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LIGHT MICROSCOPE OBSERVATIONS ON THE EXTRAFLORAL NECTARIES IN *LEUCAENA LEUCOCEPHALA* LAMK., (MIMOSACEAE)

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Abstract

Extrafloral nectaries in *Leucaena leucocephala* Lamk., were studied at the level of light microscope. Two non-porate, cup-shaped nectaries developed on the adaxial surface of the rachis from a group of initials and a nectary proper was distinguishable into secretory, sub-secretory and vascular zones. Phloem bundles appeared to be terminated at the base of the sub-secretary zone. The lifting of the cuticle appeared to occur prior to the nectar secretion and the secretion was released following the rupture of the lifted cuticle. The cells of the sub-secretory zone in a developing nectary developed massive accumulation of polyhedral crystals of calcium oxalate, which were infrequent in the secretory as well as post-secretory nectaries. However, a few crystals could be observed in such nectaries, especially in the parenchymatous cells close to the xylem traces and probably represented the poisoned crystals. It is suggested that calcium oxalate crystals are redissolved and taken back into metabolic cycles of the secretory cells.

Introduction

Extrafloral nectaries (EFNs) are the glands secreting nectar enriched in sugar, which invariably contain aminoacids (Baker & Baker, 1973). They occur on various parts viz., stem, petiole, around leaf margins and leaf surfaces of a wide variety of plants, and recently reported to have role in plant defence against various herbivores by attracting ants (Tilman, 1978; Keelar, 1980). However, it has been also recognized that the EFNs may act as bleed-valves to enhance the unidirectional flow of building materials to the developing organs (Milburn, 1975). The development, structure and ecological role of EFNs have been studied in detail, and several EFNs have been reported to contain crystals of calcium oxalate (Elias, 1983). So far nothing is known about Ca-oxalate metabolism in EFNs. The present report deals with the structure and biology of the EFNs in *L. leucocephala* (Mimosacea) with reference to the fate of Ca-oxalate crystals in the course of EFNs development.

Materials and Methods

The samples of EFNs through various stages of development were collected from plants of *Leucaena leucoceophala* Lamk., growing in the Botanical garden maintained by Sardar Patel University, Vallabh Vidyanagar and fixed on the spot in 4% gluderaldehyde (Loba) in 0.2 M PO_4 buffer (pH 7.2) for 6h at 4^{O}C. After re-

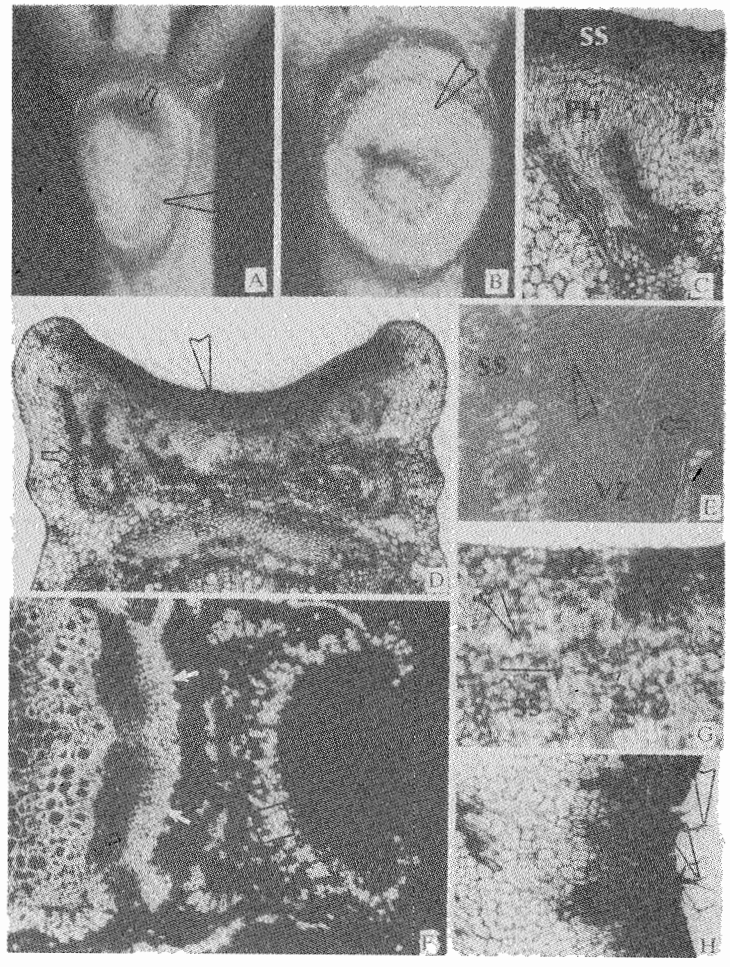

Fig.1. L.S. of extrafloral nectaries.

A,B. Tessovar photographs of secretory (Fig.A) and non-secretory (Fig.B) EFNs (both arrow heads). Arrow in Fig.A indicates nectar droplet. X 20; X 28.

C. Phloem trace (Ph) terminated below sub-secretory zone (SS). Xylem trace at XY. X105.

D. Mature EFN. Note cup-shaped nectary, primary bundles (arrows) and the secretory zone (arrow head). X 52.

E. Dark field photomicrograph showing details of vascular zone (VZ). Phloem (arrow head) closest to the sub-secretory zone (SS). Xylem trace at arrow. White contents in SS zone are Ca-oxalate crystals. X 120.

F. Developing nectary. Polarisation photomicrograph. Ca-oxalate crystals in sub-secretory cells ( ). Note a few small crystals in rachis cells (arrows). X 52.

G. Non-secreting. Lifted cuticle at arrow. Arrow head above '____' indicate secretory zone, while 'SS' below '___' the sub-secretory zone. X 95.

H. Dead Fungal hypha (?) at arrow heads. X 105.

peated washing in the same buffer at 4°C samples were dehydrated through Methyl cellasolve - N Propanol - N Butanol series at 4°C following 0'Brien & Mc Cully (1981) and embedded in tissue-prep (Melting point 56.5°C) using TBA as solvent for wax. Serial sections of 10-15 um thickness were cut on spencer rotary microtome andstained in Azur B (Bio-Rad), 1% solution in PO_4 buffer (pH 7.2); dehydrated through graded series of Ethyl alcohol, and mounted in DPX. Proteins were localized using Amido Black B (Sigma), 1% solution in 7% glacial acetic acid. Nectar gave positive reactions with Fehling and Ninhydrin reagents for sugars and amino acids, respectively.

The crystals were identified as Calcium oxalate after a convincing chemical behaviour towards dilute (10%) Hydrochloric, Acetic and Sulphuric acids. For all the preparations glass double distilled water was used.

Results

Distribution of EFNs: *L. leucocephala* bears cup-shaped EFNs (Figs.1 AB) on the adaxial surfaces of rachis. Usually two EFNs of unequal sizes, one at each end of the rachis were present. Generally, the extrafloral nectary (EFN) close to the rachis base was larger than that present on the distal end of the rachis. However, there were rachis on which either distal or both the EFNs were occasionally absent. Both the EFNs irrespective of their position on rachis and sizes had similar morphology.

Structure of the EFNs: Both the nectaries were cup-shaped & developed from a group of initials (Fig. 1 A,B,D, Fig.2 IJ) and a mature nectary was distinguishable into secretory, sub-secretory and vascular zones (Fig. ID,E,G, Fig.2I).

The cells of the secretory zone were cuboidal, 6-8 layered in cross-sections, with dense cytoplasm and contained protein (Fig.2N). The epidermis beneath which they were present was uniseriate, thinly cuticularized and without stomata and hairs.

The cells of the sub-secretory zone appeared parenchymatous with dense cytoplasm and prominent nuclei. Usually they appeared 8-12 layers thick in sections (Fig.2I) and lacked starch grains. In a mature non-secreting nectary cells of the sub-secretory zone developed polyhedral crystals (Fig. 1 E,F, Fig.2 J,K,L) of Calcium oxalate. The crystals of Ca-oxalate observed on other parts of the rachis on which a EFN was borne were few and comparatively smaller (Fig. IF).

The vascular zone consisted of thin walled cells having less dense cytoplasm as compared to the cells of sub-secretory zone and was interrupted by xylem as well as phloem traces (Fig.1CE). The phloem traces usually terminated close to the base of the sub-secretory zone (Fig.1 CE). The xylem as well as phloem traces were the direct continuation of primary petiolar bundles (Fig. 1CD, 3,4).

In a mature nectary the cuticle appeared intact with the epidermal cells. However, in some preparations the lifted cuticle was observed even though the nectary was still non-secretory (Fig.1G). At the advance stages of maturation the cells of the secretory zone appeared loose and the secretion apeared to be released by rupturing the cuticle.

In a senescent nectary crystals of Ca-oxalate were a few (Fig.2I) and mainly confined within the cells close to xylem traces (Fig.2KM) and occasionally among few

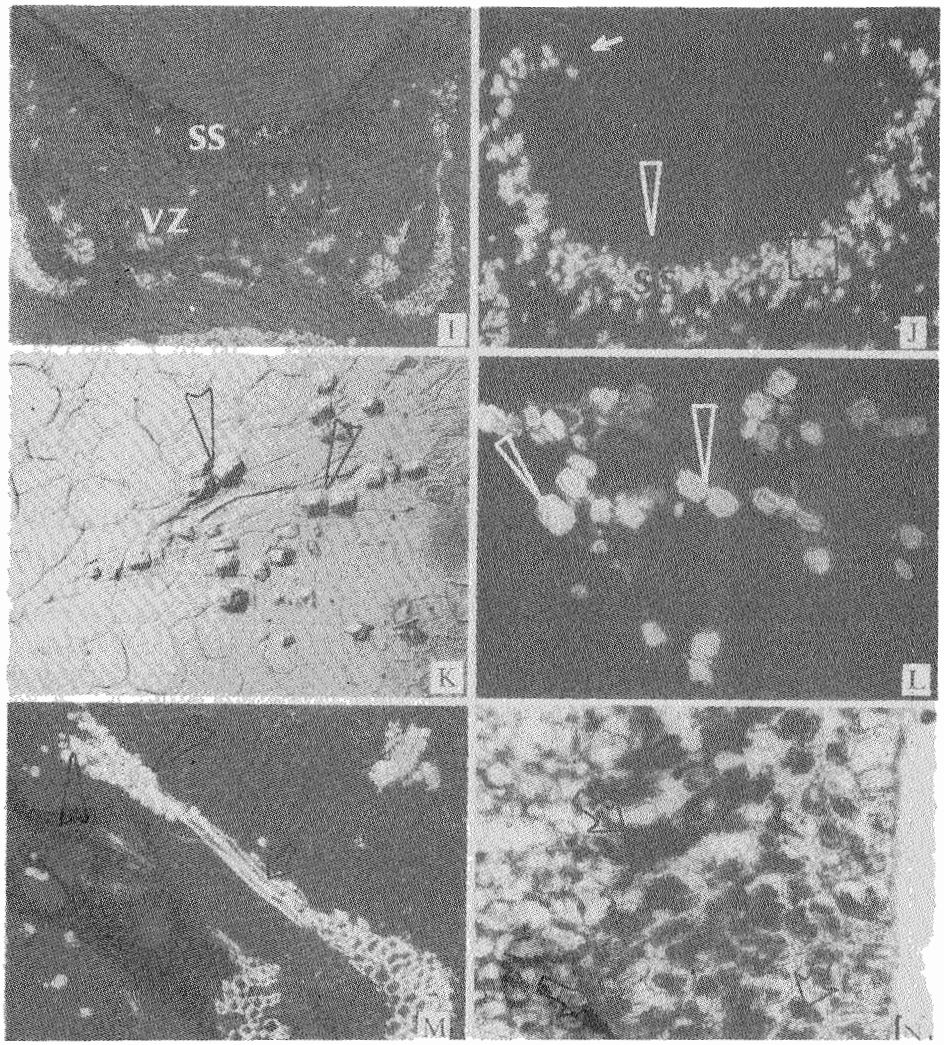

Fig.2. L.S. EFN senescent. I Polarisation photomicrograph. Ca-oxalate crystals (□) in vascular zone (VZ). Sub-secretory zone (SS) lacks crystals. Figure comparable to Figs. F & J X 52.

J. Part of the nectary containing crystals in Fig.F at higher resolution. Crystals in sub-secretory zone (SS) in □ Arrow head indicate the secretory zone, while arrows the cuticle. X 52.

K. Crystals in sub-secretory cells. Interference photomicrograph. Note the polyhedral crystals in 3-diamensional view (arrow heads). X 140.

L. Polarisation photomicrograph showing crystals at higher magnification. Note the crystal shape (arrow heads). X 140.

M. Polarisation photomicrograph. Crystals. (arrow heads) in parenchyma cells close to xylem (arrow). X 105.

N. L.S. Secretory EFN stained with Amido Black 'B'. Proteins at arrows. X 130.

cells of the sub-secretory zone (Fig.2I). In contrast to the sub-secretory cells of the active nectary the sub-secretory cells in a senescent nectary appeared devoid of contents and were lightly stained. The secretory cells appeared disorganized and dead. In one preparation fungal hyphae like structures which had emerged out from the nectary was observed (Fig.1H).

***Ecology of the EFN*:** A few black agressive ants visit the nectary under secretion but their number was very less though the several EFNs under secretion were present on the plants. At present it is difficult to draw any conclusion about the EFN - Ant relation, and the role of ant in plant defence. Most of the nectaries secreted during early morning hours only. No secretion was observed after 8.0 a.m and until the late evening hours. The secretion seemed to be more during normal bright days as compared to cloudy days.

Discussion

Bhattacharyya & Maheshwari (1973) reported bi-layered thick walled cell zone in central region of the EFN in *Leucaena*. In the present study, we failed to recognize any thick walled cell in the sub-secretory zone except those in close contact with vascular traces.

The mode of development and secretion of EFN in *Leucaena* is almost similar to the observations made on EFNs of several leguminaceous plants (Marginson *et al.*, 1985; Bhattacharyya & Maheshwari, 1971, 1973; Elias, 1972), and is in no way an exception except in the periodicity in their secretion. In *Pithecellobium, Inga* and *Acacia* nectar production has been reported to occur during morning as well as evening hours (Elias, 1972; Janzen, 1966). However, in *Leucaena* we could observe the secretion only during early morning hours similar to the observations made on EFNs of *Albizzia lebbek*.

Marginson *et al.,* (1985) working on *A. terminalis* observed lifting of cuticle when the cells were secreting actively and reported it to occur due to the outward pressure applied by secretion. In the present study lifted cuticle was observed in non-secreting mature nectaries. It seems that the lifting of cuticle in EFN, at least in *Leucaena* is in no way related to the pressure response and some other physiological aspect may be behind this phenomenon, which needs further exploration.

Since no starch grain could be detected in either secretory or sub-secretory cells in *Leucaena*, it seems that nectar produced might have been derived from sieve elements in pre-nectar form as the secretory cells are supplied by phloem (Esau, 1969; Frey-Wyssling, 1955).

The most interesting feature in the EFN of *Leucaena* through its various developmental stages was the variation in the occurrence of crystals of Ca-oxalate. The crystals of Ca-oxalate have been observed in EFNs of a wide variety of plants (Elias, 1983), but nothing has so far been reported about variations in their occurrence in EFNs.

In literature the crystals of Ca-oxalate have been reported as a waste product having no significant importance in plant metabolism (Stewart, 1960; Frey-Wyssling, 1935). However, it has also been reported that crystals of Ca-oxalate can be resolubu-

lized and again taken back into cell metabolism (Calmes & Piquemal, 1977; Hinchee, 1983; Franceschi, 1990), and Ca^{2+} has various important roles in cell metabolism (Ferguson, 1984). In a very recent study (Franceschi, 1989) it has been experimentally demonstrated that the synthesis and solubilization of Ca-oxalate crystals in plants are reversible and quick process, and Ca^{2+} is an essential material to maintain the Ca Pool in plant organs and tissues when low capacity regulatory systems are overloaded.

The irregularity in the occurrence of the crystals of Ca-oxalate in *Leucaena* EFNs can be best understood in the light of above experimental findings and the only possible explanation regarding their irregularity can be that crystals of Ca-oxalate in *Leucaena* EFN synthesized in the developing sub-secretory cells are redissolved and probably utilized by the secretory cells. The persistence Ca-oxalate crystals, a few in senescent nectaries are probably the 'Poisoned' and more resistant to dissolution (Cody *et al.*, 1982). However, more detailed studies at ultrastructure level is needed to ascertain the metabolic pathway and the role of Ca-oxalate crystals in secretion, if any.

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