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Structure, ontogeny and histochemistry of cyathial nectaries in *Euphorbia heterophylla* L. (*Euphorbiaceae*)

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Abstract

The mature cyathial nectary of *Euphorbia heterophylla* L. consists of distint secretory and sub-secretory zones. The secretory zone consists of 1-3 layer of palisade-like epidermal cells, supplied by both phloem and xylem. Distinct 'secretory pits' surrounded by thin cuticle are present in the secretory tissue. Enzymes such as succinic dehydrogenase and acid phosphatase were localized. Probable functions of cyathial nectaries and animal visitors are discussed.

Key words: cyathial nectary, Euphorbia heterophylla, ontogeny, structure, histochemistry

INTRODUCTION

Euphorbiaceae is an economically important family having a special inflorescence type – the cyathium as an adaptation to insect pollination (Stebbins 1950, Cronquist 1968). Three types of extrafloral nectaries are reported in this family: 1) patelliform, 2) glandular leaf margin, and 3) morulose (Metcalfe and Chalk 1950). They may occur at the base of petioles, stipules or at the base of lamina. These glands are found to be present in *Croton, Hevea, Macaranga, Micrada* and *Ricinus* (Metcalfe and Chalk 1950). A number of workers have studied the structure of the cyathial nectaries of *Euphorbia* (Schnepf 1964, Genc and Rauh 1984, Schnepf and Deichgraber 1984). Histochemical localization tests have been carried out in euphorbiaceous nectaries by Annigeri and Rudramuniyappa (1984). The present paper deals with the structure, ontogeny and histochemistry of the cyathial nectaries in *Euphorbia heterophylla* L.

MATERIALS AND METHODS

The cyathial nectaries at different stages were collected from University Botanical Garden and fixed in formalin-aceto-alcohol (Johansen 1940). Customary methods were followed for dehydration and embedding (Berlyn and Miksche 1976). 6-8-μm thick sections were cut and stained with tannic acid and ferric chloride followed by safranin 'O' and fast green FCF combination (Sass 1952). Bright-field photomicrographs were taken with a Carl-Zeiss Photomicroscope I. Fluorescence photomicrographs were taken with a Carl-Zeiss epi-fluorescence microscope fitted with an HBO-50 mercury lamp, using UV range filters. Histochemical tests were carried out for the enzymes acid phosphatase (Gomori 1950) and succinic dehydrogenase (Pearse 1972). Cuticle was detected by staining paraffin sections with 0.005% aniline blue in 0.067 M KH_2PO_4 buffer (Currier 1957) and examining an epi-fluorescence microscope.

RESULTS

ONTOGENY

Cyathial nectaries of *E. heterophylla* are deep cup-like structures found on the sides of the involucre (Fig. 1A). Each inflorescence possesses 1-4 nectaries. In young stages the tip of the nectary appears deep red in colour, while during secretion it becomes green. Towards the cessation of secretion the nectary turns yellow and later withers off along with the entire inflorescences. During the development of the cyathium, a group of dome-shaped cells present at the tip of the involucre function as 'nectary initials' (Fig. 1B), which can be distinguished from the neighbouring cells by their larger size and deep staining character. Parenchyma cells are present beneath the densely stained nectary initials. These initials undergo anticlinal divisions followed by oblique as well as periclinal divisions. As a result, 1-2-layered palisade-like epithelial secretory cells and a sub-secretory zone consisting of parenchyma cells are formed. The cells of the peripheral rim of the developing nectary undergo many divisions and more elongation than the cells at the centre of the nectary. Thus, a small depression is formed in the centre (Fig. 1C). Later, the peripheral rims of each nectary develop faster than the central cells and the nectary appears deep cupular at maturity (Fig. 1H).

STRUCTURE

The mature nectary is clearly distinguishable into three regions viz. 1) the secretory tissue consisting of 2-3 layers of elongated, darkly staining palisade-like cells, 2) stalk cell layer made of a single layer of parenchymatous cells

delineating the secretory and sub-secreting tissue, and 3) the sub-secretory tissue composed of parenchymatous cells embedding the vascular supply (Figs. 1G and H). The whole nectary structures is enveloped by a thick, prominent cuticular layer as observed with fluorescence microscopy (Fig. 1D).

However, a number of 'secretory pits' could be observed in the secretory tissue (Figs. 1G, H and I). Many vascular bundles composed of both xylem and phloem could be observed a few cell layers beneath the sub-secretory layer (Figs. 1G and H). Laticifers of non-articulated type are also distributed in the sub-secretory tissue, between the vascular bundles and stalk cell layer, adjacent to phloem (Fig. 1I).

Ants, bees and occasionally butterflies could be observed foraging the nectary. However, ants were present for more time than the other animal visitors.

HISTOCHEMISTRY

Acid phosphatase activity was more pronounced in the secretory cells (Fig. 1E). Succinate dehydrogenase was more intense in the stalk cells than the secretory epithelial cells (Fig. 1F).

DISCUSSION

In the family *Euphorbiaceae*, epidermal, sub-epidermal as well as deeper layers are involved in the development of nectaries (Aufrecht 1891, Lyon 1898, Dave and Patel 1975, Annigeri and Rudramuniyappa 1984, and Arumugasamy and Inamdar 1988). Hoppe and Uhlarz (1982) considered the cyathial glands of *Euphorbia* species as true interpetiolar glands. The present observations on the cyathial nectaries of *E. heterophylla* are in accordance with these authors. Cyathial nectaries display heavy cuticular insulation. 'Secretory pits' surrounded by thinner cuticular layers were reported by Radtke (1926) and Feldhofen (1933) in the glandular epithelium of some *Euphorbia* species. The cyathial nectaries of *E. heterophylla* also exhibit similar pits probably through which nectar is exuded. Subramanian and Inamdar (1986) suggested that the occurrence of heavy cuticular thickening of glands exposed to air indicates that such insulations play an important role in the control of transpiration.

High acid phosphatase activity is regarded as an important trait of nectar--secreting cells (Rachmilevitz and Fahn 1975, Mohan and Inamdar 1986). Succinic dehydrogenase is considered a marker enzyme for mitochondrial activity (Lehninger 1984). Localization of succinic dehydrogenase in the stalk cells and as well as in the secretory cells indicates that the nectariferous cells are in a higher metabolic state than neighbouring cells since active

PLATE I

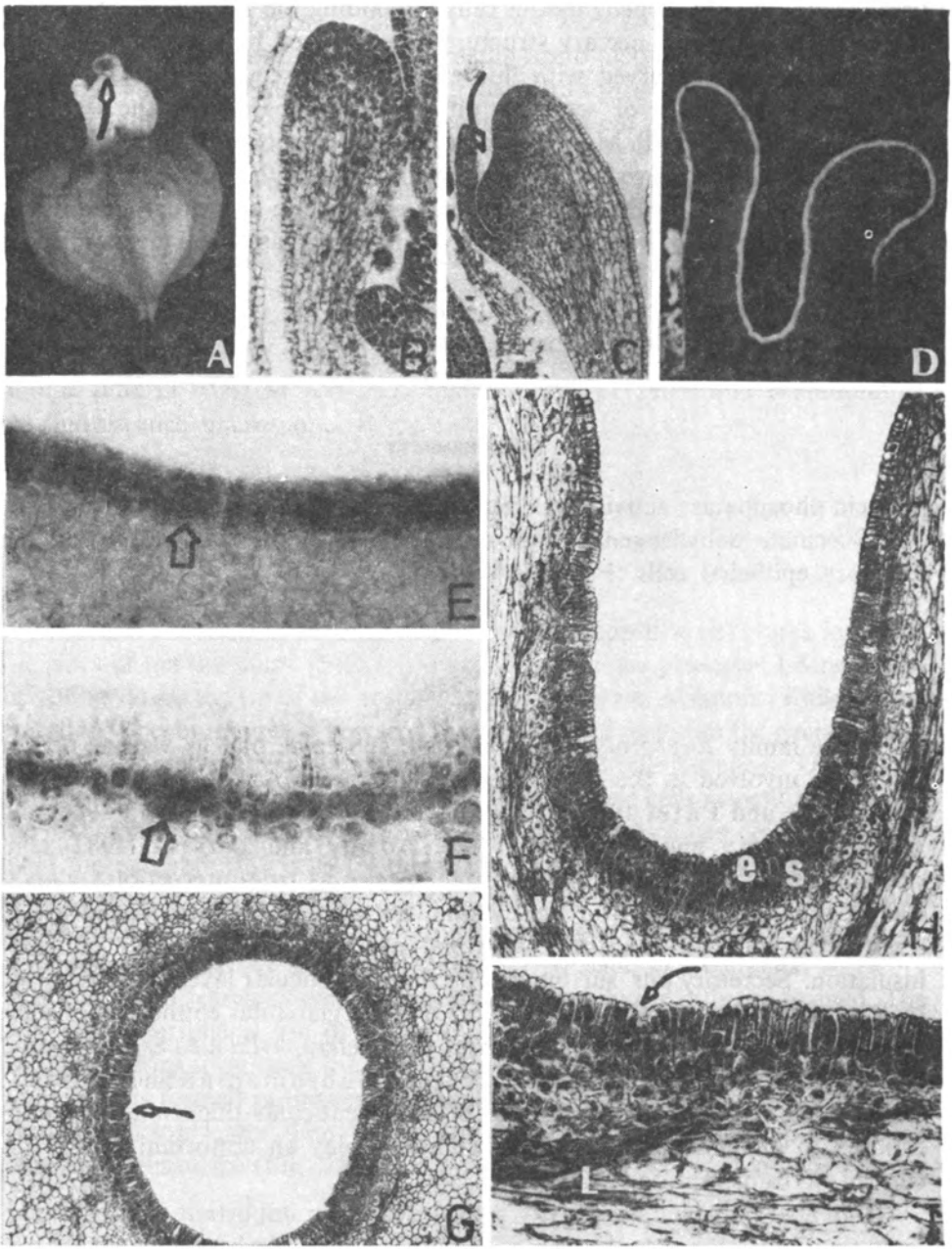

Fig. 1. A – Cyathial nectary of *Euphorbia heterophylla* (arrow). B and C – Developmental stages of cyathial nectary. Note the formation of a central depression (arrow, ×600. D – Aniline blue-induced fluorescence of cuticular layer, ×135. E – Localization of acid phosphatase activity in the secretory cells of the mature nectary (arrow), ×680. F – Succinic dehydrogenase activity in the mature nectary. Note intense reaction in the stalk cell zone (arrow), ×450. G – C.S. of mature

secretion is an energy requiring process (Arumugasamy and Inamdar 1988). Feldhofen (1933) regarded cyathial nectaries of *Euphorbia* as 'nuptial nectaries'. However, the function of the cyathial nectaries is obscure. Pistillate flowers reach maturity long before the cyathial nectaries start secreting. Therefore, the prospect of the cyathial nectaries being directly involved in pollination seems to be meagre. On the other hand, they may play a 'protective role'. The presence of numerous ants during most of the secretory phase supports this assumption. Fahn (1974) included the cyathial nectaries of *Euphorbiaceae* in the extrafloral nectary types. The present observations support the conclusion of Fahn (1974). The deep cup-shaped cyathial nectaries possess distinct differentiation of cell layer, vasculature and a deep nectar chamber to store nectar. Therefore, the cyathial nectaries of *E. heterophylla* are considered to be advanced over other extrafloral nectary types.

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cyathial nectary, showing different cell zones and secretory pits (arrow), ×100. H – L.S. of mature cyathial nectary showing vascular supply and different cell zones, ×450. I – L.S. of nectary showing the presence of laticifers and vascular elements in the sub-secretory tissue. Note secretory pit (arrow). ×240. e – epithelial cells, v – vasculature, s – stalk cells, L – laticifers

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Budowa, ontogeneza i histochemia miodników cyatium u Euphorbia heterophylla L. (Euphorbiaceae)

Streszczenie

Dojrzały miodnik cyatium *Euphorbia heterophylla* L. składa się z wyraźnych stref: wydzielniczej i subwydzielniczej. Strefa wydzielnicza zbudowana jest z 1-3 warstw palisadopodobnych komórek skórki, zaopatrzonych zarówno w floem, jak i w ksylem. W tkance wydzielniczej występują wyraźne „dołki wydzielnicze" otoczone cienką skórką. Zlokalizowano takie enzymy, jak dehydrogenazę bursztynianową i kwaśną fosfatazę. Przedyskutowano przypuszczalne funkcje miodników cyatium i rolę zwierząt odwiedzających kwiaty.