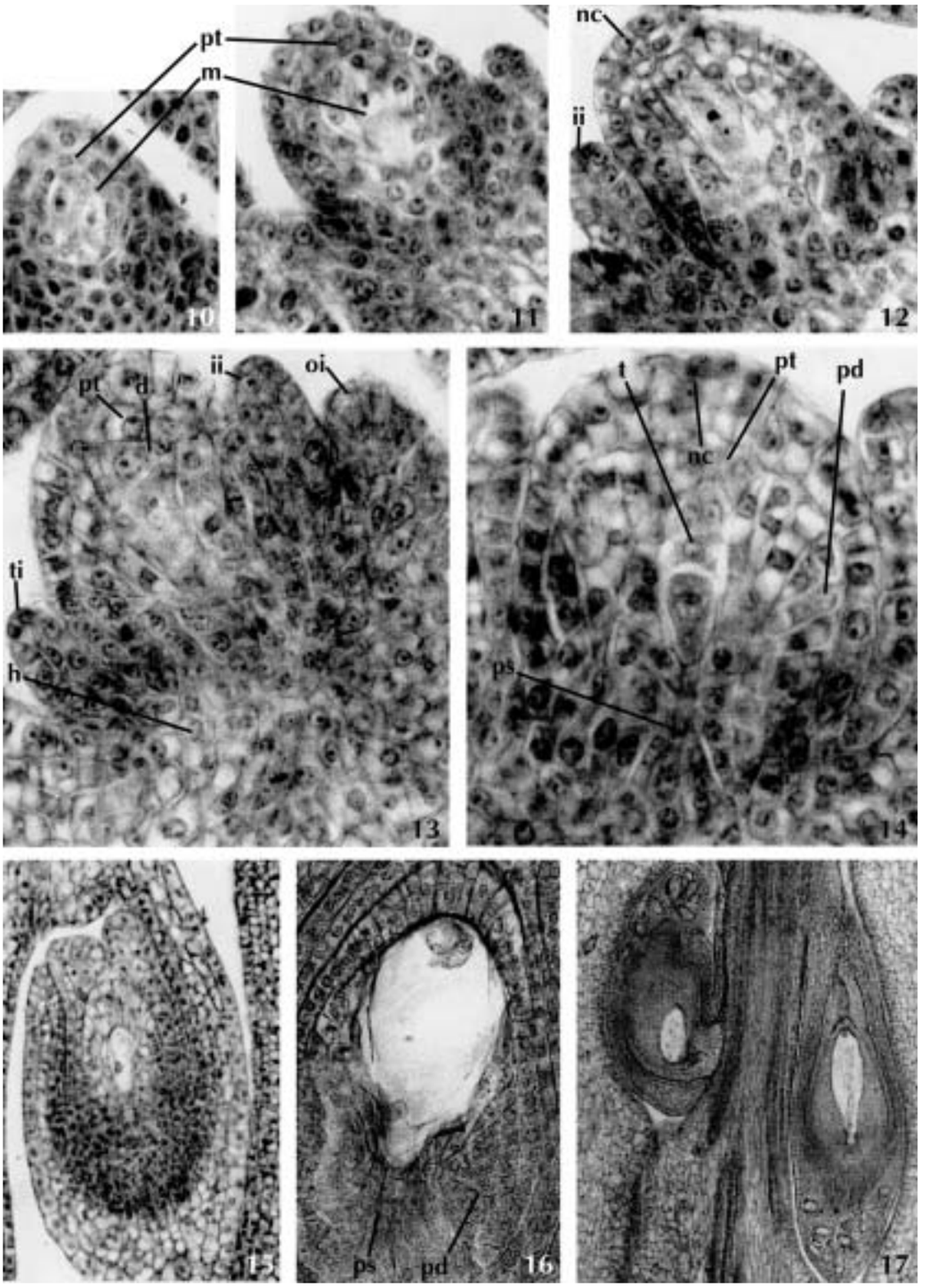
During the globular stage and the transition to organogenesis in the embryo (20–30 DAP) (Fig. 9c, d), zonality develops in the cellular endosperm: a special endospermal cavity appears around the embryo and unifies with the central vacuole; there is cell heteromorphism, with small cells in the micropyle part and large, isodiametric, vacuolated cells in the central part, and relatively tabulate cells with dense cytoplasm and transitory starch in the chalazal part (this starch is consumed at the embryo organogenesis stage). Part of the podium actively degrades, tannins accumulate in hypostase and exotegmen cells, and reticular thickening of the exotegmen cell walls occurs. Cells of the testa grow by stretching.

During embryo organogenesis (30–90 DAP) (Fig. 9e) the above-mentioned processes intensify, reserves accumulate in the endosperm (starch, proteins, lipids, reserves of thickened cell walls), and its cavity enlarges (Figs. 20, 21).

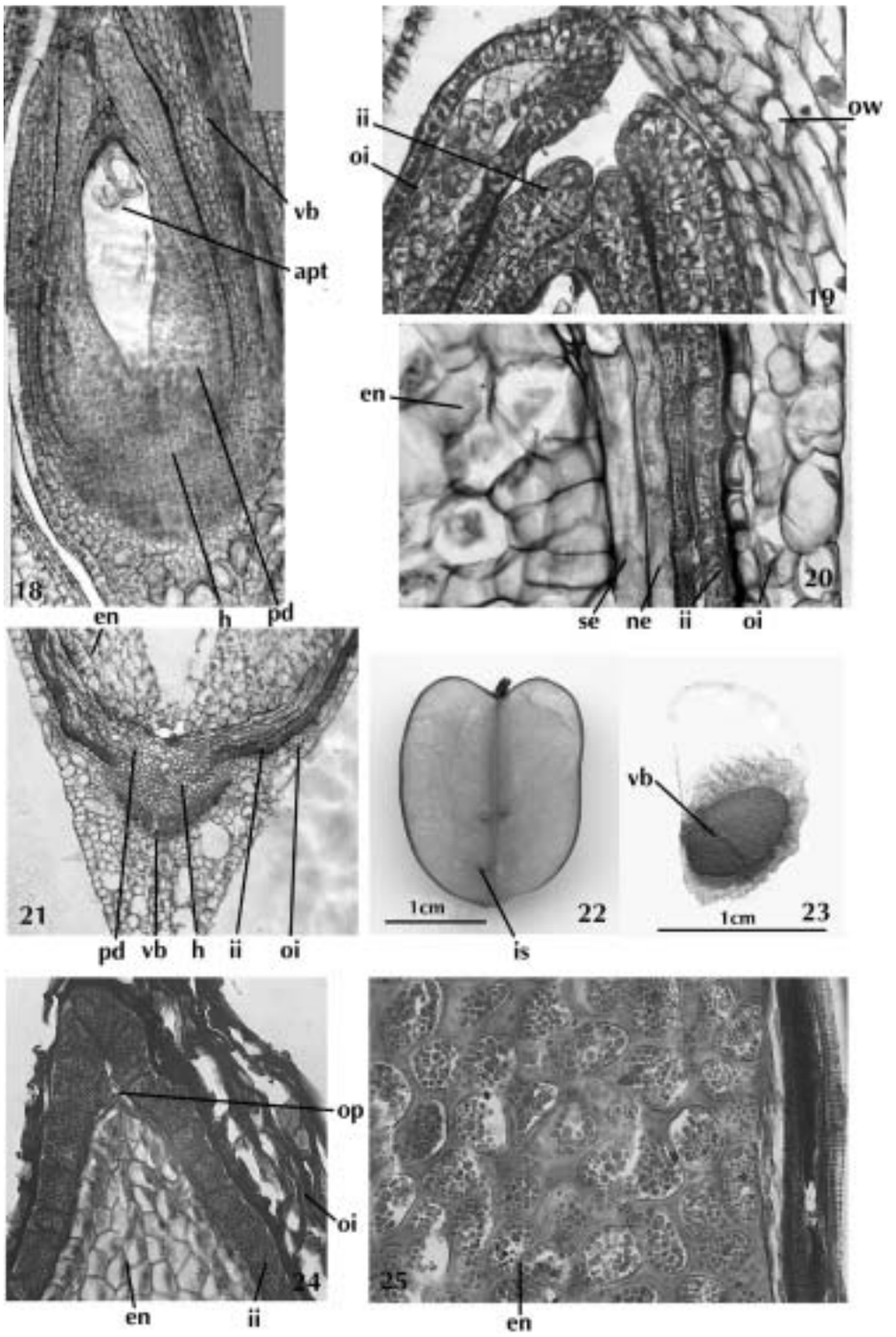
Seeds at the stage of dissemination (~100–110 DAP) (Fig. 9f) are brown, oval, flat, and 6–8 mm in diameter, with a membranous wing on one side, and are contained in a dry, 3-locular, wing capsule fruit (Figs. 22, 23). Nucellar epidermis cells in the apical and lateral parts are compressed, and 2–3 layers of podium still persist. The hypostase is partly filled with tannins. The tegmen is 2-layered, with tannins: the endotegmen is formed of narrow, stone cells, with 3–4 layers of compressed cells with thickened walls in the operculum area; the exotegmen is represented by cells with reticular thickening of the cell walls, covered by cuticle (Fig. 24). The layer of endotesta with thickened outer cell walls is filled with tannins. The mesotesta and exotesta are membranous. The endosperm is massive, solid and white, with a large endospermal cavity in the center. It has small 4–5-angular cells



Figs. 1–9. Ovule and seed structures in *D. nipponica*. **Fig. 1.** Ovular primordium. **Figs. 2–3.** Initiation of main structures of ovule. **Fig. 4.** Dyad. **Fig. 5.** Two-nucleate embryo sac. **Fig. 6.** Four-nucleate embryo sac. **Figs. 7–8.** Mature ovule with micropylar (**a**) and chalazal part (**b**). **Fig. 9.** Embryo development: (**a**) Early proembryo, (**b**) Late proembryo, (**c**) Globular embryo, (**d**) Transition to organogenesis in embryo, (**e**) Embryo organogenesis, (**f**) Dissemination, (**g**) End of embryo postdevelopment. a – archesporial cell; m – megasporocyte; d – dyad; pd – podium; ps – postament; h – hypostase; pt – parietal tissue; vb – vascular bundle; ii – inner integument; oi – outer integument; nc – nucellar cap; en – endosperm.



Figs. 10–17. Megasporogenesis and embryo sac development. **Fig. 10.** Two megasporocytes develop from multicellular archesporium. **Fig. 11.** One megasporocyte. **Fig. 12.** Meiosis I. **Fig. 13.** Dyad. **Fig. 14.** T-shaped tetrad. **Fig. 15**. Two-nucleate embryo sac. **Fig. 16.** Mature embryo sac. **Fig. 17**. Different location of ovules in ovary. Figures 10–14 and 16 × 650; 15, 17 × 250. m – megasporocyte; pt – parietal tissue; nc – nucellar cap; d – dyad; t – tetrad; pd – podium; ps – postament; h – hypostase; ii – inner integument; oi – outer integument.



Figs. 18–25. Seed structures at different stages of embryo development. **Fig. 18**. Stage of two-celled proembryo with additional pollen tube (pollen tube forms loops). × 250. **Fig. 19.** Micropylar part at 4-celled proembryo stage. × 650. **Fig. 20**. Middle part at stage of organogenesis of embryo. × 650. **Fig. 21.** Chalazal part at stage of organogenesis of embryo. × 250. **Fig. 22**. Immature fruit. **Fig. 23.** Seed at stage of dissemination. **Fig. 24**. Micropylar part at stage of dissemination. × 650. **Fig. 25**. Cell structure of endosperm at stage of dissemination. × 650. apt – additional pollen tube; pd – podium; h – hypostase; vb – vascular bundle; ii – inner integument; oi – outer integument; en – endosperm; ow – ovule wall; op – operculum; ne – nucellar epidermis; se – nucellar subepidermis; is – immature seed (stage of proembryo).

in the micropylar part, and large, oval cells with thickened cell walls with pores and reserves in the central and chalazal parts (Fig. 25).

During embryo postdevelopment (about 10 days at 23˚C in laboratory conditions) (Fig. 9g) the volume of the endospermal cavity increases due to the destruction of adjacent cells and resorption of their reserves. The nucellar cap and hypostase fill up with tannins, and the lateral part of the nucellar epidermis and podium are destroyed.

DISCUSSION

The data we obtained on ovule, embryo sac and seed development in *D. nipponica* conform with those from other species of *Dioscorea* as a whole (Rao, 1953; Takeuchi and Kimura, 1968, etc.). We gained data on the formation of the podium, postament, hypostase, pachychalaza and placental obturator, the specifics of seed growth and development after fertilization, the persistence of the epistase, hypostase, 2–3 layers of the podium and the tegmal-endotestal seed coat, the reticular thickening of exotegmen cell walls, the stone cells in the endotegmen, and endosperm zonality. Analysis of the morphogenetic correlations in seed structure development allowed us to amend some structural and functional aspects: the haustoriality of the chalazal part of the endosperm in its coenocytic and early cellular stages (resulting from heteromorphism of the nuclei and cells in the chalazal part of the endo-sperm, accompanied by the destruction of adjacent cells of the postament, and later of podium cells).

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