# DEVELOPMENT OF OVULE AND SEED-COAT OF ERYTHROXYLUM COCA LAMK.

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SUMMARY

The inner and outer integuments of *Erythroxylum coca* are both of dermal derivation. The outer integument is initially 3 cells and later 3–5 cells thick. The inner integument is at first 3 cells thick to become multiplicative and ultimately about 20 cells thick and thus forming the greater part of the mass of the seed. The inner layer is developed as an endothelium. The nucellus is oblong by mitotic activity in the part beneath the embryo sac. The testa and tegmen are crushed with the exception of the fibrous exotegmen. The flattened cells of the inner layer contain tannin. The relationship between Erythroxylaceae and Linaceae is discussed.

## 1. INTRODUCTION

The small family of the Erythroxylaceae (about 250 sp.) is chiefly of tropical distribution and contains only woody representatives. Most species belong to the genus *Erythroxylum* P.Br., whereas the other, doubtful, genera *Aneulophus* Benth., *Nectaropetalum* Engl. and *Pinacopodium* Exell & Mendonça each have only one or a few species. The family is considered to be closely related to the Linaceae.

The first publications dealing with the embryology and seed-coat anatomy of the family are those of VAN TIEGHEM (1903) and TUNMANN & JENZER (1910). Of a more recent date are the contributions of MAURITZON (1934), NARAYANA (1960, 1964, 1970), RAO (1968) and NARAYANA & RAO (1978).

According to NETOLITZKY (1926), CORNER (1976) and DAVIS (1966), the ovules of *Erythroxylum* are anatropous, bitegmic and crassinucellate with a slender nucellus and thick inner integument. The developing seed has initially a very massive inner integument, but the seed-coat becomes much compressed in later stages with the exception of the sclerotised cells of the exotegmen. These cells form the only mechanical layer of the seed-coat and are oriented in the longitudinal direction of the seed. The fruit is a one-seeded, red drupe with a well-developed endocarp.

But little chemodiagnostic studies were made on the family (HEGNAUER 1966). *Erythroxylum coca* and *E. novogranatense* contain cocain, a powerfull anaesthetic and a dangerous, habit-forming drug.

## 2. MATERIALS AND METHODS

The material of *Erythroxylum coca* Lamk. and *E. novogranatense* (Morris)

Hieron. was collected in the Hortus Botanicus, University of Amsterdam and in the hothouses of the Department of Plant Taxonomy and Plant Geography, Agricultural University, Wageningen.

Craf or Allen-Bouin mixtures were used as fixatives. Sections were cut by means of standard microtome techniques or by hand. The following stains were used for specific colour tests: Phloroglucinol-HCl, Sudan IV, ruthenium-red and IKI.

The SEM observations were made with a Cambridge Stereoscan mark 2a. The objects were stuck on Scotch thin copper-foil with conducting adhesive and mounted on the specimen stub with Demetron silver conducting paint. The fixed material was scanned after 3 minutes of sputter-coating with gold-palladium. A high voltage of 10 K.V. was consistently used.

## 3. RESULTS

*Erythroxylum coca* and *E. novogranatense* have a 3-loculed syncarpous ovary with only in one locule a fully developed ovule. The other locules often contain rudimentary ovules. Sometimes a rudimentary second ovule is present in one of the locules.

### 3.1. Ovule ontogenesis

The ovule primordium first bends down, but when the integuments are initiated it begins to curve upwards (*fig. 1A*). The archespore is hypodermal and one-celled, and it divides in a parietal cell and a megaspore mother cell. The megaspore tetrad is linear and the chalazal one becomes functional. In one case a 2-celled archesporium was observed. At first the nucellus is rather short but it soon becomes oblong as a result of mitotic activity in the part below the megaspore mother cell, producing parallel rows of cells (*fig. 1C-E, 3*). A nucellar cap is not formed. The parietal tissue becomes about 2–3 cells thick.

The inner integument (i.i.) develops as a complete ring wall resulting from periclinal and oblique divisions in a band of about three adjacent dermal cell layers (*fig. 1A*). At an early developmental stage the i.i. is about 3 cells thick, but soon it increases in width mainly by periclinal divisions of the original middle layer, but also by some cell divisions in the inner layer (*fig. 1C, D*).

The outer integument (o.i.) also develops by periclinal and oblique cell divisions in a zone of about three dermal cells high, but the ring wall is incomplete and does never develop distinctly at the raphal side. From the very beginning the o.i. is three cell layers thick. It remains much shorter than the i.i. and its development seems delayed in early stages (*figs. 1B, E*). The ovule is initially rather compact, but later an appreciable elongation in the longitudinal direction takes place (*figs. 1A-E, 3*).

### 3.2. The mature ovule

The full-grown ovule is anatropous, bitegmic and crassinucellate with an elongated nucellus (*fig. 3*). The upper portion of the nucellus is resorbed by the
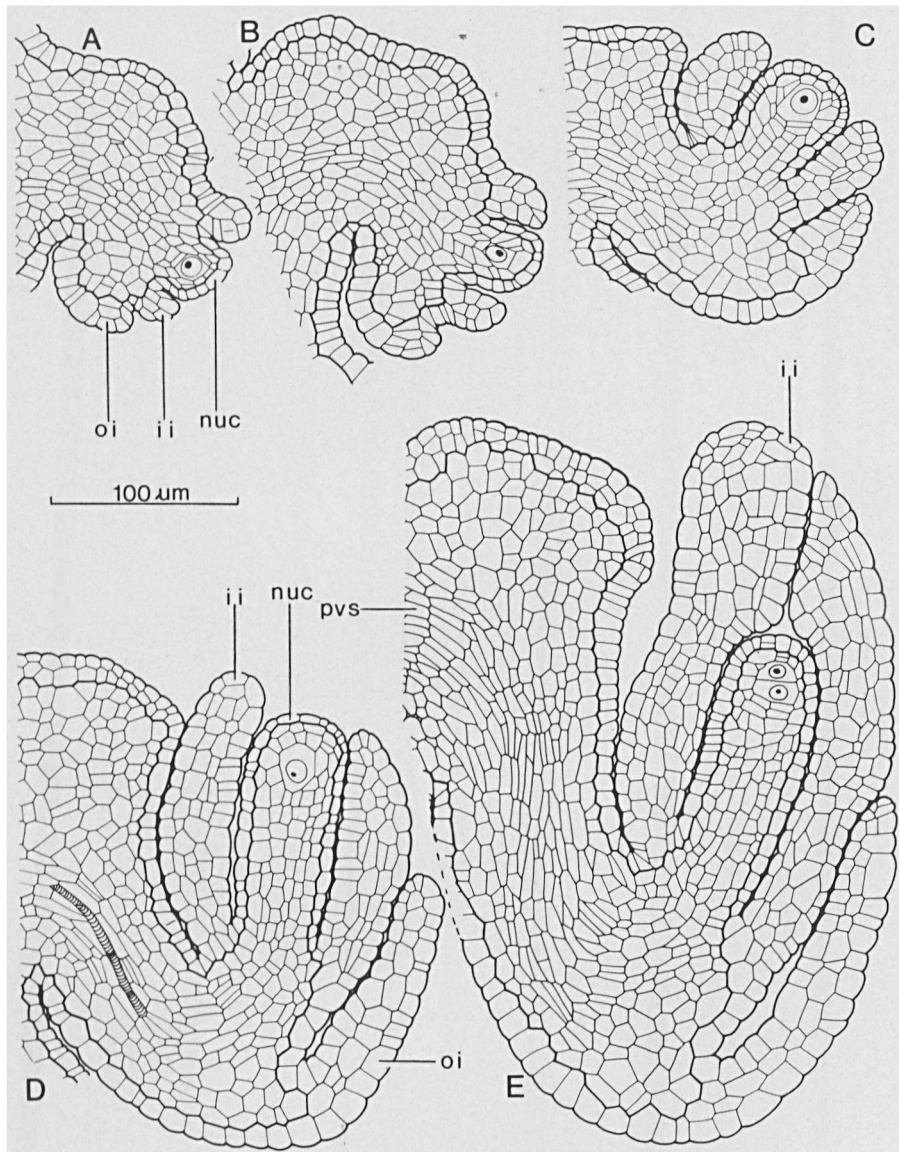
Fig. 1. *Erythroxylum coca*, l.s. of developing ovules. In all figures:
es = embryo sac, end = endosperm, ent = endothelium, nuc = nucellus, ii = inner integument, oi
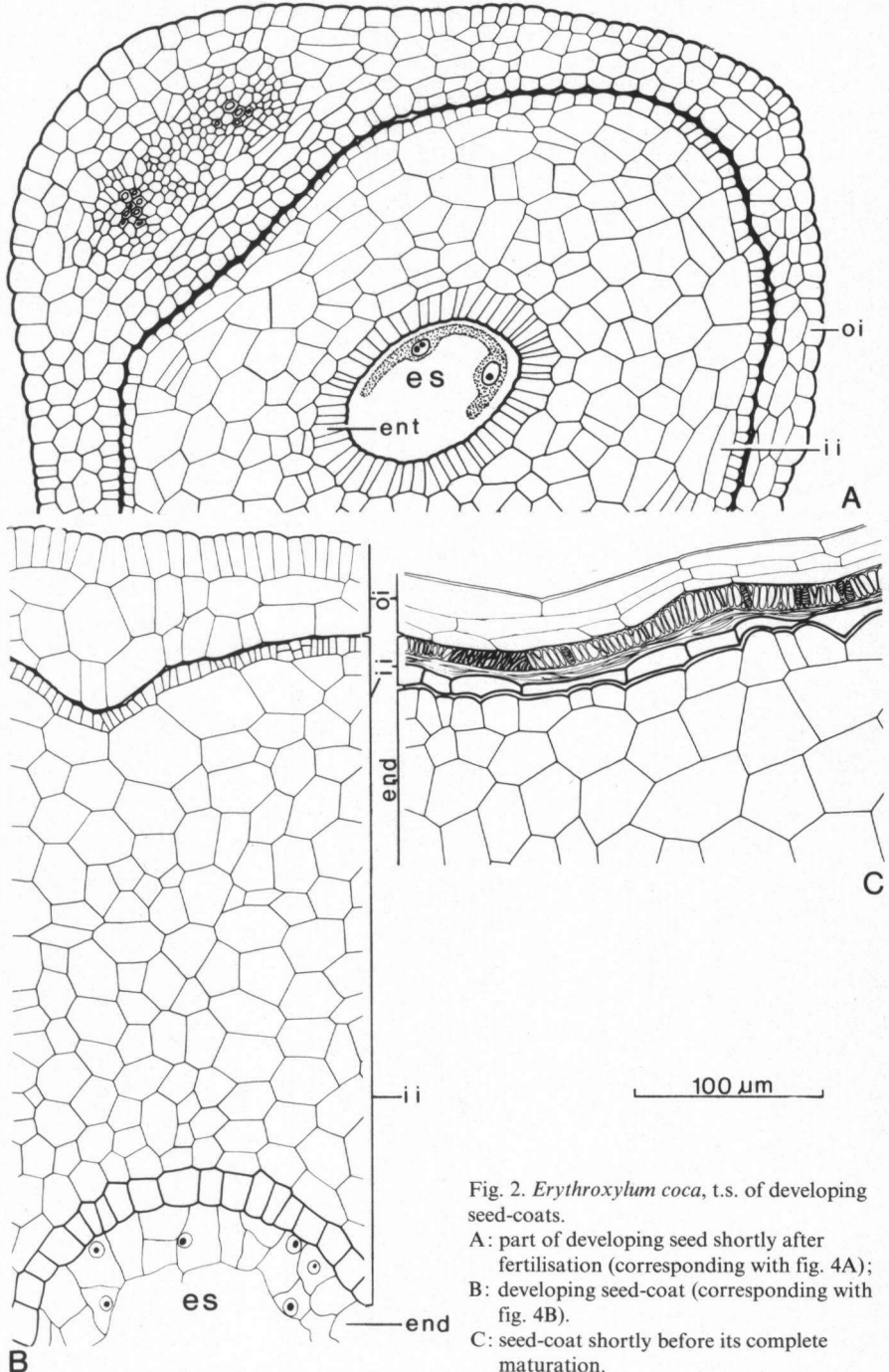= outer integument, pvs = provascular strand.

Fig. 2. *Erythroxylum coca*, t.s. of developing seed-coats.

A: part of developing seed shortly after fertilisation (corresponding with fig. 4A);

B: developing seed-coat (corresponding with fig. 4B).

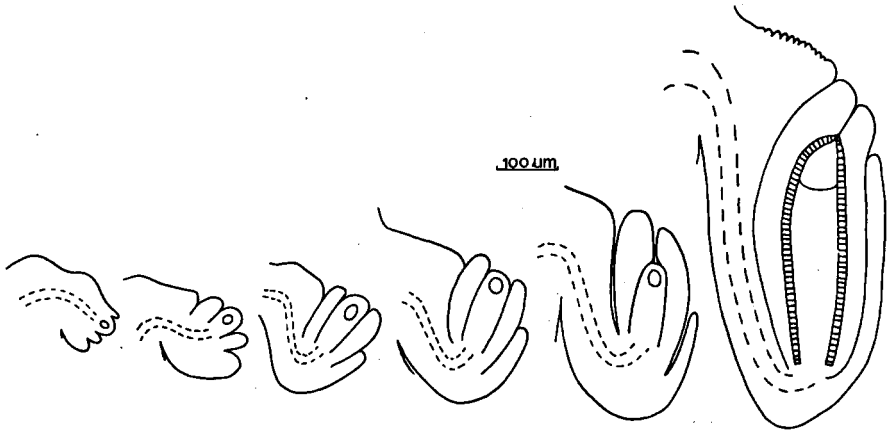C: seed-coat shortly before its complete maturation.

100 μm

Fig. 3. Development of anatropy in the ovule of *Erythroxylum coca*.

embryo sac. The i.i. is very massive and has become 5–6 cell layers thick. The inner layer is differentiated into an endothelium and consists of radially oriented cytoplasm- and tannin-rich cells. The cells of the middle layer are isodiametric and strongly vacuolated. The outer layer is rich in cytoplasm. The o.i. is not distinctly developed at the raphal side and 3–4 cell layers thick. The outer layer contains tannin. The micropyle is formed only by the i.i. and in cross section has a circular or triangular form. An obturator is developed both by dermal and subdermal divisions. The cells become only slightly papillose. Funicle and raphe contain two separate vascular bundles each surrounded by tannin deposits.

### 3.3. Seed and seed-coat development
### 3.3.1. Shape of the developing seed
Although only one ovule develops into a seed, the other two locules often each
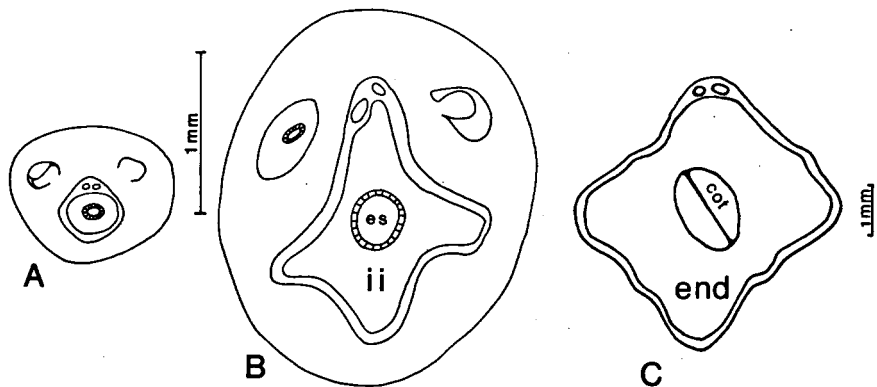


Fig. 4. *Erythroxylum coca*, seed development.
A, B: t.s. of developing fruits (with seeds);
C: t.s. of ripe seed.

contain a retarded ovule without embryo. After fertilisation these ovules may develop further for some time and even start to form a seed-coat. But these seed-coats never become fully differentiated and are crushed in later stages together with the ovules. The locules of the unfertilised seeds remain small, but around them a layer of endocarp fully develops. These two locules offer some resistance to the seed in later stages of maturation and this results in the formation of two longitudinally oriented grooves (*figs. 4B, 5A*). Also at the other side of the seed two longitudinal grooves develop but in this case as the result of the presence in the fruit wall of two lengthwise running zones in which many periclinal divisions take place. It is probable that the four grooves and ridges are partly attributable to differences in the amount of periclinal divisions in the middle layer of the i.i. The ridge through which the raphe bundles run is most pronounced (*fig. 4B*). As a result of the numerous divisions the i.i. is very thick and in early stages forms most of the bulk of the seed (*fig. 2A, 4A, B*).

### 3.3.2. Development of the seed-coat

The endosperm is initially nuclear, but later wall formation takes place centripetally (*fig. 2B*). According to RAO (1968) wall formation takes also place from the micropyle towards the chalaza. The endosperm develops vigourously and progressively replaces the i.i.

The seed-coat is gradually crushed. The inner layer of the i.i. (endothelium) at first consists of radially elongated cells (*fig. 2A*) which later become tangentially flattened. The middle layer is poor in starch and consists of parenchymatic cells. At the ridges this layer becomes up to about 20 cells thick (*figs. 2A, B, 4B*). The disintegration of the middle layer starts in the cells adjacent to the endothelium. The cell walls first become slimy before becoming compressed and resorbed. The cells of the outer layer of the i.i. are cytoplasm-rich and stretch in the longitudinal direction of the seed (*figs. 2A, B*). This layer locally becomes pluri-layered by periclinal divisions (*fig. 2B*). The cells of the o.i. are poor in starch and their walls contain large pit fields. In the zone adjacent to the raphe the o.i. is about 4–5 cells, but elsewhere mostly only 3 cells thick. The cells of the 2 or 3 innermost layers are isodiametric. The cells of the outer layer become temporarily stretched in the radial direction and more or less oriented in the longitudinal direction of the seed (*fig. 2B*). The two vascular strands in the raphe are amphicribral (*fig. 2A*).

### 3.4. The mature seed

Around the seed a well-developed endocarp is present. At the inside of the

Fig. 5. SEM photomicrographs and (A) standard photograph.
A: *Erythroxylum coca*, seed and cross sections of seed and fruit (about × 4);
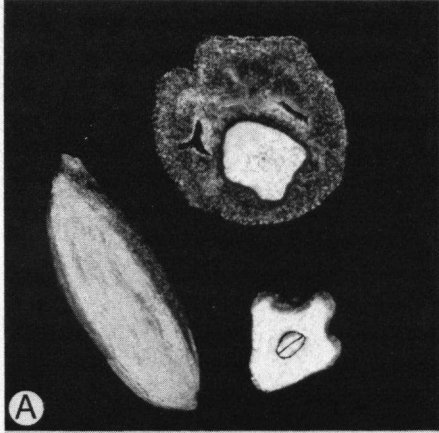B: *Erythroxylum coca*, seed-coat (about × 100);
C: *E. novogranatense*, intact seed-coat not in close contact with the endocarp (about × 250);
D: *E. novogranatense*, seed-coat slightly damaged, with impressions of endocarp layer (about × 1000);
E: *E. coca*, t.s. of seed-coat (about × 500);
F: *E. novogranatense*, exotegmen cells seen from above (about × 1000).
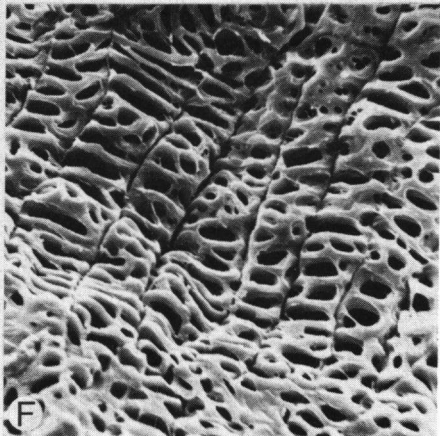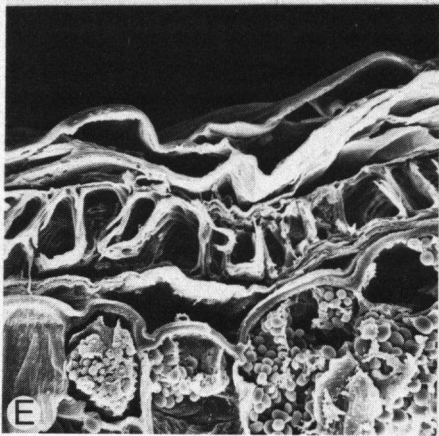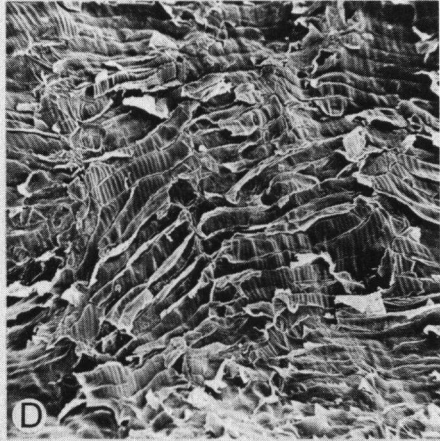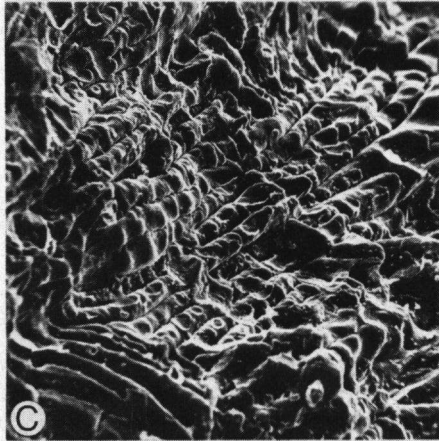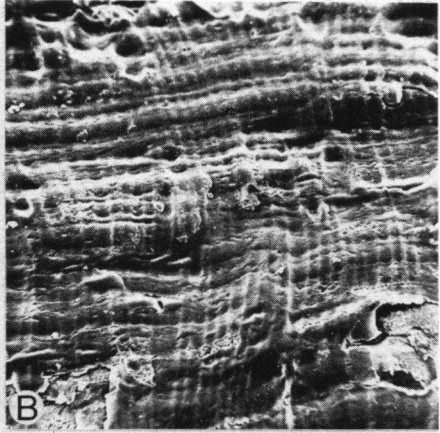
endocarp a cuticle reacting with Sudan IV is present which is tightly pressed against the seed-coat. Because the endocarp strongly adheres to the seed-coat the latter can easily become damaged by the peeling of the endocarp. Varying bits of the seed-coat, and locally even the whole o.i., can be torn off, so that in such places the exotegmen becomes visible (*fig. 5F*). With the exception of the exotegmen all layers of the seed-coat are ultimately crushed or resorbed.

From the periphery to the inside the following layers can be distinguished in cross section of the seed (*fig. 2C*):

1. The layer derived from the outer layer of the o.i., covered with an inconspicuous cuticle. The crushed cells of this layer contain tannin and are in general oriented lengthwise in the seed. When undamaged this layer is rather smooth and



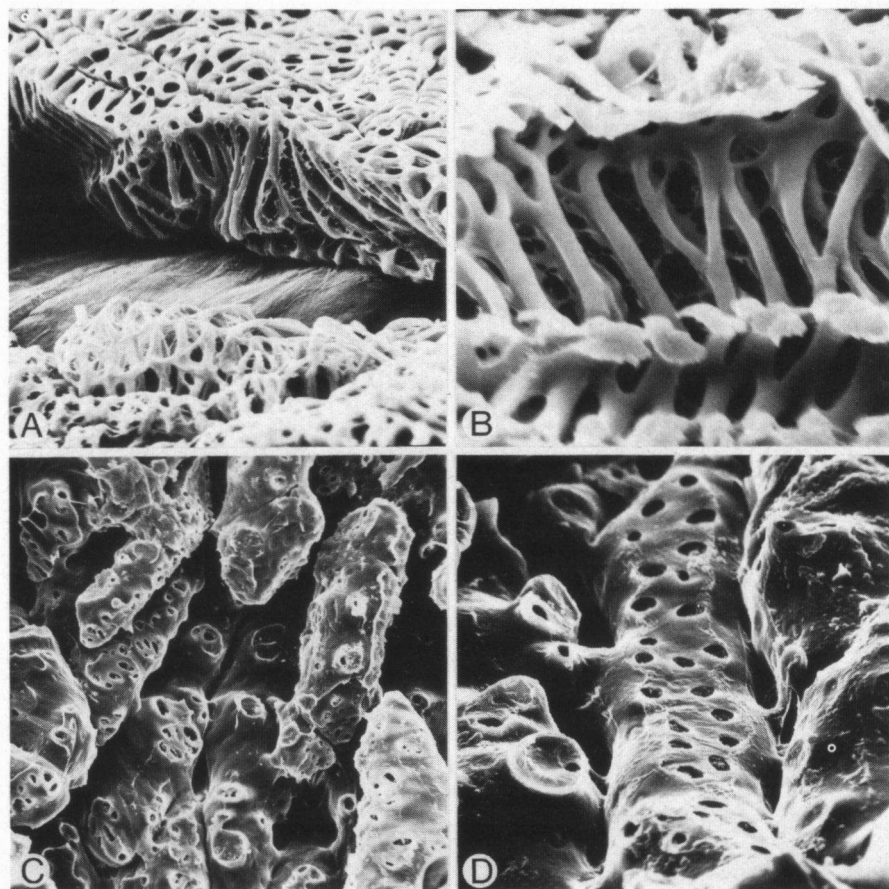Fig. 6. *Erythroxylum novogranatense*, SEM photomicrographs.
A: Rents in cell layer of exotegmen (about × 1000);
B: Side wall of exotegmen cell seen from the inside (about × 2500);
C, D: Thick-walled raphe cells (about × 500 and × 1000, respectively).

locally shows the imprints of the endocarp cells running perpendicular to the longitudinal axis of the seed (*figs. 5B, D*).

2. The two or three crushed inner layers of the o.i., which sometimes protrude into the exotegmen.

3. The exotegmen, forming the only mechanical layer of the seed-coat. The sclerotised and strongly lignified elements are oriented lengthwise in the seed (*figs. 5F, 6A*). The wall thickenings of these cells are reticulate. Especially in longitudinal sections the cell wall thickenings show ramifications (*fig. 6B*).

4. The layer derived from the middle layer of the i.i. of which the cells are nearly completely crushed and resorbed (*fig. 5E*). This original bulky layer is replaced by the developing endosperm and the embryo (*figs. 4B, C*).

5. The inner layer of the i.i. derived from the endothelium, appearing as strongly tangentially flattened and tannin-containing elements.

6. The endosperm layer, which is about 20 cell layers thick and consists of cells rich in starch and poor in lipids. The endosperm is separated from the seed-coat by a thick cuticle which strongly reacts with Sudan IV.

The raphe consists of thick-walled, pitted and non-lignified pectinous cells (*figs. 6C, D*). This type of cell may also be found in the transitional zone between the sclerotised lignified endocarp and the parenchymatous mesocarp. There is no hypostase nor is there a corky plug at the chalaza.

The light brown, grooved seed measures about 10 mm × 5 mm (*fig. 5A*) and is smooth when undamaged but mostly rough by damage to its outer layer (see above). The straight embryo has planoconvex cotyledons and a short radicle. The plane of orientation of the cotyledons varies.

*Erythroxylum coca* and *E. novogranatense* resemble each other very closely in ovule and seed structure.


## 4. DISCUSSION

The Erythroxylaceae are by almost general consensus of opinion considered to be closely related to the Linaceae but because of differences between their anatomical features (HEIMSCH 1942 and METCALFE & CHALK 1950) and embryological characteristics (NARAYANA & RAO 1978) these taxa are usually treated as separate families. Only a few authors classify the Erythroxylaceae as a tribe of the family Linaceae, for example BENTHAM & HOOKER (1862) and HALLIER (1923). According to Hallier, there are no characters to distinguish the two families unambiguously from one another. A comparison between the ovule and seed development of *Erythroxylum* with that of the Linaceae is difficult because the relevant information concerning these characteristics in representatives of the Linaceae is very scanty. One of the few more intensively investigated taxa is *Linum usitatissimum* (BOESEWINKEL 1980). However this herbaceous species is derived within the Linaceae, and the ovule morphology is probably not representative of its family. Nevertheless the ovule and the seed of *Erythroxylum* and those of *Linum* show the following striking similarities:

1. A principally bitegmic and crassinucellate ovule (only some species of the

Linaceae show a tendency towards tenuinucelly of the ovules).

2. The slender nucellus which becomes resorbed at an early stage.

3. Exclusively dermal integuments.

4. The clearly differentiated raphe bundle.

5. The multiplicative i.i.

6. The differentiation of the inner layer of the i.i. into an endothelium.

7. The fibrous exotegmen in the seed-coat.

8. The inner layer of the i.i. which develops into a pigment-containing layer.

9. The initially nuclear endosperm.

10. The presence of a conspicuous cuticle between the seed-coat and endosperm.

In the following minor characters the ovule and seed of *Erythroxylum* differ from those of *Linum:*

1. In *Erythroxylum* the i.i. is three-layered from its initiation onward, whereas in *Linum* it is two-layered.

2. In *Erythroxylum* the exotegmen has reticulate wall thickenings. This reticulation is possibly a reduction associated with the shifting of the originally protective function of the seed-coat to the endocarp. In *Linum* the wall thickenings of the exotegmen cells are homogeneous.

3. In *Erythroxylum* two vascular bundles run through the raphe. According to RAO (1965) this has something to do with the loss of function of two of the locules and with the reduction in the number of ovules.

4. In *Erythroxylum* only one seed develops per fruit, and an appreciable number in *Linum*.

The originally three layered i.i., bulky nucellus and always crassinucellate ovule of Erythroxylum are indicative of a more primitive condition as compared to that in *Linum* (not necessarily in all Linaceae). On the other hand, however, the development of only one viable seed per fruit and the much compressed seed-coat in *Erythroxylum* point to a more derived condition. From the viewpoint of embryology and seed-coat anatomy one could query the segregation of the two taxa as members of different families. Most authors place the Erythroxylaceae together with the Linaceae, in the Geraniales (SCHULZ 1931; LAWRENCE 1951). However, HUTCHINSON (1973) places them in Malpighiales and CRONQUIST (1968) in his order Linales. There is thus much disagreement as regards the taxonomic position of the Linaceae and Erythroxylaceae. Since our knowledge of ovules and seed-coat development and structure in Geraniales, Linales and Malpighiales is still rather limited, more information is needed before more definite conclusions as to the taxonomic position of the Linaceae and Erythroxylaceae can be drawn.

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