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RESEARCH ARTICLE

STUDIES ON MICRO-MORPHOLOGICAL CHARACTERISTICS OF EXTRA FLORAL NECTAR -SECRETING GLANDULAR STRUCTURES IN *CASSIA ALATA* 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 alata* are examined and presented with relevant images.

INTRODUCTION

Extrafloral nectaries (EFNs) are nectar-secreting vascularized or non-vascularized structures not directly involved with pollination (Elias, 1983; Koptur, 1992a), which are especially common on leaves, petioles, young stems, stipules and reproductive structures (e.g. buds, calyx, inflorescence axis, flower peduncles, fruit) (Rico-Gray, 1989, 1993; Rico-Gray *et al*., 2004)and not involved in pollination occurring in at least 66 families (Elias, 1983) most of the members of these families are in the tropics. Even though the debate over the role of extrafloral nectaries has lasted over a century, numerous scientists are still attracted by the role these small, fascinating structures play in the life of a flowering plant (Bentley, 1977; Koptur, 1992).Seibert 1948 made use of nectarines in a taxonomic consideration of the family Bignoniaceae. Leonard 1957 separated the genera Gilbertiodendron and Pellegriniodendron from Macrolobium Schreb on the basis of nectarines. The diversity of EFN shapes and locations has been used for taxonomic purposes (e.g. Irwin and Barneby, 1982; Randell, 1988, 1989; Marazzi *et al*., 2006*b*).Given such taxonomic value of EFNs (Bhattacharya and Maheshwari, 1970; Lersten and Brubaker, 1987), and their role in ant-plant interaction, studies on pure morphological descriptions of these nectar-secreting glands have become the concerned topic of taxonomists and ecologists. However, information on the secretory rates and gland morphology of the EFNs of different plant species have currently an aggregated value: these features influence plant attractiveness to nectar-foraging insect visitors (Apple and Feener, 2001;Hossaert-Mckey *et al*., 2001); thus affecting the outcome of the interaction among plants, their mutualistic insect visitors (i.e. ants) and herbivores (Rudgers and Gardener, 2004; Wa¨ckers and Bonifay, 2004). Bhatachria and Maheswari (1970) indicated based on phylogenetic clad that EFN is not present in *Cassia alata* But reports of Massazi *et al* (2013) is contradictory to findings of Bhatacharia and Maheswari (1970) quoting suitable evidence that *Cassia alata* has extra floral nectar secreting tissue just on the ventral side of stipules embedded within the tissue of the bearing organ. Keeping these contrasting finding on the presence of nectariferous tissues in *Cassia alata*, presently an attempt has been taken to examine the actual condition in *Cassia alata* in view of EFN.

METHODS AND MATERIALS

Description of the plant

Cassia alata, the candle bush, is an important medicinal shrub, as well as an ornamental flowering plant in the subfamily Caesalpinioideae. The plant is also known as a candelabra bush, empress candle plant, ringworm tree, or candleshrub. A remarkable species of Senna, was sometimes separated in its own genus, Herpetica. S. alata is native to Mexico, and can be found in diverse habitats. In the tropics, it grows up to an altitude of 1,200 m. It is an invasive species in Austronesia. In

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Sri Lanka, it is used as an ingredient in Sinhala traditional medicine. The shrub stands 3–4 m tall, with leaves 50–80 cm long. The inflorescence looks like a yellow candle. The fruit, shaped like a straight pod, is up to 25 cm long. Its seeds are distributed by water or animals. The leaves close in the dusk. *Cassia alata* is chosen for study of extrafloral nectar. *C.alata* a is growing within our campus itself. Observations on the extra floral nectary substances/gland are made in relation to presence and visitation of ants into such plant parts. A thorough observation on the whole plant-from base to tip; of the each branch are made; similar observation was made in 10 healthy plants. Photographs are taken on specific sites on the plant for both gland and ants. Matured and spent/withered parts of the stipule are also photographed with and without plant. The stipules are observed under Digital portable but computer guided microscope and recorded.

Fig.1 *Cassia alata* growing in the wild

RESULTS

Fig &3 There exists variation in colour and size of stipular gland within the same plant. These stipules present in the middle of the plant is healthy and one end is brightly pinkish (Fig. 2) whereas those at the base of the plant, are found to be withered and shrink with dark brown or black colour(Fig.5). The stipules on the upper growing ends/branches are slightly coloured but fresh. The colour variation and healthyness of these glandular stipules indicate matured status of the stipule and the same point is also further confirmed by the presence of nectar under the ventrally folded stipular lobe (Fig.3&4); on the other hand, the shrunk/withered stipules with dark black indicate the spent stage of the stipule; thirdly, the fresh but smaller size and slightly pinkish colour, indicate the maturing stage of the glandular stipule lobe(fig.).

Presence of such three stages of stipule in a single plant is further reinforced by the presence and frequency of ants visitation to these stipules i.e.most of the individual ant Crematogaster sp visited and tended the nectar present under the stipular lobe (Fig.). Ants visitation to such glands are also varied much; more ants both in number and variety are noticed tending on such glands and almost none of the ant visited those withered and shrink with dark colour at the base of the plant. Figures2,3 and 4 show the basal tissue of the stipule by it are attached to the main plant.

Fig. 2 Position of Stipule at one node

Fig.3 Stipules at each node

Arrows indicate stipules

Fig.4 *Cromatogaher lineatus* tending on nectar below the ventrally folded stipule

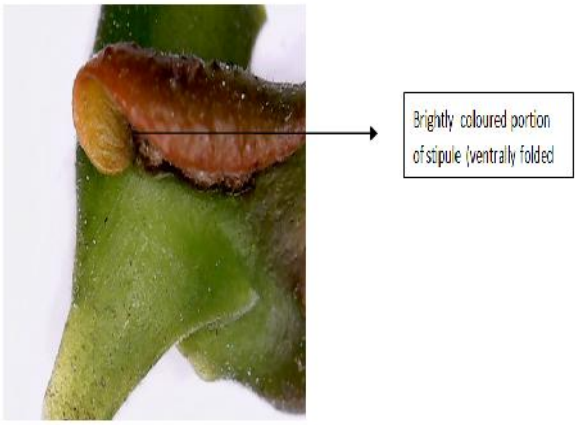

Fig.5 Ventrally folded stipule with distinct coloration (x500)

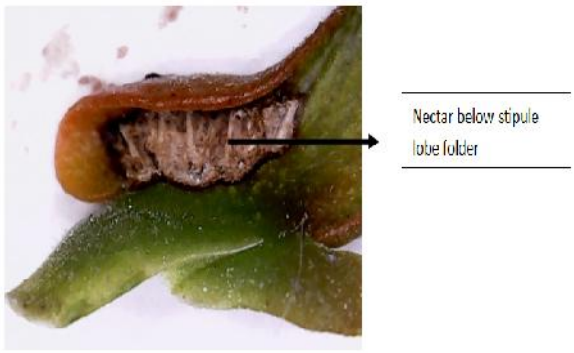

Fig.6 Nectar seen under Ventrally folded stipule lobe (x500)

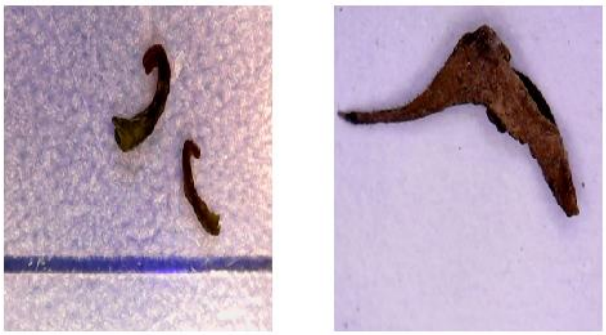

Fig.7 Spent stipule in different stages

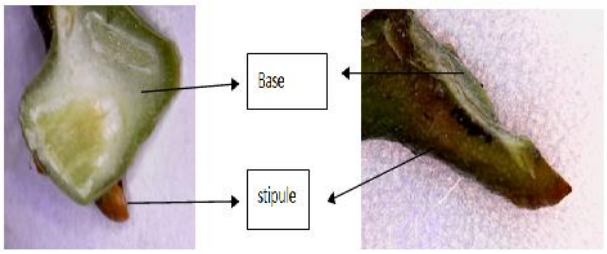

Fig. 8 Base of stipule attaching to plant

DISCUSSION

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*. A wide range of plant parts can bear EFNs. They are found on several different vegetative plant parts (e.g. leaves), as well as on inflorescences (e.g. pedicels) and the outside of the outer floral organs not directly involved in pollination (e.g. sepals). Morphologically, EFNs represent a heterogeneous multitude of secretory 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 (Elias and Gelband, 1976; Fahn, 1979; Schmid, 1988; Vogel, 1997;Bernardello, 2007). One such type of EFN tissue embedded within EFN-bearing plant parts-stipule has been found in *Cassia alata*.

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; oak *et al*. 2007). In *cassia alata* , the presence of actual gland is reported be absents (Bhatachriaa and Maheswari 1970) but reports of Massazi *et al* 2012) with relevant evidences reported that extra floral nectar secreting tissues are present in *cassia alata* just on the ventral side of stipules at each node. Keeping these contrasting finding on the presence of nectariefresous tissues in *Cassia alata*, presently an attempt has been taken to examine the actual condition in *Cassia alata* in view of EFN. The observation in 100 individual *C.alata* plant revealed obviously that *Cassia alata* possesses EFN tissues in the ventrally folded paired stipules located at both sides of base of rachis (Fig 2&3). Same type of location was also reported by Massazi *et al* (2010) embedded within the tissue of the bearing organ. One half of the fold is little bulged with bright pinkish colour and the other end is greenish (Fig. 4). There exists variation in colour and size of stipular gland within the same plant. These stipules present in the middle of the plant is healthy and one end is brightly pinkish whereas those at the base of the plant, are found to be withered and shrink with dark brown or black colour(Fig 5). The stipules on the upper growing ends/branches are slightly coloured but fresh. The colour variation and healthyness of these glandular stipules indicate matured status of the stipule and the same point is also further confirmed by the presence of nectar under the ventrally folded stipular lobe; on the other hand, the shrunk/withered stipules with dark blackness indicate the spent stage of the stipule; thirdly, the fresh but with smaller size and slightly pinkish colour, indicate the maturing stage of the glandular stipule lobe (Fig 5&7). Presence of such three stages of stipule in a single plant is further reinforced by the presence and frequency of ants visitation to these stipules i.e. most of the individual ant-Crematogaster sp. visited and tended the nectar present under the stipular lobe (Fig 4) and even this tending is done only at the brightly pinkish part of the stipule. But the visitation of ants to other two stages of maturity is very less. Thus, the field observation also confirms the presence of three stages of stipules viz. spend, matured and maturing in relation to nectar secretion. Similar observation on the different stages in terms of maturity and nectar secretion in *Cassia hirsuta* (Sharmila and Yogamoorthi, 2015) and variation in the frequency of ants' visitation. The modified stipular EFN tissue with their secretions are also visible under digital microscope at the ventral side of the stipular lobe (Fig.6). Figures 7 &8 show the basal tissue of the stipule attached to the main plant...

Further Marrazi *et al* (2013) have found out two distinct kinds of EFNs existing in two unrelated clades within Senna. 'Individualized' EFNs (iEFNs), located on the compound leaves and sometimes at the base of pedicels, display a conspicuous, gland-like nectary structure, are highly diverse in shape and characterize the species-rich EFN clade. Previously overlooked 'non-individualized' EFNs (non-iEFNs) embedded within stipules, bracts, and sepals are cryptic and may represent a new synapomorphy for clade II. Leaves bear EFNs consistently throughout. Clade II exclusively exhibits cryptic EFNs that are integrated into partly modified, existing organs. The stipule blade may be glabrous or covered by trichomes and is strongly asymmetric and cordate, forming one lobe that is modified in colour and thickness and includes the secretary tissue.Two distinct kinds of EFN morphologies exist in *Senna*, which is interpreted in relation to their degree of individualization, i.e. morphological differentiation and specialization, with respect to the organ that bears them. The newly discovered, cryptic EFNs are non-individualized EFNs (non-iEFNs), i.e. stipules, bracts and sepals that are EFN-bearing organs, in which specific zones differentiate into nectar-producing structures. Such structures consist of nectariferous tissue embedded in the coloured stipule lobe. Since EFNs in *Senna* display a remarkable diversity in location and morphology. They are found on five different parts. (1) leaves, (2) stipules, (3) along the inflorescence axis at the base of pedicel, and on the dorsal side of (4) bracts and (5) sepals and in the present study, it is confirmed that nectar secreting tissues are ventrally embedded in the stipular lobe in *Cassia alata*. Futher anatomical studies would bring out the internal architecture of nectar secreting tissues and also the mechanism behind the oozing of secretions to be fed by ants, the herbivore defender of the host plant.

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