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THE EMBRYOLOGY OF *RICHARDIA BRASILIENSIS* GOMEZ. (SYN. *RICHARDSONIA PILOSA* H.B. & K.) (RUBIACEAE) — A REINVESTIGATION

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Abstract

Flowers of Richardia brasiliensis Gomez. are trimerous. Inferior ovary is tricarpellary and trilocular. Ovules are hemianatropous, unitegmic and tennuinucellate. The so-called strophiola is well developed. Development of anther wall layer is normal. The tapetal cells are uni-nucleate and glandular. Microspores are tetrahedral. Mature pollen grains are shed at 3-nucleate stage. The nucellus is 1-3 celled. Hypodermal archesporial cell functions as the megaspore mother cell. The chalazal megaspore of a tetrad is functional. Embryo sac development is Polygonum type. Double fertilization is observed. The development of endosperm is nuclear type. The embryogeny conforms to the Solanad type. Terminal cell of the suspensor appears to be haustorial. The seeds are albuminous.

Introduction

The embryology of *Richardsonia pilosa* was described by Lloyd (1902) and Fagerlind (1937) who gave an incomplete description of its life-history. Our observations show some significant differences which are reported here.

Material and Method

Plants of *Richardia brasiliensis* Gomez., were collected from Agriculture college, Kanke, Bihar, India and fixed in FAA. After dissection the material was dehydrated in alcohol-xylol series, embedded in paraffin wax and cut at $8\text{-}12\mu$. Staining was done in safranin and fast green.

Results

Flower: The inflorescence is a cymose head of 12-15 flowers. The inflorescence axis bears 1-2 or occasionally 3-celled uniseriate trichomes. The gamosepalous calyx is

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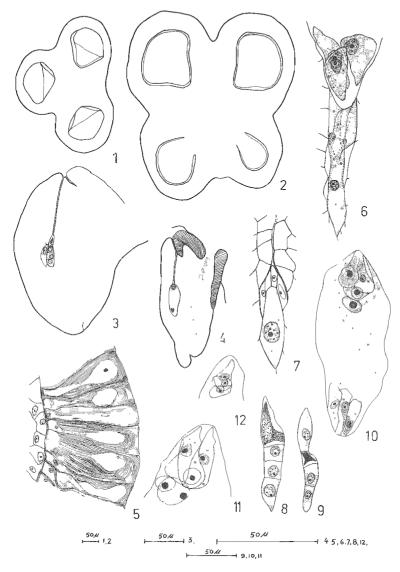
6-lobed which bears 1-celled as well as 2-celled uniseriate trichomes on their abaxial surface. The six corolla lobes bear unicellular glandular trichomes on both their surfaces. Androecium consists of six (rarely 4 or 5) epipetalous stamens. The gynaecium is tricarpellary, syncarpous and trilocular (Fig. 1) and rarely tetracarpellary and tetralocular (Fig. 2). There are a number of short, 2-celled, uniseriate glandular hairs on the outer surface of the ovary.

Ovary and ovule: A single hemianatropous unitegmic and tennuinucellar ovule is present in each ovarian locule (Fig. 3). The so-called "strophiola" is well developed and covers the entire funicular side of the ovule and extends beyoned the micropylar opening (Fig. 4). The epidermal cells of the major part of strophiola and of the funicle are radially elongated and glandular. These have a thick deposition of cellulose on the inner side of their cell walls (Fig. 5). Hypostase is differentiated at the chalazal region immediately below the antipodal cells (Fig. 6). It consists of a group of considerably elongated thickwalled cells which are densely cytoplasmic. Their location and appearance suggests that they are connected in translocation of nutrient material to the embryo sac.

Megasporangium and female gametophyte: Three to four nucellar epidermal cells cover the tip of the archesporial cell. The mound-shaped nucellus (Fig. 7) is Bouvardia type. The chalazal megaspore of a linear tetrad (Fig. 8) gives rise to the Polygonum type of embryo sac (Fig. 10). However, in the present material megaspore tetrads with more than one healthy megaspores was observed (Fig. 9). The synergids are feebly hooked (Fig. 11). Antipodal cells are variously arranged and quite conspicuous (Fig. 10, 12). They possess starch grains in their cytoplasm and are retained upto early post-fertilization stages of the sac.

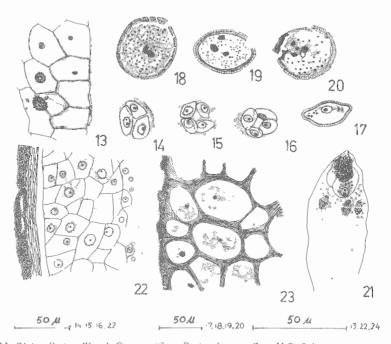
Microsporangium and male gametophyte: The development of anther wall layer is of Dicotyledonous type. Some cells of the endothecial layer may undergo one or two periclinal divisions on the side of the connective (Fig. 13). Druses may be present in some cells of the connective tissue. The glandular tapetal cells remain uni-nucleate throughout. Sometimes they may persist till the pollen grains have attained maturity. The microspore tetrads are tetrahedral and occasionally isobilateral or decussate (Figs. 14-16). The spindle shaped pollen grains are broad at the equator and taper towards the poles (Fig. 17). The mature pollen grain is of 18-colporate and 3-nucleate at shedding stage (Figs. 18-19). One exceptional pollen grain had three small sperm nuclei and a vegetative nucleus (Fig. 20).

Fertilization: The pollen tube passes between the cells of stylar tissue and destroys both the synergids (sometimes only one) during its entry into the embryo sac (Fig. 21). Double fertilization has been observed. The pollen tube inside the embryo sac is very conspicuous and persists up to the globular stage of the embryo.



Figs. 1-12. Richardia brasiliensis Gomez. (Syn. Richardsonia pilosa H.B. & K.).

1. T.s. Trilocular ovary with one ovule in each locule. 2. T.s. of a 4-chambered ovary. 3. L.s. of hemianatropous ovule. 4. L.s. ovule showing well developed strophiola with radially elongated thick-walled epidermal cell which are present all around the funicle. 5. L.s. of strophiola showing extent of cellulose thickenings in epidermal cells. 6. Showing hypostase immediately below the antipodal cells. 7. L.s. ovule showing the nucellar epidermal cells and the megaspore mother cell. 8. Linear megaspore tetrad. 9. Third megaspore from the micrpylar end has degenerated while the remaining three are healthy. 10. L.s. mature embryo sac. 11. The epical part of a mature embryo sac showing hooked synergids. 12. Showing linearly arranged antipodal cells.



Figs. 13-23. Richardic brasiliensis Gomez (Syn. Richardsonia pilosa H.B. & $\ensuremath{\mathsf{K}}$)

13. L.s. anther showing 2.3 layered endothecium with fibrous thickenings 14-16. Tetrahedral, isobilateral, decussate micro-pore tetrads respectively 17. Showing young microspore 18. 2-nucleate pollen grain with 18-germ pores, 19. Showing 3-nucleate pollen grain, 20. Pollen grain with three sperm nuclei and one vegetative nucleus, 21. Fertilized embryo sac showing persistent synergids, remains of pollen tube and dividing endosperm nuclei, 22. Part of almost a mature seed showing 4-5 layers of persistent endosperm and single layered testa, 23. Part of a mature seed. The epidermis of the integument forms the seed coat. The endosperm cells have become thick-walled due to cellulose deposition.

Endosperm: The primary endosperm nucleus is situated near the zygote where it divides. Subsequent nuclear divisions in the endosperm nuclei are simultaneous (Fig. 22). After wall formation, the endosperm cells situated near the embryo become considerably enlarged and vacuolated. These cells distinctly present a different appearance than the rest of the endosperm cells. These cells are the first to degenerate under the influence of the embryo.

Embryogeny: The development of embryo is Nicotiana variation of Solanad type.

Seed coat: As the endosperm tissue increases in size, the integumental cells degenerate and disappear. Finally the epidermis of the ovule remains and forms single layered testa (Figs. 22-23). The cells of the testa are tangentially elongated and contain abundant tanin. Occasionally some of the cells may have raphide crystals. In mature seed, there are 4-5 persistent layers of endosperm cells (Figs. 23-24) which possess consi-

derable quantity of starch grains. The entire persistent endosperm tissue becomes thick walled due to cellulose deposition (Fig. 24). The endosperm invaginates the tissue of the so-called strophiola and consumes the central part of its tissue.

Discussion

Occasional presence of tetracarpellary gynaecium suggests that tricarpellary condition in *Richardia brasiliensis* is a derived condition.

The formation of double layer endothecium at some places is an interesting feature. However, double layer endothecium was reported earlier in *Tarenna asiatica* (Periasamy & Parameswaran, 1965), *Anthocephalus chinensis, Spermadictyon suaveolens* and *Argostemma sarmentosum* (Inamuddin, 1970).

The microspore tetrads are variously arranged. It is interesting that the pollen grains in the present material have eighteen germpores while those of *R. pilosa* (syn. *R. brasiliensis*) have only nine germ pores (Fagerlind, 1937). Polyspermy is rare in Rubiaceae although it was observed in one pollen grain in the present material. Earlier one such pollen grain was reported in *Borreria hispida* (Farooq, 1959) and also some in *Oldenlandia nudicaulis* (Farooq & Inamuddin, 1969).

The presence of hypostase is worth mentioning in the Rubiaceae. However, this type of hypostase was recorded earlier in *Paediria scandens* and *Spermadictyon suaveolens* (Inamuddin, 1970).

According to Fagerlind (1937) Vaillantia type of nucellus occurs in *Richardsonia pilosa* (R. brasiliensis). However, the present material conforms to Bouvardia type.

Mostly the division in the micropylar dyad is suppressed in *Callipeltis cucularia* (Lloyd, 1902), *Hoffmannia, Bouvardia, Psychotria* and *Richardsonia* (Fagerlind, 1937). The present study, however, show regular tetrad formation in *R. brasiliensis*.

Hooked synergids are rare in the Rubiaceae. Occasionally they occur in Olden-landia nudicaulis (Farooq & Inamuddin, 1969), Anthocephalus chinensis, Argostemma sarmentosum and Paediria scandens (Inamuddin, 1970) as they were seen in R. brasiliensis.

Fagerlind (1937) reported Richardsonia type of antipodal cells in *Richardsonia* pilosa. However, in the present material the antipodal cells are similar to Leptodermis and Cephalanthus types and not to Richardsonia type because the basal antipodal cell is feebly enlarged and persists until early post fertilization stages.

One layered testa including *R. brasiliensis* is common feature in the family. However, two layered testa has been reported in *Vaillantia hispida* (Lloyd, 1902), *Oldenlandia alata* and *Dentella repens* (Raghavan & Rangaswamy, 1941). Also 10-layered testa occurs in the mature seed of *Rubia cordifolia* (Venkateswarlu & Rao, 1958) and 7-8 layered in *Hydrophylax maritima* (Ganapathy, 1956a).

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