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THE OVULE AND SEED OF HUMIRIA BALSAMIFERA (AUBL.) ST. HIL.

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SUMMARY

The ovule primordium of *Humiria balsamifera* is trizonate. The ovule is anatropous, bitegmic and crassinucellate. Both integuments are of dermal derivation and initially mainly two- to three-layered. The inner integument soon becomes three-layered by periclinal divisions of its inner layer. The seed coat structure is described for the first time. Although the endocarp has taken over the function of seed protection, the seed coat shows a clear differentiation. The lignified fibrous exotegmen is the most characteristic layer. Also the exotesta has thickened, lignified cell walls. The other layers of the seed coat remain thin-walled.

A distinct nucellar cuticle and a thin layer of adjacent nucellar cells lie inside the seed coat. The nuclear endosperm later becomes cellular. The ovule and seed characters of *Humiria* closely resemble those of the other families in the Malpighiales/Linales complex studied.

1. INTRODUCTION

The family Humiriaceae is a small very natural and homogeneous taxon comprising evergreen and woody shrubs or trees. It is a typically tropical family with 8 genera and 49 species, which are important constituents of the tropical rain forests and subhygrophytic vegetation types extending from Costa Rica to Southern Brasil (CUATRECASAS 1961).

Only one or two species of *Sacoglottis* occur outside America (*viz.* in the West-African coastal area: CHANT 1978). There are only 4 recognised species of *Humiria; Humiria balsamifera* has numerous varieties and forms and is wide-spread throughout the rain forests and savanna thickets of tropical South America.

In certain older systems the Humiriaceae are placed in the Linaceae, but in recent classifications they are treated as a separate family but always in the same order as the Linaceae (CRONQUIST 1981 and HUTCHINSON 1973).

The phylogenetic relationships with the Linaceae and Erythroxylaceae are supported by anatomical (HEIMSCH 1942 and VAN WELZEN & BAAS 1984) and palynological (OLTMANN 1971) data.

The Humiriaceae are poorly known as regards their embryological and anatomical seed characters. The ovules are anatropous, bitegmic and crassinucellate (MAURITZON 1934 and NETOLITZKY 1926). According to DAVIS (1966) the embryo sac is 8-nucleate but its development has not been recorded. The endosperm in *Vantanea* is probably nuclear (MAURITZON 1934); its embryology is unknown. The seed of Humiriaceae is oblong.

The drupaceous fruit is mostly 5-loculed with 1 or 2 seeds in each locule (NARAYANA & RAO 1973) and varies from the size of a pea to that of a mango. The endocarp is woody, usually very hard and massive, or with many resin-filled, subglobose cavities. The seeds remain enclosed by the endocarp which may have special devices to facilitate seedling liberation, such as valves or opercula which are apparently pushed out of the way by the germinating seedling (germinal dehiscence), but relevant experiments have so far not been carried out. In some genera small germination holes (foramina) are present at the apex of the endocarp.

Fruit dispersal may take place by water (buoyant endocarps with a spongy appearance caused by secretary sacs which are empty or filled with some resinous powder) or, as in some montane species of *Humiria*, the small fleshy fruit may be better suited for bird dispersal.

2. MATERIAL AND METHODS

The material of *Humiria balsamifera* (Aubl.) St. Hil. was collected in Guyana by Dr. P. J. M. Maas and Dr. L. Y. Westra (Utrecht) under numbers 5649 and 3564.

Sections were made by standard microtome technique using glycolmetacrylate as the embedding medium. The following specific staining tests were applied: Phloroglucinol-HCl, Sudan IV, Ruthenium Red and JKJ. SEM observations were made on gold-palladium sputter-coated material. The ovules were critical-point dried. Because the seed coat adheres strongly to the endocarp, the seed could hardly be taken out without suffering damage. The different seed coat layers could be separately observed after carefully peeling off the outer, compressed layers one by one.

3. RESULTS

3.1. Ovary

Humiria balsamifera has a 5-loculed, syncarpous ovary (*fig. 1G*) with two anatropous and epitropous, superposed ovules in each locule. The lignified endocarp lacks resinous cysts and is strongly tanniferous. The original locule becomes secondarily divided into two superposed locules each containing a seed, or only a single, one-seeded secondary locule remains.

3.2. Ovule ontogenesis

The ovule primordia exhibit a trizonate structure, in which a dermatogen (l_1) and subdermatogen (l_2) surround a corpus (l_3) (*fig. 1A*). The subdermal archesporium is one-celled and divides into a parietal cell and a megaspore mother cell. The megaspore tetrad is T-shaped and it is apparently the somewhat larger chalazal megaspore that develops into the embryo sac (*fig. 1B-F*).

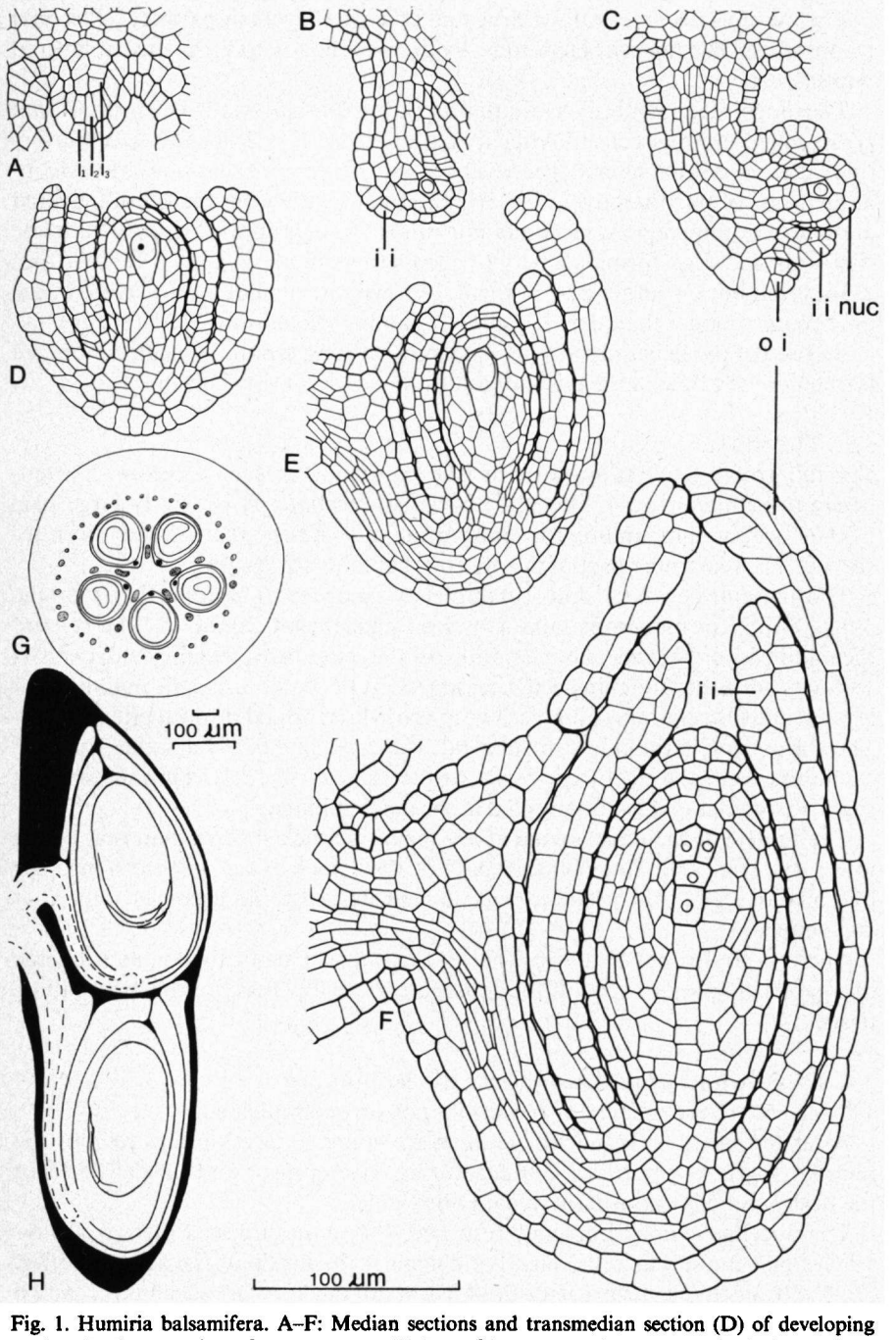

Fig. 1. Humiria balsamifera. A–F: Median sections and transmedian section (D) of developing ovules; G: Cross section of mature ovary; H: L.s. of 2 superposed mature ovules in locule. l_1, l_2 and l_3: dermal layer, subdermal layer and corpus, respectively.
o i = outer integument; i i = inner integument; nuc = nucellus.

The nucellus becomes rather large and at least 3 layers of parietal cells could be observed. The nucellar epidermis divides periclinally to form a three-layered nucellar cap.

The inner integument (ii) is initiated as a complete ring-wall. The primordium is 2-3-celled in longisection. After its initiation the ii is 2, locally 3, cell layers thick. In a somewhat later stage the ii becomes 3-layered throughout by periclinal divisions of its inner layer (*fig. 1D, E*). Finally, the ii becomes multilayered at the endostomal region. The outer integument (o.i.) is initated as an incomplete ring-wall and also forms a 2- to 3-celled primordium (*fig. 1C*). It is at first 2-3-layered but the larger part of the full-grown integument is two-layered. The o.i. becomes longer than the i.i. (*fig. 1E, F*) and is also multilayered at its tip.

Before the ovule is fully mature, strongly staining tannin-like substances are formed in the whole of the o.i. and in the outer layer of the chalaza.

3.3. The mature ovule

The full-grown ovule is anatropous (the uppermost in each locule with a tendency to hemianatropy), bitegmic and crassinucellate. A relatively large part of the nucellus surrounding the embryo sac (e.s.) has by then already been resorbed. The remaining part of the nucellus contains starch grains.

The parietal cells are almost completely resorbed. The inner layer of the 3-layered i.i. forms tannins and shows no endothelial character. The cells of the middle layer become enlarged, those of the outer layer remain small in cross section and start stretching lengthwise (*fig. 2A*). The o.i. is still mainly two-layered and also contains tannins. The micropyle is formed by both integuments. The endostome is roughly orbicular but the exostome forms a rather long slit extending from the tip of the ovule to the funicle (*fig. 3A*). This slit is often slightly irregular and is not oriented in the median plane.

The funicle of the upper ovule of the locule is much shorter than that of the lower one (*fig. 1H*). The chalazal part of the upper ovule protrudes into the micropylar region of the lower one, so that its exostome becomes somewhat cup-shaped.

There is no distinct obturator, but in the upper part of the locule trichome like papils are present around the exostome, probably functioning as a guiding tissue.

3.4. Development and structure of the mature seed

The seed coat development of *Humiria* is not very complicated.

After fertilisation the nuclear endosperm starts to develop and resorbs the nucellus further. It becomes cellular during development and the cell walls of the fleshy and oily endosperm remain thin-walled.

The nucellus is not completely resorbed. In the mature seed there is always a layer of 1-3 nucellar cells thick lying against the inside of the seed coat (*fig. 2B, C, D*) and at the chalazal side this layer is still thicker. The nucellus is covered by a thick cuticle which strongly reacts with Sudan IV.

The cells of the tannin-containing inner layer of the originally 3-layered i.i.

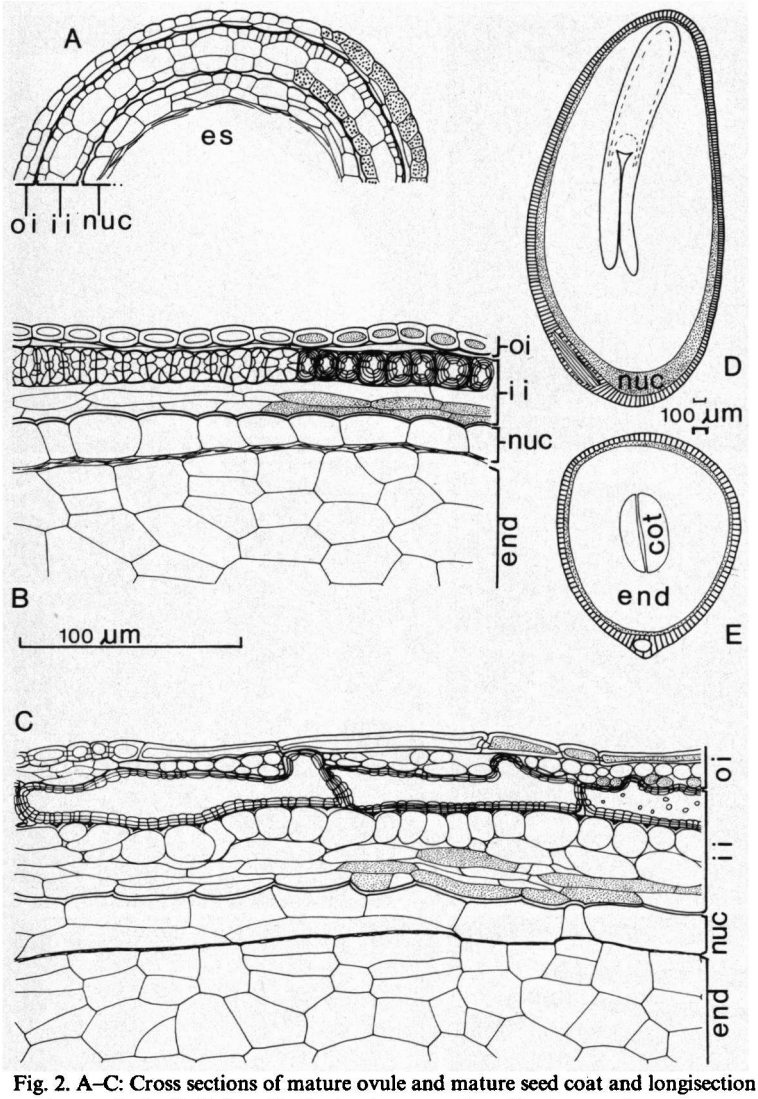

Fig. 2. A–C: Cross sections of mature ovule and mature seed coat and longisection of mature seed coat respectively; D, E: Longitudinal and cross section of mature seed.
e s = embryo sac; end = endosperm;
cot = cotyledon.

divide once or twice, which results in a 4-6-layered i.i. (*fig. 2A, B, C*). These derivatives contain mostly tannins, are isodiametric in surface view and become somewhat tangentially flattened (*fig. 4B*); they are reminiscent of the pigment layer in Linaceae. The cells of the original middle layer of the i.i. do not divide further, remain thin-walled and gradually become stretched perpendicularly to the longitudinal axis of the seed (*figs. 2A, B* and *4A*). They closely resemble

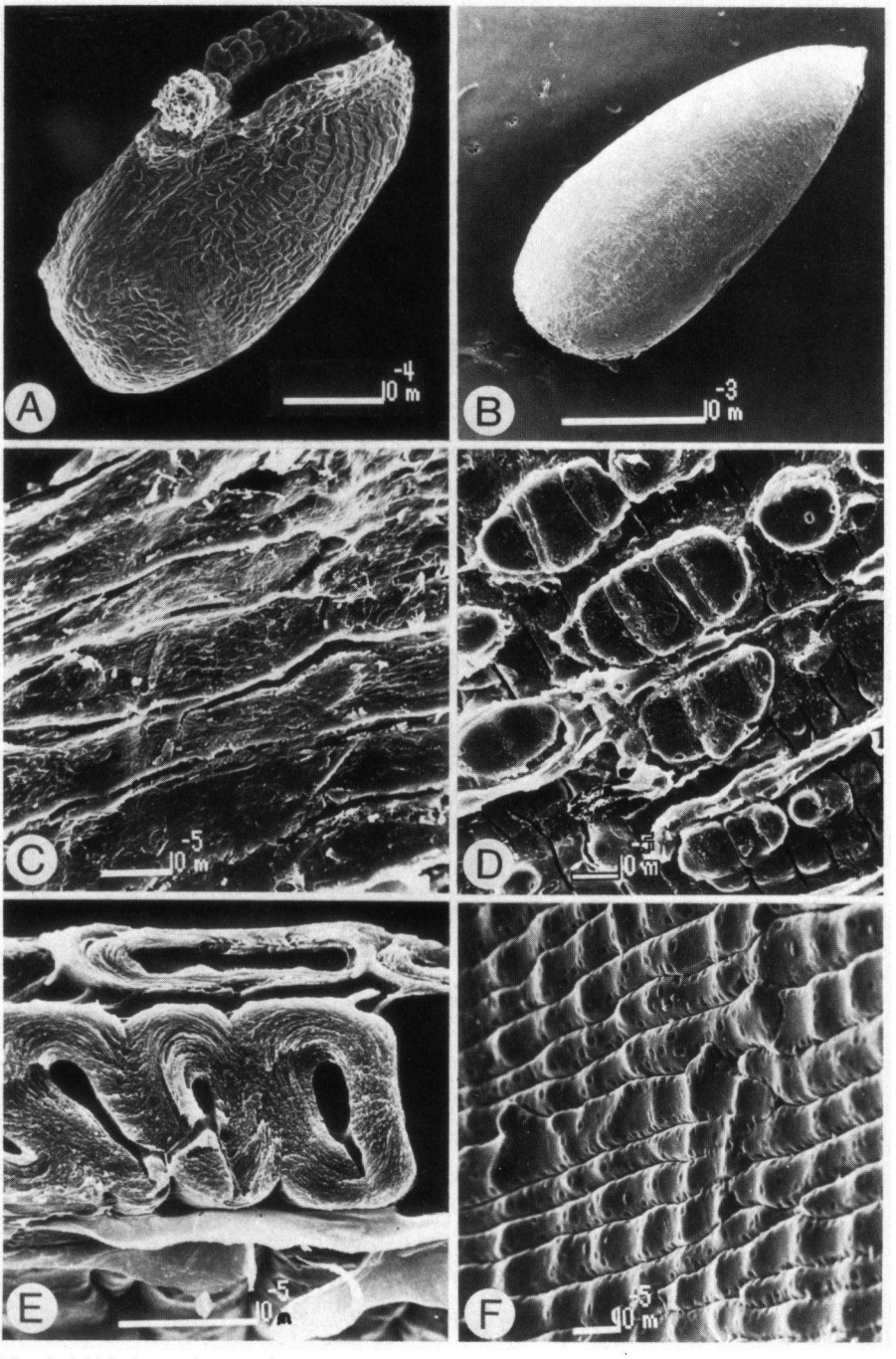

Fig. 3. SEM photomicrographs. A: Mature ovule; B: Mature seed without exotegmen and testa; C: Stretched exotestal cells; D: Outward protrusions of exotegmic cells; E: Cross section of testa and exotegmen; F: Inner side of exotegmen with wall pattern of cross cells imprinted.

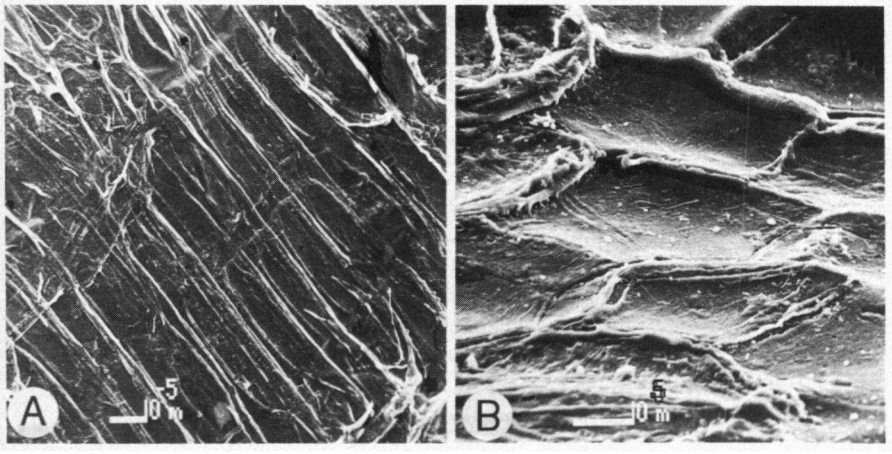

Fig. 4. A: Layer of "cross-cells" after splitting off of exotegmen and testa; B: Tanniferous cells at inside of tegmen.

the so-called "cross-cells" in *Linum*.

When the seed is taken out of the locule the seed coat often splits, because it strongly adheres to the endocarp. A part of the seed coat remains attached to the endocarp. The splitting of the seed coat may take place either between the exotegmen and the underlying layer of cross-cells (*figs. 3F, 4A*), or between this latter layer and the tannin-containing inner layer (*fig. 4B*), or between the two integuments (*fig. 3D*). The cells of the outer layer of the i.i. develop into a fibrous exotegmen consisting of thick-walled, lignified, pitted cells stretched in the longitudinal direction of the seed (*figs. 2A–C, 3E, F*). Near the chalaza the exotegmen becomes multilayered and the cells are more irregularly shaped. At the inside the extotegmen shows a rather regular structure reflecting the outer wall pattern of the layer of cross cells. At the outside the exotegmic cells locally protrude into the o.i., where they even may extend to the stretched exotestal cells (*fig. 2C*). This secondary contact leads to a striation on the exotegmic protrusions which link up with those of neighbouring cells to form small ridges (*fig. 3D*).

The cells of the original inner layer of the two layered o.i. divide once locally and the resulting thin-walled cells are stretched perpendicularly to the longitudinal axis of the seed. As a result the o.i. becomes two- to three-layered (*fig. 2A–C*). The outer layer of the o.i. (exotesta) consists of thick-walled cells with lamellate and lignified, pitted cell walls.

Locally, for instance in the raphal and chalazal region, the exotesta is two-layered. The exotestal cells contain tannins and are mostly stretched in the longitudinal direction of the seed (*figs. 2B, C, 3C*). The complicated vascular bundle is mainly amphicribral and has spiral or annular xylem elements. The raphal bundle ends in the chalaza below a thick layer of tannin cells which is continuous with the pigment layer of the tegmen.

The ellipsoid-oblong seeds measure about 3 × 1,5 mm (*figs. 2D, E, 3B*) and have a somewhat acute apex. The embryo is straight with rather short, plano-convex cotyledons. Endosperm and embryo contain lipids.

3.5. Additional embryological data

The anther is bisporangiate and its wall development conforms to the basic type. The endothecium develops fibrous thickenings, the two middle layers become crushed. The cells of the glandular tapetum are 2-nucleate. Simultaneous cytokinesis follows meiosis and the microspore tetrads are tetrahedral. The epidermis of the anther shows a very thick cuticle with pronounced cuticular folding.

4. DISCUSSION

The Humiriaceae are considered to be related with the Linaceae and Erythroxylaceae.

The embryological and structural seed characters of the Humiriaceae show many resemblances with those of Linaceae and Erythroxylaceae and support the presumed affinities of these families. The ovules are bitegmic, anatropous and crassinucellate (in *Linum* partly tenuinucellate) (BOESEWINKEL 1980a, 1980b). The i.i. becomes 3-layered, later multiplicative (mostly by repeated divisions of its middle layer). The tegmen contains the most characteristic layers of the seed coat. The outer layer develops into a fibrous exotegmen. The middle layer(s) or the outermost middle layer develop(s) into a layer of "cross-cells" and the inner one(s) into a pigment layer. The o.i. remains rather thin, as a rule is not more than 3-layered (see also BOESEWINKEL & BOUMAN 1984).

Humiria differs from *Linum* and *Erythroxylum* in having nucellar remnants in the mature seed, in lacking an endothelium and in the occurrence of periclinal divisions in the inner layer of the i.i. during seed development.

The cells of the original middle layer of the i.i. do not divide but differentiate directly into the so-called cross cells, also known from *Linum*. These cross cells are stretched perpendicularly to the longitudinal axis of the seed.

Humiriaceae seem to be more primitive than Erythroxylaceae and Linaceae, because of the large, persistent nucellus, the lack of an endothelium and the absence of repeated divisions in the original middle layer of the i.i., and perhaps also on account of the strongly lignified exotesta.

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