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FURTHER CONTRIBUTIONS TO THE EMBRYOLOGY OF *DILLENIA SUFFRUTICOSA* (GRIFF.) MARTELLI[1]

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ABSTRACT

Embryological aspects of *Dillenia suffruticosa* (Griff.) Martelli have been studied. The anther wall is four layered. The tapetum is of glandular type with binucleate cells. The endothecium is non-fibrous. Simultaneous divisions of microspore mother cells result in tetrahedral, isobilateral and decussate tetrads of microspores. Pollen grains are tricolpate, uninucleate and has a distinct rod-shaped crystal. Ovules are amphitropous, bitegmic and crassinucellate. The zygote considerably elongates within the mantle formed by the endosperm and develops into an embryo with massive suspensor. The endosperm is nuclear which later, becomes cellular. The endosperm becomes ruminated. The endosperm is heterogeneous. The endosperm cells surrounding the embryo are thinwalled and devoid of food products while the peripheral endosperm cells are smaller and filled with starch and lipid bodies. Seed coat is formed by both the integuments. The aril with thin-walled cells completely envelopes the seed.

INTRODUCTION

The genus *Dillenia* founded by Linnaeus (1753) and *Wormia* by Rottbell (1783) have undergone considerable revisions. Hoogland (1952) has given the latest revised account of the genus *Dillenia* (*Wormia* also included) and listed 55 species.

Dillenia suffruticosa (Griff.) Martelli, distributed in Sumatra, Malay Peninsula, W. Java, Borneo and Philippines can be distinguished from other species by the presence of a distinct group of staminodes on the outer side of the androecium. It grows in marshes, along streams and on the margins of forest, sometimes, immediately behind the mangrove upto 550 m. altitude.

The earliest contribution to the embryology of Dilleniaceae is that of Schnarf (1924) comparing Dilleniaceae with families like Ochnaceae, Marcgraviaceae, Theaceae, Guttiferae and to bring out the structure of the ovule and the embryo sac therein. Dilleniaceae is characterised by the presence of an aril. Paetow (1931) has given the first detailed account of the development of male and female gametophytes, endosperm and seed in *Wormia suffruticosa*. Swamy and Periasamy (1955) studied the embryology of *Acrotrema arnottianum*. Rao (1955), who reported on some phases of structure and development of anther, pollen and embryo sac in *Dillenia pentagyna*, further (1957, 1961) contributed to the embryology of Dilleniaceae. Anatomy of the

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flower, and the development of gametophytes and seed are reported in species of *Hibbertia* and *Dillenia* (Sastri, 1958). Embryological literature on the family is scanty and some of the observations are controversial. The present study is to investigate and add further information on the embryology of *Dillenia suffruticosa*.

MATERIAL AND METHODS

The material was collected from the University Botanical Garden, Calicut University, Kerala State during general flowering period (February and March). After appropriate dissections, the required parts were pickled in formalin-acetic-alcohol. Customary methods of dehydration, infiltration and embedding were employed and sections were cut from 8 to 12 microns in thickness. Heidenhain's haematoxylin and erythrosin were used for staining. Tannic acid and Ferric chloride combination (Foster, 1934), counterstained with safranin, were used to study the morphology of the zygotic mantle and embryogeny.

OBSERVATIONS

Dillenia suffruticosa is a profusely branched tree with large ovate leaves. The leaf consists of an amplexicaul petiolar wing of taxonomic importance and an expanded lamina (Fig. 1). The thick, fleshy and leathhery sepals are persistent. The bright yellow petals are thin, filmy and deciduous. The free carpels are surrounded by whorls of free stamens.

Microsporogenesis and male gametophyte :

The stamens, arranged in fascicles consist of long innermost stamens reflexing outwards over the outer whorls of stamens in bud. Peripherally is a distinct group of staminodes.

The anther consists of four wall layers inclusive of the epidermis. Epidermal cells, filled with tannin, constitute the more dominant persistent layer in the mature anther. It exhibits banded thickenings, simulating a fibrous layer, and with warty thickening on the surface (Fig. 15). The innermost wall layer functions as the tapetum of secretory type. Some of the cells of the tapetum become binucleate during the meiotic division of the microspore mother cells. Of the other two middle layers, the innermost degenerates at the time of meiosis, and the outer one remains without developing any fibrous thickening. The sporogenous cells, after repeated divisions function as microspore mother cells. Reduction divisions of the simultaneous type (Figs. 11, 13, 14), resulting in tetrahedral, isobilateral and decussate (Fig. 12) types of tetrads of microspores. The mature pollen grain is tricolpate, exine without sculpturing and with a distinct rod-shaped crystal (Fig. 15) in the middle of the cytoplasm. The pollen grain remains uninucleate at the time of dehiscence.

Ovule, megasporogenesis and female gametophyte :

The conduplicate carpels bear ovules in two vertical rows submarginally. Findings on the development of megaspore and the organisation of the female gametophyte agrees with the observations of Pactow (1931): The amphitropous, bitegmic and crassinucellate ovule with Polygonum type of embryo sac.

Endosperm and Embryo :

Following pollination, the sepals tightly envelope the ovary, the whole resembling a bud. Fruits dehiscent, with the sepals and carpels spreading star-like (Fig. 2). After fertilisation, the female gametophyte expands laterally,

leaving a single layer of nucellus peripherally and a few layered caplike nucellus at the region of the micropyle.

While the zygote is undergoing metabolic changes, the primary endosperm nucleus undergoes repeated synchronous free nuclear divisions. A dense accumulation of endosperm can be observed around the zygote upto the micropylar region (Figs. 4, 5) the nuclei of which, undergo mitotic divisions (Fig. 6). Subsequently the endosperm becomes uni-

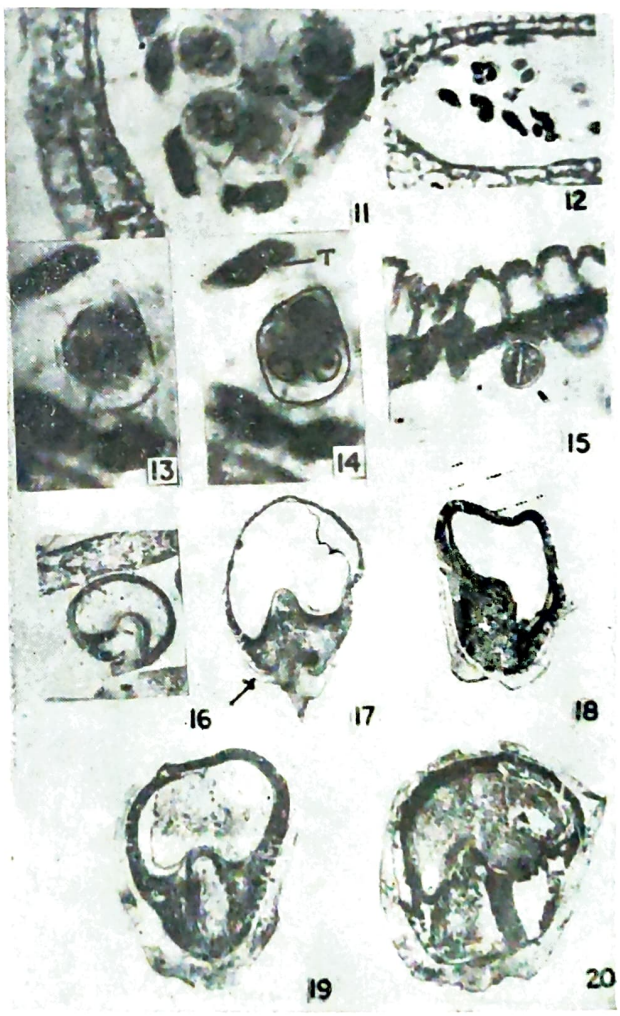
Figs. 1-10. *Dillenia suffruticosa*. Fig. 1. Habit. Fig. 2. Fruits. Young fruits enclosed by persistent calyx and a dehisced fruit. A single carpel on the right. Fig. 3. Zygote (×130). Fig. 4. Extended mantle (×190). Fig. 5. Transection of mantle (×130). Fig. 6. Free endosperm nuclei in division (×190). Figs. 7, 8. Developing suspensor enveloped by the mantle (×120). Fig. 9. Mature embryo in longisection (×120). Fig. 10. Embryo with surrounding heterogeneous endosperm (×190).

form occupying the entire region of the embryo sac.

Gradually, from the periphery towards the chalaza the endosperm becomes cellular. The cellular endosperm is heterogeneous consisting of a central elongated region surrounding the embryo and a peripheral region (Fig. 10). The former is made up of larger cells, somewhat thinwalled, and devoid of any storage material. The peripheral endosperm is made up of smaller cells, filled with starch grains and oil drops. Even in a mature seed, the two zones can easily be recognised and their physiological role is also evident, the former being physiologically active and serve as a nutritive tissue and the latter as a storage tissue.

The dense cytoplasm of the endosperm that is enveloping the zygote develops a distinctly differentiated envelope around the zygote often described as zygotic mantle (Figs. 4, 5). It is merely the cytoplasm of the endosperm, which has more affinity for the stain and is somewhat thicker in texture, than the surrounding cytoplasm. It completely envelopes the zygote and extends peripherally, as numerous elongated enucleate protrusions which often and in terminal expansions presenting a corolloid appearance. At subsequent stages of ontogeny of the embryo, it is clear, that the walls of the embryo have shrunk from the mantle and excepting the suspensor region the embryo comes out of the mantle (Figs. 7, 8). The mantle at the chalazal end is profusely branched and ramify the endosperm cells. The ramification of the mantle continues even after the endosperm has become cellular. The entire suspensor and the proembryo remain embedded within the zygotic mantle. At about the stage when the proembryo is attaining the globular configuration, it pushes through the chalazal region and remains free from the mantle.

The contour of the free nuclear endosperm at the chalazal region is undulated. When the endosperm becomes cellular, the same shape is maintained with cells of the protruding regions much denser than the other cells. In such protrusions, the cells undergo divi-



Figs. 11-20. *Dillenia suffruticosa*

Fig. 11. Microspore mother cells in meiosis I (×450). Fig. 12. Transection of anther showing tetrads of microspore (×425). Fig. 13. Microspore mother cells in meiosis II (×600). Fig. 14. Same as above : the position of nuclei marked with Indian Ink. Fig. 15. Portion of anther showing wall layers and a pollen with crystal (×320). Figs. 16-20. L. S. of seed showing the development of aril (×15).

sions to manifest itself as distinct meristematic zones, bringing about rumination.

The zygote remains without any division even after several free nuclei are formed in the endosperm. Meanwhile, the length of the zygote when compared with that of the mature egg cell is about three fourths before embarking upon its first transverse divisions (Fig. 3). The embryogeny in this species could not be interpreted in terms of cell lineage because of the unhealthy embryos. The first transverse division is followed by few more similar divisions, before the three terminal cells undergo vertical divisions. The cells at the micropylar region further undergo transverse divisions. The proembryo, at this stage, is with massive suspensor, distinguishable into two parts-uniseriate micropylar and multiseriate massive chalazal regions. The suspensor cell, adjacent to the micropyle, becomes modified into an elongate, pointed structure which pierces through the micropyle. The terminal cells of the proembryo undergo further divisions and attains maturity passing through the conventional globular and heart-shaped stages of ontogeny. The mature embryo (Fig. 9) remains embedded in the central endosperm region occupying a very small area in proportion to the large massive endosperm (also seed) (Fig. 10).

Seed : After fertilisation, the nucellus becomes replaced by the endosperm. The outer integument remains two layered, the outer layer of which is impregnated with tannin and the inner one becomes sclerified. The number of layers in the inner integument increases after fertilisation, but only the innermost layer is filled with brown contents and persists in the mature seed. The entire seed is enveloped by the aril, which, is made up of thinwalled elongated cells without any inclusions. Around the base of the funicle, the primordium of the aril arises even before fertilisation (Fig. 16). It becomes active perhaps, triggered by pollination and fertilisation and rapidly grows as a cup-like sheath around the seed. A little before the nuclear endosperm becomes cellular, the aril covers about half the size of the developing seed (Figs. 17, 18). The endosperm becomes completely cellular before the aril could envelope the entire seed (Figs. 19, 20).

DISCUSSION

The stamens exhibit uniformity in the organisation of the anther walls. The wall layers are five in *Hibbertia* (Sastri, 1958) and *Dillenia pentagyna* (Rao, 1955), but uniformly four in *Dillenia indica* (Sastri, 1958), *Acrotrema arnottianum* (Swamy and Periasamy, 1955) and in *Dillenia suffruticosa* (Present study). The endothecium is non-fibrous except in *Hibbertia stricta* (Sastri, 1958). However, some of the cells of the endothecium are said to be fibrous in *Acrotrema arnottianum* (Swamy and Periasamy, 1955) and *Hibbertia billardieri* (Sastri, 1958). In all known members of Dilleniaceae, characteristically enough, the epidermis is persistent and the cells of which are impregnated with tannin. The report on *Wormia burbidgei* (Rao, 1961) is unique, in that the cells of the epidermis develop fibrous thickening. The innermost wall layer develops into a secretory type of tapetum. It is clear from the present study, that the epidermis develops thickenings simulating a fibrous layer and remains persistent. The tapetum is of the secretory type which is a characteristic feature of the family. Paetow (1931) has described the tapetum as periplasmodial in *Wormia*

suffruticosa. The observations of Rao (1955), on the nature of tapetum in *Dillenia pentagyna* is very vague and the exact nature of tapetum could not be ascertained. The pollen grains are reported to be 2-nucleate at the shedding stage in *Dillenia pentagyna* (Rao, 1955) *D. indica* (Sastri, 1958), *Wormia suffruticosa* (Paetow, 1931) *Acrotrema arnottianum* (Swamy and Perisamy, 1955) and *Hibbertia* (Sastri, 1958). However, exceptionally enough, in the present investigation even in a mature flower in which the sepals are about to close again, the pollen grains remain uninucleate.

The development of the endosperm is of the nuclear type. To begin with, the protoplast is as a thin layer on all sides except at the micropylar region where it accumulates around the elongated zygote. The micropylar accumulation with large number of free nuclei embedded therein extends a little down towards the chalazal pole. The nuclei at the micropylar accumulation undergo active mitotic division which are more or less synchronized. The dense cytoplasm and active nuclear divisions clearly show that the endosperm at this region is more active than in the remaining part. When the endosperm occupies the entire cavity of the embryo sac it becomes cellular. A heterogeneticy in organisation is set in, the region around the embryo and a little further down, derived from the micropylar endosperm accumulation still appears to be the physiologically active region in contrast with the remaining endosperm, wherein the cells function as the storage organ, impregnated with starch grains and lipid bodies. Although the present observation agrees with that of Paetow (1931), in the micropylar accumulation and in the cellular organisation of the endosperm, the observations on the size difference between the nuclei of the micropylar, lateral and chalazal regions, as well as the chalazal accumulation of the endosperm could not be confirmed. In the earlier observations, the rumination of the endosperm at the chalazal region has not been reported. The distinct undulation of the protoplast of the endosperm, wall formation and the meristematic activity at the protrusions in random are note worthy, which have been reported (Dulcy Elizabeth and Lakshmanan, 1981). This type of rumination caused by endosperm is similar, to that reported in Acanthaceae (Mohan Ram and Masand, 1963).

An interesting and controversial feature is the occurrence of the zygotic mantle. The zygotic mantle is a characteristic feature of some members of Dilleniaceae which has not been reported in any other family of angiosperm. In *Acrotrema arnottianum* (Swamy and Periasamy, 1955) and in *Dillenia suffruticosa*, the mantle is well pronounced, and is described to be slightly developed in *Hibbertia acicularis* (Sastri, 1958) and altogether absent in *Dillenia indica* (Sastri, 1958). Swamy and Periasamy (1955) have reported that the zygotic mantle is from the plasma membrane of the zygote which throws out radially elongated protrusions which ramify the endosperm cells. In *Hibbertia acicularis* (Sastri, 1958), it is described that the zygotic membrane swells in the form of weak undulations which extend from the micropylar region towards the side but not at the chalazal end of the zygote. Fig. 41 of Sastri (1958) does not convince that the thickening at the micropylar region is actually of the zyogotic membrane. It appears that the thickened region is nothing but the cytoplasm the nuclear endosperm which has started organising itself into a weak

mantle around the zygote. It would have been convincing if the structure of the mantle is shown at the stage of the proembryo as shown in his Fig. 44. In *Dillenia indica* the zygotic mantle is absent.

Present observations agree with that of Paetow (1931), in that, the mantle is of endosperm origin rather than from the plasma membrane of the zygote as observed in *Acrotrema* (Swamy and Periasamy, 1955). The close proximity of the dense cytoplasm around the zygote, the organisation of the zygotic mantle and the considerable elongation of the zygote, clearly correlate their positional relationship and physiological activity. Therefore, it is to confirm, that the zygotic mantle in *Dillenia suffruticosa* is of endosperm origin and persists upto the heart-shaped stage of the embryo. Embryogeny of the family has not been clearly reported so far. It may be because of the high percentage of sterility, and even in the few seeds where the embryos develop, they do not appear to be healthy.

From the meagre information available, it has been observed that the "embryogeny probably follows the Onagrad type" (Davis, 1966). In contrary, Swamy and Periasamy (1955) observed that the basal cell in *Acrotrema* does not undergo divisions and at the same time a cell close to it is without vertical wall. From these observations, it is clear, that it has to be assigned to the Caryophyllad type because the basal cell remains without division. It cannot be of the Onagrad type because the cell close to the basal cell is without vertical division (Fig. 16 of Swamy and Periasamy, 1955) which should have been formed after the vertical division of the terminal cell. In all the eight Ranalian families (no report on Calycanthaceae) of Benthem and Hooker (See Willis, 1973), the terminal cell divides vertically and the embryogeny conforms to Onagrad type except in Nymphaeaceae where it follows the Asterad type (Davis, 1966).

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