

A developmental study on the cyathial nectaries in *Euphorbia thymifolia* L.

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Abstract

Four cyathial nectaries were found at the inside tip of the involucre in *Euphorbia thymifolia* L. The cyathial nectaries developed from a group of epidermal and hypodermal cells belonging to the involucre. Three regions could be distinguished in each nectary viz.: 1) a single layer of palisade-like epithelial cells, 2) one or two layers of stalk cells, and 3) basal parenchymatous cells. Starch grains and proteins were localized in the epithelial and stalk cells, but not in the basal cells. The enzymes, acid phosphatase and succinate dehydrogenase were also localized.

Key words: cyathial nectary, ontogeny, structure and histochemistry

INTRODUCTION

The cyathium is one of the notable characters of *Euphorbiaceae*. However, there are few studies on the origin of cyathial glands. Schnepf (1974) suggested that floral nectaries of *Euphorbia pulcherima* be referred to as extrafloral of cyathial nectaries. Extrafloral nectaries of *Croton*, *Hevea*, *Macarranga* and *Ricinus* were also reported in *Euphorbiaceae* (Metcalf and Chalk 1950). Dave and Patel (1975) have described extrafloral nectaries in *Pedilanthus tithymaloides* at the leaf base and leaf tip. The development, localization and distribution of insoluble polysaccharides, ascorbic acid, nucleic acids and total proteins in *Euphorbia pulcherima* was worked out by Annigeri and Rudramuniyappa (1984). These authors reported that the cyathial nectaries are present outside the involucre. However, the present study

reveals that cyathial nectaries are present inside the involucre in *Euphorbia thymifolia* and describes their origin, development, structure and histochemistry.

MATERIALS AND METHODS

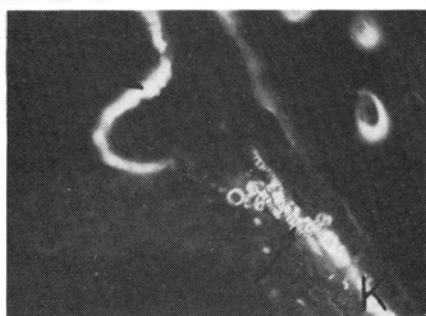
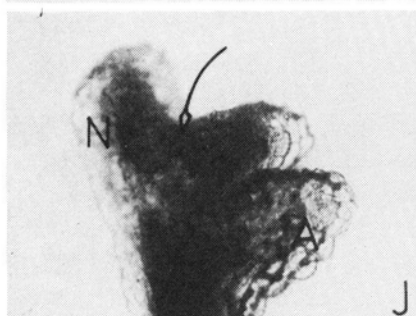
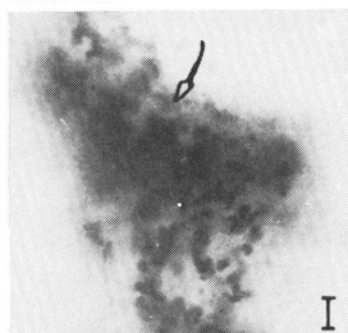
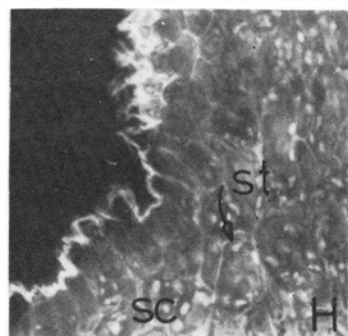
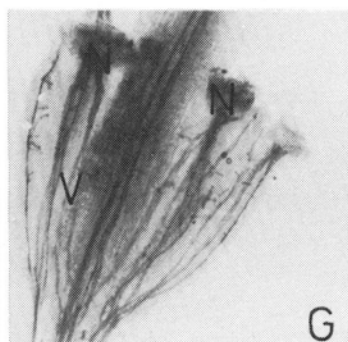
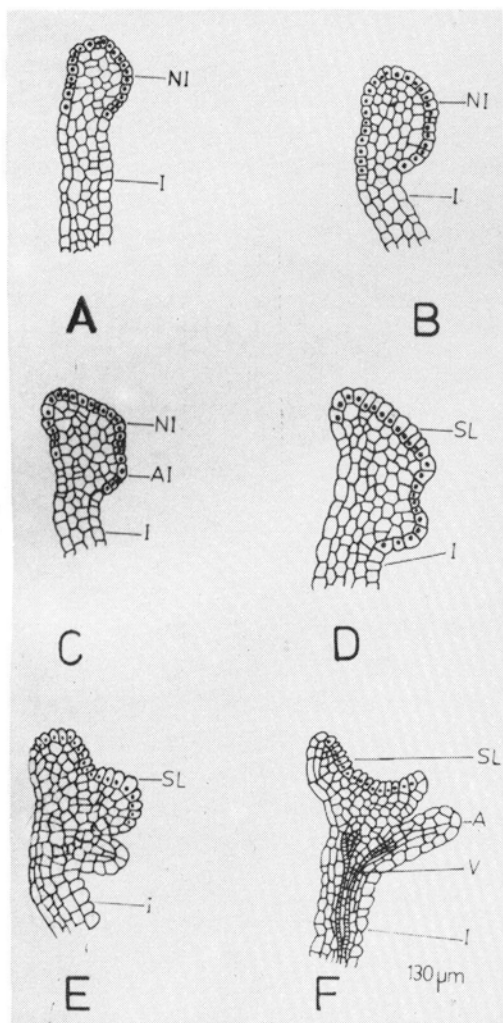
Young and mature cyathial nectaries of *Euphorbia thymifolia* were collected on the university campus and fixed in formalin-aceto-alcohol (Johansen 1940). Customary methods were followed for dehydration and embedding (Berlyn and Miksche 1976). Transverse, 6-8 μ m thick sections were cut and stained with tannic acid and ferric chloride followed by safranin O and fast green FCF combination (Sass 1952). The mature flowers were cleared following the method of Rao et al. (1980). Bright-field photomicrographs were taken with a Carl-Zeiss photomicroscope. For fluorescence photomicrographs, a Carl-Zeiss epi-fluorescence microscope fitted with a HBO-50 mercury lamp, and UV range filters, was used. Schiff's periodic acid reagent (Jensen 1962) was used to localize starch grains in bright field microscopy. Starch grains were also localized using a phase-contrast microscope. Protein and cuticles were localized using dansyl chloride (Ringertz 1968). Cryocut sections of fresh materials were used for enzyme localizations. Acid phosphatase (Gomori 1950) and succinate dehydrogenase (Pearse 1972) were localized.

RESULTS

ONTOGENY

Four red coloured, spot-like cyathial nectaries were found inside the tip of the involucre of *Euphorbia thymifolia*. The epidermal cells at the tip of the involucre were rectangular, isodiametric and stained densely exhibiting prominent nuclei. These cells functioned as nectary initials (Figs. 1A-C). At this stage,

Fig. 1. A — Tip of the involucre showing "nectary initial" cells. B, C — Involucre tip differentiated into nectary initial and appendage initial cells. D, E — Various developmental stages of *E. thymifolia* nectary. F — Mature nectary of *E. thymifolia*. G — Flower architecture showing vasculature to each nectary. H — Phase contrast photomicrograph of the starch grains in nectary. $\times 900$. I — Acid phosphatase activity in the nectariferous tissue. $\times 730$ (arrow). J — Succinate dehydrogenase activity in the nectariferous cells. $\times 830$ (arrow). K — Autofluorescence of xylem elements and cuticle. $\times 1050$ (arrow). NI — nectary initials, I — involucre, AI — appendage initials, SL — secretory layer, A — appendage, V — vasculature (xylem elements), N — nectary, SC — stalk cells, St — starch grains



the sub-epidermal cells also had a prominent nucleus. In fact, the hypodermal cells divided radially in two places, and the epidermal nectary initial cells first divided anticlinally.

As a result, the epidermal cells were pushed out and two projection-like structures were formed (Fig. 1C). The upper projection developed into a nectary, while the lower one developed into an appendage (Figs. 1D-E). Concurrently with the development of the nectary and its appendage, cells at the peripheral rim of the developing nectary divided faster than the central ones. This led to the formation of a central depression (Fig. 1E).

STRUCTURE

In longitudinal section, the entire nectary had the appearance of a cup-shaped structure. Each nectary was differentiated into three distinct regions viz.: 1) a single layer of palisade-like epithelial cells which were of a secretory nature, 2) one or two layered stalk cells, and 3) parenchymatous tissue (Figs. 1F and H). The nectaries were covered with a thick cuticle (Figs. 1K and H). The vasculature consisted of only xylem which extended up to the base of the parenchymatous tissue (Figs. 1F, G and K). Two to three separate xylem strands reached up to the base of the nectariferous tissue, where they branched further. The remaining strands entered into the appendages (Figs. 1F, G and K). Starch grains and proteins were observed in the epithelial and stalk cells (Fig. 1H). The remaining parenchymatous cells were vacuolated. Acid phosphatase gave an intense reaction (Fig. 1I), but succinate dehydrogenase showed a weaker reaction in the nectariferous tissue (Fig. 1J).

DISCUSSION

The origin, development and functions of cyathial nectaries are yet to be investigated. A host of nectaries are reported to have originated either from epidermal and/or sub-epidermal cells (Haberlandt 1914, Rao 1926, Maheswari 1954, Inamdar 1969). In *Pedilanthus tithymaloides* extrafloral nectaries develop from a group of meristematic cells arising in deeper lying ground tissues in the leaf tip and leaf base (Dave and Patel 1975). Aufrecht (1891) presented an opinion that epidermal, sub-epidermal as well as deeper layers are involved in the development of the nectary of *Ricinus communis*. Recently, Annigeri and Rudramuniyappa (1984) have reported that the cyathial nectary of *E. pulcherima* originates on the involucre as a small "protuberance". In *E. thymifolia*, nectaries develop from the epidermal and sub-epidermal cells of the tip of the involucre. The presence of starch grains and more proteins (both reserve food materials) in the secretory tissues

indicates that these cells are involved in secretion. Proteins and starch grains are known to be rich sources of energy as well as the chief constituents of the nectar (Annigeri and Rudramuniyappa 1984). Succinate dehydrogenase is a respiratory enzyme and its activity indicates a high rate of metabolic activity in the nectariferous cells. The presence of acid phosphatase activity in the secretory cells of the cyathial nectary is characteristic of the nectar secreting cells (Rachmilevitz and Fahn 1975, Mohan and Inamdar 1986). Cyathial nectaries of *E. thymifolia* are very minute and hence the smaller amount of the secretion product. Therefore, it still remain unknown whether the cythial nectaries play an important role in pollination or not. However, seed setting was observed to be high. Occasionally ants visited the blossoms. But they did not seem to play any role as pollen carriers. It is presumed that the cyathium of *E. thymifolia* functions as self-pollination promoter. The insect-attracting function is only of facultative importance to the plants. According to Ehrenfeld (1975) even this facultative importance tends to disappear as the size of the petaloid appendages decreases, as in *E. hysopifolia* which combines the lowest rate of insect visitation with the highest percentage of seed setting. The cyathial nectaries of *E. thymifolia* are considered extrafloral, since they are not directly involved in pollination.

Acknowledgment

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Badania rozwojowe miodników cyatium u Euphorbia thymifolia L.

Streszczenie

Wewnątrz wierzchołka okrywy cyatium *Euphorbia thymifolia* L. znajdują się cztery miodniki. Miodniki te rozwijają się z grupy epidermalnych i subepidermalnych komórek okrywy. W każdym miodniku wyróżnia się trzy części: 1) jedną warstwę podobnych do palisady komórek epitelialnych, 2) jedną lub dwie warstwy trzonkowe oraz 3) bazalne komórki parenchymatyczne. Ziarna skrobi i białka zlokalizowano w komórkach epitelialnych i komórkach trzonkowych, natomiast nie było ich w komórkach parenchymatycznych. Zlokalizowano również enzymy — kwaśną fosfatazę i dehydrogenazę bursztynianową.