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Embryology of two species of *Dentella* (*Dentella repens* and *Dentella serpyllifolia*)

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Abstract. Embryological features of the two tetraploid species of *Dentella*–*Dentella repens* and *Dentella serpyllifolia* are essentially identical. Anther wall development follows the Dicotyledonous type. Secretory tapetum is uniseriate with uninucleate cells. Female archesporium is single-celled. Embryo sac development follows the Polygonum type. Endosperm is nuclear. Embryogeny conforms to Hyoscyamus variation of Solanad type. Seed coat is formed by the outer epidermis of the integument.

Keywords. Embryology; Rubiaceae; *Dentella*.

1. Introduction

The genus *Dentella* comprising 10 species (Willis 1966) belongs to the tribe Hedyotideae of the sub-family Cinchonoideae of Rubiaceae. Only two species, namely *Dentella repens* J. R. and G. Forst and *D. serpyllifolia* Wall ex Airyshaw are known to occur in India. The latter differs from the former in the absence of hyaline blunt setae on the capsule. Till 1932, these two taxa were regarded as a single species—*Dentella repens*. Subramanyam and Sharma (1969) however regarded them as two distinct species. It was therefore proposed to study the embryology of the two species to find out embryological differences, if any, between them. The chromosome number and development of female gametophyte and embryo in *D. repens* was reported earlier by Raghavan and Rangaswamy (1941).

2. Materials and methods

The material collected from Kesenakurrupalem of East Godavari District (A.P.) and Indian Botanical Gardens, Howrah (W.B.) was fixed in formalin-Acetic-alcohol for embryological studies and in acetic-alcohol for cytological studies. Customary methods of dehydration, clearing and embedding were followed (Johansen 1940). Sections cut between 5–13 μ thickness were stained in Delafields haematoxylin.

3. Observations

3.1. *Microsporangium, Microsporogenesis and Male gametophytes*

The anther is tetrasporangiate (figure 1). In transverse section the hypodermal archesporium in each lobe is 4–6 celled (fiugre 2). It undergoes a periclinal division and produces an outer primary parietal layer and an inner primary sporogenous layer. The former divides periclinally to produce the inner tapetal layer and the outer secondary parietal layer which as a result of one more periclinal division gives rise to the outer endothecial layer and the inner middle layer (figure 3). The development of anther wall thus follows dicotyledonous type (Davis 1966). The secretory tapetum is uniseriate with uninucleate cells. Endothecial cells develop fibrillar thickenings ; middle layer becomes crushed as anther matures.

The primary sporogenous cells after undergoing one or two mitotic divisions develop into pollen mother cells. The meiocytes *Dentella serpyllifolia*, at the diakinesis stage of meiosis I show 18 bivalents each and thus the gametic chromosome number is 18 (figure 4). As a result of simultaneous cytokinesis in the pollen mother cells either tetrahedral or isobilateral tetrads are formed (figure 5). The divisions in pollen mother cells even within the same anther lobe are not synchronous. Pollen grains are two-nucleate and triaperturate at the shedding stage (figure 6).

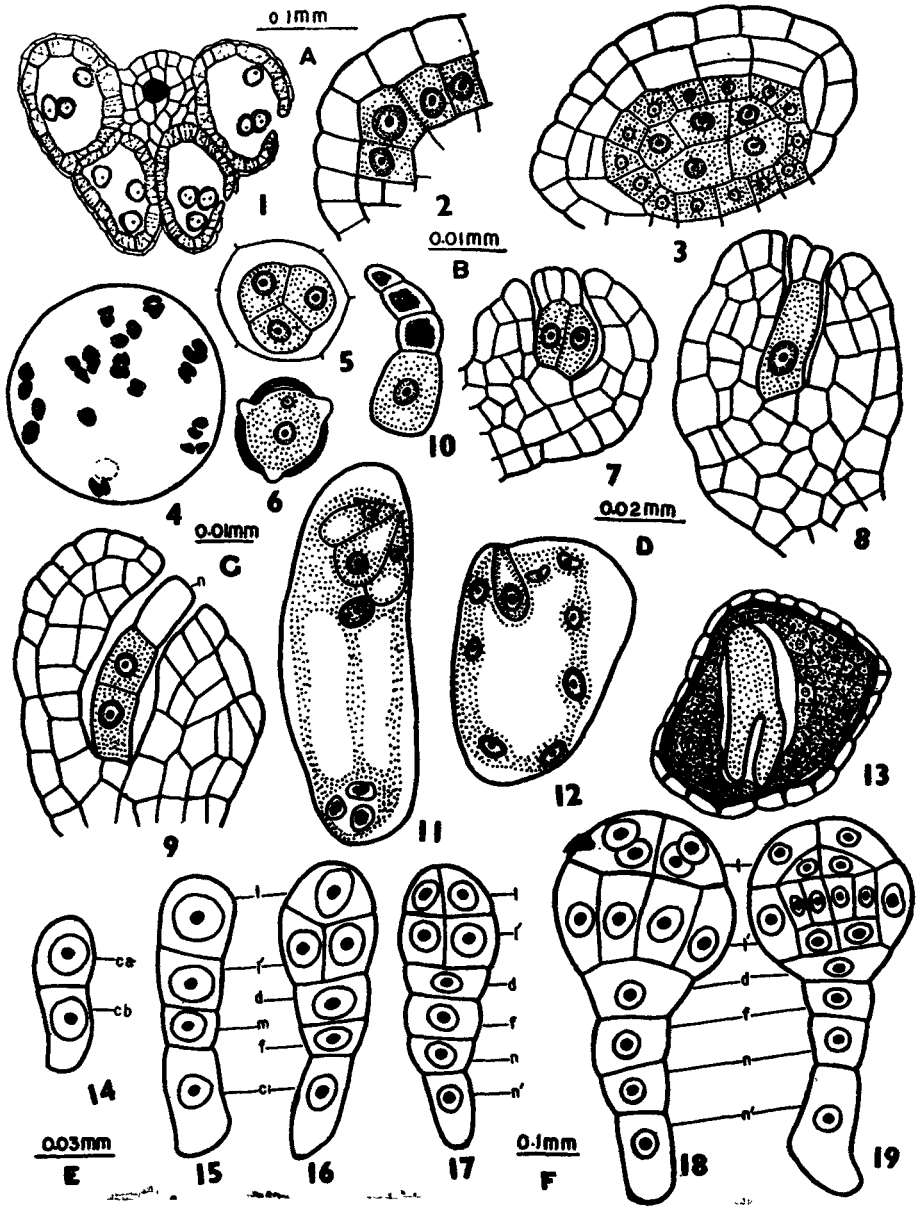
3.2. *Magasporogenesis and Female gametophyte*

Ovules are hemianatropous and unitegmic. The nucellus of *D. serpyllifolia* consists of a single epidermal cell as in *D. repens* and conforms to Oldenlandia type (Fagerlind 1937). However, in *D. repens* (present study) 2 or 3-celled nucellus is also observed as a rare feature (figures 7, 8). The archesporium is single-celled. Rarely in *D. repens*, it is two-celled (figure 7). The archesporial cell directly develops into the megaspore mother cell which after meiotic division produces a linear tetrad of megaspores (figures 7–10). The chalazal megaspore of the tetrad develops into 8-nucleate embryo sac of polygonum type (figure 11). Synergids are hooked and polar nuclei fuse near the egg apparatus. The antipodal cells are uninucleate and ephemeral.

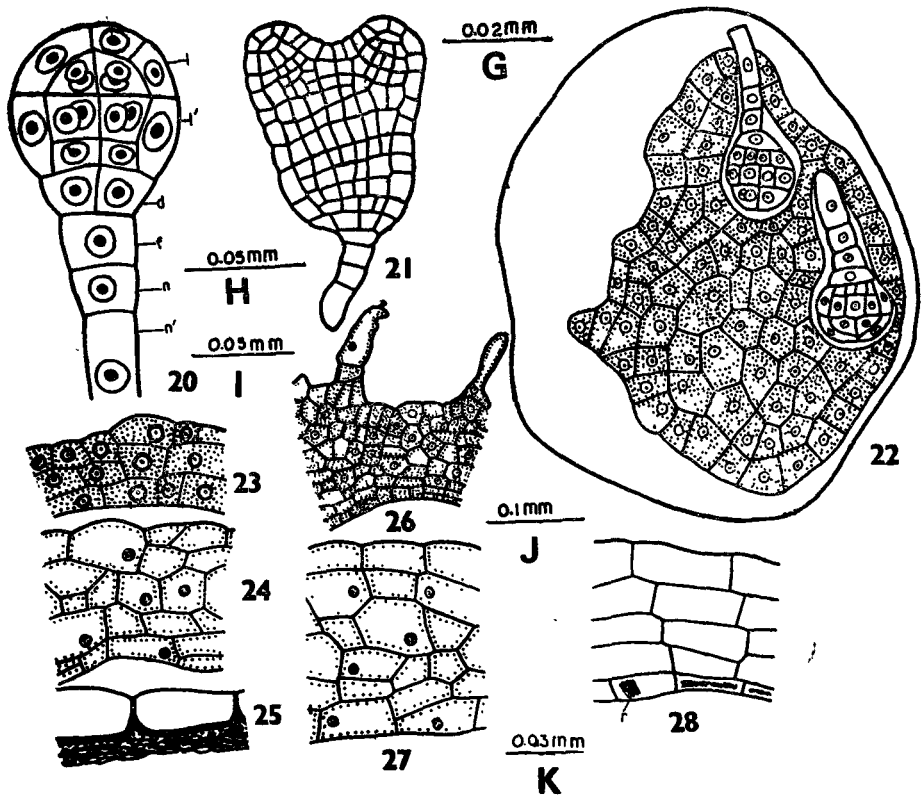
3.3. *Fertilisation, endosperm and embryo*

Fertilisation is porogamous. The endosperm is nuclear (figure 12). By the time the embryo becomes 4-celled, cell wall formation commences proceeding from periphery towards the centre and ultimately fills the entire embryo sac with cellular tissue (figures 13, 22). The seed is endospermous.

The zygote divides transversely resulting in a 2-celled proembryo (figure 14). The terminal cell *ca* and the basal cell *cb* undergo each one more transverse division to form a linear 4-celled proembryo (figure 15). The cells are termed as *l*, *l'*, *m* and *ci* from apex towards the base. The two cells *l* and *l'* divide vertically twice at right angles to each other resulting in quadrants in each tier (figure 17). Somelimes vertical division in *l'* occurs earlier than in *l* (figure 16). Meanwhile, the cells *m* and *ci* divide transversely each to form a row of four cells *d*, *f*, *n* and *n'* (figure 17). The tier *l* after undergoing furthei divisions contributes to the forma-



Figures 1–19. 1,2,7,8. *Dentella repens*; 3–6, 10–19. *D. serpyllifolia*. **1.** T.s. dehisced anther. **2.** T.s. part of anther lobe showing archesporium. **3.** T.s. anther lobe showing wall layers and sporogenous tissue. **4.** Pollen tetrad. **6.** Mature pollen grain. **7.** L.s. ovule showing archesporium. **8.** L.s. ovule showing two archesporial cells. **9.** L.s. ovule showing megaspore dyad. **10.** Megaspore tetrad. **11.** Mature embryo sac with degenerating antipodals. **12.** Embryo sac with zygote and nuclear endosperm. **13.** L.s. mature seed. **14–19.** Stages in the embryo development.



Figures 20–28. 20–25, 27,28. *Dentella serpyllifolia.* **26.** *D. repens.* **20,21.** Embryos. **22.** Embryo sac with twin embryos and cellular endosperm. **23–25.** Stages in the development of seed coat. **26–28.** Stages in the development of pericarp.
Magnifications for figures **1–19** and **20–28.**
Scale A for **1**; B for **2, 5**; C for **3, 4, 7–11, 14–19**; D for **6**; E for **12**; F for **13.**
Scale G for **20, 21**; H for **21, 22, 25**; I for **24**; J for **26, 28**; K for **27.**

tion of cotyledons and stem tip (figures 18–21). The derivatives of *l'* give rise to the hypocotyl and a part of root and those of *d* to the remaining part of the root. The rest of the cells *f*, *n*, and *n'* form the suspensor which is either three- or four-celled. The four-celled proembryo is linear and the terminal cell *ca* contributes to the formation of the embryo proper. The course of embryo development follows the Solanad type. Further, at the third cell generation the cells are arranged in five tiers and this conforms to the Hyoscyamus variation (Johansen 1950).

Twin embryos are observed in a few cases of *D. serpyllifolia* (figure 22). By virtue of its position it may be said that the second embryo is developed from the synergid.

3.4. *Seed coat*

The integument at the archesporial cell stage is 2 or 3 celled thick (figures 7–9) and remains so, even at the time when the 8-nucleate embryo sac is formed

(figure 23). The cells are richly cytoplasmic with healthy nuclei. After fertilisation the seed coat increases in its volume by an addition in the number of wall layers and the size of the cells. The seed coat which becomes somewhat thick at the globular embryo stage (figure 24) becomes single layered due to disintegration of inner wall layers by the time the dicotyledonous embryo is organised (figure 25). Thus seed coat is formed by the outer epidermis alone. At this stage inner and radial walls of epidermal cells become thickened.

3.5. *Pericarp*

The wall of the ovary at the megaspore mother cell stage consists of 6–8 layers of cells which contain dense cytoplasm and healthy nuclei (figure 26). In *D. serpyllifolia* the wall of the ovary is smooth (figure 27) whereas in *D. repens* epidermal hyaline setae are produced (figure 26). In both the cases raphides are present in the ovary wall (figure 28). During the mature embryo sac stage the cells of the ovary wall enlarge and cytoplasm becomes scanty. Simultaneously the nuclei disintegrate. After fertilisation the inner wall layers begin to degenerate and finally the outer 3 or 4 wall layers form pericarp (figure 28).

4. Discussion

As stated already the two species of *Dentella—D. repens* and *D. serpyllifolia*, which were regarded as a single species earlier were later treated as two distinct species by Wallich (1826, in Sched, in Herb. Wall, sub No. 6206 G) and now again by Airyshaw (1932, Wall. ex Airyshaw in *Kew Bull.* 289) and Subramanyam and Sharma (1969). However, the embryological features of these two tetraploid species of *Dentella* reveal that these essentially are identical in such characters as tetrasporangiate nature of the anther, dicotyledonous type of anther wall development, simultaneous cytokinesis, two-nucleate and triaperturate pollen grains, single-celled nucellus, linear megaspore tetrad, Polygonum type of embryo sac ontogeny, three ephemeral antipodal cells, hooked synergids, nuclear endosperm, development, Hyoscyamus variation of Solanad type of embryo development, single layered seed coat and pericarp made up of 3 or 4 layers. The embryological evidence, therefore is not in support of the splitting *D. repens* into two species.

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