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Development, Structure and Dehiscence of Follicles of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae)

Kuriachen, P M., Yash Dave and Vinoth Thomas
(Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120, Gujarat, India)

ABSTRACT

The atrichomatous wall of ovary in *Calotropis procera* becomes highly pubescent in the young fruit, but scabrous in the mature fruit. The single layered epicarp develops from the outer epidermis of the ovary wall. The mesocarp which develops from the mesodermis is distinguished into outer, middle and inner zones. The central mesocarp breaks up in the course of fruit development and disintegrate to form large air chambers. The 2-3 layered lignified endocarp develops from the inner epidermis as well as from the inner mesodermis layers of the ground tissue and shows a 'parquetry pattern' of cell arrangement in surface view. The parenchymatous placenta becomes aerenchymatous in the mature fruit. Fruit dehiscence in marginicidal (ventricidal).

INTRODUCTION

Calotropis procera is a common shrub bearing pale pink flowers in umbellate cymes. The bicarpellary sub-apocarpous ovary is superior. The ovary develops after fertilization into a characteristic boat shaped follicle (Fig. 1A). In most cases only one carpel matures into the fruit; rarely both carpels produce a pair of follicles.

Asclepiadaceae is an advanced angiosperm family, but almost all the genera have follicular fruit. Even though the taxonomists and fruit anatomists consider the follicle as a primitive type of fruit, anatomical studies on follicular fruits are rare (Fahn, 1967; Gill, 1976; Zala *et al.*, 1976; Roth, 1977; Wardrop, 1983; Dave and Kuriachen, 1990). *Asclepias currassavica* is the only member of the Asclepiadaceae, so in which the ontogeny and anatomy of the follicle was studied (Dave and Kuriachen, 1990). Sabet (1931) has studied the embryology and endosperm formation of *C. procera*. The floral morphology of this species was elucidated by Mulay *et al.* (1965). Dave and Kuriachen (1987) studied the development of stomata on the pericarp of *C. procera* and have described the surface pattern of the endocarp. The present paper deals with the structure of the developing and mature pericarp of *C. procera*.

MATERIALS AND METHODS

The flowers and fruits of *Calotropis procera*, growing in the environs of Vallabh Vidyanagar were collected at various developmental stages (Table 1) and fixed in formalin-aceto-alcohol mixture (Johansen 1940). The fixed ovaries and small pieces of fruit from the basal, middle and terminal parts were dehydrated through a tertiary butyl alcohol series, and infiltrated with and embedded in 'tissue prep' (m.p. 56.5; Fisher Scientific Company, New Jersey). The sections of 8-10 μm were stained with the safranin 'C' and fast green F.C.F. combination or toluidine blue 'O'. Micrographs were taken with a Zeiss Photomicroscope I. For scanning electron microscopy material was dehydrated with a graded acetone series and mounted on specimen stubs using a synthetic resin adhesive (Fevicol of Pidilite Industries, Bombay). The material was then air dried and coated with gold-palladium using a S.E.M. coating unit E5 100 (Poloron Equipment Ltd.). Samples were observed in a Cambridge Stereoscan S_4-10 microscope at ATIRA, Ahmedabad.

RESULTS

Ovary wall. The ovary wall consists of 30-40 layers

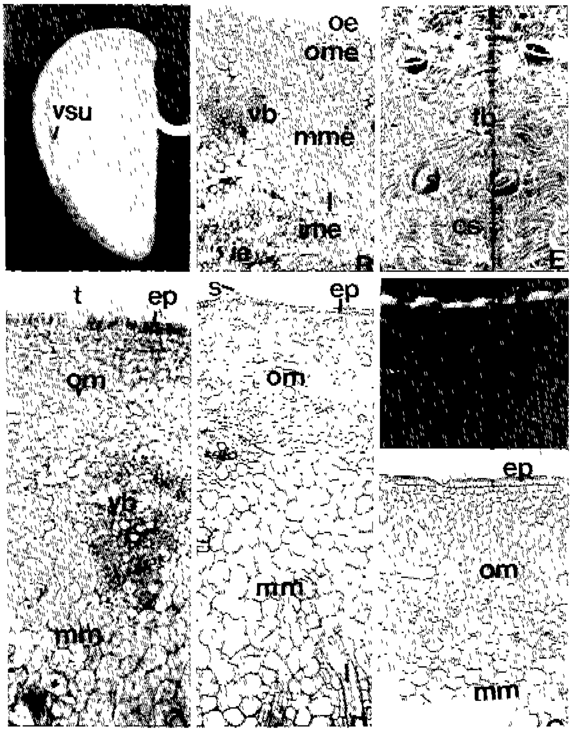

Fig. 1. 1A: Mature follicle ×1. 1B: A portion of ovary wall in transection, ×135. 1C: Transection of outer pericarpic layers from stage 2, ×198. 1C: Transection of outer pericarpic layers from stage 3, ×145. 1E: Scanning electron micrograph of mature fruit surface, ×350. 1F: Normarsky interference micrograph of outer pericarpic layers from mature fruit (see the outer tangential wall thickening of epicarpic cells at arrow) ×235. 1G: A portion of outer pericarpic layers from stage 4 ×96.
c, cuticle; cs, cuticular striation; ep, epicarp; ie, inner epidermis; ime, inner mesodermis; l, laticifer; mm, middle mesocarp; mme, middle mesodermis; oe, outer epidermis; om, outer mesocarp; ome, outer mesodermis; s, stomata; t, trichome; tb, trichome base; vb, vascular bundle; vsu, ventral suture.

Table 1. Characters of follicles of *Calotropsis procera* at various developmental stages

Stage No.	Length (mm)	Breadth (mm)	Remarks
1	2-4	1-2	Ovaries
2	5-15	3-8	
3	15-30	10-20	Immature fruits
4	30-60	20-30	Mature fruits
5	60-70	30-35	Mature fruits after dehiscence

of cells. The single layered outer epidermis has rectangular to columnar cells as seen in cross-section (Fig. 1B). Stomata and trichomes are not yet developed. The cuticle is thin. In parenchymatous ground tissue, an outer, middle and inner region or zones can be distinguished. The cells of the outer and inner mesodermis are smaller than those of the middle mesodermis (Fig. 1B). The procambium strands are seen in between the outer and middle mesoderm zones (Fig. 1B). The laticifers present in the mesodermis are non-articulated. The inner epidermal layer consists of rectangular or transversely elongated cells (Fig. 1B). These cells divide anticlinally.

Developing and mature pericarp. The pericarp which develops from the fertilized ovary wall can be distinguished into epicarp, mesocarp and endocarp.

Epicarp. The outer epidermis of the ovary wall becomes the epicarp of the fruit. In the young or immature fruit, the epicarpic cells are still thin walled with dense cytoplasm and prominent nuclei (Figs. 1C-D). At this stage, the fruit is highly pubescent due to the development of a large number of trichomes (Fig. 1C). These trichomes are uniseriate and multicellular. They arise singly or in groups of two or three. Stomata also develop in the epicarp (Figs. 1D-E). As the fruit matures, the trichomes fall off leaving roundish scars at their basal cells. The outer tangential walls of the epicarpic cells become highly thickened and the formerly thin cuticle becomes thicker (Fig. 1F) and corrugated which in surface view manifests as a beautiful ornamental pattern of the epicarp (Fig. 1E).

Mesocarp. The mesocarp, develops from the mesodermis and can be distinguished into the outer, middle and inner zones. The outer mesocarp originates from the outer mesodermis zone. The cells of this region are smaller than the middle mesocarpic cells and have slightly vacuolated cytoplasm (Figs. 1C-D). Two to four layers of cells in this zone just below the epicarp are uniformly dense and actively dividing (Fig. 1C). Vascular bundles are seen only in the outer mesocarp, just above the middle mesocarp, in the young immature fruit (Fig. 1C), but in the third and successive stages these vascular strands are seen branching towards the inner mesocarpic layers. The vascular bundles are conjoing collateral or bicollateral. The non-articulated laticifers are seen in the outer mesocarp (Figs. 1C-D). In mature fruit the outer mesocarpic cells enlarge and their cytoplasm becomes highly vacuolated (Fig. 1G).

The middle mesocarp is the largest mesocarpic zone. In the early stage of the fruit, the cells of this zone are

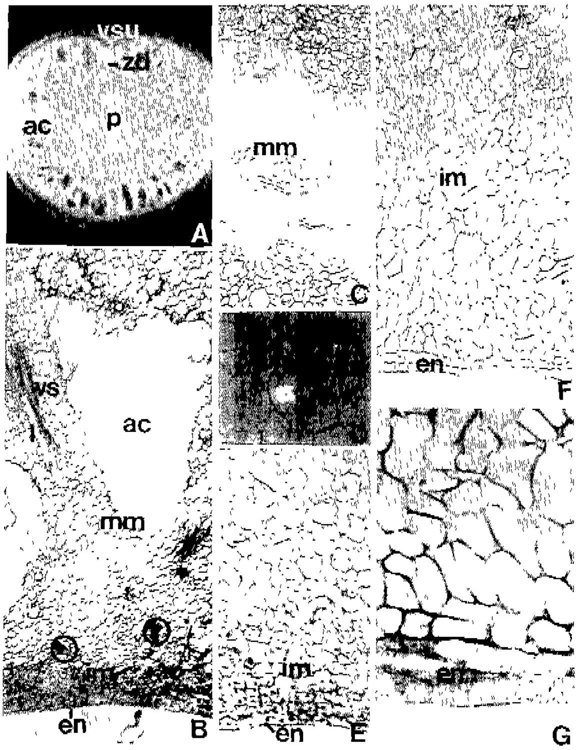

Fig. 2. 2A: An transection of entire fruit from stage 3, ×3. 2B: A portion of midole and inner pericarpic layers from stage 3, see developing vascular strands encircled in the inner mesocarp, ×72. 2C: Outer, middle and inner mesocarp at stage 6, ×105. 2D: Normarsky interference micrograph of middle mesocarpic cells with crystal, × 298. 2E: Inner pericarpic layers from stage 3, ×286. 2F: Inner mesocarp and endocarp from mature fruit, ×114. 2G: A portion of inner mesocarp and endocarp in transection from dehisced fruit ×465.
ac, air chamber; en, endocarp; im, inner mesocarp; l, laticifer; mm, middle mesocarp; p, placenta; vs, vascular strand; vsu, ventral suture; zd, zone of dehiscence.

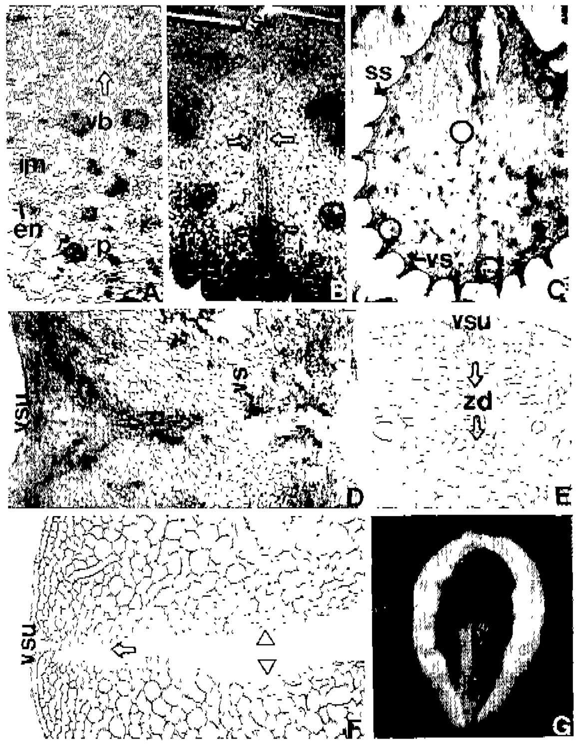

Fig. 3. 3A: Aerenchymatous part of inner mesocarp above the placenta, see the separation of cells along the zone of dehiscence at arrow, ×45. 3B: Transection of ovary from flower bud showing fusion of carpel margins from the ventral suture to the opposite end of the placenta (at arrows) and placenta (see placental bundles in circles), ×247. 3C: Placenta from stage 3, see the intercellular spaces in circles, ×41. 3D: A portion of pericarp in transection from stage 3 showing zone of dehiscence, ×48. 3E-G: Stages in dehiscence of the follicle. 3E: Transections of mature fruit from the lower part showing small and loose cells along the line of dehiscence, ×69. 3F: Showing direction of dehiscence (arrow), arrow head shows the broken cells along the separated margins, × 96. 3G: Dehisced follicle, see the detached placenta and separated valve at arrow, ×0.8.
en, endocarp; im, inner mesocarp; p, placenta; ss, seed stalk; vb, vascular bundle; vs, vascular strand; vsu, ventral suture; zd, zone of dehiscence.

found to be larger and more vacuolated than the (Fig. 1C) outer and inner mesocarpic cells. These thin walled cells enlarge more rapidly than the cells of the other two mesocarpic zones. These enlarging cells break up and disintegrate to form large air chambers during the third stage of fruit development (Figs. 2A-B). Laticifers and vascular strands traverse the unbroken strands of the tissue (Fig. 2B). When the fruit matures the middle mesocarp, except the traversing vascular strands with some accompanying parenchyma cells, disorganizes completely to form large continuous air chambers (Fig. 2C). Druse type crystals are present in the disorganizing middle mesocarpic cells (Fig. 2D). The development of air chambers in the pericarp caused compression of fruit at maturity resulting in the total reduction in thickness of the fruit wall.

The inner mesocarp consists of 10-12 layers of cells at the first developing stage of the fruit. These slightly vacuolated cells are smaller than the overlying middle mesocarpic cells (Fig. 2E). During the second developing stage of the fruit development, this zone increases in thickness due to the addition of more layers of cells and the differentiation of small vascular bundles (Fig. 2B in circles). In the mature fruit, the cells of this zone become enlarged and more vacuolated (Fig. 2F). Laticifers are also seen in this zone. One or two innermost layers of cells of the inner mesocarp seem to differentiate like the endocarpic cells and in the fully mature stage, shortly before dehiscence, these layers also become lignified to contribute to the formation of the multilayered endocarp (Fig. 2G). The inner mesocarp above the placenta in mature fruit becomes aerenchymatous. Small vascular bundles and laticifers can be seen in this region (Fig. 3A).

Endocarp. The 2-3 layered lignified endocarp of the mature fruit is the product of the inner epidermis and a part of the inner mesodermis of the ovary wall. In immature fruits, the endocarpic cells are thin walled and transversely elongated with dense cytoplasm and prominent nuclei (Fig. 2E). Towards the maturity of the fruit, these cells are distinctly elongated (Fig. 2F). This elongated inner layer of cells and the overlying one or two layers of inner mesocarp become lignified just before the dehiscence of the fruit (Fig. 2G). In surface view, this fibrous endocarp exhibits a 'parquetry' pattern in its cell arrangement (see DAVE and KURIACHEN, 1987).

Placenta. The marginal placenta, projecting into the ovary chamber from the fused carple margins, bears a number of ovules. In the ovary the parenchymatous cells of the placenta in the ovary stage are compactly arranged with two differentiating vascular bundles (Fig. 3B circles). As the fruit grows, intercellular spaces develop, which later result in the establishment of an aerenchymatous placenta (Fig. 3C). The vascular strands branch profusely and supply the developing seeds. In a fully mature fruit the placenta consists of highly compressed cells. About 350-400 seeds are present in a single follicle. The seeds are hairy and are dispersed by a 'parachute mechanism'. The basal and terminal parts of the placenta have no seeds.

Dehiscence of the follicle. The mature follicle dehiscens along the ventral suture (ventricidal dehiscence). In the young ovary a future zone of dehiscence can be easily distinguished at the union of carpel margins extending upto the opposite end of the placenta (Fig. 3B arrows). As the fruit grows 2-4 layers of cells are distinguished in this zone (Fig. 3D). The dehiscence of the follicle proceeds from the inner layers to the exterior. Before dehiscence, the inner mesocarpic tissue lying opposite of the placenta become aerenchymatous. The cells along the line of dehiscence above the aerenchyma become loose before dehiscence (Fig. 3E). Vascular strands branching through the sides of the line of dehiscence are observed (Fig. 3D). The endocarp becomes lignified and fibrous just before the dehiscence. The mature fruit becomes compressed by the development of air chambers in the pericarp. The swelling and shrinkage of the thick walled endocarpic cells create tension in the pericarp. Therefore, the unequal drying of the thin walled and thick walled cells of the pericarp and contraction of the entire fruit create a pulling force on the weak seam of the follicle margin and finally cause the opening of the follicle (Figs. 3F-G). The fruit dehisces while it is green in colour. When the dehiscence of the fruit is completed the placenta becomes separated from its place of attachment to expose the seeds (Fig. 3G). The pressure exerted by the large number of seeds also help in the process of dehiscence.

DISCUSSION

The mature fruit wall of *Calotropis* is differentiated into a single layered epicarp, parenchymatous mesocarp and 2-3 layered fibrous endocarp as Fahn (1967) also observed in the follicle of *Delphinium*. The development of various pericarpic layers is pointed out in the present study. When the epicarp develops from the smooth outer epidermis of the ovary wall, large numbers of trichomes and stomata are developed. The structure and development of stomata has been reported previously (Dave and Kuriachen, 1987). As the fruit matures, the trichomes fall off and the cuticle becomes thick and corrugated, which renders the fruit wall scabrous.

The mesocarpic zones have their origin in the respective mesodermis layers of the ovary wall. The vascular strands pass through the outer mesocarp and branches into the inner mesocarpic layers. A sclerenchymatous bundle cap is reported in the follicles of *Delphinium* (Fahn 1967) and *Asclepias curassavica* (Dave and Kuriachen, 1989), but in *Calotropis* follicle the vascular bundles are without such caps. The interesting observation on the mesocarp is the development of air chambers, formed by the disintegration of cells in the middle mesocarp. The development of air chambers in the pericarp is an advantage for the dispersal of fruits by water. But such a dispersal method is not necessary in the case of *Calotropis* because the follicle dehisces and disperses the seeds when still on the plant. Moreover, the seeds crowned with a tuft of long hairs (coma) at one end are dispersed by the well-known 'parachute mechanism'.

The 2-3 layered fibrous endocarp is developed from the inner epidermis and a few inner mesodermis. Such endocarpic development has been reported elsewhere too (Dave *et al.*, 1975, 1979, 1987; Roth 1977; Rao and Dave, 1980). The endocarp becomes lignified just before the dehiscence, which implies that the development of such a hard zone is mainly meant for the dehiscence of the fruit (Roth, 1977). This late lignification is also helpful to the growth of the seeds, because the sclerified tissue cannot adapt any more to growth in circum ference (Roth, 1977). The formation of 'parquetry pattern' in the endocarp of *C. procera* has been reported by Dave and Kuriachen (1987). The endocarp of *Asclepias curassavica* also exhibits a 'parquetry pattern' of its cell arrangement (Dave and Kuriachen, 1989).

The projecting marginal placenta is formed by the ontogenetic fusion of carpel margins. In the ovary, the placenta is composed of compactly arranged parenchyma cells with meristematic peripheral layers. In the course of fruit development, the placenta also grows in size and intercellular spaces develop between the cells resulting in the formation of an aerenchymatous placenta. Roth (1977). and Dave and Kuriachen (1989) reported aerenchymatous placenta in *Asclepias* follicle.

A special separation tissue or future zone of dehiscence, is well demaracted in the young ovary wall itself along the seam of carpel margins. According to Baum (1948), if the marginal fusion occurs earlier in the ontogeny of carpel, the line of fusion becomes less discernible. However, in *C. procera* the fusion zone is well-distinguished in the mature follicle. When the fruit matures, the cells of this zone become loose and the dehiscence proceeds from interior to exterior. The loosening of cells along the dehiscence zone in flax fruit is reported by Holden (1956). The possible factors affecting the dehiscence of the follicle can be listed as (1) the occurrence of a zone with specialized cells, (2) loosening of cells, (3) the formation of aerenchyma along the zone of dehiscence above the placental attachment, (4) formation of air chambers in the pericarp, (5) branching of the vascular strands along the dises of dehiscence zone (6) thickening of the epicarpic cells, and (7) lignification of the endocarp. Thus the unequal shrinkage of the parenchymatous mesocarp and the stresses caused by the vascular strands and thick walled epicarpic cells during the swelling and shrinkage of the fibrous endocarpic cells finally cause the opening of the follicle. The swelling and shrinkage of fibrous endocarpic cells during fruit dehiscence has been reported by several authors (Fahn and Zohary 1955; Roth 1977; Wardrop 1983).

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