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EMBRYOLOGICAL STUDIES ON RUBIACEAE III. *OLDENLANDIA VERTICILLARIS* O. KZE[1]

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ABSTRACT

Oldenlandia verticillaris O. Kze is a small marshy herb with rosette of branches. The flowers are bisexual, pentamerous and arranged in a verticillate manner. The bi-celled inferior ovary bears numerous, anatropous, unitegmic, tenuinucellate ovules on parietal placenta. Anther is tetrasporangiate with persistent epidermis followed by fibrous endothecium and a wall layer. Tapetum is uninucleate and of glandular type. Microspore tetrads show tetrahedral and decussate configurations. Pollen grains are shed at 2-celled stage. A single hypodermal archesporial cell directly functions as megaspore mother cell, which forms a linear megaspore tetrad. Endosperm is nuclear, shows wall formation at the 2-celled stage of the proembryo. Embryogeny conforms to the Solanad type. Twin embryo sacs are also reported.

INTRODUCTION

Embryological literature on the family Rubiaceae has been summarised by Vijaya and Lakshmanan (1979). *Oldenlandia* has frequently been handled by various authors, *Oldenlandia alata* (Raghavan and Rangaswamy, 1941), *O. corymbosa*, *O. nudicaulis* (Farooq, 1953, 1958; Farooq and Inammudin, 1969) ; *O. dichotoma* (Siddiqui and Siddiqui, 1968); *O. umbellata* (Shivaramaiah and Sundararajan, 1973); *O. biflora* (Prakasa Rao and Sarat babu, 1975). The authors present the embryological data on *Oldenlandia verticillaris* which grows in brackish, marshy habitat.

MATERIAL AND METHODS

The flowers at various stages of development were collected during September from Dhanushkodi, Tamil Nadu nad fixed in FAA (Formalin-acetic-alcohol). Following customary methods, microslides were prepared. The slides were stained with Heidenhain's haematoxylin, counterstained with fast green and for post-fertilisation stages, tannic acid and ferric chloride combination was used (Foster, 1934).

OBSERVATIONS

O. verticillaris is a small herb. Flowers are pentamerous with tubular calyx and corolla. Stamens 5, epipetalous and the ovary is inferior and bicelled. The ovules are numerous.

Microsporangium to male gametophyte: The anther is tetrasporangiate with persistent epidermis. It is followed by an endothecial layer, a thin walled middle layer and the tapetum. The middle layer disappears when the microspore mother cells are undergoing meiotic divisions. The endothecium develops fi-

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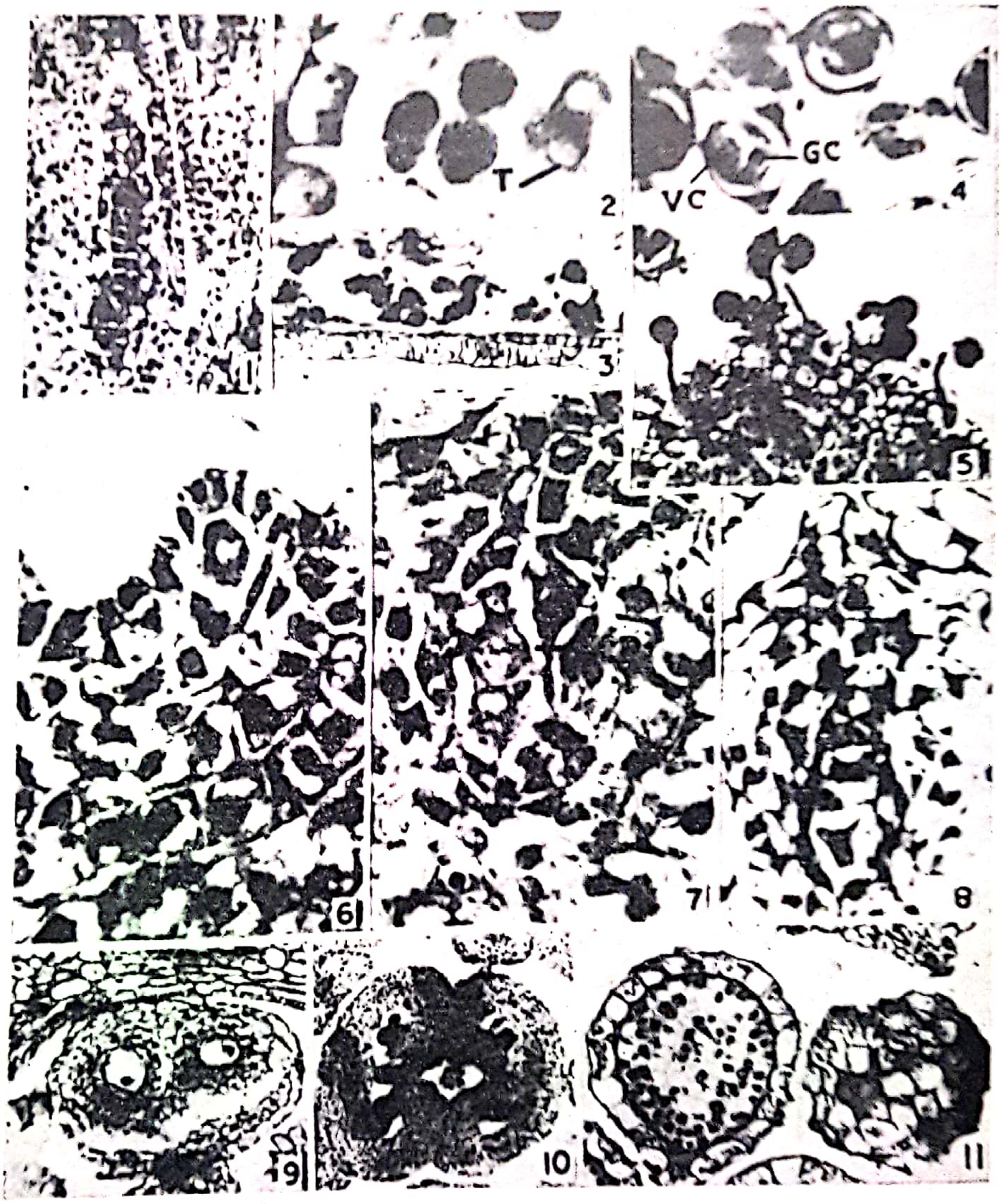
brous thickening a little prior to dehiscence (Fig. 3). The tapetal cells are uninucleate and of the glandular type. The microspore mother cells appear in 2-3 layers in longisection of the anther (Fig. 1) and undergo simultaneous quadripartitioning during meiosis resulting in tetrahedral and decussate tetrads of microspores (Fig. 2). The pollen grains are spherical with three germpores and are shed at 2-celled stage (Fig. 4). Germination of the pollen grains on the bilobed stigmatic apparatus has been shown in Fig. 5.

Megasporangium to female gametophyte: The ovules are anatropous, unitegmic and tenuinucellar borne on parietal placenta (Fig. 10). The single hypodermal archesporial cell directly functions as megaspore mother cell (Fig. 6). The nucellus is represented by a single layer of four cells. Meiotic divisions in the megaspore mother cell results in a dyad (Fig. 7) which gives rise to linear tetrad of megaspores (Fig. 8). The chalazal megaspore develops into an 8-nucleate Polygonum type of embryo sac. Few instances of twin embryo sacs developing equally are observed (Fig. 9).

Endosperm: The primary endosperm nucleus lying in the upper half of the embryo sac undergoes free nuclear divisions, prior to that of the fertilized egg. Just at the time the zygote divides or immediately after the first division, wall formation in the endosperm begins from the micropylar pole, proceeds towards the chalaza centripetally, and the entire sac becomes completely cellular. At maturity, 4-5 layers of endosperm are left at the peripheral region of the embryo sac. The cells are more or less uniformly hexagonal in shape and with deposition of large amount of starch.

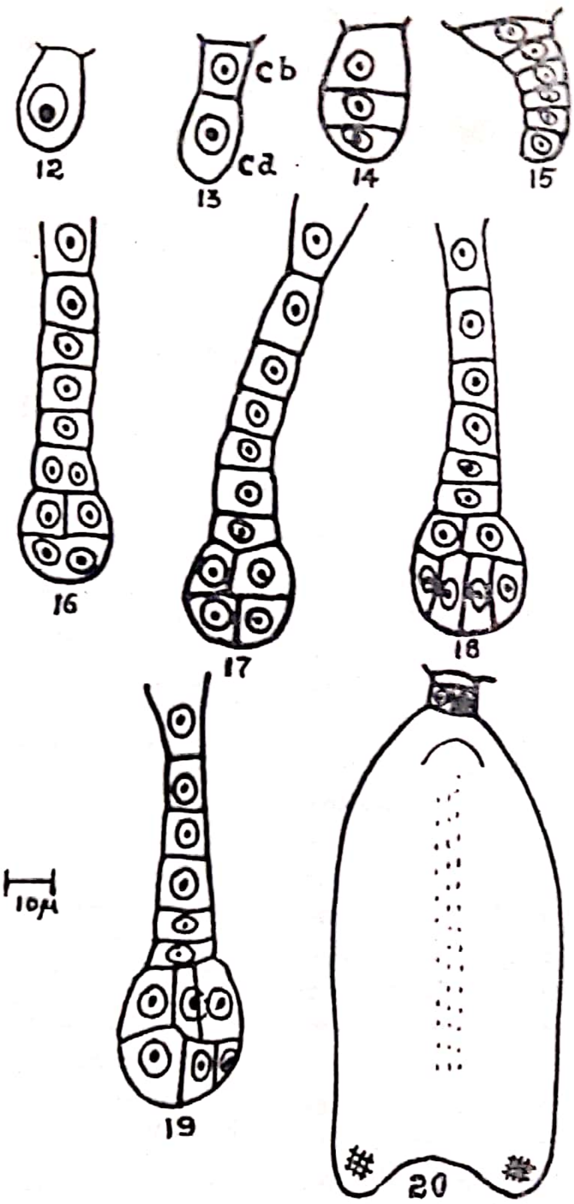
Embryo: The zygote (Fig. 12) undergoes a transverse division resulting in a basal (*Cb*) and terminal cells (*Ca*) (Fig. 13). Both the cells undergo transverse divisions either simultaneously or with belated division in the basal cell (Fig. 14). Successive transverse divisions result in a proembryo of 6-8 cells (Fig. 15) arranged in an uniseriate filament. The terminal cell of the proembryo enlarges and becomes spherical. It undergoes a transverse division followed by two vertical divisions in both the daughter cells, constituting the terminal quadrant (Figs. 16, 17). Division in similar plane in each tier results in an octant. In the terminal tier obliquely vertical walls are laid (Fig. 18) which separate a group of four axial cells from an equal number of peripheral cells. Periclinal divisions in four axial cells differentiate peripheral and central cells four each. The four peripheral cells and four daughter cells differentiatea earlier by the oblique vertical walls constitute the protoderm of the upper tier (Fig. 19). In the subterminal tier of the octant, vertical walls are laid and the peripheral cells thus differentiated become contiguous with the protoderm of the terminal tier. At this stage the terminal globular part of the proembryo exhibits well differentiated protoderm surrounding eight axial cells. Further development of the proembryo up to maturity, in all major facets of development are as is reported in other members of *Oldenlandia*. The embryogeny conforms to the Solanad type.

Seed: Seeds are small, numerous and brownish in colour. The mature seed consists of a dicotyledonous embryo ensheathed by a single layer of testa derived from the outer layer of the integument. In the surface view (Fig.



Figs. 1-11. Fig. 1. L. S. of anther showing wall layers, uninucleate tapetal cells and microspore mother cells (× 80). Fig. 2. Tetrahedral and decussate tetrads of microspores (× 285). Fig. 3. L. S. of anther showing fibrous thickening in the endothecium (× 105). Fig. 4. 2-celled pollen grains (× 245) Fig. 5. Pollen germination on the stigma (× 115). Fig. 6. L. S. of the ovule showing single hypodermal arches-porial cell (× 280). Fig. 7. Dyad (× 160). Fig. 8. Linear tetrad of megaspores (× 240). Fig 9. Twin embryo sacs (× 120). Fig. 10. C. S. of the ovary depicting the arrangement of the ovules on parietal placenta (× 55). Fig. 11. Seed coat in surface and sectional views (× 20).

(CP—Cell Plate, GC—Generative cell, T—Tapetum, VC—Vegetative cell.)



Figs. 12-20. Development of the embryo. Fig. 12. Zygote. Figs. 13, 14, 15. 2-celled, 3-celled and 6-celled proembryos respectively. Figs. 16, 17. Proembryos with terminal quadrants. Figs. 18, 19. Proembryos showing protoderm formation. Fig. 20. Heart-shaped embryo.

11) the seed coat shows hexagonal cells without any specialised thickenings.

DISCUSSION

The evolutinary trend (Fargerlind, 1937) in the organisation of the ovule in *Oldenlandia* based on the nucellar tissue can be in a reductional series beginning with *O. verticillaris* (present study) with four nucellar cells, *O. corymbosa*, *O. alata* with 1-3 cells, *O. biflora*, *O. umbellata* with 1-2 cells, and *O. nudicaulis* with one cell.

The development of the normal type of embryo sac from a single archesporial cell is an established character of *Oldenlandia*. An interesting feature is the occurrence of twin embryo sacs not reported in any other species of *Oldenlandia*. Both the embryo sacs develop uniformly and are derived from two independent archesporial cells.

The structure and development of of microsporangium exhibit a uniform pattern in *Oldenlandia*-a single fibrous endothecial layer, one middle layer, glandular uninucleate tapetum. Similarly the microspore mother cells after simultaneous meiotic division results in varied tetrad configurations. Although the pattern of development is the same amongst the species of *Oldenlandia*, the shedding stage varies. It is 2-celled in *O. umbellata* and *O. biflora*, 3-celled in *O. corymbosa*, and the present material adheres to the former category.

The family is characterised by development of nuclear type of endosperm. In *O. verticillaris* at about the 2-celled stage of the proembryo cell wall formation begins, which becomes completely cellular and occupies the entire cavity of the embryo sac. In the mature seed, the endosperm is completely used up around the embryo and 3-5 layers are left persistent at the peripheral region of the embryo sac, a stage which corresponds with the earlier report on *O. nudicaulis* (Farooq and Inamuddin, 1969).

Embryogeny conforms to the Solanad type (Johansen, 1950). The suspensor may be uniseriate as in *Oldenlandia, Dentella, Hydrophylax, Spermacoce* or multiseriate as in *Asperula, Phyllis, Vaillantia, Galium* and *Canthium*. The suspensor is 5-6 celled in *Oldenlandia alata, O. dichotoma* and 3-celled in *O. corymbosa* which is the shortest in Rubiaceae. In *O. verticillaris* the suspensor is long, uniseriate and filamentous consisting of 7 cells. In conclusion it is to be stressed that considerable variation could not be observed in embryological feature between the species growing in brackish, marshy and mesophytic ecosystems. So the embryological characters are less subjected to variations in the ecological system.

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