Structure of Seed Coat and Elaiosome in *Hybanthus nanus* (Violaceae) of Argentina

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ABSTRACT

The elaiosome is an exostomal aril or chalazal outgrowth in the micropyle of seed, this appendage contains reserves like lipids or proteins and their function is to attract ants (myrmecochory). The elaiosome had been observed in many genera of Violaceae. In this study, the anatomical structure of the seed-coat was studied for the first time in *Hybanthus nanus*. The study revealed the presence of five layers in the integument, three layers in testa and two layers in tegmen of the mature seed coat. The presence of a conspicuous elaiosome with oily drops was observed in the micropylar region; this feature is likely to be related to myrmecochory.

Key words: *Hybanthus nanus*, elaiosome, myrmecochory, seed-coat

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Introduction

The elaiosome is a lipid-rich appendage that functions to attract ants, which may disperse the seeds some distance away from the parent plant (Culver & Beattie, 1978; Lengyel et al., 2010). *Viola* presents an elaiosome which originates as an outgrowth of the micropyle at the base of the raphe (Singh, 1961; Corner, 1976; Culver & Beattie, 1978). The elaiosome is also mentioned in the congener *Hybanthus* Jacq. s.l., a genus of 100 species distributed in the tropics and subtropics (Mabberley, 2008; Lengyel et al., 2010; Seo, 2010; Seo & Fradkin, 2022). Morphologically, the ovoid to globose shape seeds of *Hybanthus* s.l. are exostegmatic in relation with the position of the mechanical layer, and possess a conspicuous linear raphe and an exostomal elaiosome (Corner, 1976). There is no anatomical study of the elaiosome in this genus. The present paper provides a detailed study of anatomical features of seed-coat and elaiosome of the Argentinian species *H. nanus* (A. St.-Hil.) Paula-Souza.

Material and methods

Plants with seeds were collected from natural habitats of the centre of Argentina and vouchers for each specimen was deposited at the Buenos Aires University Herbarium (BAFC). The collection data for the examined specimens are given in Table 1. Scanning electron microscopy (SEM) of seeds and external seed-coat microstructure were performed according to Seo (2010). For anatomical studies, the collected seeds were fixed in 3% glutaraldehyde in 0.25 M phosphate buffer (pH 6.8) for 24 h, dehydrated in a graded ethanol series and acetone for 2 h in each step and embedded in Spurr's resin. Longitudinal sections (l.s.) of seed (1- 2 mm thick) were stained with toluidine blue (O'Brien et al., 1964) and mounted in glycerol jelly. Sections

Table 1. List of localities and voucher of the collected seeds of *H. nanus* in Argentina.

Voucher	Provenance
Troncoso 1972 (SI).	Province of Entre Ríos, Dep. Colón, road from San Salvador to Concordia city
Seo 29 and 30 (BAFC)	Province of Entre Ríos, Dep. Colón, National Park "El Palmar", road to Arroyo de los Loros.
Seo 54 (BAFC)	Province of Entre Ríos, Dep. Colón, National Park "El Palmar", Camping zone.

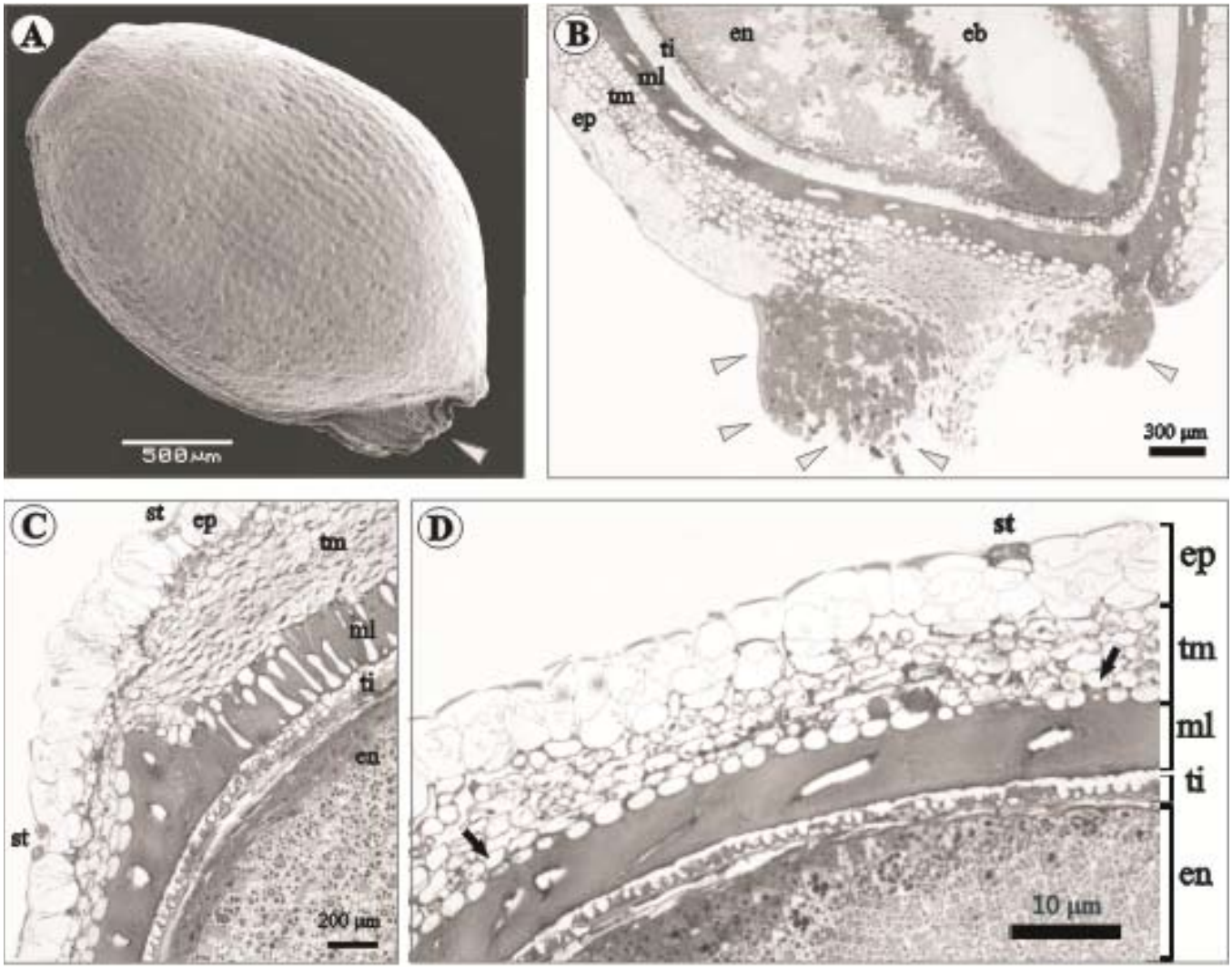

Figure 1. Seeds of *Hybanthus nanus*. (A) Dorsal view (SEM) of the seed showing a micropylar elaiosome (arrow); (B-D) Seed in l.s., showing two layers of the testa and tegmen; (B) Micropylar end showing part of the straight embryo, endosperm and the elaiosome containing presumed oil bodies (arrows); (C) Chalazal end; (D) Mid-region showing oxalate inclusion (black arrow). Abbreviations: eb – embryo, en - endosperm, ep – epidermis, ml - mechanical layer, st – stomata, ti – tegmen inner, tm – testa middle.

were observed microscopically and photographed using a photomicroscope Leica LMDB equipped with a digital camera (Leica DFC350FX) using the Leica IM50 versión 4.0 program. Description of seeds followed Corner (1976) and seed-coat layers were classified using the terminology of Singh (1961).

Results

The seeds in *H. nanus* are characterized by an ovoid to obovoid shape with a linear raphe, a wide chalazal region and conspicuous elaiosome in the micropylar region (Figs. 1A, B). In longitudinal sections of the seed a straight embryo was seen; the embryo was surrounded by a copious endosperm with inclusions inferred to be oil bodies (Fig. 1B). The mature seed coat of *H. nanus* revealed three layers of tegmen (ti) and two layers of testa (t) (Figs. 1B-D). In the chalazal region of the seed, the testa was observed to consist of more than twenty layers of parenchyma cells (t) and two to four layers of longitudinal cells with massive thickened walls in the mechanical layer (ml) (Fig. 1C).

Longitudinal sections of the seed revealed an exotesta with a cutinized epidermis with two to three layers of globose to flattened cells, as well as stomata (Figs. 1C, D). The inner side of the middle testa (tm) was formed by three to four layers of small, globose and isodiametric cells. In the tegmen, there was an outer single layer of rectangular cells with massive

thickened walls forming a mechanical layer, and an inner single layer of tegmen consisting of small and isodiametric cells with U-shaped thickenings of walls (Fig. 1D). Calcium oxalate crystals were dispersed inside some small cells of the tegmen. (Fig. 1D). In the micropylar region *H. nanus* show an emergence, the elaiosome that, in l.s. shows an external layer of exotesta and middle testa with twenty to thirty layers of small and isodiametric cells with thin walls. The cells in the outer layer of the elaiosome contain inclusions inferred to be oil bodies. These oil bodies are also found in the basal region of the exostome of the seed (Fig. 1B).

Discussion

In this, the first study of the seed coat and elaiosome of *Hybanthus nanus*, the micropylar elaiosome was found to be prominent and appeared to contain oil bodies; the seed coat consists of three layers of testa and two layers of tegmen. *Hybanthus* s.str. is one of several segregates of a polyphyletic *Hybanthus* s.l., along with genera such as *Pombalia* Vand. (Paula-Souza & Ballard, 2014) and *Afrohybanthus* Flicker (Flicker & Ballard, 2015). The seed coat structure of *Hybanthus* s.str., as observed in this study, is similar to that in the closely related *Afrohybanthus* and *Pombalia* (Raju, 1958; Singh, 1961; Seo & Fradkin, 2022). The seed coat in *Afrohybanthus* and *Pombalia* also develops from the inner and outer integument (II and OI), the middle layer of II disappears in the mature seed (Singh, 1961; Seo & Fradkin, 2022). The testa of *Pombalia* presents fewer layers of cells, which are smaller and polygonal, compared with *H. nanus*, which has more layers of cells, which are globose. As a consequence, the testa is thicker than the tegmen in *H. nanus*. The presence of calcium oxalate crystals, in the inner layer of the OI, was also reported in other genera of Violaceae – *Afrohybanthus* and *Pombalia* (Singh 1961; Seo & Fradkin, 2022) and *Viola* (Singh, 1961). The micropylar elaiosome contains inclusions inferred to be oil bodies, similar to the elaiosome in *Viola* (Culver & Beattie, 1978) and in two species of *Pombalia* (Seo & Fradkin, 2022). The putative oil bodies in *H. nanus* are more extensive than in *Pombalia* species, being present in most cells of the elaiosome. Singh (1961) did not note any elaiosome in *A. enneaspermus*; in the anatomical drawing the lack of this structure contrasts with the presence of an aril in the micropyle of seed in *V. tricolor*. Further studies of the anatomy and embryology of the seed coat of these species in *Hybanthus* s. str. are needed to understand any interaction between ants and elaiosome in myrmecochory and in colonization of new environments in nature, as was described in the genus *Viola* (Culver & Beattie, 1978; Lengyel et al., 2010).

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