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RESEARCH ARTICLE

STUDIES ON MICRO-MORPHOLOGICAL CHARACTERISTICS OF EXTRA FLORAL NECTAR GLAND OF *CASSIA HIRSUTA* LINN

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ABSTRACT

The ecological importance of the EFNs and their interaction with insects, have motivated many authors to study the morphology, distribution and frequency of these structures in different plant taxa. The diversity in nectar shape and location taxonomically valuable in addition to their ecological role in plant insect interactions and hence in the present study the location of the gland size and shape and micro-morphological characteristics of *Cassia hirsuta* are examined and presented with relevant images. Further, the attachment of gland with main stem indicated non-vascularised nature of the gland. The SEM images of the surface of the gland showed ruptures in the matured gland through which the nectar oozes out.

INTRODUCTION

Plants have evolved a myriad of traits that attract, reward and exploit animals for vital tasks, such as pollination, seed dispersal and herbivore defence (Schoonhoven *et al*., 2005;Bronstein *et al*., 2006). Extrafloral nectaries (EFNs) one of such trait in certain plants,are plant-secretory glands most commonly linked to defensive mutualisms (Doak *et al.* 2007). The ecological importance of the EFNs and their interaction with insects, have motivated many authors to study the morphology, distribution and frequency of these structures in different plant taxa (Zimmerman 1932; Bentley 1977; Metcalfe & Chalk 1979; Elias 1983; Oliveira &Leita˜o-Filho 1987; Oliveira &Oliveira-Filho 1991; Koptur, 1992; Morellato & Oliveira 1994; McDade & Turner 1997). Species of Genus Cassia bearing EFNs are widely distributed around the world, and more common in tropical than in temperate environments (Pemberton 1988, Oliveira and Leitao-Filho 1987; Oliveira and Oliveira-Filho 1991) and its role in insect attraction (Koptur 1992). Fahn (1990) mentioned the presence of nectar-secreting glandular trichomes on the stipules of *Vicia faba*.The detailed studies on the EFN glands of *Vicia faba* have been done by Devis and Gunning (1991,1992) and Davis *et al* (1988). The distributional patterns of glandular hairs on the calyx-tooth were examined in 30 species of subgenus Vicia by Endo and Ohashi (1998). They observed two types of glandular hairs; a few glandular hairs scattering on the whole surface, or many glandular hairs densely gathering at the center of teeth. Stpicznska(2000) studied extrafloral nectaries located int stipules in four *Vicia* taxa, *V.sativa* subsp.*angustifolia*. The occurrence of EFNs in leaves of *Pterodon polygalaeflorus* Benth and *Pterodon pubescens* Benth (Fabaceae: Papilionoideae) were detected in adult specimens, at the time of production of new buds and flowers. The EFNs occur in the rachis and are located under the insertion of each petiole. Each gland consists of a small elevation whose apical portion is deeply invaginated, resulting in a depression (secretary pole), a common characteristic of both species. Unicellular, non-glandular trichomes occur along the rachis, being less numerous in *P. polygalaeflorus* while in *P. pubescens* they cover entire EFNs. (Pavia.*et.al*, 2001). *Cassia hirsuta* is chosen for the present study to examine the actual location and point of attachment on the main plant and its micro-morphological characteristics of extrafloral nectar gland of *C.hirsuta*, growing widely in the University compus.

MATERIALS AND METHODS

Plants occur as weeds, especially near sites of human habitation where there has been destruction of the natural vegetation. They favour riparian situations where sandy alluvium has been deposited, but are also to be found in disturbed grassland or forest margin. Short-lived perennial shrub with erect, sparsely branched stems up to 1,5-2,7 m high. Stems ridged, villous when young with dense straight slightly upward pointing, greyish-white hairs, becoming densely pubescent.

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Fig 1 *Cassia hirsuta* Linn

Petiole 2,5-4 cm long including basal pulvinus; petiolar gland at distal end of pulvinus, sessile, cylindric, finger-like, slightly narrowed at base, blackish, 2 mm long Inflorescences in axils of middle and upper leaves, 3 cm long, 3-6-flowered. The EFN glands are collected from *Cassia hirsuta* growing in the university campus. Before collecting the glands, plants are observed for an hour to note down the movement of ants across the plants. Photographs are taken in the field itself focusing on the location and distribution of EFN gland in the plant. Mature and young glands are collected. Cross sections of the different size glands are observed under phase contrast microscope and images are photographed. To examine the surface configuration of gland, the processed gland sample were dried with co_2 for few minutes and mounted on double adhesive tape on stubs and sputter with ions, then the samples were observed in 15.0kV using Hitachi S-3400N and SEM images are obtained.

RESULT

The EFN glands in *Cassia hirsuta* is located at the base of the petiole. Each petiole beared one gland on the upper side of the base of the petiole(fig.2). The gland is spherical with dark violet in colour and the size ranged between 1.0 to 2.0±0.3mm and attached to the petiole by very short stalk (Fig.3).

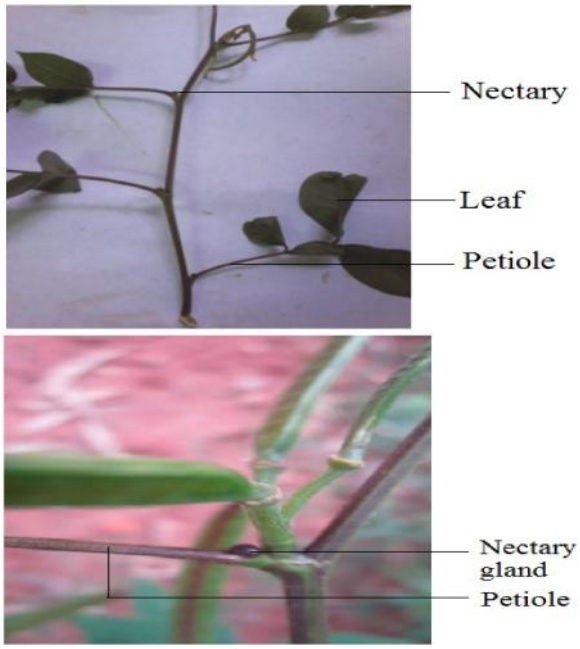

Fig 2 Location of EFN gland in *Cassia hirsuta*.

The gland is attached to the petiole but there is no vascular supply to the glandular cells; the vascular bundle is terminated before the glandular paranchymatous cells (Fig.4).The size of gland at the middle of the plant is larger than the upper/terminal and base of the plant. The surface of the gland showed variation in the surface configuration according to their position in the plant and this is also seen in SEM image of surface of the young gland located in the upper portion/terminal portion of the plant both young and mature gland(Fig.5).

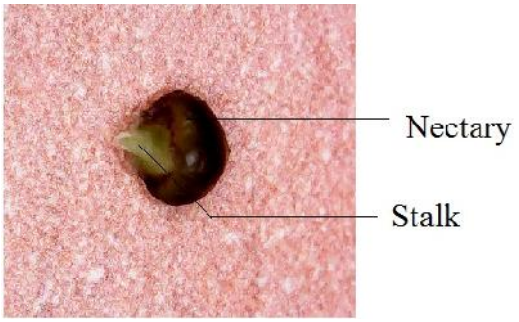

Fig 3 EFN gland with short stalk

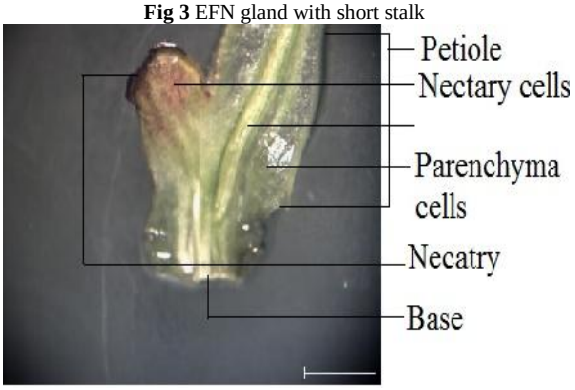

Fig 4 Microscopic view of TS of gland showing attachment of gland with petiole.

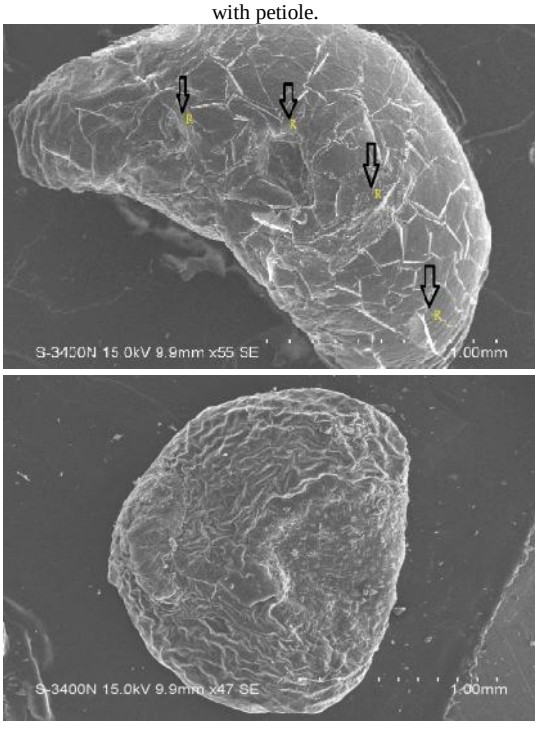

Fig.5 SEM images of Surface mature gland with and without ruptures

DISCUSSION

In general, EFNs represent a heterogeneous multitude of secretary structures, from simple glandular trichomes and cryptic secretory tissue embedded within EFN-bearing plant parts to conspicuous, complex vascularized glands, all of which produce and secrete nectar (Zimmermann, 1932; Elias and Gelband, 1976;Fahn, 1979; Schmid, 1988; Vogel, 1997; Bernardello, 2007). EFNs are found in many tropical genera of the family Caesalpiniaceae. The Genus *Cassia* Linn. comprises of 600 species occurring mostly in the tropics and subtropics, especially in India. Cassia is the only genus in Cassieae in which some species are characterized by the presence of extrafloral nectaries on the petiole, leaf-surface and rachis (Bharathi Bhattachariya. 1970). The diversity in gland shape and location taxonomically valuable in addition to their ecological role in plant insect interactions (Bentley 1977, Bentley & Elias 1983, Koptur 1992, Rudgers & Gardener 2004, Wackers & Bonifay 2004, Diaz-castelazo *et al*. 2005; doak *et al*. 2007). From the field observation on the occurrence of EFN gland in 100 plants of *Cassia hirsuta*, it is quite obvious that the position of nectar is at the base of petiole and found constant in all the plants observed in the wild (Fig1 & 2). It is understood from the available reports that the location of these glands vary conspicuously among the members of different families such as Cythraceae where the EFNs present in the leaf blade, in Vochysiaceae EFNs seen in the stem, peduncle, petiole and in Chrysobalanaceae and Malphighiaceae the EFNs located in leaf blade. In families like Ochnaceae, Fabaceae, Rosaceae, Malvaceae, Myrsinaceae, Bigoniaceae, Verbanaceae the EFNs are present in the stipules, rachis, petiole, leaf blade, stem (Machado, 2008). Besides in members belong to families Apocynaceae, Boraginaceae, Cactaceae, Compositae, Convolvulaceae, Meliaeceae, Tureraceae the EFNs are located in nodes, bracts and phyllaries also (Cecilia diaz-Castelazo, 2005). The shape of such glands varies very conspicuously in many other species. *Chamaecrista fasiculata* has cup shaped while *Senna marilandica* and *S.hepecarpa* are clavate (Lenore T. Durkee, 1999); as Hair like structures (trichomes) in *Hibiscus pernambucensis* (Joecildo Francisco Rocha, 2009). Elevated EFN in plants such as *Terminalia argentea*, *T.brasilium*, *Lafoensia pacari* and *Enterolobium gummiferum*. Flattened type of glands are present in *Licania humilis*, *Ourateaspectabilis*, and in *O.castanaefolia*it whereas in *Bauhinia rufa*, *Rapanea guianensis*, and *R.lancifolia*, it is has glandular trichomes (Machado, 2008). The macromorphological features viz. shape, size and colour revealed that the EFN of *Cassia hirsuta* is spherical in shape which is attached in the basal portion of the petiole through a very short stalk. The colour of the gland are dark brown to black. With regard to size of these glands, as such there is no report on the size range of glands in the species hence, presently an attempt was made to know about the size range of EFN in *cassia hirsuta* and found that the maximum size of nectar gland ranged between 1-2mm diameter. Further the size also could be categorized into 3 types namely the glands in the middle portion of the whole plant is little larger than the other two i.e. lower and terminal portion. The number of glands in each plant differ from one another as glands are present at the base of each petiole evenly. The no. of glands is depending on the number of compound leaf in a plant which in turn vary according to the age of the plant. Further it is also observed that attachment/emergence of these glands from petiole indicated that the vascular bundle of the main stem is extending in to the glandular part; rather it terminates at the base of the gland itself and hence EFN gland in *Cassia hirsuta* becomes non-vascularised. In most of the members in Caesalpeniaceae, the EFN glands are vascularised i.e. the gland has the supply from the vascular system of the plant and they are also involved in secretion and transport of such secretion ; but in few members of Malvaceae such as *Eriotheca gracilipes*, are non-vascularised similar to a condition found in *Cassia hirsuta* studied presently.

SEM images were examined to understand how nectar from the gland comes out as a food/attractant and made available to foraging ants at the surface. SEM images showed ruptured regions on the surface of the epidermis and these ruptures are more in the larger gland in a single plant; it is also noticed that the surface of the gland from terminal portion of the plant is not having such rupture and then ruptures are not very clear in the glands from basal portion of the plant (spent gland); the former condition may be due to immature nature of the gland; later is the one that has shrunk due to age. Visiting of ants to such larger glands with ruptured epidermis are more frequent than in smaller glands (spent and immatured). Therefore, in view of the SEM images and frequency of ants visit noticed in the field, it is presumed that the epidermis of EFN gland on its maturation the size of the gland increases resulting ruptures thro which nectar oozes out. To feed on the nectar, ants move around such matured glands for their food. Such observations are in close conformity with the observations made in *Cucurbita maxima by* Fernando Lopez-Anido (2007) that when the gland matures cuticle rupture occurs through which the nectar ooze out. It is to be highlighted presently that though present investigation has recorded ecologically and taxonomically important morphological structural attributes of EFN gland in *Cassia hirsuta* for the first time, still there is a need to study the anatomical/histological features of glandular cuticle/epidermis in relation to different phases/stages in secretion in *Cassia hirtusa.* Thus, present findings on relating to gland location in the plant, size, shape, colour of EFN and its maturity based distribution along the plant and the non-vascularised characteristic of *Cassia hirsuta* would help to understand not only evolutionary significance and taxonomical studies but also to understand whether EFN and its characteristics, are species specific among the members of the Genus Cassia.

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