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Structural Design of the Developing Fruit of *Nicotiana tabacum*

By

Y. S. Dave, N. D. Patel and K. S. Rao *)

With 3 Figures (1 Plate)

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Abstract

Dave Y. S., Patel N. D. & Rao K. S. 1981. Structural design of the developing fruit of *Nicotiana tabacum*. — Phyton (Austria). 21 (1): 63—71, 3 figures (1 plate). — English with German summary.

The 8—12 layers thick mature pericarp of an indehiscent capsular fruit of tobacco is the direct result of increment in size of the cells of ovary wall layers. The epicarp is single layered and possesses an anomocytic and contiguous stomata surrounded by 5—7 epicarpic cells. The 4—7 layers thick mesocarp is resulting from 4—7 layered mesoderm of the ovary wall when its cells enlarge, become vacuolated and then separated in the mature fruit. The basic vascular ring of the ovary wall consists of 30 vascular bundles, the middle region has 20—25 and the terminal region has 16—18 vascular bundles.

The multilayered, lignified endocarp has developed from the parenchymatous inner epidermis and the two inner hypodermal layers of the ovary wall. The lignified endocarpic cells are simple, forked or branched. The placental cells show increment in their size in the mature fruit.

Zusammenfassung

Dave Y. S., Patel N. D. & Rao K. S. 1981. Strukturmuster in der sich entwickelnden Frucht von *Nicotiana tabacum*. — Phyton (Austria) 21 (1): 63—71, 3 Abbildungen (1 Tafel). — Englisch mit deutscher Zusammenfassung.

Das 8—12 Zellagen zählende Pericarp einer nichtaufgesprungenen Kapsel von *Nicotiana tabacum* entsteht unmittelbar durch Vergrößerung der Zellen der

*) Dr. Y. S. Dave, Dr. N. D. Patel, K. S. Rao, Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120, Gujarat, India.

Fruchtknotenwandschichten. Das Epicarp besitzt anomocytische, häufig paarweise liegende, von 5—7 Zellen umgebene Stomata. Das 4—7 Zellagen mächtige Mesocarp entsteht aus dem Mesoderm der Fruchtknotenwand unter Zellvergrößerung, die Zellen vakuolisieren und zerfallen bei der Reife. Der Gefäßbündelring an der Basis der Fruchtknotenwand besteht aus 30 Bündeln, ihre Zahl nimmt gegen die Spitze auf 16—18 Bündel ab. Das vielschichtige verholzte Endocarp entwickelt sich aus der parenchymatischen inneren Epidermis und den zwei inneren Hypodermschichten, die Zellen sind einfach, gegabelt oder verzweigt. Die Zellen der Plazenta vergrößern sich bei der Reife.

(Editor transl. and abbrev.)

Introduction

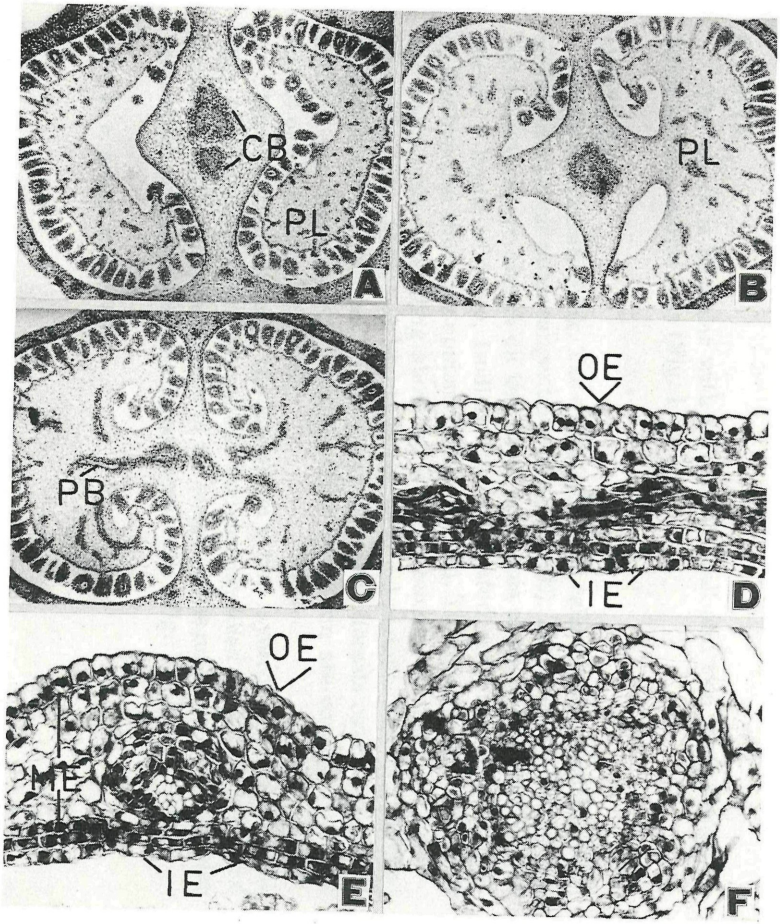
Little attention seems to have been given to the morpho-histological studies of the developing fruit walls. Along with the studies of physiological changes occurring in ripening fruit, the associated anatomical changes appear to have been somewhat neglected (CUTTER 1978). Very few morpho-histogenic studies on capsular fruit walls have been made (KARAWYA *et al.* 1973a, b; DAVE *et al.* 1974, KRISHNAKUMAR *et al.* 1975, ZALA *et al.* 1976, DAVE & RAO 1980). The general anatomical features of some capsules have been described by ESAU (1965), KAPOOR (1973), FAHN (1977) and SWAMY & KRISHNAMURTHY (1980). In this light the detailed structural study of the developing fruit of *Nicotiana tabacum* will be proved useful.

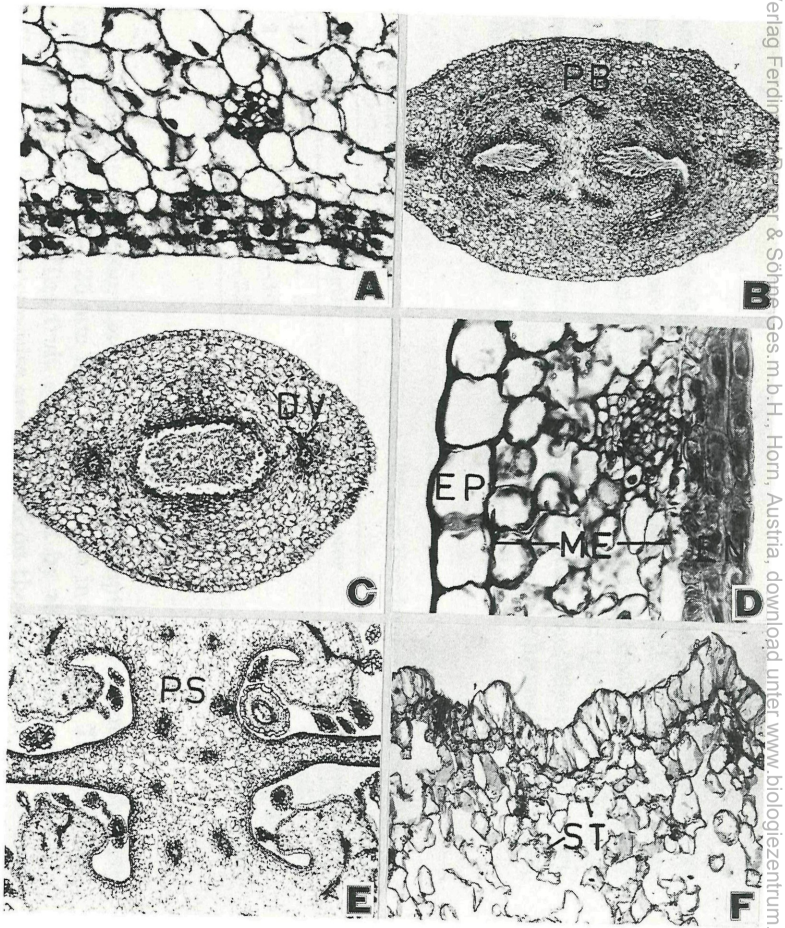
Structurally, the fruit consists of the pericarp, placental tissue and the seeds but the present work deals mainly with the developmental and anatomical studies of the pericarp and the placentae.

The term 'mesoderm' is used here to designate the ground tissue interspersed with vascular tissue between the outer epidermis and the inner epidermis of the ovary wall before ripening. In the ripening ovary or mature fruit the more or less differentiated tissue in the middle region of the pericarp is called as mesocarp.

Materials and Methods

The flowers and fruits of *Nicotiana tabacum* L. of various developmental stages as shown in the table 1 were collected from the fields of Napad (Gujarat). To facilitate the studies the fruits were cut into basal, middle and terminal parts and then fixed in FAA (BERLYN & MIKSCHE 1976). The fixed materials from different parts of fruits were cut again in small pieces and washed 2—3 times in 70% alcohol and dehydrated in TBA series and embedded in Tissueprep (BERLYN & MIKSCHE 1976). Transections at 8—12 μm thickness were cut with the help of Spencer-820 microtome. Temporary mounts of free hand sections were also employed for general anatomical studies. The sections were stained with safranin and fastgreen staining combination. For the epidermal structural studies the peels were taken from the outer and inner epidermis. The maceration of the pericarp





Explanation of Fig. 1 and Fig. 2 on p. 71

was done in JEFFREY's fluid (10% chromic acid & 10% nitric acid). The epidermal peels and macerated tissues were stained with safranin or DELAFIELD's haematoxylin and mounted in glycerine jelly. Starch was localized by I_2KI test (JOHANSEN 1940). Drawings were made at the level of the base of a ZEISS microscope with its drawing apparatus. Photomicrographs were taken on a ZEISS photomicroscope I, using planapochromatic objectives, fortpan and ORWO NP 15 films and yellow filters.

Table 1

Measurements of ovary and fruit at different developmental stages (mm)

Stage	Length	Diameter	Remarks
1	4— 5	1—2	Ovaries
2	8—10	3—5	Young fruits
3	5—25	6—8	Mature fruits

Observations

The fruit of *Nicotiana tabacum* is narrowly elliptic, ovoid and indehiscent capsule which is 15—25 mm long and 6—8 mm in diameter. The fruit tips are acute or blunt. The fruit is surrounded by the gamosepalous calyx. Usually the *Nicotiana* capsules are retained on the plant itself but in very few cases the capsules have a tendency to fall off together with pedicels when ripe (PATEL 1960).

OVARY:

The ovary is bilocular with thin ovary wall and two large placentae borne on the central axis (Fig. 1A—C).

Ovary wall:

It is 8—12 cells thick. The outer epidermal cells appear tabular, thinly cutinized on their outer tangential walls (Fig. 1E). The stomata are present but trichomes are absent on the outer epidermis of the ovary wall. There are 4—7 layers of large, isodiametric vacuolated cells with intercellular spaces beneath the outer epidermis (Fig. 1D, E). This is called mesoderm of the ovary wall embedding about 30 vascular bundles in the basal region, 20—25 in the middle and 16—18 in the terminal regions. The two ventrals disappear in the terminal region and only two dorsals extend up to the extreme tip. The vascular bundles of the mesoderm are conjoint and collateral or bicollateral (Fig. 1E).

The next zone beneath the mesoderm is two layered inner hypodermis (Fig. 1D, E). Its cells are rectangular and densely filled with cytoplasm and large nuclei. The innermost layer of the ovary wall is made up of rectangular cells which simulate the inner hypodermal cells (Fig. 1D, E).

Placentae:

There are two large placentae borne on the central axis with their pendulous lobes in the basal region, so in transection they are seen away from the central axis in the extreme basal region of the ovary (Fig. 1A). They are so large that they almost fill up the entire chambers in the upper, basal and middle regions. In transection they appear peltate with thick placental stalk and the broad, convex and marginally incurved placentae bearing ovules on their periphery (Fig. 1A—C). In the terminal region the placentae become deeply notched in the centre (Fig. 1D). The cells of the placentae and the septa are parenchymatous with large intercellular spaces. The peripheral 2—3 layers (i. e. the outer epidermis and the hypodermal layers) of the placentae are made up of densely stained meristematic tissue. They continue on the stalk of the placentae, the septa and the inner side of the ovary wall (Fig. 1B, C).

The two main centric bundles of the placental axis of the extreme basal region appear to fuse at a higher level (Fig. 1A, B). Towards the middle region they branch again and supply to the placental stalk from which ovular traces depart (Fig. 1C). In the terminal region only one central axial bundle and few placental bundles are observed, but little higher up towards the extreme tip the central axial vascular bundle is not found to be extended. The vascular bundles are hadrocentric and embedded in the loosely arranged placental tissue (Fig. 1F).

DEVELOPING PERICARP:

There is no increment in the number of layers of the pericarp towards the maturity.

Epicarp:

It is the outermost single layer of the pericarp. Its cells are largely vacuolated and cutinized on their outer and inner tangential walls (Fig. 2D). In surface view the epicarpic cells appear large and polygonal with small nuclei. The anomocytic and contiguous stomata are common in the epicarp. The anomocytic stomata are surrounded by 5 or 7 epicarpic cells (Fig. 3A—C). Sometimes a single epidermal cell is found parallel to the guard cell (Fig. 3B), or a common epidermal cell inbetween two stomata (Fig. 3D, G). The contiguous stomata are surrounded by 5—7 epicarpic cells Fig. 3F, H—J). Sometimes one of the two nearly laced stomata has single guard cell (Fig. 3E). The contiguous stomata appear parallely or obliquely placed (Fig. 3H, I). Sometimes a group of two mature stomata and a meristemoid is observed in which one of the stomata is with double the length of the other mature small stomata (Fig. 3K). Sometimes the abnormal cell wall thickening amongst the epicarpic cells is observed (Fig. 3L).

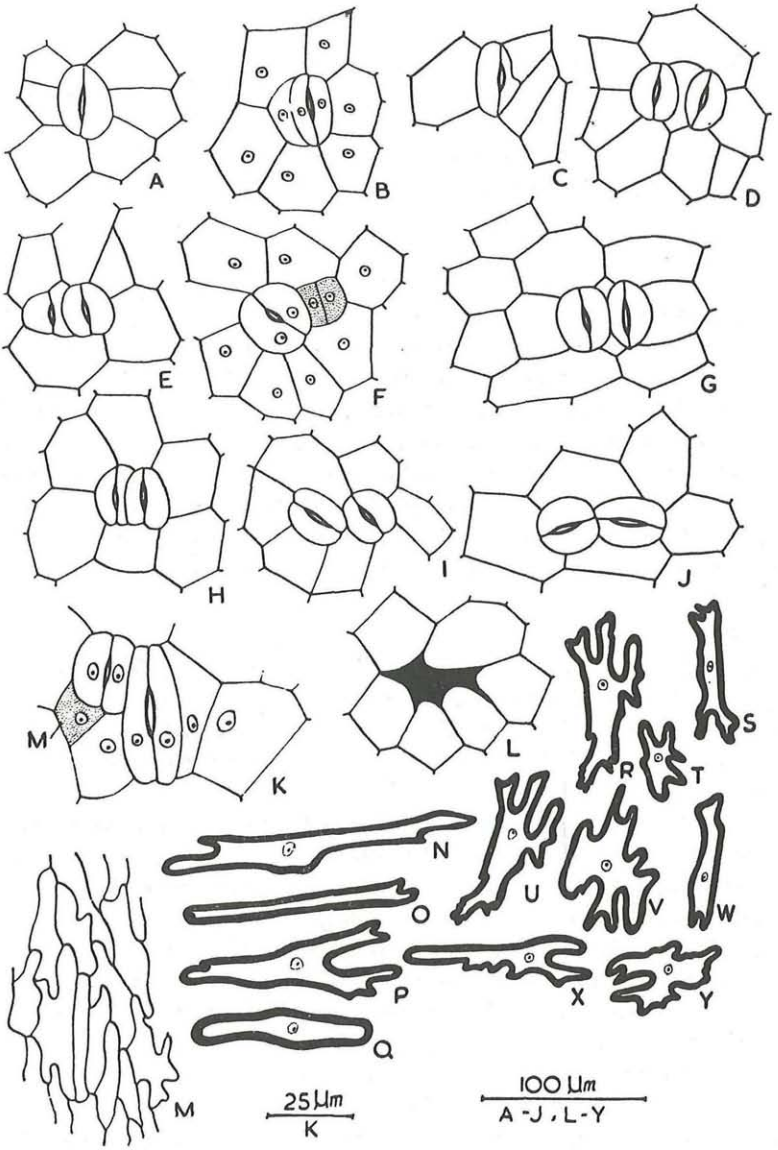


Fig. 3. A: Anomocytic stomata in the epicarp; B: Epidermal cell parallel to the stomata in the epicarp; C: Stomata with one of its degenerating guard cells; D: Common epidermal cell between the stomata; E: Complete stomata and stomata with single guard cell; F—J: Contiguous stomata; K: Group of large stomata, small stomata and a meristemoid in the epicarp; L: Wall thicking among the epicarpic cells; M: Inner epidermis in surface view from pericarp; N—Y: Different shapes of lignified endocarpic cells with cytoplasm and nucleus; (M = meristemoid)

Mesocarp:

The mesodermal cells of the ovary wall develop as mesocarpic cells in the pericarp. The mesocarpic cells of the younger fruit become enlarged, somewhat irregular in shape and largely vacuolated. They are separated or with large intercellular spaces in the mature fruit (Fig. 2D). The vascular bundles are conjoint and collateral or bicollateral (Fig. 2A, D).

Endocarp:

It is three layered thick consisting of inner epidermis and two inner hypodermal layers. In the young fruit their cells appear slightly larger and more vacuolated than those of the ovary wall (Fig. 2A). In surface view they appear sinuous and with protruding cell walls (Fig. 3M). In mature fruit they have become lignified and still contain cytoplasm and nuclei (Fig. 2D). Thus the mature endocarp is made up of compactly arranged sclerenchyma. The macerated endocarpic sclerenchyma are observed in their various shapes. They are simple (Fig. 3 Q), forked at one or two ends (Fig. 3P, R—V, X, Y). Their lobes or branches are so nicely fitted in each other that a compact endocarpic cell pattern is formed.

Placentae:

The placentae in the terminal region of the young fruit are seen as deeply lobed and without seeds. Their lobes appear to fuse with the pericarp towards the tip of the fruit (Fig. 2B) and finally the central axis also disappears and the fruit tip becomes monolocular without placentae and seeds (Fig. 2C).

The enlargement of the placentae in the fruit is more due to the enlargement of their outer epidermal and the inner or central cells than the increment in number of their cells (Fig. 2E, F). Their outer epidermal cells appear columnar or wedge shaped and largely vacuolated. The inner cells are irregular in their size and shape and are loosely arranged parenchyma (Fig. 2E, F). The middle cells of the septa and the placental stalk in the young and mature fruit also appear separated (Fig. 2E). Their outer epidermal layers have not become lignified.

The placental vascular bundles decrease in number in the terminal region of the fruit (Fig. 2B, C). They are hadrocentric bundles embedded in the loosely arranged tissue. The starch is present in the placentae and the septa of the fruit (Fig. 2F).

Discussion

The ovary wall of *N. rustica* is composed of outer and inner epidermis enclosing inbetween a four layered mesocarp (mesoderm) composed of parenchymatous tissue, which is traversed by numerous vascular strands and scattered prisms of calcium oxalate (Karawya *et al.* 1973a, 1973b).

The ovary wall of *N. tabacum* is found to be 8—12 layered thick including outer and inner epidermis. In *N. tabacum* the 4—7 layered thick mesoderm beneath the outer epidermis embeds about 30 vascular bundles in the basal region, 20—25 in the middle and 16—18 in the terminal region of the ovary.

In *N. rustica* stomata of both cruciferous and ranunculaceous types and prisms of calcium oxalate are present in both epidermes of the ovary wall and in the inner epidermis of the fruit but the epicarp shows only prisms and no stomata (KARAWYA *et al.* 1973a, 1973b). The epicarps of *Datura innoxia* and *D. metel* (PATEL & DAVE 1976) also showed no stomata but their inner epidermes revealed anomocytic stomata.

In contrast to *N. rustica* no prisms of calcium oxalate are found in the ovary wall of *N. tabacum* and the anomocytic stomata are observed in its outer epidermis of ovary wall and epicarp but in the inner epidermes of ovary wall and of pericarp they are found to be absent. PATEL & INAMDAR (1971) also observed anomocytic and contiguous stomata in the epicarp of *N. tabacum*.

The pericarp of *N. rustica* (KARAWYA 1973b) is six layered thick and *N. tabacum* pericarp also remained 8—12 layered thick at maturity without any increment in the number of its layers. ESAU (1965) mentioned that an ovary wall maturing into the pericarp of a capsule may have but little increase in the number of cells, as in tobacco. According to her the pericarps of capsules have both sclerenchymatous and parenchymatous tissues in variable distributions, for example pericarp of *Linum usitatissimum* has an exocarp of highly lignified cells and a mesocarp and an endocarp of parenchymatous cells. But the pericarp of *N. tabacum* shows, in contrast, 2—3 layered thick and lignified endocarp and parenchymatous lacunose exocarp and mesocarp. SWAMY & KRISHNAMURTHY (1980) mentioned that the pericarp in follicle, legume and capsule is largely made up of sclerenchymatous cells of deverse morphological shapes.

The lignified endocarp of *N. tabacum* consists of the inner epidermis and the two inner hypodermal layers of the pericarp. In *Capsicum* (DAVE *et al.* 1979) and *Moringa* capsule (DAVE *et al.* 1974) the endocarp is single layered and consists of parenchyma and macrosclereids. The multilayered endocarp of *Datura* capsule is found to be completely parenchymatous (DAVE *et al.* 1980). As in *N. tabacum* the multilayered endocarp is also reported to be sclerenchymatous in the capsule of *Tecoma* — 2 to 3 layered endocarp — (KRISHNAKUMAR *et al.* 1975) and in a follicle of *Catharanthus* — 3 to 4 layered endocarp — (ZALA *et al.* 1976). The siliques of *Brassica* and *Raphanus* (DAVE & RAO 1980) showed single layered parenchymatous endocarps associated with a hypodermal layer of very small sclerenchyma.

The placentae are peculiarly lobed from base to the apex. In transection the broad and convex placentae with incurved margins bearing ovules on their periphery are found borne on thick placental ridges. The placentae appear deeply notched in the terminal region. The enlargement

of the placentae in the fruit is more due to the increment in size of their outer epidermal and the inner or central cells than the increment in number of cells. Unlike many Solanaceous berries eg. *Withania*, *Lycopersicon*, *Solanum indicum*, *S. tuberosum* (PATEL 1977) and *Solanum melongena* (DAVE *et al.* 1979), but in accordance with *Datura* (DAVE *et al.* 1980) the placentae of *Nicotiana* do not show any meristematic activity and no placental outgrowths are formed.

Acknowledgements

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Fig. 1. A—C: Transections of the ovary from basal to terminal region showing the arrangement of vascular bundles (×26); D: Cellular details of ovary wall in longisection (×258); E: Cellular details of ovary wall from dorsal side (×258); F: Cellular details of placental vascular bundles (×258); (CB = central bundles, PL = placentae, IE = inner epidermis, OE = outer epidermis, PB = placental bundle)

Fig. 2. A: Cellular details of mesocarp and endocarp of young fruit (×240); B, C: Transections from terminal region of pericarp to the tip of it showing narrowing of the chambers and absence of seeds (B: ×40; C: ×64); D: Transection of pericarp from dorsal side of mature fruit (×240); E: Transection of mature fruit showing the separated cells of the placentae, placental stalk and the septa (×24); F: Part of placenta showing large peripheral cells (×96); (DV = dorsal bundle, EP = epicarp, EN = endocarp, ME = mesocarp, PB = placental bundle, PS = placental stalk, ST = starch)